ACUTE HAEMODYNAMIC EFFECTS OF THREE CARDIOACTIVE AGENTS : METOPROLOL, SOTALOL AND MILRINONE. INFLUENCE OF MYOCARDIAL CONTENT AND SYSTOLIC INTERVAL.

by

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DECLARATION

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PUBLICATIONS AND COMMUNICATIONS TO LEARNED SOCIETIES IN SUPPORT OF THESIS

Some of this thesis has been accepted for publication, or communicated to learned societies within Australia or overseas.

Publications

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SUMMARY

The acute effects of cardioactive agents are determined, at least in part, by the process of their uptake into the heart. However, for several drugs at least, there is evidence that effects are also modulated by inter-beat interval, leading to accentuation of haemodynamic effects of some agents during tachycardia. The potential for this phenomenon to be beneficial or deleterious is largely unknown, except for a few agents.

Utilizing a paired transcoronary sampling technique, serial determination of myocardial drug content was determined following intravenous bolus injection in patients undergoing diagnostic cardiac catheterization for the investigation of chest pain. During this procedure, serial determinations of various haemodynamic, electrocardiographic, and electrophysiological parameters were obtained. Examination of the potential for rate-related inotropic effects involved construction of a component of the mechanical restitution curve (MRC), utilizing atrial pacing with insertion of premature beats at progressively shorter diastolic intervals. MRC construction permits evaluation of a surrogate of tachycardia without significant risk of induction of ischaemia. A curve-fitting model for the component of the MRC associated with diastolic intervals shorter than those at spontaneous heart rate in man was developed, to facilitate accurate quantitation of drug effects.

Three cardioactive agents were chosen for study :

(i) metoprolol, as a classical $ß_1$-selective adrenoceptor antagonist, which has been associated with reduced mortality after acute myocardial infarction;

(ii) sotalol, a non-selective ß-adrenoceptor antagonist which also possesses class III antiarrhythmic effects, and which appears to be the most effective agent currently available for the management of ventricular tachyarrhythmias; and

(iii) milrinone, a phosphodiesterase III inhibitor, which exerts both positive inotropic and vasodilatatory effects. Milrinone appears to increase risk of cardiac death during long-term administration. However, the mechanism of these deleterious effects is uncertain.

Metoprolol, a relatively lipophilic ß-adrenoceptor antagonist, has a short elimination half-life (3-4h) and a reasonably high apparent volume of distribution. Following intravenous bolus administration (4mg), peak myocardial content was achieved within three minutes of injection. Conversely, peak haemodynamic and electrocardiographic effects were not observed until 5-10mins post administration, indicating a time lag between content and effect. Significant reductions in spontaneous heart rate and LV +dP/dt at constant heart rate were observed, accompanied by prolongation of PR intervals. However, the negative inotropic effects of metoprolol became progressively diminished with reductions in extrasystolic interval, indicating "reverse use-dependent" negative inotropic effects. Utilizing Langendorff-perfused rat hearts, the influence of a period of hypoxia on the uptake process of metoprolol was assessed : while myocardial uptake was not significantly modified by the hypoxic perfusion conditions, the efflux of the drug from the heart was impaired.

Sotalol, a relatively hydrophilic ß-adrenoceptor antagonist, has a longer elimination half-life than metoprolol (≥7h) and a lower apparent volume of distribution. Despite this, following intravenous bolus administration (20mg), peak myocardial content was achieved less than one minute after injection. Peak haemodynamic, electrocardiographic and electrophysiologic effects were not observed until 10minutes post administration. Significant reductions in spontaneous heart rate and LV +dP/dt at constant heart rate were observed, accompanied by prolongation of PR and AH intervals, and atrioventricular nodal effective refractory periods. However, the negative inotropic effects of sotalol tended to be augmented during pacing-induced tachycardia, suggesting a small degree of use-dependence of sotalol's negative inotropic effects, in contrast to the reverse use-dependence exhibited for the electrophysiological effects of both the racemate and the d-enantiomer *in vitro* .

Milrinone, a phosphodiesterase inhibitor, has a short elimination half-life (1-2h) and a low apparent volume of distribution. Following intravenous bolus administration (1mg), peak myocardial content was also achieved less than one minute after injection. Again, peak haemodynamic effects were not observed until 7 - 10mins post administration. Significant in -

creases in spontaneous heart rate and LV+dP/dt at constant heart rate were observed, accompapnied by reductions in PR intervals, LV systolic and mean arterial pressures. The positive inotropic effects of milrinone were significantly less marked with progressive reductions in systolic interval in isolated premature beats.

Thus, for all agents studied, acute drug uptake into the human heart precedes attainment of maximal effects. There was significant modulation of the haemodynamic effects of all three drugs according to changes in systolic interval. Therefore for some cardioactive agents, the relationship between myocardial drug content and acute effects is determined by baseline haemodynamic status.

CHAPTER 1 : GENERAL INTRODUCTION

1.1 Overview

Determination of the short-term myocardial drug content profile permits a better understanding of the basis of a cardioactive agent's acute effects, in terms of both the time course of these effects, and accurate differentiation between cardiac and extracardiac effects. This determination also facilitates examination of the validity of conventional pharmacokinetics as an accurate tool for monitoring a drug's acute pharmacodynamic effects. The disposition of cardioactive drugs within the myocardium has, in previous studies in isolated tissues (Gillis and Kates 1986; Gillis and Keashly 1991), and in *in vivo* studies in experimental animals including dogs (Anderson *et al* 1980a,1980b; Keefe and Kates 1982) and sheep (Upton *et al* 1988), and in humans with ischaemic heart disease (Horowitz *et al* 1986; Powell *et al* 1990b), been identified as a major determinant of the acute effects of the agent under investigation.

Accumulation of a drug by the myocardium, and the resultant acute haemodynamic, electrocardiographic (ECG), and electrophysiological (EP) effects of cardioactive drug administration also require accurate determination, in order to maximally optimise therapeutic management of cardiovascular disease. Additionally, the pharmacodynamic actions of many drugs can potentially be influenced by other factors, one of which is modulation of heart rate, as demonstrated in a number of studies (Sharma *et al* 1990; Hammermeister 1990; Akiyama *et al* 1991; Stewart *et al* 1987; Rankin *et al* 1987; Buxton *et al* 1987; Switzer *et al* 1986). As a specific example, use-dependent effects of both sodium and calcium channel antagonists have been demonstrated in various investigations, whereby drug effects are augmented during tachycardia, but less marked during sinus rhythm (Sharma *et al* 1990; Hammermeister 1990; Akiyama *et al* 1991; Stewart *et al* 1987; Rankin *et al* 1987; Buxton *et al* 1987; Switzer *et al* 1986).

Thus, understanding of the acute myocardial drug uptake profile, the time course of subsequent pharmacodynamic effects, and the influence of increased heart rates on these effects may assist the evaluation of the optimal clinical roles of cardioactive drugs, so they may be administered more wisely.

1.2 Acute effects of cardioactive drugs

There a number of key issues warranting consideration when discussing the acute effects of cardioactive agents. Drug administration to patients in the setting of systolic dysfunction and/or ischaemia may create situations in which otherwise beneficial effects may be accentuated or impaired, or potentially harmful doses less well tolerated. For example, despite adequate initial responses in patients with cardiac disease with only moderately reduced ejection fractions and mildly elevated pulmonary capillary wedge pressures, the haemodynamic responses to verapamil in patients with severe ventricular dysfunction were considerably augmented to the point of acute haemodynamic deterioration (Chew *et al* 1981).

Further examples of altered haemodynamic responsiveness to cardioactive agents in a variety of disease states, particularly ischaemia / hypoxia, have been explored in animal studies (Gillis and Keashly 1991; Ku and Lucchesi 1979; Culling *et al* 1984; Nayler *et al* 1985; Anderson *et al* 1983; Cobbe *et al* 1985). These conditions have been demonstrated to augment pharmacodynamic effects of quinidine in isolated perfused rabbit hearts (Gillis and Keashly 1991) and the incidence of digitalis-induced arrhythmias (Ku and Lucchesi 1979). Conversely, the class III antiarrhythmic effects of sotalol were diminished in isolated Langendorff-perfused guinea pig hearts, and isolated arterially perfused interventricular septum of rabbit hearts during both ischaemia and the subsequent reperfusion (Culling *et al* 1984; Cobbe *et al* 1985a), and in rabbit papillary muscles exposed to severely hypoxic conditions (Cobbe *et al* 1985).

The extent of pharmacodynamic effects of a cardioactive drug are also subjected to modulation of receptor number and affinity (Nayler *et al* 1985; Anderson *et al* 1983). Examples of this phenomenon include the ischaemia-induced reduced affinity of low affinity binding sites, the decreased density of both low and high affinity dihydropyridine binding sites (Nayler *et al* 1985), and reduced ventricular fibrillation threshold (Anderson *et al* 1983). Thus, in addition to determining influence of changes in heart rate on acute myocardial effects, fundamental myocardial health must also be considered when attempting to identify the major determinants of drug-induced changes in pharmacodynamics.

1.2.1 Drug effects

Acute cardioactive drug administration can induce significant changes in a wide range of haemodynamic, electrocardiographic (ECG) and electrophysiologic (EP) effects. Systemic haemodynamic parameters that may be affected include : mean arterial pressure (MAP); cardiac output (CO); systemic (SVR) and pulmonary vascular resistances (PVR). Coronary haemodynamic indices include : coronary blood flow (CBF; often determined using thermodilution techniques : Ganz *et al* 1971); coronary vascular resistance (CVR); and myocardial oxygen consumption (MVO_2).

While optimal methodology remains controversial, myocardial contractility can be crudely measured as left ventricular ejection fraction, and/or more accurately, utilizing a micromanometer-tipped catheter positioned in the left ventricle, for determination of the sensitive contractile index LV +dP/dt, the peak rate of rise of LV pressure (Broughton and Korner 1980; Seed and Walker 1988; Barnes *et al* 1979; Quinones *et al* 1976; Zimpfer *et al* 1981). Other contractile indices include :

(i) E_{max} (maximum slope of the line of the nexus between ventricular pressure and volume during systole);

(ii) V_{CF} (circumferential fibre shortening velocity);

(iii) dP/dt/P (dP/dt relative to simultaneously determined LV pressure);

(iv) dP/dt_{DP40} (dP/dt when LV pressure 40mmHg);

and (v) LV+dD/dt (maximum rate of change of LV diameter).

Examination of the pressure-volume relation of the left ventricle, from which E_{max} is determined, is possibly a more reliable index of changes in systolic function than LV+dP/dt (Sagawa 1981; Starling 1989; Kass *et al* 1987; Suga *et al* 1973), but construction of pressure-volume loops is not suitable for serial measurement of myocardial contractility. In most circumstances, peak LV+dP/dt is attained prior to ejection, and therefore represents a measure of contractile force : contractile indices based on changes in ventricular volume or length (eg LV internal diameter, V_{CF}), flow, or pressure during ejection, do not. These indices are also considerably more sensitive than LV+dP/dt to fluctuations in both afterload and preload (Seed

and Walker 1988; Barnes *et al* 1979; Quinones *et al* 1976; Zimpfer *et al* 1981; Mahler *et al* 1975; Kass *et al* 1987). The only major limitation of LV+dP/dt as an index of inotropic state is its dependence on heart rate (Broughton and Korner 1980; Barnes *et al* 1973; Quinones *et al* 1976) : this is easily overcome by measuring dP/dt while maintaining a constant heart rate with atrial pacing

ECG parameters include PR, QT intervals, ST segment changes, QRS duration and sinus cycle length, all measurable from a surface ECG. AH and HV intervals, and the refractory periods of atria, ventricles, and the atrioventricular node all constitute EP parameters that can potentially be altered by cardioactive drugs and readily determined employing intracardiac catheters.

Precise knowledge of the acute time course of a cardioactive drug's acute effects is of relevance also. Examples include pharmacological reversion of tachyarrhythmias, acute myocardial ischaemia, and low cardiac output syndrome following cardiac surgery, where the selection of a fast-acting drug may be highly critical. Of additional importance, the haemodynamic, ECG and EP effects elicited by cardioactive drug administration may not all be beneficial. Toxic effects, including arrhythmogenesis, haemodynamic deterioration, exaggerated bradycardia and ischaemia, may result, limiting well-being and prognosis. Understanding of the myocardial concentration profile of the drug concerned might aid in the prediction of the duration and extent of these effects.

The chronic effects of a cardioactive drug may not necessarily be predicted from the acute effects. Factors including tolerance development, changes in receptor density and/or affinity for particular cardioactive agents, as well as changes in autonomic tone could all potentially modify long-term cardioactive agent-induced effects. Additionally, chronic drug therapy permits a role for conversion of the parent drug to one or more pharmacologically active metabolites, ie the parent compound may act as a pro-drug.

1.2.2 Determinants of drug effects

The major determinants of cardioactive drug effects include

(i) the acute uptake process,

(ii) the myocardium as a substrate for drug effects,

(iii) instantaneous cardiac functional and metabolic state, and finally,

(iv) the factors contributing to the receptor sensitivity to the drug.

The concentration of cardioactive drug within the myocardium as a major determinant of acute effect (Anderson *et al* 1980a, 1980b; Gillis and Kates 1986; Keefe and Kates 1982; Horowitz *et al* 1986; Upton *et al* 1988; Powell *et al* 1990b; Gillis and Keashly 1991), has been demonstrated for a variety of compounds, including : propafenone (Gillis and Kates 1986) and quinidine (Gillis and Keashly 1991) in the isolated perfused rabbit heart; verapamil (Keefe and Kates 1982) and bretylium (Anderson *et al* 1980a, 1980b) in dogs; and for lignocaine, mexiletine and verapamil in sheep and humans (Upton *et al* 1988; Horowitz *et al* 1986; Powell *et al* 1990b). As an example, the antiarrhythmic effects of bretylium were more closely related to myocardial than serum concentrations (Anderson *et al* 1980a, 1980b).

The myocardium as a substrate for direct drug effects has, for example, been studied in the comparison of an intravenous milrinone bolus with an intracoronary infusion in patients with severe congestive heart failure (Colucci *et al* 1985; Ludmer *et al* 1986) : milrinone was demonstrated to possess direct cardiotonic activity independent of its vasodilatatory actions (Colucci *et al* 1985; Ludmer *et al* 1986).

Instantaneous cardiac functional state, as distinct from resting cardiac state, may also be influential in the determination of acute cardiac effects. For example, tachycardia augmented myocardial accumulation of both mexiletine and lignocaine by the human myocardium *in vivo* (Horowitz *et al* 1986). Similarly, increased mechanical activity accelerated myocardial accumulation of a range of drugs by isolated guinea pig atria (Lullmann *et al* 1979) and of ouabain in open-chested dogs (Lloyd and Taylor 1978).

The presence of ischaemia may also impair accumulation of drugs by the myocardium. This phenomenon of reduced myocardial cardioactive drug concentrations in regions of impaired blood flow has been demonstrated for a number of agents, including : metoprolol, atenolol (Ablad *et al* 1987), procainamide (Avitall *et al* 1990; Wenger *et al* 1978, 1980), digoxin (Ku 1983), and lignocaine (Patterson *et al* 1982) in canine; and metoprolol and atenolol in both feline (Ablad *et al* 1987) and porcine models of myocardial ischaemia / infarction (Ablad *et al* 1987; Ryden *et al* 1990, 1991). In addition to impairment of maximal myocardial concentrations (Wenger *et al* 1980; Gillis and Keashly 1991), ischaemia / hypoxia also slows the rate of myocardial accumulation (Wenger *et al* 1980). Furthermore, accumulation of cardioactive drugs by regions of ischaemic myocardium is not uniform for all compounds, but is influenced by a number of factors, possibly including lipophilicity (Ablad *et al* 1987). The greater lipophilicity of metoprolol for example, may, at least in part, explain its more extensive distribution to ischaemic myocardium than another ß1-adrenoceptor antagonist atenolol, in canine, porcine, and feline models of ischaemia *in vivo* (Ablad *et al* 1987).

The determinants of receptor sensitivity to a drug can also influence the acute effects. These include up- or down-regulation, rate-related receptor interactions, and ischaemia-related receptor interactions. The down-regulation of ß-adrenoceptors observed in the clinical settings of old age, and chronic heart failure (Vestal *et al* 1979; Colucci *et al* 1981; Waagstein *et al* 1989; Fowler *et al* 1986; Heilbrunn *et al* 1989) for example, results in reduced sensitivity to both ß-adrenoceptor agonists and antagonists (Vestal *et al* 1979; Colucci *et al* 1981; Fowler *et al* 1986; Heilbrunn *et al* 1989), although in severe heart failure, ß-adrenoceptor affinity may be slightly improved (Waagstein *et al* 1989).

The protracted time course of interaction between inotropic agents and cellular effector mechanisms has previously been shown to persist in the absence of significant diffusion barriers within myocardial cells and the perfusate and thus represents a true "biochemical lag phase" (Horowitz *et al* 1982; Barry *et al* 1985; Horowitz and Powell 1986). Briefly, the time course of accumulation of calcium antagonists was not parallel to the time course of negative inotropic effects in a monolayer culture of isolated myocardial cells, a situation in which

minimal obstacles exist between drug uptake and effect, aside from diffusion across the cell membrane (Horowitz *et al* 1982; Barry *et al* 1985; Horowitz and Powell 1986).

An illustration of a rate-related receptor interaction is the augmented inhibition of the slow calcium channel current induced by verapamil at high stimulation frequencies, demonstrated in voltage-clamp experiments in isolated cat papillary muscles (Ehara and Kaufmann 1978). This is termed use-dependent block, and suggests that the antagonist (or indeed an agonist) interacts preferentially with its receptor when the membrane is depolarized (Ehara and Kaufmann 1978). Receptor / substrate interactions can also potentially be influenced by ischaemia, as demonstrated by : the enhanced negative inotropic effects of nifedipine; and the impaired negative inotropic effects of verapamil; in canine models of acute myocardial ischaemia (Urquhart *et al* 1984).

1.3 Myocardial drug uptake of cardioactive agents

As discussed above, myocardial drug content has been correlated with inotropic and antiarrhythmic effects of a number of cardioactive agents, in both whole animal and *in vitro* studies (Anderson *et al* 1980a, 1980b; Gillis and Kates 1986; Keefe and Kates 1982; Horowitz *et al* 1986; Upton *et al* 1988; Powell *et al* 1990b; Gillis and Keashly 1991). It has also been demonstrated that accumulation of drugs by the heart is influenced by : beating rate (Lullman *et al* 1979; Horowitz *et al* 1986; Lloyd and Taylor 1978); presence of ischaemia (Avitall *et al* 1990; Wenger *et al* 1978, 1980; Patterson *et al* 1982; Ablad *et al* 1987; Ryden *et al* 1990, 1991; Gillis and Keashly 1991); and also the mode of administration (Ryden *et al* 1990, 1991). However, extrapolation of these findings from animal studies is not necessarily appropriate to the clinical situation. Antiarrhythmic, antiischaemic and positive inotropic agents are often administered to patients intravenously in the in-hospital phase, with the assumption that a predictable relationship exists between administration of the drug and subsequent cardiac effects. This assumes however, the predictability of acute myocardial accumulation of a range of cardioactive agents, and its relation to acute cardiac effects *in vivo* .

1.3.1 Methods of assessment in humans *in vivo*

Because of logisitic difficulties, measurement of myocardial drug content is not usually conducted during emergencies, despite its desirability in theory, and therefore these circumstances need to be simulated. There are four major ways to determine myocardial drug content in humans. Firstly, myocardial tissue can be collected from patients at autopsy (Latini *et al* 1987). This technique is questionable however, because of the randomness of drug therapy, the single sampling timepoint and obvious selection bias. Nevertheless, very high levels of flecainide have been reported, particularly in the left ventricle, at the autopsy of a patient who had died suddenly after acute onset of dyspnoea following four months of chronic oral flecainide therapy (Latini *et al* 1987).

Myocardial biopsy, either while the patient is undergoing cardiac surgery or diagnostic cardiac catheterization for the investigation of chest pain, is a second option (Jogestrand 1980), which unfortunately is extremely invasive, time-consuming and regional. However, Jogestrand (1980) successfully detected significant digoxin concentrations in samples obtained from serum, right atrial myocardium and skeletal muscle in patients in either sinus rhythm or atrial fibrillation at the time of open heart surgery. Similar studies examining myocardial right atrial tissue biopsy samples have also been published for propranolol, verapamil, and digoxin (Plachetka *et al* 1981; Padrini *et al* 1985; Hartel *et al* 1976).

Imaging techniques such as positron emission tomography (PET) or thallium scanning, incorporating administration of a radiolabelled drug, offer a more indirect means of assessing myocardial drug concentrations (Charbonneau *et al* 1986). Myocardial concentrations of an ^{11}C-labelled benzodiazepine receptor antagonist in three regions of interest have been demonstrated with PET in human volunteers (Charbonneau *et al* 1986). While accurate determination of regional myocardial drug uptake is possible with such scanning techniques, there is no capacity for serial sampling, attributable to the acquisition time of the camera. Furthermore the technique is extremely expensive, and imposes an inevitably considerable radiation exposure on the subject.

Finally, there is the technique of paired transcoronary sampling at the time of cardiac catheterization (Horowitz *et al* 1986). Despite the labour intensity and invasiveness of this approach, instantaneous myocardial drug uptake or efflux at any time point up to 30min after an intravenous bolus can be obtained. The instantaneous myocardial drug uptake is then calculated as the product of the coronary arteriovenous concentration gradient and simultaneously determined coronary sinus blood flow.

1.3.2 Results of previous studies

A limited number of studies in man have previously been reported utilizing the technique of coronary sinus catheterization for determination of myocardial drug uptake (Horowitz *et al* 1986; Powell *et al* 1990a, 1990b). Maximal myocardial content of lignocaine and mexiletine in humans was demonstrated to occur at 2.4±0.2 and 5.5±0.6min after intravenous bolus injection respectively : in both cases, a period of nett myocardial efflux followed for at least 30min post administration (Horowitz *et al* 1986). When heart rate was increased via coronary sinus pacing to 100beats/min prior to injection, and continued for the first 2min of determination of myocardial drug uptake, time of attainment of peak myocardial drug content did not change (Horowitz *et al* 1986). However, the extent of drug accumulation by the myocardium was significantly enhanced for both mexiletine and lignocaine, demonstrated by approximately 80% and 45% higher values of peak myocardial drug content for the two antiarrhythmic agents respectively (Horowitz *et al* 1986). This period of pacing did not influence the later myocardial drug efflux phase of either agent (Horowitz *et al* 1986), although a different finding may have resulted had the faster heart rate been maintained for the duration of the research protocol.

Similarly, following a 4mg intravenous bolus injection, peak myocardial verapamil content (expressed relative to resting coronary sinus flow) of 475±31ng per ml/min, representing 1.2±0.2% of the total injected dose, was achieved 5.4±0.4min after injection (Powell *et al* 1990b). Gradual nett verapamil efflux from the myocardium was then observed, with approximately 70% of peak myocardial content still present 30min post bolus administration (Powell *et al* 1990b). While not influenced by baseline systolic function, peak myocardial

verapamil content was inversely proportional to the extent of fixed coronary artery disease in this study (Powell *et al* 1990b). The extent of maximal myocardial verapamil content was positively correlated with the extent of AH and PR interval prolongation, although a lag was observed between the time course of the two (Powell *et al* 1990b).

Two phases of myocardial digoxin uptake were observed in an additional study examining the time course of myocardial uptake of a 500μg intravenous bolus digoxin dose (Powell *et al* 1990a). A rapid early phase of myocardial uptake, indicated by an early large difference between femoral artery and coronary sinus concentrations in whole blood, was followed by a a subsequent reduction in the transcoronary digoxin concentration gradient indicative of a second, slower phase of nett uptake (Powell *et al* 1990a). In many patients, coronary sinus blood digoxin concentrations failed to reach those in the femoral artery, therefore not attaining a maximum even after 30mins of sampling (Powell *et al* 1990a). The extent of myocardial digoxin content was not influenced by systolic function or the degree of coronary artery disease prior to administration (Powell *et al* 1990a). As for many of the changes induced by verapamil, the augmentation of LV +dP/dt induced by digoxin was not correlated with simultaneous myocardial digoxin content (Powell *et al* 1990a), but this may have been influenced by the failure to reach a maximum level of content in many patients.

1.4 Influence of cardioactive drugs on contractile state

1.4.1 Conventional indices

As previously indicated (see section 1.2.1), despite the availability of a number of potential techniques for the accurate measurement of left ventricular (LV) systolic function in humans, few are suitable for serial studies. Therefore, when studying the effects of drugs on inotropic state invasively in man, the isovolumic index LV+dP/dt is commonly utilized. This peak rate of rise of LV pressure at constant heart rate is a conventional measure of systolic function, requiring placement of a micromanometer-tipped catheter into the left ventricle (Broughton and Korner 1980; Seed and Walker 1988; Barnes *et al* 1979; Quinones *et al* 1976; Zimpfer *et al* 1981). The extent and direction of the acute effects of a cardioactive drug on this index at

baseline heart rate do not, however, provide information regarding the influence of changes in systolic interval on the effects of such drugs on contractile state : for example, how the induction of tachycardia might result in exaggeration or attenuation of the drug's effects.

1.4.2 The staircase phenomenon

It has been known for many years that cardiac muscle alters its contractile behaviour as a result of changes in heart rate. Isolated cardiac muscle rapidly responds to fluctuations in stimulation frequency : termed the staircase or "Treppe" phenomenon, this manifestation, whereby each train of progressively increasing frequency displays a matching increase in muscle tension to a new plateau, has been described in both *in vitro* and *in vivo* experiments (Bowditch 1871; Woodworth 1902; Koch-Weser and Blinks 1963; Pidgeon *et al* 1982; Seed and Walker 1988). Increased intracellular calcium concentrations (from internal stores and / or influx) and catecholamine release have been attributed as possible mechanisms for this observation (Seed and Walker 1988; Koch-Weser and Blinks 1963).

1.4.3 The mechanical restitution curve

The inotropic effects of many cardioactive agents may not be apparent at normal heart rates, but may become unmasked by changes in heart rate. For example, in patients prone to tachyarrhythmias, acute haemodynamic deterioration following initiation of intravenous therapy with several class 1A and 1C antiarrhythmic agents (Sharma *et al* 1990; Gottlieb *et al* 1990; Hammermeister 1990; Akiyama *et al* 1991), and calcium antagonists (Stewart *et al* 1987; Rankin *et al* 1987; Buxton *et al* 1987; Switzer *et al* 1986) has been reported, and in some cases, deterioration has occurred specifically during tachycardia (Sharma *et al* 1990; Switzer *et al* 1986). The potential for this deterioration would therefore influence long-term prognosis. Such problems have not been reported for similar patients treated with ß-adrenoceptor antagonists.

Some cardioactive agents, such as verapamil, may undergo rate-dependent interactions with their receptor mechanisms (Davis *et al* 1986; Chappell *et al* 1985), and this might be the basis

for accentuation of the negative inotropic effects of the drug during tachycardia. Rate-dependent effects of verapamil were demonstrated in voltage clamp experiments in isolated cat papillary muscles (Ehara and Kaufmann 1978). The parameters of a slow channel current were not altered by an increased discharge rate in control preparations, but, in the presence of verapamil, the partial inhibition of this current induced by the drug at slower rates of discharge was considerably augmented at rapid discharge rates (Ehara and Kaufmann 1978).

The force-interval relationship can be examined by construction of mechanical restitution curves (MRC), obtained by inserting isolated test stimuli every few beats during steady-state trains of beats. The relationship between LV+dP/dt of the test stimulus is then plotted as a function of the interval between beats. Construction of the "left half" of the MRC, the component associated with extrasystolic intervals shorter than the cycle length at spontaneous heart rate, permits examination of the force-interval relationship without the requirement for inducing sustained tachycardia, which could change the loading conditions, alter the ventricular dimensions, and may even induce ischaemia. This component of the MRC, in which LV+dP/dt is progressively decreased as the isolated extrasystole is of increasing prematurity, has previously been examined in humans *in vivo* (Seed and Walker 1988; Franz *et al* 1983; Pidgeon *et al* 1982), and a quantitative description in patients with and without ischaemic heart disease has been developed, and is discussed in chapter 3 of this thesis. While the mechanism of this phenomenon is more widely discussed in that chapter, suffice to say that an isolated premature beat is weaker than a steady-state contraction, because of the absence of the haemodynamic or reflex effects normally observed in the setting of increased heart rate *per se* .

1.5 The present study

1.5.1 Current relevant knowledge of the acute haemodynamic effects of the cardioactive drugs under investigation

Metoprolol was selected for investigation as a classical $ß_1$-selective adrenoceptor antagonist, exerting a negative inotropic effect in humans at constant heart rate following an intravenous bolus (Dell-Italia and Walsh 1989; Bourdillon *et al* 1979; Reale *et al* 1979). However, the effect of changes in heart rate on these negative inotropic effects has not previously been described. Metoprolol has been associated with reduced cardiac mortality in patients with acute myocardial infarction, possibly due to a reduction in sudden death (Herlitz *et al* 1983, 1986; Hjalmarson *et al* 1981; The MIAMI Trial Research Group 1985; Lopressor Intervention Trial Research Group 1987). Despite frequent intravenous administration of metoprolol for the management of acute myocardial ischaemia (The MIAMI Trial Research Group 1985; McBoyle *et al* 1983; Halinen *et al* 1989) or tachyarrhythmias (The MIAMI Trial Research Group 1985; Ryden *et al* 1983; Moller and Ringqvist 1979; Wasir et al 1977; Stroobandt and Kesteloot 1981), its immediate myocardial kinetics have not previously been examined in humans.

Sotalol was selected as a ß-adrenoceptor antagonist with additional class III antiarrhythmic properties. These properties are attributable to action potential duration prolongation, resulting from inhibition of the outward delayed rectifier potassium current (Carmeliet 1985; Singh and Nademanee 1987) This prolongation is more marked than observed with conventional ß-adrenoceptor antagonists (Edvardsson and Olsson 1981; Singh and Nademanee 1987). Sotalol also exerts a negative inotropic effect in humans at constant heart rate following an intravenous bolus (Hutton *et al* 1972). The influence of changes in heart rate on these negative inotropic effects, and the acute myocardial sotalol uptake profile, have not previously been reported. Furthermore, sotalol has recently been identified as more efficacious than other antiarrhythmic agents, although the mechanism for this reduction is unclear (Mason *et al* 1993a, 1993b).

The third cardioactive agent chosen for study in this thesis was a positive inotropic / vasodilator agent, milrinone, potentially more useful than ß-adrenoceptor agonists in the management of acute heart failure, because a marked positive inotropic effect can be elicited in the presence of the elevated sympathetic tone (Skoyles and Sherry 1992). The observation of increased mortality associated with milrinone therapy versus placebo in patients with severe heart failure in the recent PROMISE trial (Packer *et al* 1991a, 1991b) could be partially explained by the influence of changes in cycle length on its positive inotropic effects, although arrhythmogenic effects *per se* have been postulated as a mechanism. However, the myocardial uptake profile of milrinone has not previously been examined.

1.5.1.1 Metoprolol

Metoprolol (H 93/26, 1-[4-(2-methoxyethyl)phenoxyl-3-[(1-methylethyl)amino]-2-propanol) is a competitive $ß_1$-selective adrenoceptor antagonist of comparable potency to propranolol without intrinsic sympathomimetic activity (Ablad *et al* 1973). The drug, whose chemical structure is illustrated in Figure 1.1, has been widely used in the past in the treatment of mild to moderate hypertension, and is now in frequent clinical use in the management of acute and chronic myocardial ischaemia and tachyarrhythmias in man. Its beneficial effects in ischaemic heart disease are attributable, at least in part, to a reduction in heart work, thereby decreasing myocardial oxygen demand.

(i) Results of in vitro studies with metoprolol

Studies of metoprolol *in vitro* have been performed in isolated uteri (Abrahamsson *et al* 1988), trachea (Toda *et al* 1978), left (Abrahamsson *et al* 1988; Barrington and Ten Eick 1990), and right ventricle (Manley *et al* 1986), left (Toda *et al* 1978; Doggrell 1991) and right atrium (Toda *et al* 1978; Johansson 1979; Abrahamsson *et al* 1988), coronary (Toda *et al* 1978), pulmonary (Toda *et al* 1978), portal (Johansson 1979; Doggrell 1991), and mesenteric (Toda *et al* 1978) arteries, aorta (Toda *et al* 1978) and soleus muscle preparations (Abrahamsson *et al* 1988) from rat, rabbit, cat, guinea pig, dogs and humans. Of the populations of

ß-adrenoceptors in a variety of cardiac tissues, including left ventricles from rat, guinea-pig, and man, as well as right atrium from rat, 60% have been identified as the $ß_1$-subtype (Abrahamsson *et al* 1988). Conversely, up to 100% of ß-adrenoceptors in the variety of non-cardiac muscles, including rat and human uteri and guinea pig soleus muscles, were identified as the $ß_2$-subtype (Abrahamsson *et al* 1988). The ß-adrenoceptor antagonist metoprolol is approximately 37-fold more selective for $ß_1$- than $ß_2$-adrenoceptors (Abrahamsson *et al* 1988).

Metoprolol induced a shift of the isoprenaline dose / chronotropic response curve to the right in isolated rat atria (Johansson 1979; Doggrell 1991), without affecting the responses to cardiac electrical stimulation, or to stimulation combined with isoprenaline (Doggrell 1991). Conversely, only high concentrations had a slight influence on spontaneous contractile activity in the isolated rat portal vein (Johansson 1979; Doggrell 1991). Additionally, metoprolol tended to influence the isoprenaline inhibition of spontaneous activity following initial α-adrenoceptor blockade, demonstrating a degree of cardioselectivity of the drug (Johansson 1979; Doggrell 1991). Metoprolol reversibly decreased duration and voltage of the action potential plateau, without influencing other action potential parameters in isolated feline patch-clamped ventricular myocytes (Barrington and Ten Eick 1990), and also slowed the beating rate in isolated rat atria (Johansson 1979), with minimal effects on effective fracory period in rabbit right ventricular papillary muscles (Manley *et al* 1986).

The effects of the individual (+)- and (-)-metoprolol enantiomers on various isolated tissues have also been examined (Toda *et al* 1978; Doggrell 1991). In isolated atria, spontaneous beating rate and contractile force were reduced, the isoprenaline dose-chronotropic and dose-inotropic response curves were shifted to the right, and the effects of propranolol were antagonized by lower concentrations of (-)-metoprolol than those required for the (+)-enantiomer (Toda *et al* 1978; Doggrell 1991). However, neither enantiomer influenced the responses to cardiac stimulation, or stimulation combined with isoprenaline (Doggrell 1991). Both enantiomers tended to slightly prolong the atrial functional refractory period (Toda *et al* 1978). Conversely, in a variety of non-cardiac vascular preparations, the same concentrations failed to influence spontaneous contractile activity (Doggrell 1991). In the presence of isoprenaline, (-)-metoprolol was more potent than (+)-metoprolol, and both isomers were much

less potent than propranolol in this regard (Doggrell 1991; Toda *et al* 1978). These results implied that the (+) - enantiomer was virtually devoid of ß-adrenoceptor antagonistic activity (Doggrell 1991).

Thus, metoprolol is a $ß_1$-adrenoceptor antagonist which slows spontaneous beating rate and reduces contractile force *in vitro* . The drug exists as a racemic mixture, but the (-)-enantiomer is more potent.

(ii) Results of in vivo studies with metoprolol in animals

Metoprolol (0.025-25.6mg/kg iv) has been demonstrated to dose-dependently antagonize the effects of isoprenaline or sympathetic nerve stimulation on heart rate and contractile force in anaesthetized cats (Ablad *et al* 1973; Borg *et al* 1975a), and at doses $\geq$ 1mg/kg iv, metoprolol exerts significant negative chrono- and ino-tropic effects on basal cardiac function (Ablad *et al* 1973). However, the $ß_1$-selective adrenoceptor antagonist exerted less than 1% of the potency of propranolol in inhibition of the hypotensive effect of isoprenaline (Ablad *et al* 1973), consistent with the much higher doses of intra-arterially injected metoprolol required to block the hind-limb vasodilator response in the anaesthetized cat (Ablad *et al* 1973).

Following intravenous metoprolol administration in a variety of animal models, the predominant acute haemodynamic effects (summarized in Table 1.1) are reductions in spontaneous heart rate and LV+dP/dt, with negligible effects on systolic and diastolic blood pressure (Gross *et al* 1984; Ryden *et al* 1990, 1991; Nanki *et al* 1987; Buck *et al* 1981a; Hatori *et al* 1991; Midol-Monnet *et al* 1991). When injected into the lateral cerebral ventricle, a low metoprolol dose significantly reduced heart rate, cardiac output, and stroke volume, and increased peripheral vascular resistance, with minimal influence on mean arterial pressure (Midol-Monnet *et al* 1991).

Reductions in heart rate at rest can also induced by the metoprolol metabolites :

(i) 4-hydroxy-metoprolol and H 105 / 22, only at doses five-fold, and plasma concentrations ten-fold higher;

and (ii) H 104 / 83 and H 117 / 04 plasma concentrations 100-fold higher than required

for the parent compound (Regardh *et al* 1979; Borg *et al* 1975). Thus, the only metabolites to exert significant ß-adrenoceptor blocking activity were 4-hydroxy-metoprolol and H 105 / 22, and these were roughly only 10% as potent as the parent compound in this regard (Borg *et al* 1975).

In a number of experimentally-induced animal models of ischaemia, metoprolol induced : marked, significant anti-fibrillatory effects; reduced the number of ventricular ectopic beats; and completely prevented ventricular tachycardia (Wainwright and Parratt 1985; Anderson et al 1983; Cobbe *et al* 1983; Ablad *et al* 1987). In addition, the reductions in LV+dP/dt and spontaneous heart rate (Ryden *et al* 1990, 1991; Hatori *et al* 1991; Buck *et al* 1981a; Nanki *et al* 1987; Gross *et al* 1984) were preserved, cardiac output remained stable, and marked antiischaemic effects were demonstrated (Ablad *et al* 1987), without influence on ventricular effective refractory periods in the infarcted ventricular zone (Cobbe *et al* 1983). The preserved cardiac output and pressure may have reflected an improvement in the ischaemia-impaired contractile and relaxation mechanisms (Ablad *et al* 1987). Metoprolol has also been demonstrated to improve subendocardial blood flow and the ratio of endocardial / epicardial flow in ischaemic regions, while reducing overall nonischaemic myocardial flow, thus favourably redistributing flow (Buck *et al* 1981a; Nanki *et al* 1987; Gross *et al* 1984). Segmental function in a partially ischaemic but not occluded area is also significantly improved by metoprolol (Gross *et al* 1984).

Longer-term studies of both oral and intravenous metoprolol administration to spontaneously hypertensive rats demonstrated significant reductions in blood pressure (Ljung *et al* 1976). In dogs with surgically-induced systemic hypertension and left ventricular hypertrophy, chronic metoprolol therapy significantly reduced early mortality associated with coronary occlusion, which was not dependent on changes in arterial pressure (Dellsperger *et al* 1990).

In summary, metoprolol has been demonstrated in a number of experimental animal models *in vivo*, as a cardioselective ß-adrenoceptor antagonist which exerts negative inotropic and chronotropic effects, as well as displaying antiarrhythmic and antiischaemic actions. The drug favourably redistributes coronary blood flow during ischaemia, and has two less potent active metabolites.

(iii) Results of in vivo studies following oral metoprolol administration in humans

The acute haemodynamic and ECG effects of metoprolol following either oral or intravenous metoprolol administration in humans is summarized in Table 1.2. In healthy volunteers and in patients with ischaemic heart disease or hypertension, a single metoprolol oral presentation dose-dependently reduces spontaneous heart rate (Johnsson *et al* 1975; Kendall *et al* 1977; Koch and Fransson 1987; Bengtsson *et al* 1975; Myers and Thiessen 1980), with minor reduction of systolic blood pressure in the nonhypertensive patients (Johnsson *et al* 1975; Kendall *et al* 1977; Koch and Fransson 1987). In this latter group, more significant reductions in both systolic and diastolic blood pressures accompanied the fall in heart rate (Bengtsson *et al* 1975; Myers and Thiessen 1980). Significant reductions in oxygen uptake, cardiac output, stroke work, and rate-pressure product, accompanied by increases in arteriovenous oxygen differences, systemic and pulmonary vascular resistances have also been observed (Koch and Fransson 1987). Twenty-four hours after an intravenous metoprolol bolus followed by oral administration, the bradycardiac and PR interval prolonging effects of the ß-adrenoceptor antagonist persisted in patients with acute myocardial infarction, although the actions on blood pressure, cardiac index, systemic vascular resistance and pulmonary capillary wedge pressure were less pronounced, or even attenuated (Held 1986; Murray *et al* 1987; Davila *et al* 1989). Chronic oral metoprolol therapy in healthy young volunteers and in patients with ischaemic heart disease, elicits significant reductions in heart rate, with moderate prolongation of ventricular effective refractory periods and monophasic action potential duration (Edvardsson and Olsson 1981; Imperi *et al* 1987; Willich *et al* 1989). Additional effects include significantly reduced total silent ischaemic time, and reduced frequency of silent ischaemic episodes (Imperi *et al* 1987; Willich *et al* 1989), without influencing platelet aggregability (Willich *et al* 1989). In patients with moderate to severe heart failure, an increased cardiac index, significantly increased peak oxygen uptake, oxygen pulse, ejection fraction, functional heart failure classification, heart failure symptoms, improved LV contractility, diastolic function, and reduced LV mass were also observed, with a reduction in plasma catecholamines and an accompanied moderate up-regulation of ß-adrenoceptors (Waagstein *et al* 1989;

Nemanich *et al* 1990; Heilbrunn *et al* 1989; Engelmeier *et al* 1985). In patients with hypertension, metoprolol reduced systolic and diastolic blood pressure, spontaneous heart rate, plasma renin activity, and cardiac index with no influence on myocardial oxygen consumption, total peripheral resistance, or stroke volume (Lund-Johansen and Ohm 1977; von Bahr et al 1976; Myers and Thiessen 1980; Hansson *et al* 1977; Wallin *et al* 1984). While the number of sympathetic bursts per 100 beats was not significantly influenced in patients with essential hypertension, long-term metoprolol therapy did significantly reduce the number of sympathetic bursts per minute in peroneal nerve muscle branches, and the blood pressure reduction was at least in part attributed to this reduction in activity (Wallin *et al* 1984).

In patients with cardiac disease and complex ventricular rhythm disturbances, chronic low dose metoprolol therapy significantly reduced the incidence of premature ventricular beats, ventricular couplets, and ventricular tachycardia, with a further reduction ithe frequencies of both observed after chronic high-dose metoprolol therapy, without influence on ventricular function (Pratt *et al* 1983). Initiation of therapy early post acute myocardial infarction tended to reduce overall mortality, which was most marked in patients in the highest risk and sudden mortality categories (The MIAMI Trial Research Group 1985; Hjalmarson *et al* 1981; Herlitz *et al* 1983, 1984a, 1984b; Lopressor Intervention Trial Research Group 1987). Metoprolol also significantly reduced the incidence of ischaemic and tachyarrhythmic episodes (The MIAMI Trial Research Group 1985; Herlitz *et al* 1983, 1984a), reduced the lactate dehydrogenase activity (Hjalmarson *et al* 1981; Herlitz *et al* 1983, 1984a, 1984b), accompanied by reductions in enzymatically-determined infarction size (McBoyle *et al* 1983; Halinen *et al* 1989), and increased global LV ejection fraction (Halinen *et al* 1989). However, other comparable studies have failed to support these findings of reduced infarct size and increased ejection fraction (Murray *et al* 1987).

Following oral administration, metoprolol thus reduces spontaneous heart rate, with minor reductions in systolic and diastolic blood pressures, accompanied by antiischaemic and antiarrhythmic actions, and possibly a reduction in cardiovascular mortality.

(iv) Results of in vivo studies following intravenous metoprolol administration in humans

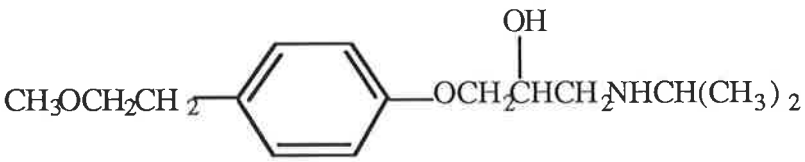
As indicated in Table 1.2, intravenous metoprolol bolus administration in healthy volunteers generally elicits significant reductions in heart rate, accompanied by minor fluctuations in systolic blood pressure, and increased systemic vascular resistance and arteriovenous oxygen difference, without change in myocardial oxygen consumption (Johnsson *et al* 1975; Stenberg *et al* 1975; Edvardsson and Olsson 1981; Steingart *et al* 1989). Some investigators also reported reduced cardiac output and ejection fractions, and increased right atrial pressure (Stenberg *et al* 1975; Steingart *et al* 1989).

Similar effects of metoprolol have been observed in patients with stable coronary artery disease, hypertension, advanced heart failure, cardiac arrhythmias, acute myocardial infarction, or patients undergoing routine otolaryngological surgery, clinical cardiac electrophysiological studies, or following coronary artery bypass surgery (Silke *et al* 1985a, 1985b, 1986; Hendry *et al* 1981; Bourdillon *et al* 1979; Nelson *et al* 1984; Reale *et al* 1979; Astrom and Jonsson 1977; Waagstein *et al* 1989; Saarnivaara *et al* 1984; Camm *et al* 1982; Rizzon *et al* 1978; Marchlinski *et al* 1984; Pechan *et al* 1988; Sannerstedt and Wasir 1977; Wallin *et al* 1984; Salmenpera *et al* 1983; Wasir *et al* 1977; Stroobandt and Kesteloot 1981; Moller and Ringqvist 1979; Held 1986; Davila *et al* 1990; MIAMI 1985; Dell'Italia and Walsh 1989; Murray *et al* 1987; Waagstein and Hjalmarson 1975; Heublein *et al* 1991). Other effects include dose-dependent increases in pulmonary capillary wedge pressure (Hendry *et al* 1981), reductions of: LV+dP/dt and / or ejection fraction at rest (Bourdillon *et al* 1979; Reale *et al* 1979; Heublein *et al* 1991); circumferential fibre shortening; myocardial wall stress (Heublein *et al* 1991); and antagonism to both isoprenaline- and pacing-induced positive inotropic effects (Bourdillon *et al* 1979). Additionally, significant reductions in the number of patients in whom sustained supraventricular tachycardia was inducible (Rizos *et al* 1984), augmentation of total peripheral resistance (Pechan *et al* 1988), significant increase in the number of sympathetic bursts per 100 beats, but not the number per minute in peroneal nerve muscle branches (Wallin *et al* 1984), and reverted a high proportion of patient arrhythmias to sinus rhythm without eliciting

hypotension were observed (Moller and Ringqvist 1979; Wasir et al 1977; Stroobandt and Kesteloot 1981).

A series of three 5mg intravenous bolus doses of metoprolol in patients early after onset of acute myocardial infarction reduced mean heart rate, systolic blood pressure and the rate-pressure product, without eliciting heart failure or cardiogenic shock, demonstrating satisfactory toleration of the drug in these patients (The MIAMI Trial Research Group 1985; Kronenberg *et al* 1990; Held 1986; Steingart *et al* 1989; Murray *et al* 1987; Steingart *et al* 1989; Davila *et al* 1989; Waagstein and Hjalmarson 1975; Grines *et al* 1991). Shortly after the dose, significant reductions in cardiac output, stroke volume, stroke work, chest pain, LV+dP/dt, LV ejection fraction, LV relaxation, peak systolic circumferential wall stress and ST segment, and increases in right atrial pressure, PR interval duration, and systemic vascular resistance were also observed, with little or no influence on LV ejection fraction, diastolic blood pressure or pulmonary artery pressure (The MIAMI Trial Research Group 1985; Kronenberg *et al* 1990; Held 1986; Steingart *et al* 1989; Murray *et al* 1987; Steingart *et al* 1989; Davila *et al* 1989; Waagstein and Hjalmarson 1975; Grines *et al* 1991; Dell'Italia and Walsh 1989). Additionally, wall motion of the noninfarct zone was reduced, while that of the infarct zone was enhanced or at least not significantly impaired (Grines *et al* 1991; Steingart *et al* 1989) despite the reduced global ejection fraction (Steingart *et al* 1989). When patients were subdivided according to heart rate prior to injection, the bradycardiac and antiischaemic effects were more pronounced in those patients with initial heart rate greater than 65 beats/min (Held 1986).

Intravenous metoprolol administration in humans is therefore associated with reductions in spontaneous heart rate and LV+dP/dt, increases in systemic vascular resistance, arteriovenous oxygen difference, PCWP, and PR interval duration, with minor reductions in blood pressures and cardiac output.



metoprolol

FIGURE 1.1 Structural formula of metoprolol

TABLE 1.1 Acute effects of intravenous bolus metoprolol administration in a variety of animal models (% change)

Dose	HR	SBP	DBP	LV+dP/dt	n	Preparation	Reference
0.075µmol ICV	-17%	-2%	-2%		8	anaesth rats	Midol-Monnet *et al* 1991
0.10mg/kg	-19%	-10%		-19%	7	dogs with LAD occlusion + Cx stenosis	Gross *et al* 1984
0.20mg/kg	-6%		0	0 -17%	22	pigs with LAD occlusion	Ryden *et al* 1991
0.20mg/kg	-22%	0			8	dogs with LAD occlusion	Nanki *et al* 1987
0.30mg/kg	-7%	-5%			6	pigs with LAD occlusion	Ryden *et al* 1990
0.30mg/kg	-23%	0		-23%	6	dogs with Cx stenosis	Buck *et al* 1981a
0.30mg/kg	-7%	-5%	0		6	pigs with LAD occlusion	Ryden *et al* 1990
0.20mg/kg + 0.20mg/kg CVR	-19%	-9%		-38%	7	pigs with Cx occlusion	Hatori *et al* 1991
0.50mg/kg	-29%	0			8	dogs with LAD occlusion	Nanki *et al* 1987

CVR, coronary venous retroinfusion; Cx, circumflex branch of left main coronary artery; DBP, diastolic blood pressure; HR, heart rate; ICV, intracerebroventricular; LV+dP/dt, peak rate of left ventricular pressure rise; LAD, left anterior descending branch of left main coronary artery; SBP, systolic blood pressure.

TABLE 1.2 Acute effects of metoprolol administration in humans (% change)

Dose	HR	SBP	DBP	PR	QT	CO	SVR	dP/dt	n	Pt type	Reference
Oral :											
20mg	-4%	-6%							5	normal	Johnsson *et al* 1975
50mg	-10%	-11%							5	normal	Johnsson *et al* 1975
50mg	-18%	-19%	-22%						8	HT	Bengtsson *et al* 1975
80mg	-20%	-25%	-19%						6	HT	Bengtsson *et al* 1975
100mg	-14%	-8%							5	normal	Johnsson *et al* 1975
100mg	-10%	-8%	-5%			-17%	+16%		14	IHD	Koch & Fransson 1987
100mg	-30%	-18%	-18%						14	HT	Myers & Thiessen 1980
Intravenous bolus :											
1.25mg	-6%	+4%	+4%			-3%	+5%	-7%*	12	stable IHD	Silke *et al* 1986
0.02mg/kg	-10%	-2%	0		0				17	OLG surgery	Saarnivaara *et al* 1984
0.03mg/kg	-6%	-0	0			-3%	-5%		10	immediately post CABG surgery	Salmenpera *et al* 1983
0.03mg/kg	-14%	-5%	0		0				22	OLG surgery	Saarnivaara *et al* 1984
2.5mg	-8%	-4%	-2%			-6%	+3%		12	stable IHD	Hendry *et al* 1981
2.5mg	-10%	+3%	+4%			-6%	+7%	-8%*	12	stable IHD	Silke *et al* 1986
0.04mg/kg	-20%	-3%	0		0				24	OLG surgery	Saarnivaara *et al* 1984
5.0mg		-4%							5	normal	Johnsson *et al* 1975
5.0mg	-6%	-6%				+3%	-11%		7	1st stage HT	Pechan *et al* 1988
5.0mg	-17%	-3%				-27%	+33%#		7	2nd stage HT	Pechan *et al* 1988
5.0mg	-8%	+3%	+4%			-9%	+14%	-10%*	12	stable IHD	Silke *et al* 1986
5.0mg	-13%	-6%	-4%			0	-15%		12	stable IHD	Hendry *et al* 1981
0.10mg/kg	0	-5%				0	-4%	-10%	10	stable IHD	Bourdillon *et al* 1979

TABLE 1.2 (Continued)

Dose	HR	SBP	DBP	PR	QT	CO	SVR	dP/dt	n	Pt type	Reference
0.10mg/kg	-9%			+6%					16	normal	Rizzon *et al* 1978
7.1mg	-32%	-4%	-3%						23	AF_1 + AF_2	Wasir *et al* 1977
7.5mg	-47%	+5%	+20%						4	AF_1	Stroobandt & Kesteloot 1981
7.5mg	-49%	-4%	-4%						12	paroxysmal AT	Wasir *et al* 1977
9.2mg	-24%	-4%	0						9	VT	Wasir *et al* 1977
10mg		-6%							5	normal	Johnsson *et al* 1975
10mg	-11%	0	+5%			-16%	+25%	-29%	11	stable IHD	Reale *et al* 1979
10mg	-11%	+4%	+6%			-12%	+17%	-13%*	12	stable IHD	Silke *et al* 1986
10mg	-15%	-7%	-6%			-13%	+6%		10	stable IHD	Nelson *et al* 1984
10mg	-16%	-7%	-4%			-14%	+11%		12	stable IHD	Hendry *et al* 1981
10mg	-17%	-4%	-4%			-15%	+13%		10	stable IHD	Silke *et al* 1985a
10mg	-19%	0	0			-13%	+10%		11	stable IHD	Silke *et al* 1985b
10mg	-43%	-15%	-2%						6	paroxysmal AT	Moller & Ringqvist 1979
0.15mg/kg	-7%	-4%	-4%			-14%	+15%#		5	IHD+HT	Heublein *et al* 1991
0.15mg/kg	-14%	-3%	0			-17%	+19%		5	normal	Stenberg *et al* 1975
0.15mg/kg	-10%	-9%	-10%			-19%	+13%		5	HT	Sannerstedt & Wasir 1977
0.15mg/kg	-17%	0	0	+22%		0			12	normal	Marchlinski *et al* 1984
11mg	-33%	-8%	-10%						7	AF_1	Moller & Ringqvist 1979
13mg	-25%	-14%	-15%						8	AF_2	Moller & Ringqvist 1979
0.20mg/kg	-12%			+28%					10	normal	Camm *et al* 1982

TABLE 1.2 (Continued)

Dose	HR	SBP	DBP	PR	QT	CO	SVR	dP/dt	n	Pt type	Reference
15mg		-5%							5	normal	Johnsson *et al* 1975
15mg	-9%								16	normal	Edvardsson & Olsson 1981
15mg	-14%	-6%	+3%	+11%		+2%			16	normal	Edvardsson *et al* 1984
15mg	-17%	-5%				-20%			19	HF	Waagstein *et al* 1989
15mg	-12%					-21%			12	AMI initial HR < 65beats/min	Held 1986
15mg	-18%	-12%	-9%			-21%	+16%		95	AMI initial HR > 65beats/min	Held 1986
15mg	-13%	-8%	-8%	+8%					20	AMI	Davila *et al* 1990
15mg	-13%	-7%						-5%*	10	AMI	Kronenberg *et al* 1990
15mg	-16%	-11%	-5%			-26%	+21%		190	AMI	MIAMI 1985
15mg	-18%	-6%				-27%	+26%	-28%	16	AMI	Dell'Italia & Walsh 1989
15mg	-18%	-9%	-3%			-27%	+27%		61	AMI	Murray *et al* 1987
15mg	-24%	-9%	0						9	AMI	Waagstein & Hjalmarson 1975
17mg	-49%	+2%	+6%						7	SVT	Stroobandt & Kesteloot 1981
20mg	-13%	-4%							5	normal	Johnsson *et al* 1975
20mg	-16%					-18%	+11%		10	stable IHD	Astrom & Jonsson 1977
20mg	-17%	-8%	-5%			-17%	+13%		12	stable IHD	Hendry *et al* 1981
21mg	-48%	0	+11%						9	AF_2	Stroobandt & Kesteloot 1981

AF_1, atrial flutter; AF_2, atrial fibrillation; AMI, acute myocardial infarction; AT, atrial tachycardia; CABG, coronary artery bypass graft; CO, cardiac output; DBP, diastolic blood pressure; dP/dt, peak rate of left ventricular pressure rise; HF, heart failure; HR, heart rate; HT, hypertension; IHD, ischaemic heart disease; OLG, otolaryngeal; PR, PR interval at fixed heart rate; Pt, patient; QT, QT interval at fixed heart rate; SBP, systolic blood pressure; SVR, systemic vascular resistance; SVT, supraventricular tachycardia; VT, ventricular tachycardia.

* actually left ventricular ejection fraction # actually total peripheral resistance

1.5.1.2 Sotalol

Racemic sotalol (MJ1999), N-[4-[-hydroxy-2-[(1-methylethyl)amino)ethyl]phenyl]methane-sulfonamide, is a nonselective ß-adrenoceptor antagonist devoid of intrinsic sympathomimetic activity. This compound, whose chemical structure is illustrated in Figure 1.2, also possesses unique class III antiarrhythmic effects in addition to its ß -blocking activity (Singh and Nadermanee 1987). Sotalol's haemodynamic effects are therefore probably the result of combined activity of ß-adrenoceptor blockade, and potassium channel blockade, leading to myocardial action potential duration prolongation. Post infarction, a lack of either significant improvement or deterioration in prognosis with sotalol administration has been described (Julian *et al* 1982, 1983). However, the results of the recently published ESVEM study are very favourable for long-term administration of sotalol in patients with ventricular tachyarrhythmias (Mason *et al* 1993a, 1993b). Clinical interest in the potential for a therapeutic role for d-sotalol has also recently emerged with the beneficial finding of antiarrhythmic action of the dextrorotatory isomer in the absence of significant ß-adrenoceptor-antagonism (Sahar *et al* 1987; Schwartz *et al* 1987).

(i) Results of in vitro studies with sotalol

Sotalol has been demonstrated to competitively antagonize the ß-adrenergic effects of both isoprenaline and adrenaline, in a number of tissues in *in vitro* studies, including the smooth muscle of rat uterus, guinea pig trachea (Lish *et al* 1965), rabbit sinoatrial node (Strauss *et al* 1970), and spontaneously beating rat atria (Lish *et al* 1965). The antagonism of adrenergic-induced increases in both the rate and force of contraction by sotalol have been described by ED_{50}'s ranging from 0.17 to 0.84µg/ml (Lish *et al* 1965).

Direct negative inotropic and chronotropic effects of sotalol *in vitro* were demonstrated by 10-20% reductions of muscle force in guinea-pig right ventricular papillary muscles (Ezrin *et al* 1992) and increases in spontaneous cycle length in rabbit sinoatrial node (Kato *et al* 1986). However, in both isolated rabbit atria and cat hearts, only at very large doses does sotalol slow

rate and depress contractile force (Stanton *et al* 1965). Sotalol has also been demonstrated to exert weak local anaesthetic action on stripped frog sciatic nerve, but its potency in this regard was almost one hundred fold less than that of procaine (Singh and Vaughan Williams 1970).

Class III antiarrhythmic effects of this unique ß-adrenoceptor antagonist have also been reported *in vitro* . Action potential duration (APD) is significantly prolonged by sotalol in a number of tissues, including : guinea pig, rabbit, and kitten papillary muscles (Carmeliet 1985; Kaumann & Olson 1968; Cobbe *et al* 1985); guinea pig and human atrial muscle fibres (Campbell 1987); canine, sheep, guinea pig and rabbit ventricular fibres (Strauss *et al* 1970; Kato *et al* 1986; Carmeliet 1985; Manley *et al* 1986; Campbell 1987); rabbit and guinea pig sinoatrial node (Kato *et al* 1986; Campbell 1987); rabbit and guinea pig atria (Singh and Vaughan Williams 1970; Campbell 1987); and guinea pig hearts (Culling *et al* 1984; Gwilt *et al* 1991). This is reflected in other *in vitro* studies by prolongation of effective refractory periods (ERP) in canine Purkinje fibres (Strauss *et al* 1970; Kato *et al* 1986), canine ventricular muscle fibres (Strauss *et al* 1970; Kato *et al* 1986), rabbit ventricular fibres (Manley *et al* 1986) and guinea pig hearts (Culling *et al* 1984; Gwilt *et al* 1991). However, the class III antiarrhythmic effects of sotalol were lost during both severely hypoxic and global myocardial ischaemic conditions in rabbit papillary muscles and interventricular septum respectively (Cobbe *et al* 1985a, 1985b). No influence of sotalol on action potential amplitude or membrane resting potential has been observed at pharmacological concentrations (Kato *et al* 1986; Strauss *et al* 1970).

Sotalol does not elicit appreciable changes in inward rectifier, transient outward, or inward calcium currents *in vitro* (Varro *et al* 1991; Wettwer *et al* 1992), hence its APD prolongation is attributable to depression of the outward delayed rectifier potassium current, directly demonstrated in guinea-pig myocytes (Varro *et al* 1991; Connors *et al* 1992; Wettwer *et al* 1992) and rabbit Purkinje fibres (Carmeliet 1985; Connors *et al* 1992). Only minimal influence of sotalol on the backgound inward potassium current have been described (Fitton and Sorkin 1993; Carmeliet 1985; Varro *et al* 1991). A concentration-dependent, sotalol-induced, action potential duration prolongation was observed in guinea-pig ventricular cells (Connors *et al* 1992), a preparation lacking significant transient outward current (Fedida *et al*

1989). Conversely, demonstrating the tissue selectivity of sotalol, this effect on rectifier potassium currents could not be obtained in mouse neuroblastoma x rat glioma hybrid cells (Reeve and Peers 1992).

Antiarrhythmic effects of sotalol in *in vitro* studies have also been described. For example, in isolated perfused Langendorff guinea pig hearts, sotalol reduced the incidence of ventricular tachycardia and fibrillation during both ischaemia and the subsequent reperfusion (Culling *et al* 1984). The influence of myocardial ischaemia on the effects of sotalol *in vitro* have not been widely investigated, although its effect on action potential duration have been shown to be impaired (Culling *et al* 1984). Direct class I actions of sotalol on cardiac intracellular potentials have also been reported in isolated guinea-pig papillary muscles and rabbit atria, but only at higher concentrations, and these actions are only weak (Carmeliet 1985; Singh and Vaughan Williams 1970).

The presence of a chiral carbon dictates that sotalol exists as two enantiomers, levo- and dextro-rotatory, and is usually administered as the racemate of these. The individual effects of d- and l-sotalol on isoprenaline dose / response curves, action potential characteristics, the outward rectifier potassium current, and effective refractory periods have been examined (Kato *et al* 1986; Carmeliet 1985; Manley *et al* 1986). The isoprenaline dose / chronotropic response curve in a spontaneously-beating rabbit sinoatrial node preparation was either not altered, or was shifted to the right, by d-sotalol, but only at high concentrations (Kato *et al* 1986).

Conversely, no significant differences between the two isomers in their prolongation of spontaneous cycle length, action potential duration, effective refractory period, or impairment of the time-dependent outward potassium current were observed in rabbit sinoatrial node, atrial or ventricular muscle preparations (Kato *et al* 1986; Carmeliet 1985; Manley *et al* 1986). Specific blockade of a rapidly activating component of the delayed rectifier K^+ current by d-sotalol has been demonstrated in guinea pig cardiac cells (Sanguinetti and Jurkiewicz 1990; Wettwer *et al* 1992) with less inhibition of the rapidly activating inwardly rectifying potassium current (Wettwer *et al* 1992). In phasically active rat portal vein smooth muscle preparations, d-sotalol markedly increased phasic contractile activity at similar concentrations required to increase effective refractory periods of isolated ferret right ventricular papillary muscles, with

no influence on electrically quiescent vascular smooth muscle preparations, suggesting the spasmogenic activity of d-sotalol was not non-specific (Baskin *et al* 1992). Thus, the stereoisomers of sotalol exert similar electrophysiological effects, while only l-sotalol displays significant ß-adrenoceptor antagonist activity at pharmacological concentrations.

In summary, sotalol has been demonstrated in a number of *in vitro* models as a competitive ß-adrenoceptor antagonist displaying negative inotropic, negative chronotropic and antiarrhythmic actions, accompanied by outward potassium channel blockade, resulting in prolonged action potential duration. The drug exists as d- and l-enantiomers : only l-sotalol displaying significant ß-adrenoceptor antagonism, but both possessing potassium channel blocking characteristics.

(ii) Results of in vivo studies with sotalol in animals

Much of the *in vivo* investigations of sotalol in animal models have employed intravenous administration, and these studies have predominantly been in dogs, although a small number have utilized rat, rabbit and guinea-pig models. Dose-dependent competitive antagonism by sotalol of isoprenaline effects has been demonstrated (Lish *et al* 1965; Hoffmann and Grupp 1968; Gomoll and Bartek 1986; Ishizaki and Tawara 1979; Nattel *et al* 1989) *in vivo* .

Direct haemodynamic effects of sotalol on experimental animals *in vivo* are summarized in Table 1.3. The predominant finding in these studies is the dose-dependent reduction in heart rate, accompanied by small reductions or no changes in mean arterial and left ventricular systolic pressures (Lish *et al* 1965; Gross *et al* 1984; Gomoll and Bartek 1986; Stanton *et al* 1965; Hoffmeister *et al* 1991b; Ishizaki and Tawara 1979; Kaumann and Aramendia 1968; Ezrin *et al* 1992; Buck *et al* 1981b; Weissenburger *et al* 1991; Singh and Vaughan Williams 1970; Gwilt *et al* 1991; Twidale *et al* 1993).

A limited number of studies also observed reductions in LV+dP/dt, and possibly also minor reductions in cardiac index, although these parameters were not as extensively studied (Stanton *et al* 1965; Buck *et al* 1981b; Gross *et al* 1984; Hoffmeister *et al* 1991b; Ezrin *et al* 1992; Hoffmann & Grupp 1968; Twidale *et al* 1993). Furthermore, in all but one of these studies (Hoffmann & Grupp 1968), heart rate was not held constant during determination of LV+dP/dt

(Stanton *et al* 1965; Buck *et al* 1981b; Gross *et al* 1984; Hoffmeister *et al* 1991b; Ezrin *et al* 1992; Twidale *et al* 1993). Stroke volume (Stanton *et al* 1965; Hoffmeister *et al* 1991b) and LV end-diastolic pressure (Hoffmeister *et al* 1991b; Ezrin *et al* 1992) may be increased or decreased, while ejection fraction (Hoffmeister *et al* 1991b) and circumflex blood flow are reduced (Ezrin *et al* 1992). Systemic and coronary vascular resistances are reported to increase (Ezrin *et al* 1992), while total peripheral resistance, renal blood flow, renal or pulmonary resistance, or pulmonary arterial pressure do not appear to be significantly modified (Stanton *et al* 1965; Hoffmeister *et al* 1991b; Ezrin *et al* 1992; Twidale *et al* 1993). Similar haemodynamic effects of sotalol were observed in anaesthetized dogs following induction of ischaemia (Buck *et al* 1981a; Buck *et al* 1981b; Gross *et al* 1984).

Additionally, significant increases in subendocardial blood flow, and the ratio of endocardial / epicardial flow, have been observed in regions of ischaemia (Buck *et al* 1981a; Buck *et al* 1981b; Gross *et al* 1984). Conversely, in nonischaemic regions, sotalol elicited a significant reduction in tissue blood flow, without altering the ratio of endocardial / epicardial flow (Buck *et al* 1981a; Buck *et al* 1981b; Gross *et al* 1984). Segmental function in partially ischaemic but not completely occluded regions was significantly improved with sotalol administration (Gross *et al* 1984).

Table 1.4 summarizes the ECG and EP effects of sotalol on experimental animal preparations *in vivo* (Gomoll and Bartek 1986; Kaumann and Aramendia 1968; Singh and Vaughan Williams 1970). Sotalol predominantly induced significant prolongation of the PR interval, with a smaller increase or no change in the QT interval (Gomoll and Bartek 1986; Kaumann and Aramendia 1968). The changes in EP intervals induced by sotalol were less extensively examined, although dose-dependent increases in AH intervals, as well as atrial and ventricular refractory periods and atrioventricular conduction time, with no change in HV intervals, was reported in anaesthetised dogs (Gomoll and Bartek 1986; Nattel *et al* 1989; Hoffmann & Grupp 1968). However, the ED_{50} for sotalol's ß-adrenoceptor antagonism is considerably lower than that for effects on these refractory periods (Nattel *et al* 1989).

Additional increases in ventricular cycle length, QT interval and ventricular effective refractory period have recently been described in the setting of hypokalaemia and atrioventricular block in

conscious dogs with sotalol (Weissenburger *et al* 1991). Ventricular effective refractory periods were further prolonged by sotalol in infarcted ventricular zones in conscious dogs post myocardial infarction (Cobbe *et al* 1983; Patterson *et al* 1984), which was not observed with metoprolol, implying sotalol's effect in this regard may not have been due to ß-adrenoceptor blockade (Cobbe *et al* 1983). Sotalol's beneficial effects in this regard in the presence of ischaemia occur despite no effect on coronary thrombus formation or ischaemia progression (Patterson *et al* 1984).

The antiarrhythmic effects of sotalol in animals *in vivo* have also been investigated (Stanton *et al* 1965; Singh and Vaughan Williams 1970; Somani *et al* 1966; Kaumann and Aramendia 1968; Schmid and Hanna 1967; Patterson *et al* 1984; Weissenburger *et al* 1991). Ouabain-induced arrhythmias and persistent ventricular tachycardia were significantly suppressed by sotalol in anaesthetized guinea-pigs (Stanton *et al* 1965; Singh and Vaughan Williams 1970), but other studies failed to demonstrate sotalol-induced suppression of non-sympathomimetic amine-induced arrhythmias (Somani *et al* 1966). Suppression of atrial flutter and fibrillation (Stanton *et al* 1965) and coronary occlusion-induced ventricular fibrillation were observed in anaesthetized dogs (Kaumann and Aramendia 1968; Schmid and Hanna 1967; Patterson *et al* 1984). Similar findings were obtained when the dogs were pretreated with reserpine prior to both sotalol and ligation (Kaumann and Aramendia 1968). Sotalol also suppressed both aconitine- and sympathomimetic amine-induced experimental arrhythmias in anaesthetized dogs (Schmid and Hanna 1967; Somani *et al* 1966).

However, in one study, five of six dogs developed severe ventricular arrhythmias post commencement of sotalol infusion, resulting in death in two of these dogs (Weissenburger *et al* 1991), while in another investigation, all except one dog treated with sotalol died within 24h after ligation (Kaumann and Aramendia 1968). The arrhythmogenic effects were not reduced when bradycardia was limited to 40beats/min, although a 50% reduction in dose reduced the severity of the arrhythmias observed (Weissenburger *et al* 1991).

Again addressing the issue of enantioselectivity of sotalol's effects, both d- and l-sotalol displayed competitive antagonism of the isoprenaline dose / heart rate response in anaesthetized dogs (Gomoll and Bartek 1986), although l-sotalol was far more potent (Gomoll and Bartek

1986). This was also true for the haemodynamic, ECG and EP effects (Gomoll and Bartek 1986), and their antagonism of adrenergically-induced arrhythmias (Gwilt *et al* 1991; Somani and Watson 1968). However, d-sotalol significantly decreased heart rate and increased the QT interval, in doses not dissimilar from those required of l-sotalol (Mortensen *et al* 1992; Gwilt *et al* 1991; Wallace *et al* 1991; Duker *et al* 1992). The dextro-isomer of sotalol has also been demonstrated to exert negative inotropic effects in anaesthetized rats and dogs (Hoffmeister *et al* 1991b; Duker *et al* 1992). In anaesthetized dogs, d-sotalol predominantly induced significant dose-dependent increases in ventricular relative and effective refractory periods, with parallel dose-dependent prolongation of paced QT intervals, suggesting that the increased refractoriness was secondary to delayed ventricular repolarization (Wallace *et al* 1991; Duker *et al* 1992).

Following sotalol administration to animals *in vivo* , the predominant effects are reductions in heart rate and LV+dP/dt, increases in systemic and coronary vascular resistances, PR and AH intervals, and ventricular effective refractory periods, with minor fluctuations in pressures and QT intervals. These effects are accompanied by antiarrhythmic actions, and a favourable redistribution of coronary blood flow during ischaemia. L-sotalol is the more potent ß-adrenoceptor antagonist of the two isomers.

(iii) Results of in vivo studies following oral sotalol administration in humans

A summary of sotalol's acute haemodynamic, ECG, and EP effects after either oral or intravenous administration in humans is provided in Table 1.5. In healthy volunteers, sotalol elicited decreases in mean arterial blood pressure and spontaneous heart rate (Anttila *et al* 1976; Tjandramaga *et al* 1976). In clinical settings ranging from patients with previous myocardial infarction (Myburgh *et al* 1979) to sustained ventricular tachycardia (Kopelman *et al* 1987) and chronic renal failure (Tjandramaga *et al* 1976; Sundquist *et al* 1975), sotalol significantly prolonged sinus cycle length, QT and AH intervals, and right atrial and ventricular effective refractory periods, with no effect on HV intervals. The drug is devoid of significant central nervous system effects at standard antihypertensive doses (Bender *et al* 1979). Chronic oral sotalol administration reduced supine and standing systolic and diastolic blood pressures

and spontaneous heart rate in patients with moderate to moderately severe renal impairment and elevated diastolic pressure (Berglund *et al* 1980).

The antiarrhythmic effects of sotalol *in vivo* in humans following oral administration have also been extensively investigated (Julian *et al* 1982, 1983; Lidell *et al* 1985; Burckhardt *et al* 1984; Myburgh *et al* 1979; Mohama *et al* 1991; Sotalol vs amiodarone study group 1989; Trappe *et al* 1990; Jordaens *et al* 1989; Simon and Berman 1979). These are manifested as a reduced number of frequent chronic premature ventricular contractions (PVC's) in patients prone to such arrhythmias (Lidell *et al* 1985; Burckhardt *et al* 1984; Myburgh *et al* 1979). The incidence of ventricular tachycardia and / or fibrillation is also reduced (Lidell *et al* 1985; Mohama *et al* 1991; Trappe *et al* 1990). Oral sotalol has been demonstrated to be at least as effective, if not more so, as amiodarone (Burckhardt *et al* 1984; Sotalol vs amiodarone study group 1989) and procainamide (Lidell *et al* 1985; Jordaens *et al* 1989) for abolition of repetitive PVC's. Oral sotalol administration for one year post myocardial infarction failed to demonstrate any significant influence of the drug on either overall or cardiac mortality (Julian *et al* 1982, 1983). Recently however, long-term sotalol administration was demonstrated to exert considerable efficacy in reducing the incidence of ventricular tachyarrhythmias and death relative to a range of antiarrhythmic drugs, which also included imipramine, mexiletine, pirmenol, procainamide, propafenone, and quinidine (Mason *et al* 1993a, 1993b).

Oral sotalol administration in humans is therefore associated with reductions in spontaneous heart rate and mean arterial pressure, and increases in AH, PR, and QT intervals and ventricular effective refractory periods. The antiarrhythmic actions of the drug are particularly favourable in comparison with other available antiarrhythmic agents.

(iv) Results of in vivo studies following intravenous sotalol administration in humans

The acute haemodynamic, ECG, and EP effects of sotalol after a single intravenous injection has been more extensively studied than after oral administration, and these effects are also summarized in Table 1.5. Generally speaking, a reduction in spontaneous heart and prolongation of PR and QT intervals at fixed heart rates, as well as increases in AH intervals

and atrioventricular nodal effective refractory periods, have been observed following intravenous bolus administration in man, with little or no changes in mean arterial pressure and HV intervals. These effects apply across the spectrum of humans studies, from healthy volunteers to patients prone to chronic arrhythmias (Anttila *et al* 1976; Hutton *et al* 1972; Nathan *et al* 1982; McComb *et al* 1987; Ward *et al* 1979; Touboul *et al* 1984; Senges *et al* 1984; Echt *et al* 1982; Nademanee *et al* 1985; Huikuri *et al* 1992; Edvardsson *et al* 1980; Touboul *et al* 1987; Kopelman *et al* 1987). Other reductions in aortic systolic blood pressure, cardiac output, external cardiac work and LV+dP/dt at constant heart rate have also been observed (Hutton *et al* 1972).

Atrial and ventricular effective refractory periods (Nathan *et al* 1982; McComb *et al* 1987; Ward *et al* 1979; Senges *et al* 1984; Echt *et al* 1982; Nademanee *et al* 1985; Huikuri *et al* 1992; Edvardsson *et al* 1980; Touboul *et al* 1987), and also the retrograde functional refractory period of the His-Purkinje system (Touboul *et al* 1984; Touboul *et al* 1987) may also be prolonged. Prolongation of atrial and ventricular monophasic action potential duration at various stages of repolarization have also been reported in patients following intravenous sotalol administration (Echt *et al* 1982; Edvardsson *et al* 1980).

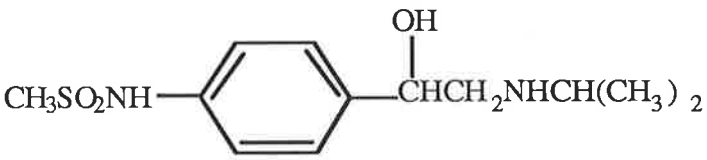
Similar effects of sotalol have been observed following intravenous sotalol infusion (Kopelman *et al* 1987; Kopelman *et al* 1988; Astrom *et al* 1990). All of these acute effects of intravenous sotalol, with the exception of sinus cycle length prolongation and blood pressure reduction, could not be mimicked by propranolol, thus distinguishing the ß-adrenoceptor antagonistic from non-ß-adrenoceptor antagonistic effects of dl-sotalol (Echt *et al* 1982).

The antiarrhythmic effects of sotalol *in vivo* in humans following intravenous administration have also been investigated (Senges *et al* 1984; Nademanee *et al* 1985; Huikuri *et al* 1992). These effects probably account for the reduced incidence of : ventricular tachycardia in patients with sustained ventricular tachycardia or fibrillation (Senges *et al* 1984; Nademanee *et al* 1985); and of supraventricular tachycardia, particularly in patients with atrioventricular nodal or intra-atrial re-entry (Huikuri *et al* 1992). Significant reductions in the number of patients in whom sustained supraventricular tachycardia was inducible was observed in patients prone to

this dysrhythmia following iv sotalol (Rizos *et al* 1984). In the remaining patients, the tachycardia cycle length was significantly prolonged (Rizos *et al* 1984).

Again addressing the issue of enantioselectivity of sotalol's effects, significant prolongation of sinus cycle length and paced PR, QT, AH intervals were observed, without marked changes in HV intervals, following intravenous d-sotalol administration (Sahar *et al* 1987; McComb *et al* 1987; Schwartz *et al* 1987). The atrial and ventricular effective refractory periods, and the atrioventricular nodal functional refractory periods were also significantly prolonged by d-sotalol, but less pronounced than elicited by the racemic parent compound (McComb *et al* 1987). The dextrorotatory isomer also effectively reduced the incidence of supraventricular (Sahar *et al* 1987) and ventricular arrhythmias (Schwartz *et al* 1987) in patients prone to such rhythm disturbances.

Thus, sotalol induces reductions in spontaneous heart rate and LV+dP/dt, increases in AH, PR, and QT intervals and atrioventricular nodal effective refractory periods, and antiarrhythmic effects with intravenous administration. Injection of the d-sotalol enantiomer alone is associated with similar ECG, EP, and antiarrhythmic effects without significant ß-adrenoceptor blockade.



sotalol

FIGURE 1.2 Structural formula of sotalol

TABLE 1.3 Haemodynamic effects of intravenous sotalol administration in animals (% change)

Dose	CI	HR	LV+dP/dt	LVP	MAP	n	Preparation	Reference
Intravenous bolus :								
0.25µmol/kg		-10%	-23%#		-5%	5	anaesth dogs	Stanton *et al* 1965
0.50µmol/kg		-11%	-17%#		-4%	5	anaesth dogs	Stanton *et al* 1965
1.00µmol/kg		-12%	-11%#		-2%	5	anaesth dogs	Stanton *et al* 1965
0.3mg/kg	-1.3	-250	-4000	-20	-40	5	anaesth dogs	Ezrin *et al* 1992*
0.3mg/kg		-19%	-19%		-10	7	anaesth dogs LAD occlusion + Cx stenosis	Gross *et al* 1984
0.5mg/kg		-12%			0	5	anaesth dogs	Kaumann & Aramendia 1968
0.5mg/kg		-17%			-3%	4	anaesth dogs	Gomoll & Bartek 1986
0.75mg/kg		-16±4%				10	anaesth guinea pigs	Singh & Vaughan Williams 1970
1mg/kg	-1.8	-400	-5500	-10	-50	5	anaesth dogs	Ezrin *et al* 1992*
1mg/kg		-22%	-14%		0	7	anaesth dogs Cx stenosis	Buck *et al* 1981b
1mg/kg		-24%	-22%		0	6	anaesth dogs Cx stenosis	Buck *et al* 1981a
1.5mg/kg		-20±4%				10	anaesth guinea pigs	Singh & Vaughan Williams 1970
2mg/kg		-28%			-7%	4	anaesth dogs	Gomoll & Bartek 1986
2mg/kg	-34%	-20%	-70%	-27%	-27%**	12	anaesth rats post LV ischaemia	Hoffmeister *et al* 1991b
3mg/kg		-24±3%				10	anaesth guinea pigs	Singh & Vaughan Williams 1970
3mg/kg		-36%			-2%	7	anaesth dogs	Ishizaki & Tawara 1979
3mg/kg	-2.25	-600	-6500	0	-40	5	anaesth dogs	Ezrin *et al* 1992*

TABLE 1.3 (Continued)

Dose	CI	HR	LV+dP/dt	LVP	MAP	n	Preparation	Reference
6mg/kg		-28±4%				10	anaesth guinea pigs	Singh & Vaughan Williams 1970
8mg/kg		-31%			-3%	4	anaesth dogs	Gomoll & Bartek 1986
10mg/kg		-44%			-16%	10	anaesth dogs	Kaumann & Aramendia 1968
10mg/kg	-3.5	-800	-9000	-90	-120	5	anaesth dogs	Ezrin *et al* 1992*
10mg/kg		-24%			-6%	10	anaesth dogs reserpine PT	Kaumann & Aramendia 1968
10mg/kg		87			-7	8	rats with coronary occlusion	Gwilt *et al* 1991
10mg/kg		-50%			-24%	10	anaesth dogs LAD occlusion	Kaumann & Aramendia 1968
10mg/kg		-30%			-6%	10	anaesth dogs reserpine PT+ LAD occlusion	Kaumann & Aramendia 1968
Intravenous infusion :								
1µmol/kg/min		+6%	-24%		-9%	3	anaesth dogs	Stanton *et al* 1965
4.5mg/kg then 1.5mg/kg/h			-41%			6	consc dogs AV block	Weissenburger *et al* 1991

* AUC time-response curves over 8-10min, with the units of L/min, bpm, mmHg/s, mmHg, mmHg for CI, HR, LV+dP/dt, LVP and MAP respectively

contractile force ** mean aortic pressure

anaesth, anaesthetized; AV, atrioventricular; CI, cardiac index; Cx, circumflex; LV+dP/dt, peak rate of pressure rise in left ventricle; HR, heart rate; LAD, left anterior descending; LV, left ventricle; LVP, left ventricular pressure; MAP, mean arterial pressure; PT, pretreatment.

TABLE 1.4 Electrocardiographic and electrophysiologic effects of intravenous sotalol administration in various animal models (% change)

Dose	PR	QT	AH	HV	n	Preparation	Reference
Intravenous bolus :							
0.5mg/kg	+				5	anaesth dogs	Kaumann & Aramendia 1968
0.5mg/kg	+22%	+8%	+31%	0	4	anaesth dogs	Gomoll & Bartek 1986
0.75mg/kg	0	0			10	anaesth guinea pigs	Singh & Vaughan Williams 1970
1.5mg/kg	0	0			10	anaesth guinea pigs	Singh & Vaughan Williams 1970
2mg/kg	+47%	+12%	+67%	0	4	anaesth dogs	Gomoll & Bartek 1986
3mg/kg	0	+13%			10	anaesth guinea pigs	Singh & Vaughan Williams 1970
6mg/kg	0	+19%			10	anaesth guinea pigs	Singh & Vaughan Williams 1970
8mg/kg	+67%	+22%	+119%	0	4	anaesth dogs	Gomoll & Bartek 1986
10mg/kg	+				10	anaesth dogs	Kaumann & Aramendia 1968
10mg/kg	+				10	anaesth dogs reserpine PT	Kaumann & Aramendia 1968
Intravenous infusion :							
4.5mg/kg then 1.5mg/kg/h		+27%			6	consc dogs AV block	Weissenburger *et al* 1991

+, increased; AH, AH interval; anaesth, anaesthetized; AV, atrioventricular; HV, HV interval; LAD, left anterior descending; LV, left ventricle; PR, PR interval; QT, QT interval.

TABLE 1.5 Effects of sotalol administration in humans (% change)

Dose	HR	MAP	PR	QT	AH	HV	AVN	n	Pt type	Reference
Oral :										
160mg	-23%	-13%						8	normal	Anttila *et al* 1976
612±206 mg/day	-26%		+45%	+30%	+59%	+13%		13	VT	Kopelman *et al* 1987
Intravenous bolus :										
20mg	-16%	-6%						8	normal	Anttila *et al* 1976
0.2mg/kg	-17%	-3%#						6	normal	Hutton *et al* 1972
0.3-0.6mg/kg		-12%	-12%		+12%			9	normal	Echt *et al* 1982
0.4mg/kg	-11%				+15%	+2%	+14%	10	normal	Ward *et al* 1979
0.4mg/kg	-15%			+3%	+15%	+2%	+14%	24	normal	Nathan et al 1982
0.6mg/kg	-18%		+15%	+5%	+24%	0	+16%	12	normal	Touboul *et al* 1984
0.6mg/kg					+26%	0	0	14	AV AP	Touboul *et al* 1987
1mg/kg	-19%	0	+15%	+15%	+29%	0	+18%*	8	normal	McComb *et al* 1987
1mg/kg	-11%		+5%	+11%	+11%	0	+18%	14	AV re-entry	Huikuri *et al* 1992
1mg/kg	-11%		+13%	+7%	+22%	0	+19%	16	AVN or intra-atrial re-entry	Huikuri *et al* 1992
92.5mg	-18%	-7%						8	AF	Edvardsson*et al* 1980
1.5mg/kg	-19%			+24%				18	VF/VT	Senges *et al* 1984
1.5mg/kg	-23%		+11%	+11%	+18%	0	+25%	33	VF/VT	Nademanee*et al* 1985
Intravenous infusion :										
0.5mg/kg then 0.6mg/kg then 0.2mg/kg/h for up to 12h	-13%			+10%			+15%	10	AMI + VF/VT	Astrom *et al* 1990
1.5mg/kg then 0.008mg/kg/min	-21%		+24%	+21%	+34%	+2%		13	VT	Kopelman *et al* 1987
1.5mg/kg then 0.008mg/kg/min	-24%	-12%		+23%				9	VT	Kopelman *et al* 1988

AF, atrial fibrillation; AH, AH interval; AMI, acute myocardial infarction; AP, accessory pathways; AV, atrioventricular; AVN, AV node; CI,cardiac index; ERP, effective refractory period; HR, heart rate; HV,HV interval; MAP, mean arterial pressure; PR, PR interval at fixed heart rate; Pt, patient; QT, QT interval at fixed heart rate; VF, ventricular fibrillation; VT, ventricular tachycardia.

* actually AVN functional refractory period # actually mean aortic pressure

1.5.1.3 Milrinone

Milrinone, 1,6-dihydro-2-methyl-6-oxo-(3,4-bipyridine)-5-carbonitrile, is a recently developed analogue of amrinone, and its chemical structure is illustrated in Figure 1.3. This compound is a non-glycoside, non-catecholamine positive inotrope with additional vasodilatatory actions. Both of these characteristics combine to improve cardiac haemodynamics in patients with heart failure. Milrinone's mechanism of action is attributed to inhibition of the phosphodiesterase enzyme responsible for intracellular degradation of cyclic adenosine -3'5'-monophosphate (cAMP), resulting in accumulation of the nucleotide. Originally, the drug was developed as an alternative positive inotrope to digoxin and ß-adrenoceptor agonists for the management of heart failure. However, the recent observation of increased mortality associated with long-term milrinone therapy versus placebo in patients with severe heart failure in the recent PROMISE trial has limited the drug's indications to management of acute heart failure only (Packer *et al* 1991a, 1991b).

(i) Results of in vitro studies with milrinone

Milrinone specifically inhibits phosphodiesterase III, with an IC_{50} of approximately 1μM in isolated guinea pig cardiac muscle (Silver *et al* 1989), canine left ventricle (Vandenplassche *et al* 1992), and spontaneously beating reserpine-treated and non-treated guinea pig atria (Komai *et al* 1991; Wilhelm *et al* 1992). Enhanced calcium influx has been demonstrated in the presence of milrinone, predominantly via augmentation of the magnitude of the slow channel current, in voltage-clamped calf cardiac Purkinje cells (Sutko *et al* 1986; Endoh *et al* 1986). This enhanced influx, a combined result of increased calcium channel opening events and binding to sarcoplasmic reticulum, was reinforced by the observation of additive effects of elevated external calcium concentrations on milrinone's positive inotropic effects in isolated cardiac muscle (Alousi and Johnson 1986; Holmberg and Williams 1991; Farah and Frangakis 19??).

Significant concentration-dependent cardiac effects of milrinone have been demonstrated *in vitro* (Sys *et al* 1986; Alousi and Johnson 1986; Silver *et al* 1989; Farah and Frangakis 19??). These include increases in tension development, rate of contraction, peak shortening, velocity of shortening, peak rate of rise of force (+dF/dt) and increased peak rate of fall of force (-dF/dt) in : isolated human, feline, and guinea pig papillary muscles (Sys *et al* 1986; Alousi and Johnson 1986; Farah and Frangakis 1989); spontaneously beating atria from non-pretreated and reserpine-treated guinea pigs; and isolated Langendorff-perfused rat and guinea pig whole hearts (Alousi and Johnson 1986; Steffen and Wastila 1992; Komai *et al* 1991; Rapundalo *et al* 1986; Wilhelm *et al* 1992). In isolated coronary artery and aorta preparations, milrinone exerted concentration-dependent relaxation of these arteries when pre-contracted with potassium chloride (Lindgren and Andersson 1991a; Lindgren and Andersson 1991b; Lebedinsky *et al* 1992). Other haemodynamic effects of milrinone observed *in vitro* include augmented action potential duration in normal canine cardiac Purkinje fibres (Davidenko and Antelevitch 1984) and increased coronary blood flow and consequently reduced coronary vascular resistance in a nonfailing canine heart-lung preparation (Kabela *et al* 1986).

Potency of milrinone has been demonstrated in the micromolar range for phosphodiesterase III inhibition (Silver *et al* 1989; Vandenplassche *et al* 1992; Komai *et al* 1991), vasodilatation (Lindgren and Andersson 1991a; Lebedinsky *et al* 1992; Lindgren and Andersson 1991b), and the positive inotropic (Silver *et al* 1989; Komai *et al* 1991) and chronotropic effects (Komai *et al* 1991). Milrinone exerted a three to five-fold greater potency than amrinone in these studies on a weight-for-weight basis (Sys *et al* 1986; Alousi and Johnson 1986).

Milrinone increased contractile force in isolated dog right ventricular trabeculae with a biphasic dose / response curve, with one phase sensitive to calcium antagonists and the other to ryanodine (Farah and Frangakis 19??; Brown *et al* 1986). However, under conditions of high extracellular calcium, the biphasic nature of this curve was eliminated (Farah and Frangakis 1989). The positive inotropic effects of the drug were correlated with increases in cAMP content (Silver *et al* 1989; Farah and Frangakis 1989). However, the positive inotropic effects of milrinone occurred prior to the increase in tissue cAMP concentrations in canine ventricluar muscle (Endoh *et al* 1986). Involvement of cAMP in the positive inotropic effects of milrinone

was also indirectly demonstrated with carbachol-induced inhibition of these effects demonstrated in guinea pig right ventricular papillary muscles (Endoh *et al* 1986; Wilhelm *et al* 1992; Brown *et al* 1986).

In isolated rat heart models of ischaemia and reperfusion, milrinone exerted significantly improved recovery of left ventricular pressure, adenosine triphosphate, substrate metabolism, and augmented the cardiac output of stunned myocardium (Buser *et al* 1991; Komai *et al* 1991). In canine cardiac Purkinje fibres, milrinone markedly improved conduction in areas of depressed conductivity (Davidenko and Antelevitch 1984). In a pentobarbital-induced failing heart-lung preparation, the phosphodiesterase inhibitor significantly improved cardiac output, while continuing to improve coronary blood flow (Kabela *et al* 1986).

Addition of milrinone to the bathing medium is thus associated with inhibition of the phosphodiesterase III enzyme and increased calcium influx resulting in increased intracellular calcium concentrations *in vitro* . Other predominant effects include vasodilatation, accompanied by increased tension development, dP/dt, action potential duration and coronary blood flow, and improved contractile performance of *in vitro* models of heart failure.

(ii) Results of in vivo studies in animals with milrinone

Investigations of milrinone in animal studies *in vivo* have focussed predominantly on dogs (Alousi and Johnson 1986; Rapundalo *et al* 1986; Shaffer *et al* 1986; Gosgnach *et al* 1991; Lee *et al* 1991b; Steffen and Wastila 1992; Abe *et al* 1992; Trolese-Mongheal *et al* 1992; Vandenplassche *et al* 1992; Lebedinsky *et al* 1992; Lee *et al* 1991a), although a number of studies in guinea pigs (Ortiz *et al* 1992; Lin and Chen 1991) and rats (Jain *et al* 1991a,1991b) have also been described. The majority of these studies examined the haemodynamic effects of intravenous milrinone administration, and these are summarized in Table 1.6. Milrinone predominantly induced significant increases in cardiac output, heart rate, LV+dP/dt and contractile force, often dose-related, accompanied by small decreases in mean arterial pressure (Gosgnach *et al* 1991; Lee *et al* 1991a, 1991b; Alousi and Johnson 1986; Rapundalo *et al* 1986; Shaffer *et al* 1986; Steffen and Wastila 1992; Lebedinsky *et al* 1992; Abe *et al* 1992;

Vandenplassche *et al* 1992; Lin and Chen 1991; Alousi *et al* 1984). Increased LV-dP/dt, circumflex and left anterior descending blood flow, and decrements in total peripheral, renal and coronary vascular resistances, left ventricular end-diastolic, right atrial and pulmonary diastolic blood pressures have also been observed (Lee *et al* 1991a, 1991b; Alousi and Johnson 1986; Rapundalo *et al* 1986; Shaffer *et al* 1986; Steffen and Wastila 1992; Lebedinsky *et al* 1992; Vandenplassche *et al* 1992; Lin and Chen 1991) These effects persist in the setting of acute pentobarbital-induced cardiac depression (Steffen and Wastila 1992). Milrinone was approximately 30-fold as potent as amrinone in this regard (Alousi and Johnson 1986).

Direct cardiotonic and vasodilatating effects of milrinone have been demonstrated in animal studies *in vivo* , where neither effect is dependent on the other (Gosgnach *et al* 1991; Steffen and Wastila 1992; Lebedinsky *et al* 1992). Utilizing direct milrinone injection into the canine iliac artery, no positive ino- or chrono-tropic effects were observed, despite similar increases in iliac blood flow and diameter to intravenous administration, indicating direct vasodilatatory actions of milrinone (Gosgnach *et al* 1991; Steffen and Wastila 1992). Direct cardiotonic activity of milrinone was demonstrated with intracoronary milrinone injections, inducing dose-related improvement in regional contractility and reduction in coronary vascular resistance, despite an absence of significant effects on LV+dP/dt, systolic and end-diastolic pressures, mean arterial pressure, and spontaneous heart rate (Lebedinsky *et al* 1992).

Pro-arrhythmic effects of milrinone have been demonstrated as ventricular rhythm disturbances and a reduced number of sinus beats, in coronary occlusion-induced myocardial infarction in conscious dogs (Trolese-Mongheal *et al* 1992). Conversely, in another model of ligation-induced infarction, milrinone elicited no change in infarction size, significantly improved left ventricular end-diastolic pressure, and reduced scar thinning and infarct expansion (Jain *et al* 1991a, 1991b). Positive inotropic and chronotropic effects are still elicited by milrinone under these circumstances (Trolese-Mongheal *et al* 1992; Abe *et al* 1992; Vandenplassche *et al* 1992), but fail to restore the impaired segment shortening and myocardial pH in the ischaemic region (Abe *et al* 1992).

Nonselective ß-adrenoceptor blockade prior to phosphodiesterase inhibitor administration attenuated the hypotensive and chronotropic effects, but failed to eliminate the positive inotropic effect, the reduced cardiac preload or improved cardiac output, indicating the directly cardioactive effects of milrinone (Lee *et al* 1991a). Autonomic blockade induced by hexamethanonium in anaesthetized dogs enhanced the effects of milrinone on heart rate, LV+dP/dt and end-diastolic pressure, but attenuated its effects on blood pressure (Shaffer *et al* 1986), implicating some role for the autonomic nervous system in the actions of milrinone.

Milrinone administration in a variety of animal preparations is therefore associated with increases in cardiac output, spontaneous heart rate, LV+dP/dt, contractile force and coronary blood flow, and small decreases in mean arterial pressure. Both the positive inotropic and the vasodilatatory effects can be distinctly exerted without the other. Also, some indication for pro-arrhythmic effects have been demonstrated.

(iii) Results of in vivo studies in humans following oral milrinone administration

A number of studies have examined the acute haemodynamic effects of milrinone following oral administration in humans, particularly in the setting of moderate to severe congestive heart failure, as summarised in Table 1.7. Oral milrinone predominantly exerts significant increases in cardiac index, with smaller increases in heart rate and LV+dP/dt. Pulmonary capillary wedge pressure (PCWP), systemic vascular resistance and LV end-diastolic pressure are consistently reduced after oral doses, with smaller reductions in mean arterial pressure (Larsson *et al* 1986; Likoff *et al* 1985; Remme *et al* 1992; Maskin *et al* 1983, 1984; Le Jemtel et al 1985;Cody et al 1984a). However, milrinone induced hetereogenous effects on cardiac pump function, depending on PCWP prior to administration (Remme *et al* 1992). Cardiac index increased significantly in patients with baseline PCWP greater than or equal to 18mmHg, while it tended to be reduced in patients with PCWP less than 18mmHg (Remme *et al* 1992), and may therefore be of greater benefit in patients with more severe heart failure.

Acute oral milrinone administration has also been demonstrated to improve LV diastolic performance in patients with severe congestive heart failure, as demonstrated by : increased

LV-dP/dt, coronary sinus flow, peak filling rate; decreased minimal LV diastolic pressure, LV end-diastolic pressure, end-diastolic volume, time constants of relaxation; and a leftward shift of pressure-volume curves (Piscione *et al* 1987). This study observed no influence of the drug on arterial or coronary sinus plasma catecholamine concentrations (Piscione *et al* 1987).

Long-term oral administration of milrinone in patients with severe congestive heart failure has also been extensively evaluated (Baim *et al* 1983; Likoff *et al* 1985; Colucci *et al* 1991; Packer *et al* 1991a; Packer *et al* 1991b). Milrinone administration as adjunctive therapy for moderate to severe heart failure was associated with improved heart failure symptoms (including improvement by almost one unit in the New York Heart Association classification), enhanced ejection fraction, and better exercise capacity than placebo (Baim *et al* 1983; Likoff *et al* 1985; Colucci *et al* 1991). However, milrinone was also associated with vasodilatation-related adverse effects, trends towards proarrhythmia and significantly increased mortality. This mortality was attributed to a 69% increase in cardiac death, so much so that two randomized, placebo-controlled trials of oral milrinone for heart failure were prematurely terminated, including the recent PROMISE (Prospective Randomized Milrinone Survival Evaluation) study (Baim *et al* 1983; Colucci *et al* 1991; Packer *et al* 1991a, 1991b).

Thus, milrinone elicits increases in cardiac index, spontaneous heart rate, LV+dP/dt, relaxation, and functional heart failure classification, and decreases in PCWP, systemic vascular resistance, LV end-diastolic pressure, and mean arterial pressure following oral administration. However, long-term therapy is associated with increased cardiovascular mortality in patients with severe heart failure, limiting its indications.

(iv) Results of in vivo studies in humans following intravenous milrinone administration

The haemodynamic effects of milrinone following intravenous administration in humans have been extensively examined, in normal volunteers and in patients, in the setting of moderate to severe congestive heart failure, low cardiac output syndrome and ischaemic heart disease, as also summarised in Table 1.7. Similar to effects observed following oral administration, intravenous milrinone injection predominantly exerts significant increases in cardiac index, with

smaller increases in heart rate and LV+dP/dt. PCWP, systemic vascular resistance and left ventricular end-diastolic pressure are consistently reduced after these doses, with smaller reductions in mean arterial pressure. In many cases, the effects were dose-dependent. Similar effects are induced after either bolus or infusion modes (Cody *et al* 1984a, 1984b; Colucci *et al* 1985; Benotti *et al* 1984; Cody *et al* 1984; Baim *et al* 1983; Benotti *et al* 1985; Benotti and Hood 1984; Boesch *et al* 1992; Le Jemtel *et al* 1984; Likoff *et al* 1985; Grose *et al* 1986; Monrad *et al* 1984; Sonnenblick *et al* 1986; Maskin *et al* 1983; Jaski *et al* 1985; Monrad *et al* 1986; Hasking *et al* 1987; Dubois-Rande *et al* 1991; Muir and Nolan 1991; Klocke *et al* 1991; Mager *et al* 1991; Anderson *et al* 1991; Villari *et al* 1991; Braunwald 1991; Pflugfelder *et al* 1991; Ludmer *et al* 1986; George *et al* 1992; Feneck *et al* 1991, 1992a; Wright and Sherry 1991; Borow *et al* 1986; Nolan *et al* 1992; Goldstein *et al* 1986; Mitrovic *et al* 1991).

The improvement in cardiac output was particularly augmented in patients with low initial cardiac index (less than or equal to 1.6L/min/m^2) or cardiogenic shock (Klocke *et al* 1991; Feneck *et al* 1992b). Other effects influenced by baseline status included : enhanced reduction in pulmonary vascular resistance (PVR) in patients with high initial PVR; and no fall in mean arterial pressure (MAP) in patients with low initial MAP (Feneck *et al* 1992b).

Coronary vascular resistance and arteriovenous oxygen gradient can also be significantly decreased following milrinone administration (Baim *et al* 1983; Grose *et al* 1986; Sonnenblick *et al* 1986; Jaski *et al* 1985; Braunwald 1991). No significant changes in coronary blood flow, myocardial oxygen consumption or myocardial lactate extraction were observed, although coronary flow tended to be elevated by milrinone (Grose *et al* 1986). Improved diastolic function, as manifested by increased LV-dP/dt and peak filling rate, and decreased time constant of LV isovolumic relaxation and mean aortic pressure, have also been associated with milrinone administration(Monrad *et al* 1984), as has increased venous volume (Muir and Nolan 1991). Significantly increased percent fractional shortening and end-diastolic wall stress : diameter ratio, reduced end-systolic diameter and end-systolic wall stress, with no change in end-diastolic diameter have also been described (Dubois-Rande *et al* 1991). Increased atrioventricular maximal conduction, frequency, and incidence of ventricular tachycardia (spontaneous or inducible), with no significant changes in ECG or EP intervals, or

refractory periods may also be observed (Ludmer *et al* 1986; Pflugfelder *et al* 1991). Frequency of PVC's and ventricular couplets may not change (Ludmer *et al* 1986), or be increased (Pflugfelder *et al* 1991).

The extent of milrinone's augmentation of cardiac index was similar in magnitude to that elicited by the ß-adrenoceptor agonist dobutamine. This was accompanied by a greater improvement in PCWP and a lower myocardial oxygen consumption than with the catecholamine, while eliciting similar haemodynamic improvement as nitroprusside, with less hypotension (Monrad *et al* 1986; Grose *et al* 1986). In recent years, milrinone has been employed as a useful tool in patients with a low cardiac output syndrome following cardiac surgery (George *et al* 1992; Feneck *et al* 1991; Wright and Sherry 1991).

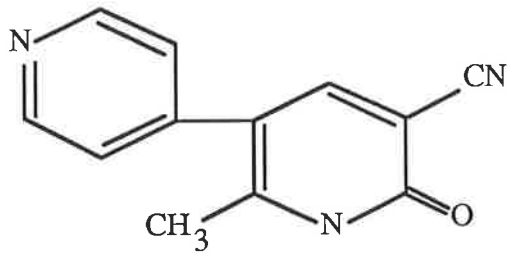
Significant improvement in heart failure symptoms, including dyspnea, orthopnea, oedema and fatigue, have been demonstrated after milrinone infusion, with a tendency for greater improvement in patients sicker prior to infusion (Anderson *et al* 1991; Villari *et al* 1991), although some arrhythmias were observed (Anderson *et al* 1991). Milrinone eliminated exercise-induced ischaemia and decreased exercise-induced ST segment depression, and, at both rest and during maximal exercise, increased cardiac output, myocardial oxygen consumption, and heart rate, and reduced PCWP and systemic vascular resistance, although the positive chronotropic effect observed during milrinone infusion at maximal exercise was less marked than that occurring during infusion at rest (Mitrovic *et al* 1991)

In an attempt to elucidate the direct positively inotropic and vasodilatatory effects of intravenous milrinone in humans, a 50µg/min intracoronary infusion was compared to a 75µg/kg intravenous bolus in patients with severe congestive heart failure (Colucci *et al* 1985; Ludmer *et al* 1986; Arnold *et al* 1986). Both doses were chosen to achieve coronary artery milrinone concentration of roughly 400ng/ml. However, the direct coronary administration resulted in only 50ng/ml systemic arterial concentrations, and failed to markedly improve cardiac output, LV end-diastolic pressure, or systemic vascular resistance observed following intravenous injection, although LV+dP/dt and stroke volume were still significantly elevated, and plasma noradrenaline reduced, by intracoronary milrinone (Colucci *et al* 1985; Ludmer *et al* 1986; Arnold *et al* 1986). The direct coronary administration also failed to exert significant effects on

heart rate or mean arterial pressure (Colucci *et al* 1985; Ludmer *et al* 1986; Arnold *et al* 1986). Milrinone therefore possesses cardiotonic activity in patients with congestive heart failure independently of its vasodilatatory actions. The direct vasodilator effect of milrinone independent of inotropic activity was demonstrated in a complementary study : brachial artery administration of a subsystemic dose of the drug in patients with moderate to severe congestive heart failure significantly increased forearm blood flow and reduced forearm resistance without influence on systemic haemodynamics (Cody *et al* 1986).

Following complete autonomic blockade in normal volunteers, milrinone's effects on systemic vascular resistance with intravenous injection persisted, the increased heart rate still occured but to a lesser extent, while the reduction in mean arterial pressure was enhanced and the augmented cardiac index was completely abolished (Hasking *et al* 1987), implicating cardiovascular reflexes in milrinone's action on cardiac index (Hasking *et al* 1987).

In summary, milrinone has been demonstrated to increase cardiac index, spontaneous heart rate, and LV+dP/dt, decrease PCWP, systemic and coronary vascular resistances, LV end-diastolic and mean arterial pressures, with no significant effect on ECG or EP parameters following intravenous administration. Therapeutic response to the drug is partially determined by baseline haemodynamics. Direct positive inotropic effects distinct from vasodilatation of milrinone, and *vice versa* , have been demonstrated.



milrinone

FIGURE 1.3 Structural formula of milrinone

TABLE 1.6 Haemodynamic effects of intravenous milrinone administration in animals (% change)

Dose	CO	HR	dP/dt	Contr F	MAP	Preparation	Reference
Intravenous bolus :							
3-30µg/kg		+0-8%		+20-110%	-0-6%	anaesth dogs	Lebedinsky *et al* 1992
6µg/kg		+9%	+81%	+180%	-14%	anaesth dogs	Rapundalo *et al* 1986
10µg/kg		+3%	+15%		-2%	anaesth dogs	Shaffer *et al* 1986
10µg/kg		+4%	+12%		0	anaesth dogs + auto block	Shaffer *et al* 1986
10-100µg/kg		+8-14%		+30-97%	-6-9%	anaesth dogs	Alousi *et al* 1984
10-300µg/kg		+9-19%	+13-59%		-3-15%	conscious dogs	Lee *et al* 1991b
10-300µg/kg	+40±6%	+41±6%	+131±14%		-27±5%	anaesth dogs	Lee *et al* 1991a
10-300µg/kg	+15±5%	+26±4%	+131±25%		-15±3%	anaesth dogs with ß-blockade	Lee *et al* 1991a
10-1000µg/kg		+2-71%	+20-130%		-16-44%	anaesth dogs	Steffen & Wastila 1992
30µg/kg		+3%	+45%		-3%	anaesth dogs	Shaffer *et al* 1986
30µg/kg	+29±6%	+11±1%	+69±6%		0	anaesth dogs	Alousi & Johnson 1986
30µg/kg		+6%	+35%		-5%	anaesth dogs + auto block	Shaffer *et al* 1986
40µg/kg		+5±2%	+21±3%		-5±4%	conscious dogs	Gosgnach *et al* 1991
40-320µg/kg		+5-19%	+20-84%#		-3-24%*	anaesth dogs 30mins post reperfusion	Vandenplassche *et al* 1992
0.05-10mg/kg		+0-28%	+15-212%			anaesth guinea pig	Lin & Chen 1991

TABLE 1.6 Continued

Dose	CO	HR	dP/dt	Contr F	MAP	Preparation	Reference
100µg/kg		+36%	+114%		0	anaesth dogs	Shaffer *et al* 1986
100µg/kg		+15%	+62%		-15%	anaesth dogs + auto block	Shaffer *et al* 1986
100µg/kg	+40%		+115%			dogs with HF pentobarbital-induced	Steffen & Wastila 1992
300µg/kg		+117%	+232%		0	anaesth dogs	Shaffer *et al* 1986
300µg/kg		+24%	+106%		-22%	anaesth dogs + auto block	Shaffer *et al* 1986
1mg/kg		+193%	+218%		-21%	anaesth dogs	Shaffer *et al* 1986
1mg/kg		+36%	+153%		-29%	anaesth dogs + auto block	Shaffer *et al* 1986
3mg/kg		+204%	+194%		-34%	anaesth dogs	Shaffer *et al* 1986
3mg/kg		+45%	+183%		-40%	anaesth dogs + auto block	Shaffer *et al* 1986
5mg/kg		+45%	+203%			anaesth dogs + auto block	Shaffer *et al* 1986
Intravenous infusion :							
10µg/kg/min for 6mins				+130%		anaesth dogs	Alousi *et al* 1984
10µg/kg then 1µg/kg/min for 1h		+6%	+17%		-11%	anaesth dogs partial LAD occlusion	Abe *et al* 1992

anaesth, anaesthetized; auto block, autonomic blockade; CO, cardiac output; Contr F, contractile force; Cx, circumflex; dP/dt, peak rate of LV pressure rise; HF, heart failure; HR, heart rate; LAD, left anterior descending; LV, left ventricle; MAP, mean arterial pressure

* mean aortic pressure # LV dP/dt / LVP

TABLE 1.7 Acute haemodynamic effects of milrinone administration in man (% change)

Dose	CI	PCWP	LV dP/dt	HR	MAP	SVR	LV EDP	n	Patient type	Reference
Oral :										
25µg/hg	+38%	-28%		0	-3%	-24%		26	CHF	Le Jemtel *et al* 1984
2.5mg	+22%	-22%		+6%		-22%		12	CHF	Cody *et al* 1984a
2.5-5mg	+36%	-28%						10	CHF	Maskin *et al* 1983
2.5-5mg	+5%	-24%		+5%	-2%	+10%		18	CHF	Remme *et al* 1992
2.5-7.5mg	+28%	-34%		0	+2%	-20%		12	CHF	Maskin *et al* 1984
5mg	+25%	-26%						12	CHF	Likoff *et al* 1985
5mg	+5%	-47%		-3%	+4%	+2%		18	CHF	Remme *et al* 1992
5mg	+28%	-23%		+10%		-23%		12	CHF	Cody *et al* 1984a
5mg	+32%	-28%						22	CHF	Le Jemtel *et al* 1984
7.5mg	+40%	-36%		+8%		-30%		12	CHF	Cody *et al* 1984a
10mg	+5%	-43%		+13%	-7%	-12%		18	CHF	Remme *et al* 1992
10mg	+51%	-48%		+11%		-40%		12	CHF	Cody *et al* 1984a
Intravenous bolus :										
12.5µg/kg	+29%	-19%		0	0			13	CHF	Benotti *et al* 1984
12.5µg/kg	+33%	-19%		0	0	-20%		13	CHF	Benotti *et al* 1985
12.5µg/kg	+32%	-22%		+3%	-3%	-29%		11	CHF	Benotti & Hood 1984
12.5µg/kg	+3%		+1%					6	CHF	Sonnenblick*et al* 1986
12.5-75 µg/kg	+76%		+32%	+8%	-13%	-15%	-31%	11	CHF	Jaski *et al* 1985
25µg/kg	+27%	-24%			+2%			13	CHF	Benotti *et al* 1984
25µg/kg	+36%	-11%		+3%	+4%	-20%		13	CHF	Benotti *et al* 1985
25µg/kg	+35%	-29%		+8%	-6%	-31%		11	CHF	Benotti & Hood 1984
25µg/hg	+38%	-28%		0	-2%	-34%		26	CHF	Le Jemtel *et al* 1984
25µg/hg	+17%	-24%		+8%	-3%			12	CHF	Likoff *et al* 1985
25µg/kg	+21%		+1%					6	CHF	Sonnenblick*et al* 1986
25-75µg/kg	+32%		+10%	+6%	-13%	-31%	-54%	11	CHF	Grose *et al* 1986

TABLE 1.7 (Continued)

Dose	CI	PCWP	LV dP/dt	HR	MAP	SVR	LV EDP	n	Patient type	Reference
25-75µg/kg	+49%	-40%		-2%	-4%	-33%		11	CHF	Maskin *et al* 1983
25-175 µg/kg	+53%	-38%	+28%	+8%	-6%	-35%	-33%	20	CHF	Baim *et al* 1983
50µg/kg	+22%	-37%		+12%	-12%	-24%		10	Low CO after surgery	Wright & Sherry 1991
50µg/kg	+30%	-25%		+14%	-9%	-29%		15	Low CO after surgery	Wright & Sherry 1991
50µg/kg	+35%	-33%		+14%	-10%	-31%		99	Low CO after surgery	Feneck *et al* 1991
50µg/kg	+35%	-33%		+14%	-11%	-31%		99	Low CO after surgery	Feneck *et al* 1992a
50µg/kg	+53%	-18%		+16%	-7%	-38%		10	Low CO after surgery	Wright & Sherry 1991
50µg/kg	+36%	-38%			-5%			13	CHF	Benotti *et al* 1984
50µg/kg	+45%	-35%		+4%	-6%	-33%		13	CHF	Benotti *et al* 1985
50µg/kg	+45%	-36%		+8%	-8%	-38%		11	CHF	Benotti & Hood 1984
50µg/hg	+13%	-29%		+9%				12	CHF	Likoff *et al* 1985
50µg/kg	+35%		+7%	+3%	-10%			6	CHF	Sonnenblick*et al* 1986
50µg/kg	+43%	+34%						12	CHF	Dubois-Rande*et al* 1991
50µg/kg	+62%	-27%		0	-4%	-37%		40	CHF	Klocke*et al* 1991
50µg/kg	+62%	-29%		0	-5%	-38%		20	CHF	Mager*et al* 1991
50µg/kg	+47%	-29%		+10%	-4%			95	CHF	Anderson *et al* 1991
50µg/kg	+30%	-39%		0	-7%	-28%		10	CHF	Villeri*et al* 1992
50µg/kg	+35%	-27%		+2%	-2%	-25%		96	CHF	Pflugfelder*et al* 1991
50µg/kg	+49%	-36%		+2%	-6%	-30%		26	CHF	Boesch *et al* 1992
5mg	+32%	-28%						22	CHF	Le Jemtel *et al* 1984
75µg/hg	+45%	-	+32%	+3%	-14%	-40%	-52%	8	CHF	Colucci *et al* 1985
75µg/kg	+53%	-38%	+28%	+8%	-5%	-35%	-33%	20	CHF	Baim *et al* 1983
75µg/kg	+44%	-52%			-7%			13	CHF	Benotti *et al* 1984
75µg/kg	+47%	-48%		+11%	-5%	-31%		13	CHF	Benotti *et al* 1985

TABLE 1.7 (Continued)

Dose	CI	PCWP	LV dP/dt	HR	MAP	SVR	LV EDP	n	Patient type	Reference
75µg/kg	+54%	-45%		+8%	-14%	-42%		11	CHF	Benotti & Hood 1984
75µg/hg	+28%	-38%		+6%				8	CHF	Likoff *et al* 1985
75µg/hg post ic infusion	+38%	-46%	0	0	-14%	-40%	-42%	8	CHF	Ludmer *et al* 1986
125µg/hg		-33%		+8%	-11%		-32%	17	CHF	Monrad *et al* 1984
Intravenous infusion :										
37.5µg/min then 0.375µg/kg/min 24h	+39%	-23%		0	-2-6%	-22%		26	CHF	Anderson *et al* 1991
50µg/min then 0.5µg/kg/min 2h	+22%	-52%		-3%	-9%	-22%		10	CHF	Nolan *et al* 1992
50µg/min then 0.5µg/kg/min 24h	+28%	-52%		0	0	-26%		10	CHF	Muir & Nolan 1992
50µg/min then 0.5µg/kg/min 24h	+61%	-26%		-2%	+2%	-35%		40	CHF	Klocke*et al* 1991
50µg/min then 0.5µg/kg/min 24h	+63%	-24%		0	0	-36%		20	CHF	Mager*et al* 1991
50µg/min then 0.5µg/kg/min 24h	+31%	-27%		+2%	-4%	-24%		12	CHF	Dubois-Rande*et al* 1991
50µg/min then 0.5µg/kg/min 48h	+30%	-27%		+11%	5%	-22%		96	CHF	Pflugfelder*et al* 1991
50µg/min then 0.5µg/kg/min 24h	+37%	-21%		0	-2-6%	-22%		95	CHF	Anderson *et al* 1991
50µg/kg then 0.5µg/kg/min 24h	+49%	-36%		+2%	-6%	-30%		26	CHF	Boesch *et al* 1992
50-75 µg/min then 0.5µg/kg/min 24h	+50%	-27%		+3%	-8%	-34%		10	CHF	Monrad *et al* 1986
50µg/min then 5µg/kg/min 24h	+30%	-32%		0	-3%	-25%		10	CHF	Villeri*et al* 1992
75µg/min then 0.75µg/kg/min 24h	+73%	-25%		0	-8-16%	-25%		15	CHF	Anderson *et al* 1991
? dose 2h	+59%	-46%		0		-38%	-35%	25	CHF	Braunwald 1991
? dose 48h	+49%	-39%		+5%		-29%		25	CHF	Braunwald 1991

TABLE 1.7 (Continued)

Dose	CI	PCWP	LV dP/dt	HR	MAP	SVR	LV EDP	n	Patient type	Reference
3-50µg/min i.c.	-7%	+4-20%		-3%	0	0	-3%	11	CHF	Ludmer *et al* 1986
50µg/kg then 0.375µg/kg/min	+58%	-15%		+11%		-9%	-39%	34	Low CO after surgery	Feneck *et al* 1991
50µg/kg then 0.375µg/kg/min	+40%	-33%		+15%	-7%	-31%		34	Low CO after surgery	Feneck *et al* 1992a
50µg/kg then 0.375µg/kg/min	+23%	+17%		+4%	+7%	-14%		10	Low CO after surgery	Wright & Sherry 1991
50µg/kg then 0.375-0.750 µg/kg/min 48h	+47%	-33%		+5%	-4%	-35%		24	Low CO after surgery	George *et al* 1992
50µg/kg then 0.5µg/kg/min	+5%	-17%		+23%	0	-5%		20	Stable IHD	Mitrovic *et al* 1991
50µg/kg then 0.5µg/kg/min	+49%	-20%		+12%	-13%	-39%		34	Low CO after surgery	Feneck *et al* 1991
50µg/kg then 0.5µg/kg/min	+28%	-33%		+14%	-13%	-30%		34	Low CO after surgery	Feneck *et al* 1992a
50µg/kg then 0.5µg/kg/min	+31%	+13%		+6%	+5%	-20%		15	Low CO after surgery	Wright & Sherry 1991
50µg/kg then 0.5µg/kg/min 48h	+33%	-27%		+2%	-10%	-30%		10	CHF	Goldstein *et al* 1986
50µg/kg then 0.75µg/kg/min	+66%	-15%		+17%	-13%	-43%		31	Low CO after surgery	Feneck *et al* 1991
50µg/kg then 0.75µg/kg/min	+36%	-36%		+13%	-15%	-33%		31	Low CO after surgery	Feneck *et al* 1992a
50µg/kg then 0.75µg/kg/min	+28%	0		+9%	+3%	-7%		10	Low CO after surgery	Wright & Sherry 1991
45 or 60 µg/kg then 0.99µg/kg/min	-7%			-5%	-12%			8	Normal	Borow *et al* 1986

?, dose not reported; CHF, moderate-severe congestive heart failure; CI, cardiac index; CO, cardiac output; dP/dt, peak rate of pressure rise; EDP, end-diastolic pressure; HR, heart rate; IHD, ischaemic heart disease; i.c., intracoronary; LV, left ventricle; MAP, mean arterial pressure; PCWP, mean pulmonary capillary wedge pressure; SVR, systemic vascular resistance

1.5.2 Current relevant knowledge of the short-term pharmacokinetics of the cardioactive drugs under investigation

1.5.2.1 Metoprolol

The pharmacokinetics of metoprolol have been thoroughly investigated. The drug is not significantly bound to plasma and serum proteins (Sundquist *et al* 1980), and undergoes significant hepatic metabolism, to a variety of active and inactive metabolites (Borg *et al* 1975b). Metoprolol is relatively lipophilic in comparison to other ß-adrenoceptor antagonists (log partition coefficient octanol : water 2.15).

(i) Pharmacokinetic studies of metoprolol in animals

Conventional pharmacokinetic studies of metoprolol in animal studies have been reported in a number of models, including anaesthetized cats (Borg *et al* 1975a), and conscious rats (Borg *et al* 1975b) and dogs (Borg *et al* 1975, 1975b; Regardh *et al* 1979), summarized in Table 1.8. Oral metoprolol administration to conscious rats and dogs resulted in 33% and 62% of the dose excreted in urine as H 117 / 04, 21% and 1% as H 104 / 83, and 9% and 25% as 4-hydroxy-metoprolol respectively (Borg *et al* 1975). Following intravenous administration, metoprolol elimination from the plasma follows either a mono- or biexponential time course, described by a two-compartment pharmacokinetic model (regardless of species), depending on the time period of observation (Borg *et al* 1975a; Regardh *et al* 1979). Elimination half-life is 1-2h, with apparent volume of distribution up to 6L/kg (Regardh *et al* 1979; Borg *et al* 1975a). Two hours after intravenous administration, plasma metoprolol concentrations are roughly two-fold those after oral administration of the same dose, indicating oral bioavailability of metoprolol of 40-50% (Borg *et al* 1975a): about 5-10% of the dose is transformed to 4-hydroxy-metoprolol, and slightly less to O-demethyl-metoprolol (Regardh *et al* 1979).

A limited number of investigations of metoprolol distribution to tissues including the heart have been undertaken in animals. Initial investigations in rodents indicated significant concentrations

of ^{3}H-metoprolol in murine myocardium five minutes after either intravenous or oral administration. Concentrations then progressively declined to nondetectable levels at 8h, although the extent of accumulation was reduced following oral dosing (Bodin *et al* 1975). Similar results were obtained in rats administered intravenous metoprolol (Bodin *et al* 1975). More recently, metoprolol distribution to the myocardium following intravenous administration has been examined in dogs (Ablad *et al* 1987), cats (Ablad *et al* 1987), and pigs (Ablad *et al* 1987; Ryden *et al* 1990, 1991; Hatori *et al* 1991). Metoprolol was extensively distributed to canine myocardium, to the extent that myocardial tissue concentrations were roughly five-fold those in arterial plasma (Ablad *et al* 1987). A similar degree of tissue accumulation of metoprolol has recently also been demonstrated into the brain of rats following intravenous oolus injection, with peak uptake 10min post administration (Nakazono *et al* 1992).

Metoprolol concentrations were approximately 30% lower in ischaemic myocardium than in nonischaemic myocardium in dog, cat, and pig models (Ablad *et al* 1987; Ryden *et al* 1990, 1991), although one group was unable to detect such a gradient (Hatori *et al* 1991). Hetereogenous metoprolol distribution across the wall of the ischaemic myocardium has also been reported, which is probably influenced by the rate of collateral blood flow in the region (Ablad *et al* 1987; Ryden *et al* 1990, 1991). The influence of collateral flow was excluded in additional studies in a pig model of ischaemia, an animal with extremely low collateral blood flow (Ablad *et al* 1987). Despite the low level of blood flow, significant concentrations of metoprolol could still be demonstrated across the wall of the ischaemic myocardium (Ablad *et al* 1987).

Coronary venous retroinfusion has been employed in a number of studies, to achieve substantial accumulation of metoprolol in the ischaemic myocardium, which has been demonstrated as significantly and markedly greater in all regions of ischaemic than nonischaemic myocardium, despite not achieving equivalent plasma metoprolol concentrations (Ryden *et al* 1990, 1991; Hatori *et al* 1991). Again, distribution of metoprolol across the ischaemic myocardial wall after retrograde infusion were hetereogenous, with levels decreasing from subepimyocardium to subendomyocardium (Ryden *et al* 1990, 1991; Hatori *et al* 1991).

Myocardial metoprolol concentrations, conversely, in nonischaemic regions were not altered by the mode of administration (Ryden *et al* 1990, 1991; Hatori *et al* 1991).

(ii) Pharmacokinetic studies in humans following oral metoprolol administration

The pharmacokinetics of metoprolol following oral administration in humans have been widely studied, and the results of these studies are summarised in Table 1.9 (Regardh *et al* 1974, 1975, 1981a, 1981b; Bengtsson *et al* 1975; Borg *et al* 1975b; Regardh and Johnsson 1980; Johnsson *et al* 1975; Williams *et al* 1976; Kendall *et al* 1977, 1980; Melander *et al* 1977; Jordo *et al* 1980; Myers and Thiessen 1980; Quarterman *et al* 1981; Jack *et al* 1982; Briant *et al* 1983; Godbillon *et al* 1983; Lucker *et al* 1990). As demonstrated by virtually complete recovery of a radioactive oral metoprolol dose, the drug is completely absorbed from the gastrointestinal tract, attaining peak concentrations in plasma (C_{max}) within 1-2h of administration (Regardh *et al* 1974, 1975; Bengtsson *et al* 1975; Borg *et al* 1975b; Sundquist *et al* 1980; Johnsson *et al* 1975; Williams *et al* 1976; Kendall *et al* 1977; Melander *et al* 1977; Jordo *et al* 1980; Kendall *et al* 1980; Myers and Thiessen 1980; Quarterman *et al* 1981; Regardh *et al* 1981a, 1981b; Jack *et al* 1982; Briant *et al* 1983; Godbillon *et al* 1983; Lucker *et al* 1990). First-pass metabolism limits the proportion of metoprolol reaching the systemic circulation to 60% of the dose (Regardh *et al* 1974; Kendall *et al* 1977; Sundquist *et al* 1980). Thus, the oral bioavailability of the drug is roughly half that of an intravenous dose (Regardh *et al* 1974; Johnsson *et al* 1975; Kendall *et al* 1977; Jordo *et al* 1980; Regardh *et al* 1981a; Lucker *et al* 1990), although food enhances the bioavailability of metoprolol (Melander *et al* 1977). Subsequently, there is rapid extensive metoprolol distribution to various body tissues, with approximately a 12min distribution phase half-life ($t_{1/2}$) and high steady-state apparent volume of distribution (V_d) of 3.2 L/kg in normal subjects (Regardh *et al* 1974; Jordo *et al* 1980). This rapid distribution following oral administration of metoprolol permits the plasma concentration : time profile to be described by either one-compartment (Regardh *et al* 1974) or two-compartment models (Jordo *et al* 1980), depending on the time frame of sample collection.

Presence of a number of disease states may influence the oral pharmacokinetics of metoprolol. In elderly healthy volunteers, the time of maximum plasma concentrations (T_{max}) is

significantly delayed, occurring 2.5±0.4h post administration, but C_{max} is not influenced (Briant *et al* 1983). In patients with hepatic cirrhosis, bioavailability of an oral metoprolol dose is significantly increased, to 84±10% (Regardh *et al* 1981a). Metoprolol tends to be more slowly distributed to a larger volume of rapidly accessible tissues in patients with chronic renal failure (Jordo *et al* 1980).

The major metabolic pathways of metoprolol are represented in Figure 1.4. The predominant metabolites are the non-adrenergically active H 104 / 83 and H 117 / 04, with only small amounts of O-demethyl-metoprolol (Borg *et al* 1975b; Regardh and Johnsson 1980; Quarterman *et al* 1981). The fourth metabolite, 4-hydroxy-metoprolol, exerts approximately one tenth the efficacy of the parent drug (Regardh and Johnsson 1980). Following a single oral metoprolol dose, 4-hydroxy-metoprolol concentrations were approximately 8ng/ml, gradually rising to a peak of 67±3 and 112±13ng/ml at 3.1±0.8 and 2.4±0.4h after administration, with elimination phase half-lives of 6.7±1.0 and 9.9±1.8h in young and elderly healthy volunteers respectively (Quarterman *et al* 1981). Approximately 10% of the dose was excreted in the urine at 24h as this active metabolite, 10% as H 104 / 83, 0.2% as H105 / 22, and approximately 60% as H 117 / 04 (Borg *et al* 1975b; Quarterman *et al* 1981). The extensive hepatic metabolism limits the amount of drug excreted as the parent compound to only about 5-15% of the initial dose (Regardh *et al* 1974; Kendall *et al* 1977; Jordo *et al* 1980; Quarterman *et al* 1981; Regardh *et al* 1981), and even less in patients with chronic renal failure (Jordo *et al* 1980).

The elimination $t_{1/2}$ of metoprolol in healthy subjects, and a variety of patient groups, ranges from 2.5-5h generally, is not influenced by patient age (Bengtsson *et al* 1975; Regardh *et al* 1974, 1975; Kendall *et al* 1977; Regardh and Johnsson 1980; Johnsson *et al* 1975; Williams *et al* 1976; Melander *et al* 1977; Jordo *et al* 1980; Kendall *et al* 1980; Myers and Thiessen 1980; Quarterman *et al* 1981; Regardh *et al* 1981b, Jack *et al* 1982; Briant *et al* 1983; Godbillon *et al* 1983), but is positively correlated with serum bilirubin (Regardh *et al* 1981a). However, significantly prolonged elimination half-lives (roughly 8h) have been reported in humans with an autosomal recessive oxidative polymorphism, who display impaired formation of the 4-hydroxy-metabolite (Lennard *et al* 1982; Silas *et al* 1985). Stereoselective metabolism

of metoprolol has been demonstrated following oral metoprolol administration in extensive (EM) and poor debrisoquine metabolizers (PM) in man : S-metoprolol plasma concentrations and elimination half-lives were higher than R-metoprolol in EM, while the reverse was true in PM; and renal clearance of R-metoprolol was consistently greater than for S-metoprolol, regardless of phenotype (Lennard *et al* 1983).

The renal excretion of metoprolol is predominantly determined by the rate of glomerular filtration, but this does not necessarily imply altered elimination of the drug in the settings of depressed renal function and / or abnormal urinary pH, because of the minor contribution of renal excretion to the elimination of metoprolol from the body in humans (Regardh *et al* 1974; Jordo *et al* 1980). Additionally, alterations in gastric emptying and gut motility do not influence metoprolol bioavailability, but may influence the time course and extent of maximal plasma concentrations (Briant *et al* 1983). Total body clearance of metoprolol is weakly but linearly directly related to galactose clearance (Regardh *et al* 1981a).

When incubated with heparinized whole blood from healthy volunteers for one hour, metoprolol is concentrated in the red blood cells (RBC), to the extent that concentrations within the RBC are approximately 50% higher than in plasma (Regardh *et al* 1974). As a consequence, metoprolol concentrations are about 20% higher in whole blood than plasma, and are not equal, as previously assumed (Regardh *et al* 1974).

(iii) Pharmacokinetic studies in humans following intravenous metoprolol administration

Intravenous metoprolol pharmacokinetics have also been widely explored in humans, and these are also summarized in Table 1.9 (Johnsson *et al* 1975; Regardh *et al* 1974, 1980, 1981a; Jordo *et al* 1980). In healthy volunteers, an intravenous metoprolol dose shows a distribution phase half-life of 5-15min, an elimination phase half-life of 4h, and volume of distribution at steady-state of 3.2kg (Jordo *et al* 1980; Regardh *et al* 1980, 1981). Total body clearance was approximately 1L/min, and 14.5% of the dose was excreted in the urine unchanged at 48h (Jordo *et al* 1980; Regardh *et al* 1981). Thirty minutes after the iv dose, concentration of 4-hydroxy-metoprolol was approximately 25nM, which increased to a peak of roughly 41nM 2h

post injection (Regardh *et al* 1981). O-demethyl-metoprolol was not detectable at any time point (Regardh *et al* 1981). Depending on the time scale of observation of plasma metoprolol concentrations following intravenous bolus administration (5-20mg), concentrations decay either monoexponentially (6h monitoring) or biexponentially (≥24h follow up). This decay was described by a one or two-compartment model (Johnsson *et al* 1975; Regardh *et al* 1974; Jordo *et al* 1980; Regardh *et al* 1980, 1981). The longer period of observation provides more reliable information, as the shorter period only examines ≤ 2 complete elimination half-times. The short distribution-phase $t_{1/2}$ of 0.2h indicates rapid and extensive distribution of metoprolol to the tissues, while the average elimination phase $t_{1/2}$ of 3-5h implied less than 1% of the dose remained in the body at 24h (Regardh *et al* 1974; Johnsson *et al* 1975; Jordo *et al* 1980; Regardh *et al* 1980, 1981). Metabolism and elimination of an intravenous metoprolol dose is essentially the same as following oral administration, without the first-pass effect (Borg *et al* 1975a). Metoprolol is predominantly hepatically excreted (see Figure 1.4), with 62% of an intravenous dose excreted in urine as H 117 / 04, 10% as H 104 / 83, and 10% as 4-hydroxy-metoprolol (Borg *et al*, 1975a).

In patients with hepatic cirrhosis, the intrinsic elimination rate constant was significantly reduced, and the volume of the central compartment augmented (Regardh *et al* 1981). Additionally, the extent of metabolism of metoprolol to the 4-hydroxy-derivative was reduced two to three fold (Regardh *et al* 1981).

In summary, the disposition of metoprolol following oral or intravenous administration in animals or humans is represented in Figure 1.5. The drug undergoes extensive hepatic metabolism, with very little excreted unchanged in urine. Significant cardiac concentrations have been reported in animal studies, with lower concentrations in ischaemic myocardium. In man, metoprolol exhibits an elimination-phase $t_{1/2}$ of 3-4h, but the acute time course of myocardial metoprolol accumulation has not been previously studied.

TABLE 1.8 Metoprolol pharmacokinetics in animals after oral and intravenous administration

Dose	C_{max} ng/ml	T_{max} h	$t_{1/2}$ h	V_d L/kg	CL	n	Preparation	Reference
Oral :								
0.40 mg/kg	17 ng/g	1.0	1.7			3	conscious dogs	Borg *et al* 1975a
1.00 mg/kg	70 ng/g	0.5	1.7			3	conscious dogs	Borg *et al* 1975a
Intravenous bolus :								
1.10 µmol/kg	310	0.15*	2	3.4	19 ml/kg/min	2	dogs	Regardh *et al* 1979
0.40 mg/kg	70 ng/g	0.25*	1.7			3	conscious dogs	Borg *et al* 1975a
0.80 mg/kg	320 ng/g	0.03*	1.4±0.1	5.5±0.6		2	anaesth cats	Borg *et al* 1975a
1.00 mg/kg	210 ng/g	0.25*	1.7			3	conscious dogs	Borg *et al* 1975a

anaesth, anaesthetized; CL, total plasma clearance; C_{max}, peak plasma concentration; h, hours; $t_{1/2}$, elimination half-life; T_{max}, time of peak plasma concentrations; V_d, apparent volume of distribution

* Note that peak concentrations following intravenous administration are the first sampling time point

TABLE 1.9 Metoprolol pharmacokinetics in humans after oral and intravenous administration

Dose	C_{max} ng/ml	T_{max} h	$t_{1/2}$ h	V_d L/kg	CL	% 24h urine	n	Patient type	Reference
Oral :									
5mg	5	1	3.1±0.1			5%	5	Normal	Regardh *et al* 1974
20mg	16	1.6	2.9				5	Normal	Johnsson *et al* 1975
50mg	50	1.6	2.9				5	Normal	Johnsson *et al* 1975
50mg	80±16	1.7±0.3	4.3±0.9				6	Normal	Jordo *et al* 1980
50mg	85	1.0	3.3			4.4%	6	Normal	Kendall *et al* 1977
50mg	237±52	1.7±0.3	4.3±0.9			11.0%#	6	Normal	Regardh *et al* 1981a
50mg	72±13	1.4±0.2	3.8±0.3				8	HT	Bengtsson *et al* 1975
50mg	80±16	1.8±0.2	5.0±1.1				6	RF	Jordo *et al* 1980
50mg	429±71	1.8±0.3	6.2±1.1			16.2%#	10	HC	Regardh *et al* 1981a
80mg	113±22	1.2±0.1	4.3±0.7				6	HT	Bengtsson *et al* 1975
100mg	90±10	2.1±0.3	2.7±0.2				8	Normal + fasting	Melander *et al* 1977
100mg	103±20	2.0±0.2	3.3±0.2				11	Normal females	Jack *et al* 1982
100mg	105	1.5	3.4			3.5%	6	Normal	Kendall *et al* 1977
100mg	115±24	2.4±0.4	4.2±0.3			5.6±0.9%	8	Normal	Quarterman *et al* 1981
100mg	117	1.3	2.9				5	Normal	Johnsson *et al* 1975
100mg	118±18	1.7±0.3	2.5±0.2				8	Normal + non-fasting	Melander *et al* 1977
100mg	125	1.7±0.3	3.3±0.2				6	Normal	Regardh *et al* 1981b
100mg	135±34	1.5±0.3	4.0±0.6			3%##	6	Normal	Regardh *et al* 1975
100mg	140±17	2.3±0.3	3.6±0.4				12	Normal females taking OC	Jack *et al* 1982
100mg	152±100	1.0	1.7±0.3				6	Normal	Williams *et al* 1976

TABLE 1.9 (Continued)

Dose	C_{max} ng/ml	T_{max} h	$t_{1/2}$ h	V_d L/kg	CL	% 24h urine	n	Patient type	Reference
100mg	157±90	2.0	3.1±0.9				6	Normal	Godbillon *et al* 1983
100mg	208±36	1.5	2.9±0.3				6	Normal	Kendall *et al* 1980
100mg	214±56	1.3±0.6	3.1±0.4				5	Normal males	Jack *et al* 1982
100mg	216±45	1.2±0.1	4.1±0.9				8	Normal	Briant *et al* 1983
100mg	189±45	4.6±0.6	4.4±1.5				5	Normal with probanthine PT	Briant *et al* 1983
100mg	229±45	0.7±0.1	3.5±0.8				8	Healthy with metoclopramide PT	Briant *et al* 1983
100mg	106±24	1.2±0.1	2.8±0.4			2.8±0.5%	7	Elderly	Quarterman *et al* 1981
100mg	217±43	2.5±0.4	4.5±0.8				7	Elderly	Briant *et al* 1983
100mg	321±63	1.7±1.0	4.1±0.5				7	Elderly with metoclopramide PT	Briant *et al* 1983
100mg	199±38	1.7±0.2	4.1±0.6				14	HT	Myers & Thiessen 1980
200mg	280	2.0	3.2			3.6%	6	Normal	Kendall *et al* 1977
Chronic oral :									
100 mg/day	606±64	1.0					16	Normal	Lucker *et al* 1990
200 mg/day	959±85	1.0					18	Normal	Lucker *et al* 1990
300 mg/day	1287±117	1.2					18	Normal	Lucker *et al* 1990
400 mg/day	1111±63	1.2					18	Normal	Lucker *et al* 1990

TABLE 1.9 (Continued)

Dose	C_{max} ng/ml	T_{max} h	$t_{1/2}$ h	V_d L/kg	CL	% 24h urine	n	Patient type	Reference
Intravenous bolus :									
5mg	11.5	0.33*	2.9				5	Normal	Johnsson *et al* 1975
5mg	21	0.07*	3.2±0.1	5.6±0.2		10%	5	Normal	Regardh *et al* 1974
10mg	31	0.33*	2.9				5	Normal	Johnsson *et al* 1975
10mg	170	0.08*	3.6±0.3	4.4±0.4	1.2L/min		6	Normal	Regardh *et al* 1980
15mg	44	0.33*	2.9				5	Normal	Johnsson *et al* 1975
20mg	56	0.33*	2.9				5	Normal	Johnsson *et al* 1975
20mg	200	0.17*	4.2±1.1	3.2±0.2	0.80±0.11 L/min	14.5± 0.5%##	6	Normal	Jordo *et al* 1980
20mg	317	0.20*	4.2±1.1	3.2±0.2	0.80±0.11 L/min	14.5%#	6	Normal	Regardh *et al* 1981a
20mg	130	0.17*	3.5±0.6	3.5±0.3	1.00±0.13 L/min	3.0± 0.7%##	6	RF	Jordo *et al* 1980
20mg	223	0.20*	7.2±1.2	4.0±0.3	0.61±0.13 L/min	19.4%#	10	HC	Regardh *et al* 1981a

CL, total plasma clearance; C_{max}, peak plasma concentration; h, hours; HC, hepatic cirrhosis; HT, hypertension; OC, oral contraceptives; PT, pretreatment; RF, renal failure; $t_{1/2}$, elimination half-life; T_{max}, time of peak plasma concentrations; V_d, apparent volume of distribution; % 24h urine, amount of parent drug excreted unchanged at 24h

* Note that peak concentrations following intravenous administration are the first sampling time point
\# actually 48h ## actually 72h

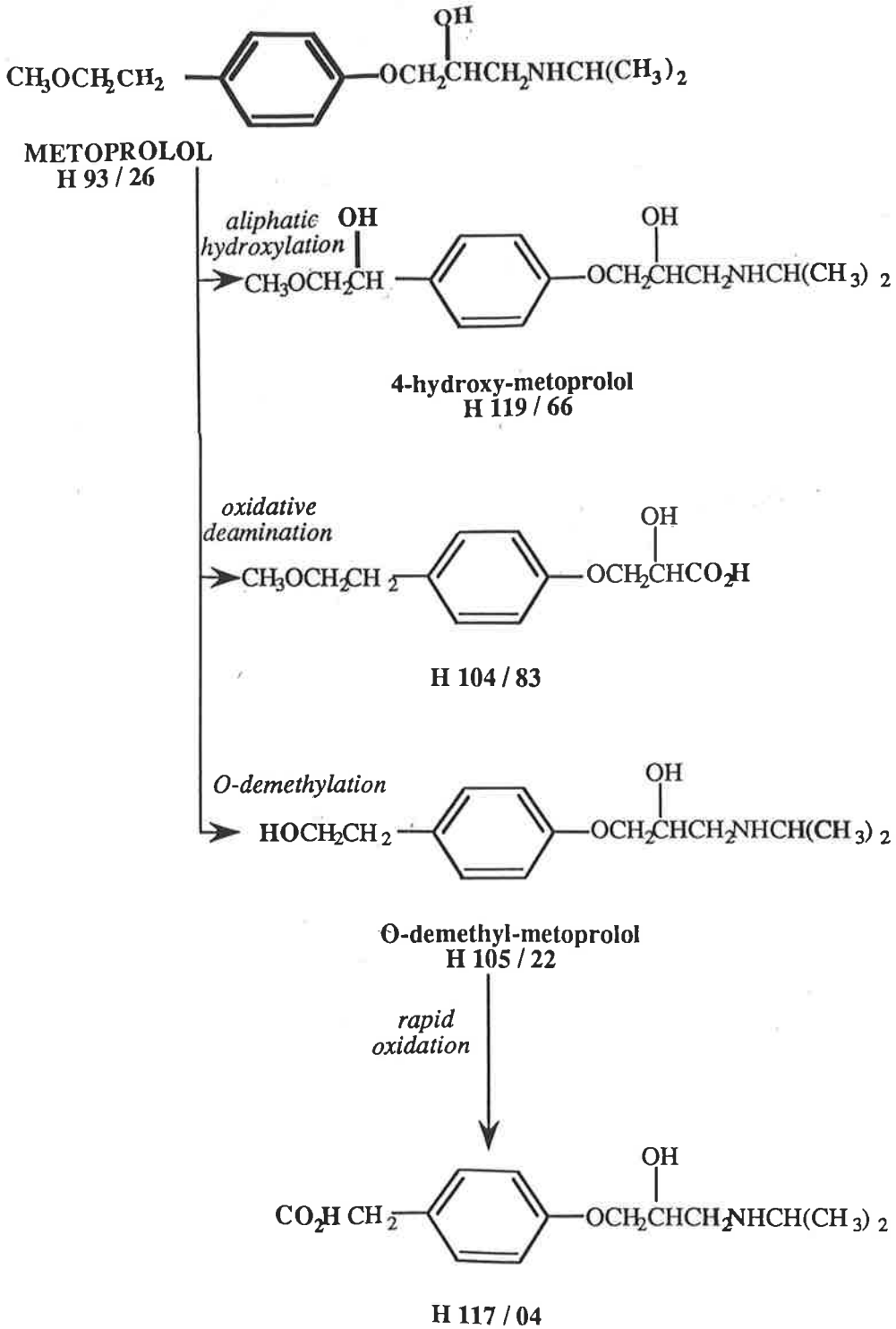


FIGURE 1.4 The major metabolic pathways of metoprolol in humans (Borg *et al* 1975b; Regardh and Johnsson 1980; Quarterman *et al* 1981)

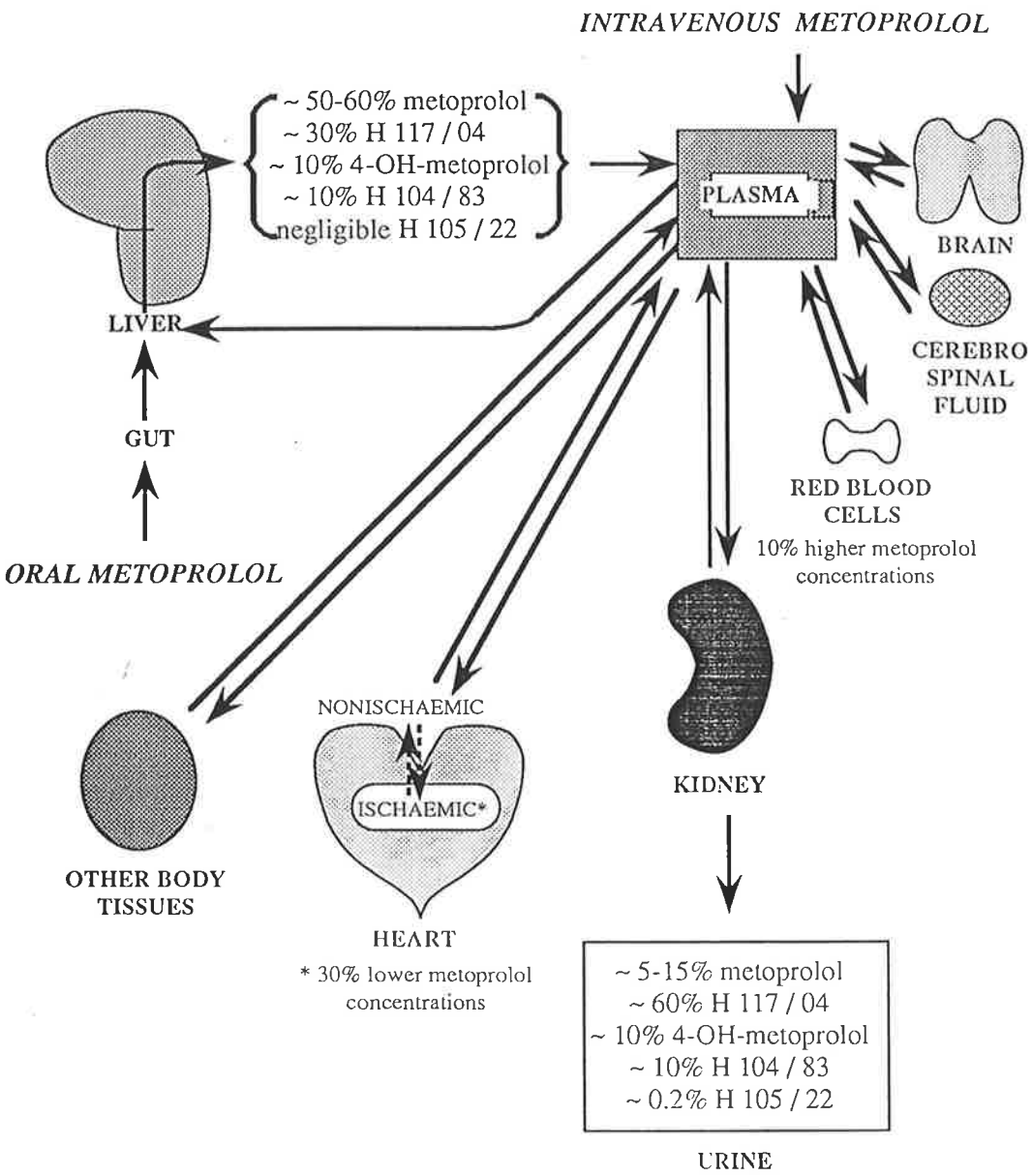


FIGURE 1.5 An outline of the disposition of metoprolol in animals and man after either oral or intravenous administration (Borg *et al* 1975a, 1975b; Regardh and Johnsson 1980; Regardh *et al* 1974, 1979, 1980, 1981; Kendall *et al* 1977; Sundquist *et al* 1980; Jordo *et al* 1980; Quarterman *et al* 1981; Nakazono *et al* 1992)

1.5.2.2 Sotalol

The pharmacokinetics of sotalol have been extensively examined. The drug is not significantly metabolized, and is devoid of active metabolites. Sotalol is relatively hydrophilic, at least in comparison to other ß-adrenoceptor antagonists (Arendt *et al* 1984).

(i) Pharmacokinetic studies of sotalol in animals

The pharmacokinetics of sotalol in animals *in vivo* have not been as extensively examined as those in humans. However, a number of animal kinetic studies have been published (Schnelle and Garrett 1973; Kato *et al* 1986; Ishizaki and Taawara 1979; Arendt *et al* 1984; Sundquist *et al* 1980). Significantly higher concentrations of sotalol in rat hearts have been observed compared to plasma, implying significant myocardial sotalol uptake (Sundquist *et al* 1980). After intravenous sotalol bolus administration, plasma concentration-time profile has been demonstrated to fit a two-compartment model, with an elimination half-life of between 3-5h, mean residence time of 5.21±0.73h, apparent volume of distribution between 1-2L/kg and total plasma clearance between 2.7-7.6ml/min/kg, (approximately 90% of which was renal clearance), and 72±12% was excreted unchanged in the urine at 8h, in conscious dogs, in anaesthetized dogs, in anaesthetized cats (Schnelle and Garrett 1973; Kato *et al* 1986; Ishizaki and Taawara 1979; Arendt *et al* 1984). Sotalol undergoes no major metabolism, and is only negligibly bound to plasma proteins and red blood cells (Schnelle and Garrett 1973). Pharmacokinetics of sotalol in animals, at least in dogs, are not enantioselective (Kato *et al* 1986).

The pharmacokinetics of sotalol have also been examined in cerebrospinal fluid (CSF) in anaesthetized cats (Arendt *et al* 1984). Peak CSF sotalol concentrations were achieved 27.0±3.0min following a 10mg/kg intravenous injection. The half-lives of entry into and disappearance from CSF of this ß-adrenoceptor antagonist were 12min and 3h respectively, considerably later than those observed with either propranolol or acebutolol (Arendt *et al* 1984). Uptake of sotalol by the cerebral cortex was considerably lower than that of the more

lipophilic propranolol (Arendt *et al* 1984), indicating less potential for central nervous system side effects with sotalol administration.

(ii) Pharmacokinetic studies in humans following oral sotalol administration

Sotalol pharmacokinetics in humans after oral administration, in both health and disease, are summarized in Table 1.10. The pharmacokinetics of sotalol following oral administration in healthy volunteers have been extensively examined (Anttila *et al* 1976; Tjandramaga *et al* 1976; Kahela *et al* 1979; Ishizaki *et al* 1980; Blair *et al* 1981; Sundquist *et al* 1980; Carr *et al* 1992). Generally, following a single 160mg dose, peak plasma sotalol concentrations (C_{max}) of 900-2000ng/ml are achieved 2-3h (T_{max}) after administration. The elimination phase half-life ($t_{1/2}$) ranged from 5-17h, with apparent volume of distribution (V_d) 1-3L/kg, total plasma clearance (CL) between 2-6ml/min/kg, and 50-80% of the dose was excreted unchanged in the urine at 24h (Anttila *et al* 1976; Tjandramaga *et al* 1976; Kahela *et al* 1979; Ishizaki *et al* 1980; Blair *et al* 1981; Sundquist *et al* 1980; Carr *et al* 1992), implying a lack of significant metabolism, and predominantly renal clearance. A lower dose of 80mg behaved similarly, but with peak plasma concentrations of 420±60ng/ml (Ishizaki *et al* 1980). In healthy volunteers, peak plasma sotalol concentrations were significantly delayed and lower following food (Kahela *et al* 1979; Sundquist *et al* 1980). Sotalol was not bound to plasma proteins in the 0.5-50μg/ml concentration range (Anttila *et al* 1976; Sundquist *et al* 1980). Plasma half-life and the clearance rate were not correlated with any indirect or direct reflectors of hepatic drug metabolizing enzyme activity (Sotaniemi *et al* 1979). The pharmacokinetics of d- and l-sotalol in plasma were not enantioselective in any of the above pharmacokinetic parameters, the plasma concentration versus time profiles, or in trough plasma samples (Carr *et al* 1992; Sallustio *et al* 1993).

The pharmacokinetics of sotalol following oral administration in patients with a range of conditions have also been examined (Tjandramaga *et al* 1976; Sotaniemi *et al* 1979; Blair *et al* 1981; Ishizaki *et al* 1980; Sundquist *et al* 1975; Berglund *et al* 1980). In patients with hypertension or angina pectoris, who are often elderly, the pharmacokinetics of sotalol were not markedly different from those described in healthy volunteers following a single oral dose

(Sotaniemi *et al* 1979; Ishizaki *et al* 1980). Conversely, despite no change in peak plasma concentrations, time to peak concentrations or apparent volume of distribution in patients with moderate to severe renal failure (Tjandramaga *et al* 1976; Blair *et al* 1981; Sundquist *et al* 1979), a single 160mg oral sotalol dose resulted in significantly longer elimination half-lives (20-60h), impaired plasma sotalol clearance (only approximately 20% of that in healthy volunteers) and reduced percent excreted in urine unchanged at 24h (only 4-7%; Tjandramaga *et al* 1976; Blair *et al* 1981; Sundquist *et al* 1975). Sotalol plasma clearance and elimination half-life were clearly dependent on creatinine clearance (Tjandramaga *et al* 1976; Blair *et al* 1981; Sundquist *et al* 1975), also indicating renal clearance as the predominant elimination pathway for sotalol in humans.

The pharmacokinetics of sotalol have also been examined in cerebrospinal fluid (CSF) in humans (Sundquist *et al* 1980). Sotalol concentrations in CSF were only 10% of those in plasma (Sundquist *et al* 1980), consistent with the lack of centrally-mediated side effects of sotalol in humans.

(iii) Pharmacokinetic studies in humans following intravenous sotalol administration

Sotalol pharmacokinetics in humans after intravenous administration are also described in Table 1.10. The pharmacokinetics of sotalol following intravenous administration in man have been less extensively examined than after oral administration, but a small number of studies have been reported (Anttila *et al* 1976; Poirier *et al* 1981). Generally, the elimination phase half-life, apparent volume of distribution, total plasma clearance and percent of the dose excreted unchanged in the urine at 24h in healthy volunteers after intravenous bolus administration were similar to those after oral doses (Anttila *et al* 1976; Poirier *et al* 1981), although peak plasma concentrations are obviously more rapidly achieved (Anttila *et al* 1976; Poirier *et al* 1981).

Figure 1.6 summarizes the distribution of sotalol following oral or intravenous administration in animals or humans. The drug is not significantly metabolized, and therefore predominantly undergoes renal elimination. Although significant cardiac sotalol concentrations have been reported in animal studies, only small amounts were apparent in cerebrospinal fluid. Sotalol has

an elimination-phase $t_{1/2}$ of 5-17h in humans, but its acute time course of myocardial content has not been previously studied.

TABLE 1.10 Sotalol pharmacokinetics in humans after oral and intravenous administration

Dose	C_{max} ng/ml	T_{max} h	$t_{1/2}$ h	V_d L/kg	CL	% 24h urine	n	Patient type	Reference
Oral :									
80mg	420±60	2.5±0.3	7.1±0.9	1.8±0.3	5.9±1.0 ml/min/kg	80±5%	6	Normal	Ishizaki *et al* 1980
160mg	890±130	2.5±0.3	7.1±0.9	1.8±0.3	5.9±1.0 ml/min/kg	80±5%	6	Normal	Ishizaki *et al* 1980
160mg	1234±117	3.1±0.2	8.0±0.3	2.0±0.1	12.1±0.8 L/h	76%	8	Normal	Carr *et al* 1992
160mg	1250±140	3.0	17.2	2.7	150 ml/min	56%	8	Normal	Anttila *et al* 1976
160mg	1590±80	2.2±0.2					5	Normal	Kahela *et al* 1979
160mg	1600±200	2.3±0.3	5.2±1.3			54±1%	4	Normal	Tjandramaga *et al* 1991
160mg	2000±700	3.0±0.8	8.1±3.4	1.4±0.4	71±31 ml/min/m^2	64±20%	8	Normal	Blair et al 1991
160mg	720-1190	4.0	12±5		129±37 ml/min		32	HT or angina	Sotaniemi *et al* 1979
160mg	1420±120	2.9±0.5	11±2	0.9±0.2	3.3±0.2 ml/min/kg	70±8%	9	HT and elderly	Ishizaki *et al* 1980
160mg	2000±600	3.4±0.8	24±8	1.3±0.6	24±7 ml/min/m^2	55±14%	6	moderate RF	Blair *et al* 1991
160mg	1330±170		10				8	RF (SC < 0.2mM)	Sundquist *et al* 1975

TABLE 1.10 (Continued)

Dose	C_{max} ng/ml	T_{max} h	$t_{1/2}$ h	V_d L/kg	CL	% 24h urine	n	Patient type	Reference
160mg	1270±180		27				7	RF (SC 0.2-0.6mM)	Sundquist *et al* 1975
160mg	1120±110		56				10	RF (SC > 0.6mM)	Sundquist *et al* 1975
160mg	1900±400	3.8±1.7	34±27	1.6±1.1	15±11 ml/min/m^2	7±7%	6	RF requiring dialysis	Blair *et al* 1991
160mg	1900±200	3.0±0.5	41±5			4±2%	6	RF requiring dialysis	Tjandramaga *et al* 1991
Intravenous bolus :									
20mg	320	0.25*	7.33	1.5	159 ml/min	80%	8	Normal	Anttila *et al* 1976
1.2mg/kg	2750±380	0.08*	7.3±1.1	1.1±0.1	2.0±0.3 ml/min/kg	87% at 72h	6	Normal	Poirier *et al* 1981

CL, total plasma clearance; C_{max}, peak plasma concentration; h, hours; HT, hypertension; RF, renal failure; SC, serum creatinine; $t_{1/2}$, elimination half-life; T_{max}, time of peak plasma concentrations; V_d, apparent volume of distribution; % 24h urine, amount of parent drug excreted unchanged at 24h.

* Note that peak concentrations following intravenous administration are for the first sampling time point

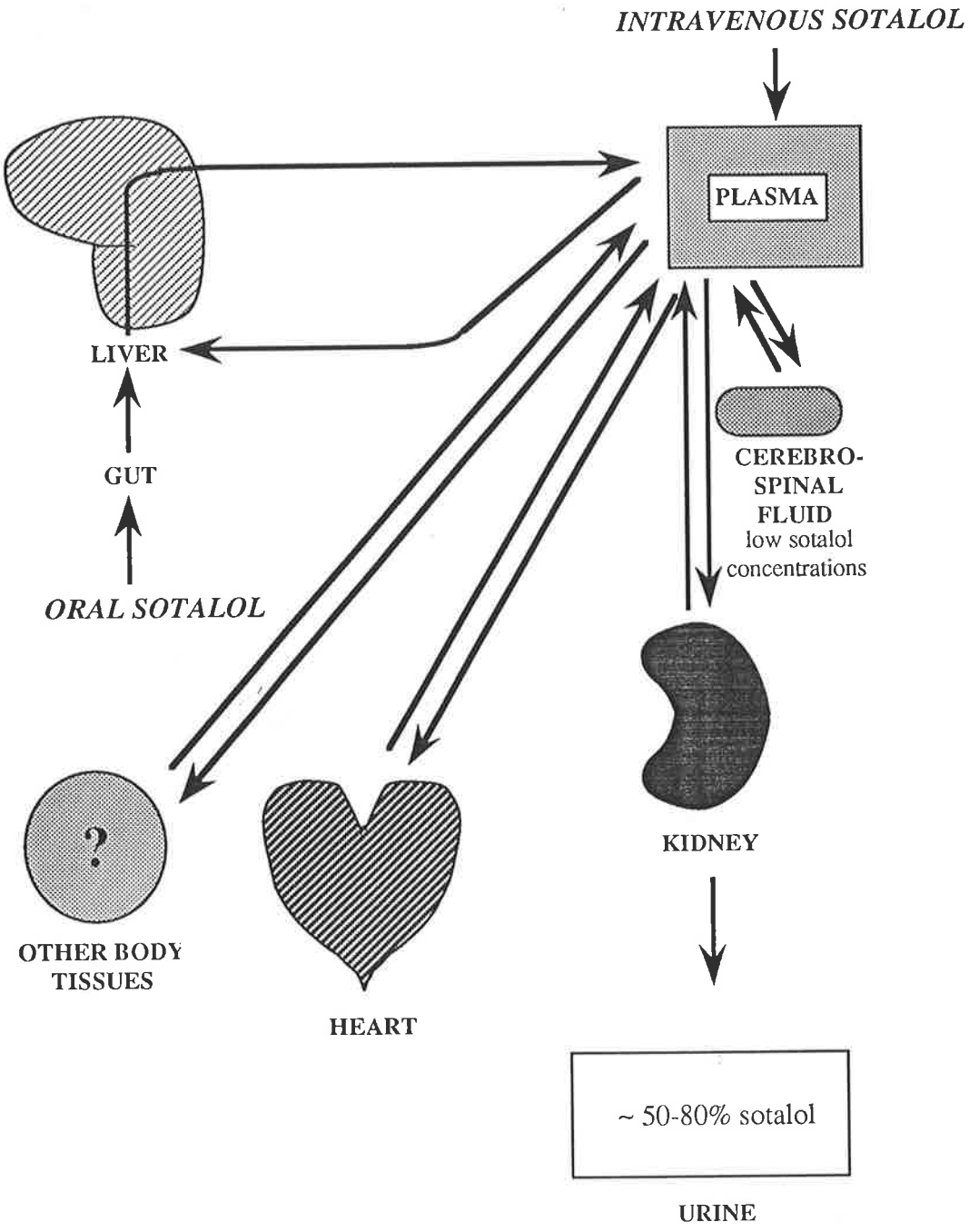


FIGURE 1.6 An outline of the disposition of sotalol in animals and humans after either oral or intravenous administration (Sundquist *et al* 1975a, 1980; Tjandramaga *et al* 1976; Anttila *et al* 1976; Blair *et al* 1981; Arendt *et al* 1984; Carr *et al* 1992)

1.5.2.3 Milrinone

A considerable volume of information regarding the conventional pharmacokinetics of milrinone in humans has been published in the past decade, and a small amount in animals *in vivo* . The majority of this information concerns intravenous administration, although some pharmacokinetic studies of oral milrinone administration in humans, and a limited number of pharmacokinetic syudies in animals, have been reported.

(i) Pharmacokinetic studies in animals following milrinone administration

The pharmacokinetics of milrinone have been investigated in rats, dogs, and monkeys (Baker and Edelson 1984). Following oral administration of ^{14}C-milrinone, 60-80% of the dose was recovered in the urine at 18h, 70-90% of which was the parent compound (Baker and Edelson 1984). An elimination half-life of 3.6h, and an apparent volume of distribution of 1.3h were observed following intravenous administration of 5mg/kg ^{14}C-milrinone (Baker and Edelson 1984).

(ii) Pharmacokinetic studies in humans following oral milrinone administration

In both healthy volunteers and patients (with either congestive heart failure or renal impairment), the pharmacokinetics of milrinone following oral milrinone administration have been investigated (Larsson *et al* 1986; Edelson *et al* 1986; Stroshane *et al* 1984). Following a single oral dose in healthy subjects, milrinone was rapidly absorbed, achieving maximal plasma concentrations between 0.6-1.1h, with a terminal elimination half-life of approximately 55min (Larsson *et al* 1986; Stroshane *et al* 1984). Somewhat longer half-lives were observed in patients with severe congestive heart failure or moderate renal impairment (1.8 and 2.7h respectively), and peak concentrations in plasma were observed 1-1.2h in both patient groups (Larsson *et al* 1986; Remme *et al* 1992; Edelson *et al* 1986).

(iii) Pharmacokinetic studies in humans following intravenous milrinone administration

Intravenous milrinone pharmacokinetics in humans are summarised in Table 1.11. Bolus milrinone injection generally achieved peak milrinone concentrations in plasma between 60-1500ng/ml, elimination half-life ranged from 1.5-2.0h in these patients (but was shorter in healthy volunteers), apparent volume of distribution of milrinone was approximately 0.35L/kg, clearance was roughly 0.15 L/kg/hr, regardless of patient type, and 85% was excreted in the urine unchanged (Cody *et al* 1984b; Stroshane *et al* 1984a, 1984b; Benotti *et al* 1984, 1985; Edelson et al 1986; Baim *et al* 1983; Likoff *et al* 1985). First-order, two-compartment pharmacokinetics were observed, with plasma concentrations declining biexponentially with time (Stroshane *et al* 1984).; Cody *et al* 1984; Benotti *et al* 1984; Benotti *et al* 1985).

In conclusion, the conventional pharmacokinetics milrinone have been predominantly investigated following intravenous administration. An approximate summary of the disposition of milrinone in either animals or humans is illustrated in Figure 1.7. The drug is not significantly metabolized, and therefore predominantly undergoes renal elimination. Milrinone has an elimination-phase $t_{1/2}$ of only 1h in normal humans, which may be delayed up to 2.5h in patients with moderate to severe congestive heart failure. The acute time course of myocardial milrinone uptake has not been previously studied.

TABLE 1.11 Milrinone pharmacokinetics in humans after intravenous administration

Dose	C_{max} ng/ml	$t_{1/2}$ h	V_d L/kg	CL	% 24h urine	n	Patient type	Reference
Intravenous bolus :								
10-125µg/kg	63-640	0.9	0.3±0.1	26±6 L/h	85±10%	21	Normal	Stroshane *et al* 1984b
12.5-75µg/kg	57-329	1.7	0.4±0.0	0.2±0.0 L/m/kg		13	CHF	Benotti *et al* 1984
12.5-75µg/kg	81-454	1.7±0.0	0.4±0.0	0.2±0.0 L/m/kg		13	CHF	Benotti *et al* 1985
12.5-75µg/kg	155-446	1.7	0.4	0.16 L/kg/h		8	CHF	Stroshane *et al* 1984a
12.5-125 µg/kg	133-994	2.3	0.3±0.1	113±7 ml/kg/h		26	CHF	Edelson *et al* 1986
12.5-175 µg/kg	392±23					20	CHF	Baim *et al* 1983
25-75µg/kg	540-1480					32	CHF	Likoff *et al* 1985
2.5-10mg	63-294	2.1	0.5			10	CHF	Cody *et al* 1984b
Intravenous infusion :								
0.2-0.7 µg/kg/min	96-355	2.6	0.5	0.14 L/kg/h		26	CHF	Edelson *et al* 1986
0.2-0.7 µg/kg/min	81-277		0.5	0.15 L/kg/h		8	CHF	Stroshane *et al* 1984a

CHF, moderate to severe congestive heart failure; CL, total plasma clearance; C_{max}, peak plasma concentration; h, hours; $t_{1/2}$, elimination half-life; V_d, apparent volume of distribution; % 24h urine, amount of parent drug excreted unchanged at 24h

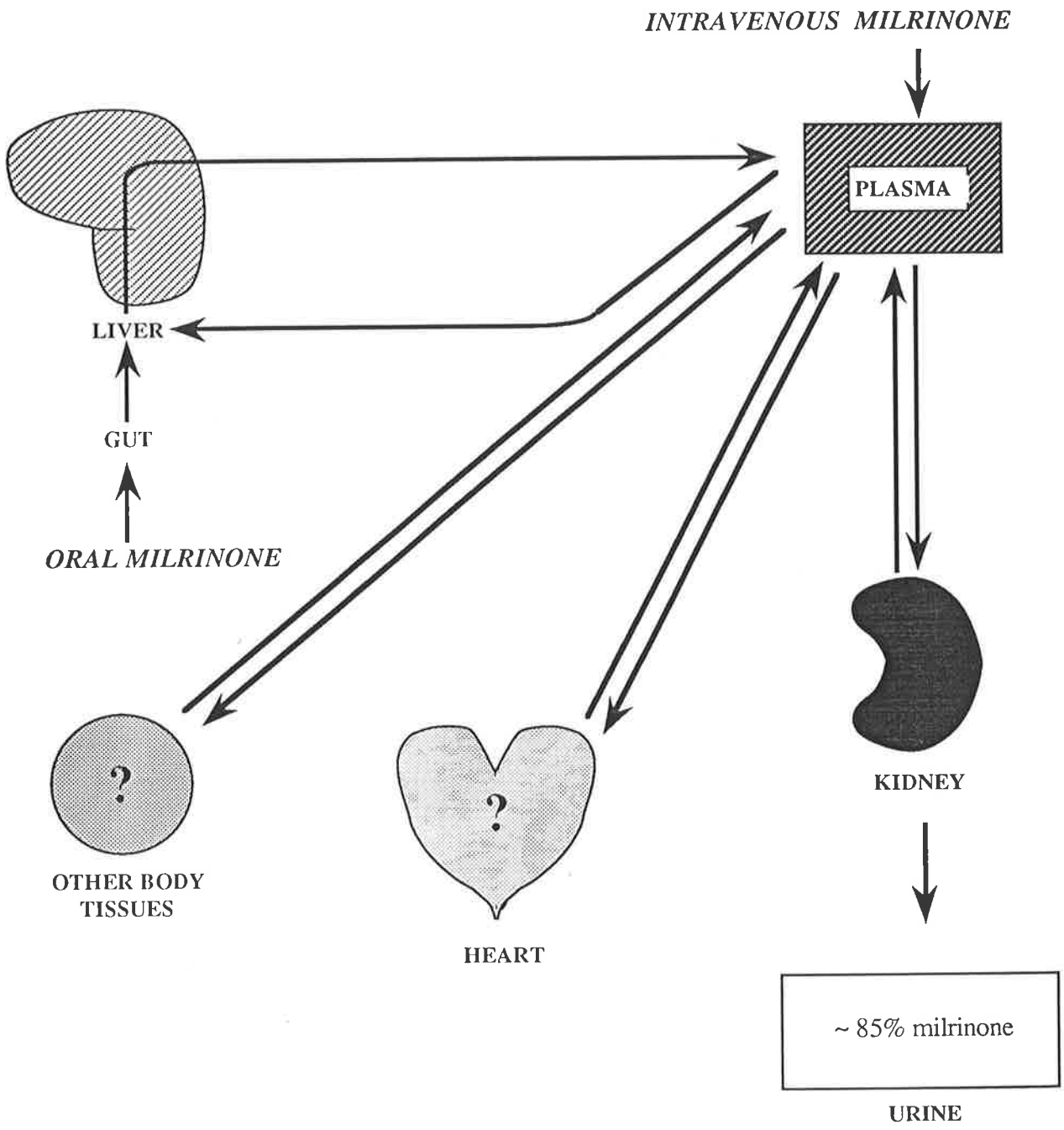


FIGURE 1.7 An outline of the disposition of milrinone in animals or humans after either oral or intravenous administration (Cody *et al* 1984b; Stroshane *et al* 1984a, 1984b; Benotti *et al* 1984, 1985; Edelson et al 1986; Baim *et al* 1983; Likoff *et al* 1985; Baker and Edelson 1984)

1.5.3 Current relevant knowledge of the potential for rate-dependence of the effects of these cardioactive drugs on contractile state

1.5.3.1 Metoprolol

Metoprolol, a cardioselective ß-adrenoceptor antagonist, significantly reduces the peak rate of rise of left ventricular pressure (LV+dP/dt), a conventional measure of systolic function (Seed and Walker 1988; Barnes *et al* 1979; Quinones *et al* 1976; Zimpfer *et al* 1981), at constant heart rate following an intravenous bolus in man (Dell-Italia and Walsh 1989; Bourdillon *et al* 1979; Reale *et al* 1979). However, the effect of changes in heart rate on these negative inotropic effects has not been extensively reported. In one study, in which LV+dP/dt was examined in the absence of fixed heart rate (although a significant effect on heart rate was not observed), the impairment of contractile performance by metoprolol was increased from 10% at rest, to 43% during one minute of rapid atrial pacing in patients with coronary artery disease (Bourdillon *et al* 1979). These investigators interpreted these results as rate-dependent, with augmented negative inotropic effects of metoprolol during pacing-induced tachycardia : it should however be noted that, when results where expressed in absolute rather than relative values, metoprolol induced a 158±52mmHgs^{-1} reduction in LV+dP/dt compared with 197±114mmHg^{-1} during pacing, which was probably not a significant difference. Thus, depending on how the results were analysed, metoprolol exhibited either rate-dependent or rate-independent negative inotropic effects in this patient group (Bourdillon *et al* 1979).

1.5.3.2 Sotalol

The nonselective ß-adrenoceptor antagonist sotalol, which also possesses important class III antiarrhythmic properties (Schmitt *et al* 1991, 1992; Singh and Nadermanee 1987; Deedwania 1990), also exerts a negative inotropic effect following intravenous administration in patients (Hutton *et al* 1972). Despite these findings, the effect of changes in heart rate on the negative inotropic effect has not previously been reported. However, reverse use-dependent class III

electrophysiologic effects of both dl-sotalol and d-sotalol have been demonstrated in canine Purkinje fibres, and canine ventricular muscle fibres *in vitro* , and in human right ventricle *in vivo* , in which the drug's prolongation of action potential duration was greater at slower rates of stimulation (Strauss *et al* 1970; Huikuri and Yli-Mayry 1992). Despite these findings, sotalol's prolongation effects on atrial and ventricular effective refractory periods in anaesthetized autonomically blocked dogs were not dependent on frequency (Nattel *et al* 1989).

1.5.3.3 Milrinone

Milrinone, a phosphodiesterase inhibitor with dual cardiotonic and vasodilator properties, significantly enhances LV+dP/dt following an intravenous bolus in patients with moderate to severe heart failure (Jaski *et al* 1985; Baim *et al* 1983; Colucci *et al* 1985). However, the effect of changes in heart rate on the positive inotropic effects has not been extensively examined. Frequency-dependent increases in both the tension development and the time to peak tension were observed in isolated guinea pig papillary muscle (Alousi and Johnson 1986). Additionally, during intravenous milrinone infusion in patients with ischaemic heart disease, the positive chronotropic effect induced by the phosphodiesterase inhibitor was less marked than that occurring at rest (Mitrovic *et al* 1991).

1.6 Objectives of the current investigation

The aims of this investigation were :

(i) **to further examine the process of acute myocardial drug uptake** following intravenous administration for the three cardioactive drugs - metoprolol, sotalol and milrinone;

(ii) **to correlate acute myocardial drug uptake with simultaneous haemodynamic, electrocardiographic and electrophysiological effects**, in order to test the hypothesis that :

"myocardial drug content is a direct determinant of acute effect" - although considerable information is available regarding the acute effects of these cardioactive drugs (see section 1.5.1), the acute drug uptake profile of these agents into the human myocardium, or its relationship to effect, have not previously been studied;

(iii) **to examine the potential** for the effects of these drugs on **contractile state** of the myocardium to be influenced by **systolic interval**, utilizing the short-cycle- length component of the MRC, in humans *in vivo* , employing a mathematical model;

(iv) **to examine the effects** of these cardioactive agents on **postextrasystolic potentiation** (PESP), where the postextrasystolic interval is equal to the baseline cycle length, which has not previously been investigated.

Chapter 2 describes all materials and methods utilized for the project, with the exception of determination of rate-related inotropic effects. This includes the protocol for the research component of the cardiac catheterization procedure in humans with ischaemic heart disease, and the determination of global myocardial drug content in the isolated perfused Langendorff rat heart. Analysis of biological samples via high-performance liquid chromatography is described

for each drug, as are quantitation of effects, myocardial content, the pharmacokinetic / pharmacodynamic models and statistical procedures employed.

The methods utilized for determination of the influence of systolic interval on contractile state are extensively described in chapter 3. Mechanical restitution curve construction and other methods of exploring the rate-dependence of the inotropic effects of cardioactive drugs are included, as is the model developed for the purpose of quantitating the influence of heart rate on these effects.

Myocardial uptake of each of metoprolol, sotalol, and milrinone and their simultaneous haemodynamic, electrocardiographic and electrophysiological effects following intravenous bolus injection in humans are reported in chapters 4, 5 and 6 respectively. The relationship between myocardial drug levels and effects were further explored for both metoprolol and sotalol utilizing pharmacokinetic / pharmacodynamic link models. Additionally, the potential for modulation of the effects of each drug on contractile state by heart rate are reported. Finally, in the case of metoprolol, the influence of myocardial hypoxia on global myocardial drug accumulation is defined.

The general discussion of chapter 7 utilizes information gained in the preceding chapters to make some general conclusions as to the nexus between the pharmacodynamics of cardioactive drugs and their uptake and efflux from the myocardium subsequent to acute intravenous bolus administration. The differing susceptibility of effects on inotropic state of the three cardioactive drugs studied to changes in systolic interval are compared and discussed, drawing general conclusions about the force-interval relationship in humans.

CHAPTER 2 : MATERIALS AND METHODS

2.1 Methods utilized for *in vivo* (human) and *in vitro* (animal) experimentation

2.1.1 Protocol for cardiac catheterization for determination of myocardial drug uptake and measurement of acute effects in humans

Patients undergoing diagnostic cardiac catheterization and coronary arteriography for the investigation of chest pain were selected. Exclusion criteria included severe and / or unstable angina pectoris, left main coronary artery stenosis, electrocardiographic evidence of abnormal conduction intervals, clinically significant valvular heart disease, recent transmural myocardial infarction, severe impairment of left ventricular systolic function (ejection fraction <30%), clinically significant renal or hepatic disease, previous adverse effects with the agent to be investigated and, in the case of the ß-adrenoceptor antagonists, a past history of asthma. The research procedure commenced at the end of the routine catheterization, with all ß-adrenoceptor and calcium channel antagonists stopped at least five halflives beforehand. Oral diazepam and diphenhydramine were administered approximately 30min prior to cardiac catheterization as premedication. The protocol was approved by the Ethics of Research Committee of The Queen Elizabeth Hospital and prior informed consent was obtained.

Cardiac catheterization and coronary arteriography were performed, under local anaesthesia (1% lignocaine) utilizing the Judkins approach via femoral arterial (Barry *et al* 1979) and venous sheaths. Cardiac output was measured via the thermodilution technique, utilizing a 7F Swan-Ganz catheter in the pulmonary artery. Following the routine catheterization, an 8F Webster thermodilution coronary sinus catheter was inserted via the left cubital fossa, through the right atrium and positioned in the coronary sinus for measurement of coronary blood flow (Ganz *et al* 1971) and coronary sinus sampling throughout the procedure. Correct position was confirmed by determination of coronary sinus oxygen saturation and screening. The catheter was then fixed in position externally. A 4F Millar micromanometer-tipped catheter was inserted via a femoral artery sheath into the left ventricle for measurement of left ventricular (LV)

pressure and determination of its first time derivative, +dP/dt. Catheter placement is illustrated in Figures 2.1. Patients' electrocardiograms (ECG) were monitored utilizing leads I and II throughout.

The protocol involved determination of haemodynamic parameters, ECG and EP conduction intervals at constant heart rate. A rate just greater than that occurring spontaneously was chosen, and maintained via coronary sinus pacing. Parameters measured in this way included cardiac output, coronary sinus flow, mean femoral arterial pressure, LV pressure and +dP/dt, ECG and EP conduction intervals and effective refractory periods, as well as serial mechanical restitution curve and postextrasystolic potentiation curve construction. During transient interruption of pacing (approximately 5s), spontaneous ECG conduction intervals (including heart rate) were also recorded.

After duplicate baseline measurements of the above parameters had been made, and baseline blood samples drawn from coronary sinus, femoral artery and femoral vein, a bolus dose of the agent under investigation was injected into a rapidly flowing intravenous line. Blood samples were drawn from the femoral artery and coronary sinus every 30 seconds for the first three minutes, then at 4, 5, 7.5, 10, 15 and 20min, with an additional 15s sample from the femoral artery, as previously described (Horowitz *et al* 1986). Femoral vein samples were collected every two minutes. Serial measurements of cardiac output and coronary sinus flow were made at 3, 8, 12, and 18min, and of LV pressure and +dP/dt, mean femoral arterial pressure, ECG and EP conduction intervalsand effective refractory periods and mechanical restitution curve and postextrasystolic potentiaion curve construction at 2, 5, 10, 15 and 20min. The influence of 1min of rapid pacing (~30beats/min > spontaneous rate) on LV+dP/dt was also investigated prior to and 10-20min post administration.

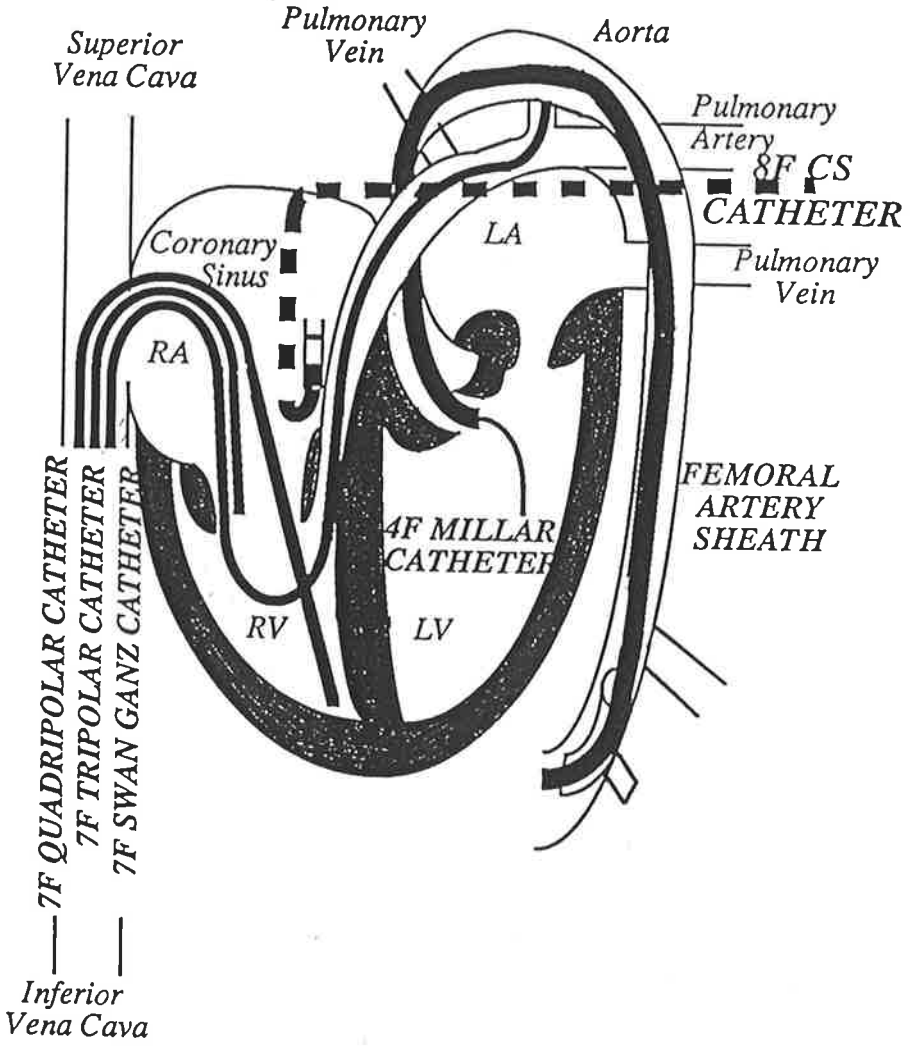


FIGURE 2.1 Placement of the coronary sinus, Swan Ganz, Millar, tri- and quadripolar catheters for determination of myocardial drug uptake and quantitation of drug effects on haemodynamic and electrophysiologic parameters

2.1.2 Protocol for isolated perfused rat hearts

Male Sprague-Dawley rats (440±7g) were anaesthetized with 70±1mg/kg ip sodium pentobarbitone (Ceva Chemicals, Hornsby, Australia), and hearts were rapidly excised and immersed in ice-cold Krebs buffer. This consisted of : 119mM NaCl, 5.36mM KCl, 1.20mM KH_2PO_4, 25.0mM $NaHCO_3$, 5.56mM glucose, 2.50mM$CaCl_2$, 0.95mM$MgCl_2$, and 0.01mM EDTA (di-sodium salt). The buffer was bubbled with 95% O_2/5% CO_2, resulting in a final pH of 7.4. Following removal of connective and fatty tissues, the aorta was cannulated. A small nick was made in the right ventricle, and additional ice-cold Krebs buffer flushed through the heart prior to perfusion with Krebs buffer at 37°C via the Langendorff mode (Langendorff 1895). A constant flow of Krebs buffer from a 1L reservoir of 10.0ml/min was delivered by a Minipuls 3 rotary pump (Gilson, Villiers Le Bel, France), in spontaneously beating hearts. A heating coil and bubble trap were also incorporated into the perfusion apparatus, shown in Figure 2.2. The time course of metoprolol uptake into normoxic and hypoxic isolated rat hearts was examined.

(i) Metoprolol uptake into isolated perfused rat hearts : normoxic conditions

Following 10min equilibration period, a 20µg metoprolol bolus (0.2ml x 100µg/ml, made fresh daily in Krebs buffer) was injected just above the aortic cannula. The hearts were allocated to perfusion periods of 2, 5, or 10min following bolus injection, with 6 hearts in each group. An additional 2 hearts were perfused with Krebs buffer alone. At the end of the perfusion period, hearts were blotted dry, weighed, and snap-frozen prior to storage at -80°C until time of assay.

(ii) Metoprolol uptake into isolated perfused rat hearts : hypoxic conditions

The protocol for perfusion of isolated rat hearts under hypoxic conditions was similar to that for normoxia, with only one modification : following the first 8min of the equilibration period, the preparation was perfused with Krebs buffer gassed with 95% N_2/5% CO_2. This continued for the remaining 2min of the equilibration period and the duration of the post-injection period.

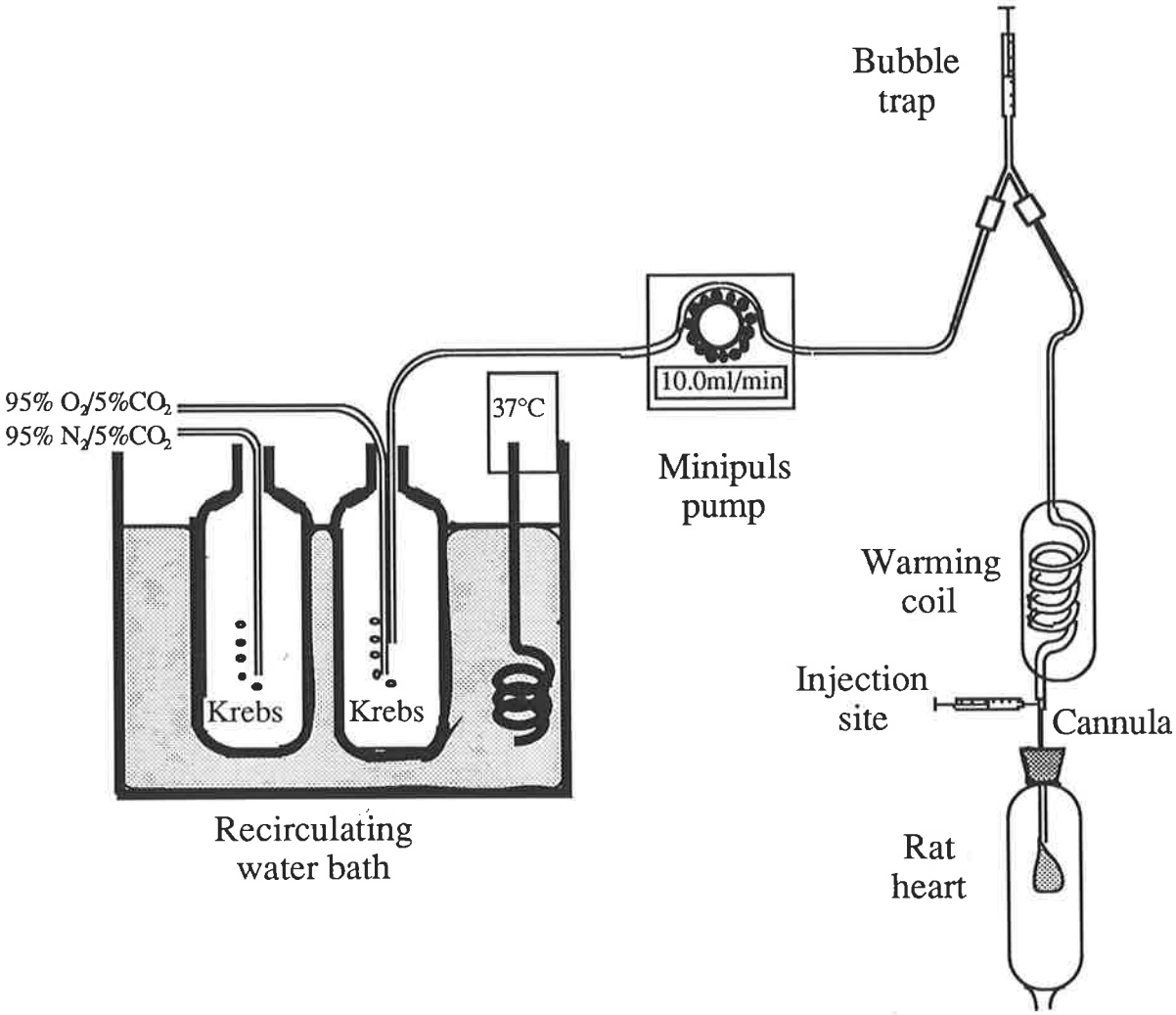


FIGURE 2.2 Perfusion apparatus for determination of myocardial drug uptake in isolated rat hearts

2.2 Analytical methods

2.2.1 HPLC quantitation of metoprolol in human whole blood and rat heart homogenates

Whole blood patient samples were collected into 10ml screw-capped lithium heparin polypropylene tubes (Disposable Products Pty Ltd, Adelaide, Australia). Samples were then transferred to 5ml screw-capped polypropylene tubes (Johns Laboratory Disposables, a division of Hardie Health Care Products, South Oakleigh, Australia) and stored at -20°C until time of assay. Frozen rat hearts from the isolated perfusion experiments were thawed at room temperature. A portion of LV free wall was removed, weighed, and homogenized as finely as possible in two volumes of glass-distilled water. Homogenates were then stored at -20°C until time of assay. A sensitive HPLC assay was developed for determination of metoprolol in these samples, described below (Stafford *et al* 1993).

All reagents, including sodium hydroxide, disodium tetraborate and orthophosphoric acid (BDH, Kilsyth, Australia), were of analytical grade, and solvents of HPLC grade, including dichloromethane (BDH, Poole, England) and acetonitrile-190nm (Ajax, Sydney, Australia). Metoprolol and pindolol (Sigma Chemical Co, St Louis, USA) were of British Pharmacopoeial grade, and stock solutions were made in glass-distilled water and methanol respectively. Serial dilutions of both compounds were made up in glass-distilled water. Metoprolol concentrations were expressed as metoprolol tartrate rather than base. Standard curves in drug free whole blood and blank rat heart homogenate ranged from 5 to 2000ng/ml.

One ml of whole blood was aliquotted into screw-capped disposable 16 x 125mm borosilicate glass tube (Kimble, a division of Owens-Illinios, Toledo, USA). To this was added 0.5ml borate buffer (0.06M disodium tetraborate / 1N sodium hydroxide; prepared fresh daily) and 100µl of internal standard (5µg/ml pindolol). Tubes were briefly vortex-mixed, 5ml dichloromethane was added and samples were then shaken for 20min at 160opm. Separation of aqueous and organic phases was achieved via centrifugation at 2000g for 10min at 4°C prior to

aspiration of the upper aqueous phase to waste. The lower organic phase was transferred to 12x75mm disposable borosilicate glass tubes (Kimble), and evaporated under N_2 at 35°C. Samples were then reconstituted in 200µl mobile phase prior to injection of 60µl onto the column. The extraction process for the tissue homogenates was essentially identical, except that only 0.5ml of the rat heart homogenate was extracted, with the addition of 50µl internal standard.

The high performance liquid chromatography (HPLC) system utilized comprised a dual piston pump, automatic injector, column oven and temperature control module (Waters Assoc, Milford, USA) with a fluorescence detector (model LS-40, Perkin-Elmer, Beaconsfield, USA) and a dual-pen chart recorder (Rikadenki Kyogo Co Ltd, Tokyo, Japan). A Brownlee 5µm 22cm x 4.6mm C_{18} reverse-phase column and 3cm x 4.6mm precolumn (Applied Biosystems Inc, San Jose, USA) were employed. Mobile phase, consisting of 3 : 2 acetonitrile : ortho-phosphoric acid (5mM adjusted to pH 3.5 with 1N sodium hydroxide), at a flow rate of 2.0ml/min and 50°C resulted in a back-pressure of 15MPa. Excitation and emission wavelengths of 220nm and 291nm were utilized, to obtain optimal detection of metoprolol and the internal standard in extracted samples, illustrated in Figure 2.3. The 4-hydroxy-metabolite could also be detected with this assay, eluting before pindolol at approximately 8.8min.

A total of 21 calibration curves in whole blood were run over a period of 12 months. Inter-assay reproducibility was determined using the individual mean peak height ratios (PHR) of five consecutive curves, obtained as the ratio of the peak heights of metoprolol and pindolol. The coefficient of variation (CV) of the slope was 5.49%, and mean slope was 0.0091±0.0002. The CV of the regression coefficient (r) was 0.04%, and mean r was 0.9995±0.0002. The mean calculated concentration of the quality control (QC) standard over these 5 assays, and of 5 replicates of spiked whole blood within one assay, the inter- and intra-assay coefficients of variation, are represented in Table 2.1. Assay threshold was 2.5ng/ml. A typical calibration curve in blood is shown in Figure 2.4.

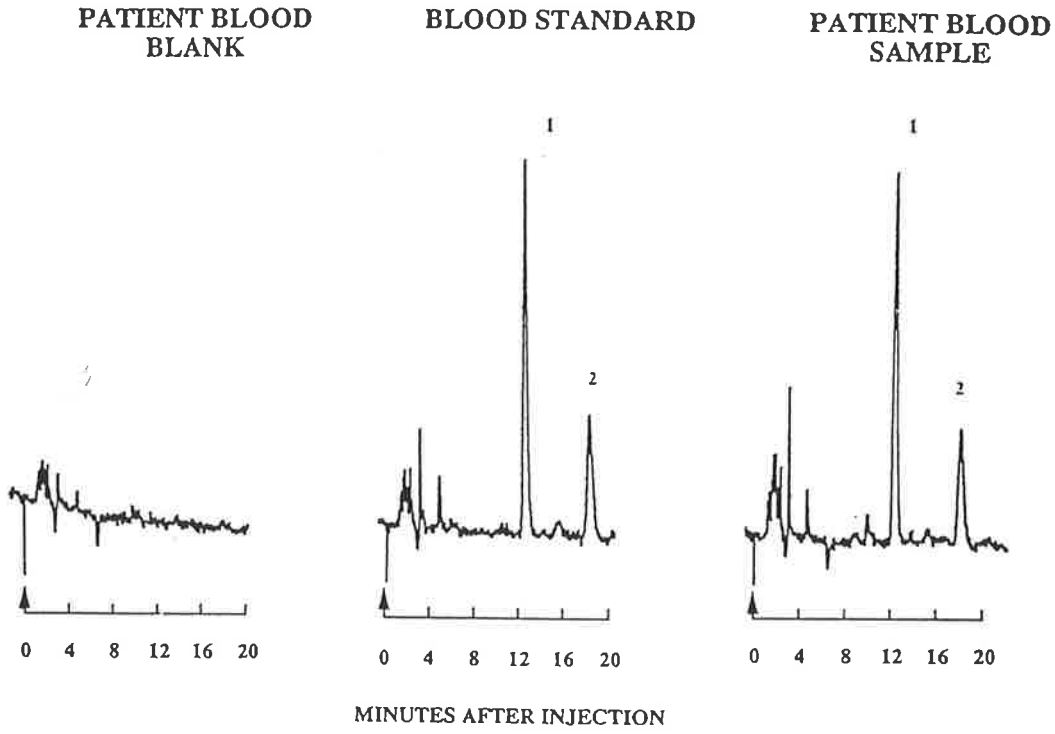


FIGURE 2.3 Typical HPLC chromatograms of patient blank blood sample, (1) pindolol and (2) metoprolol in a standard containing metoprolol (50ng/ml), and pindolol (0.5ug/ml) and a patient blood sample

TABLE 2.1. Coefficients of variation of the metoprolol whole blood assay

INTER-ASSAY

CONCENTRATION	10ng/ml	150ng/ml	750ng/ml
Mean	31.089	158.288	735.394
n	4	4	4
SD	1.783	4.495	55.550
SE	0.891	2.248	27.775
CV	5.734 %	2.840 %	7.554 %

INTRA-ASSAY

CONCENTRATION	20ng/ml	100ng/ml	500ng/ml
Mean	21.472	98.628	513.869
n	5	5	5
SD	0.959	2.548	9.074
SE	0.429	1.140	4.058
CV	4.468 %	2.584 %	1.766 %

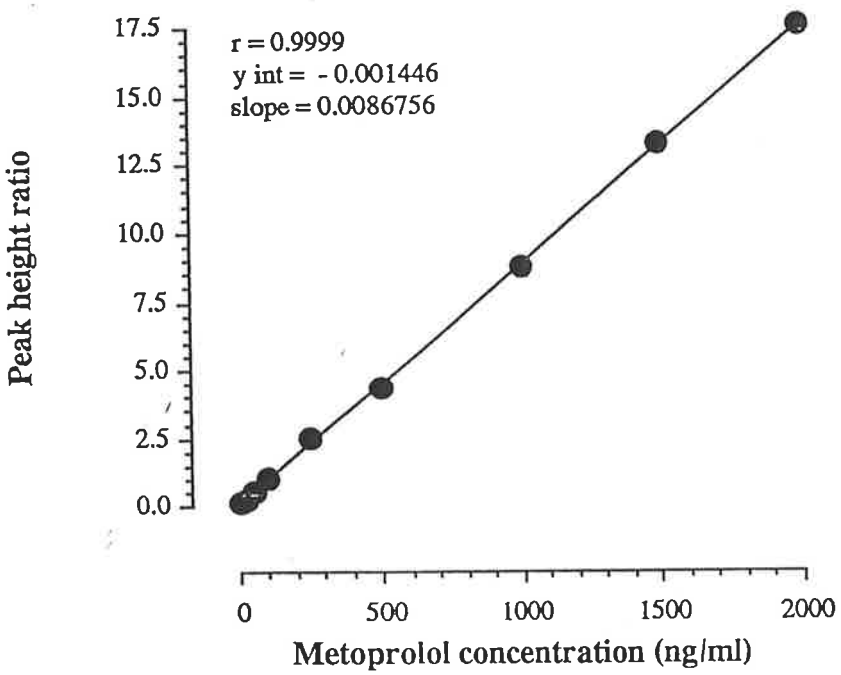


FIGURE 2.4 Typical calibration curve for metoprolol : pindolol peak height ratios over 5-2000ng/ml in whole blood

2.2.2 HPLC quantitation of sotalol enantiomers in human whole blood

Whole blood patient samples were collected into 10ml screw-capped lithium heparin polypropylene tubes (Disposable Products Pty Ltd). Samples were then transferred to 5ml or 10ml screw-capped polypropylene tubes (Johns Laboratory Disposables), depending on sample size, and stored at -20°C until time of assay. These samples were extracted essentially according to a recently published plasma method for sotalol enantiomers (Sallustio *et al* 1992), with a few modifications, necessary for whole blood.

All reagents, including sodium hydrogen carbonate, anhydrous disodium carbonate (BDH, Kilsyth, Australia), and anhydrous disodium sulphate (May and Baker, West Footscray, Australia), were of analytical grade and solvents of HPLC grade, including dichloromethane, propan-2-ol, methanol, acetic acid (BDH, Poole, England) and chloroform (Ajax, Sydney, Australia). dl-Sotalol (donated by Bristol-Myers Squibb, Noble Park, Australia), and atenolol (Sigma Chemical Co, St Louis, USA) stock solutions and serial dilutions were made in glass-distilled water. S-(-)-α-methylbenzyl isocyanate was purchased from Aldrich Chemical Co (Milwaukee, USA). Standard curves in drug free whole blood ranged from 25-10000ng/ml for each of the sotalol enantiomers.

One ml of whole blood was aliquotted into screw-capped disposable 16x125mm borosilicate glass tube (Kimble). To this was added 100µl of internal standard (10µg/ml racemic atenolol) and 2.0ml bicarbonate buffer (1.0M sodium hydrogen carbonate adjusted to pH 9.0 with 1.0M disodium carbonate). Tubes were briefly vortex-mixed, 5ml dichloromethane : propan-2-ol (3 : 1 v/v) was added, and samples were then gently shaken for 20min at 100opm. Separation of aqueous and organic phases was achieved via centrifugation at 1000g for 30min at 4°C prior to aspiration of the upper aqueous phase to waste. Upon removal of the precipitous layer between the two phases, the lower organic phase was transferred to fresh disposable 16x125mm borosilicate glass tubes (Kimble). To this was added approximately 1g of anhydrous sodium sulphate to remove any residual water. Following a brief vortex mix and several minutes settling time, samples were recentrifuged for 5min at 1000g. The organic phase

was then transferred into clean 12x75mm disposable borosilicate glass tubes (Kimble), and evaporated under N_2 at 40°C. Samples were then reconstituted in 200µl derivatizing solution (20µl S-(-)-α-methylbenzyl isocyanate in 10ml Na_2SO_4-dried chloroform), briefly vortex-mixed and allowed to stand overnight at 4°C. Samples were evaporated under N_2 at room temperature and reconstituted in 200µl mobile phase. The tubes were micro-centrifuged to remove any particulate matter prior to injection of 30µl onto the column.

The high performance liquid chromatography (HPLC) system utilized comprised a dual piston pump, automatic injector, column oven and temperature control module (Waters Assoc, Milford, USA) with a fluorescence detector (model LS-40, Perkin-Elmer, Beaconsfield, USA) and a dual-pen chart recorder (Rikadenki Kyogo Co Ltd, Tokyo, Japan). A Brownlee Velosep 3µm 10cm x 3.2mm C_{18} reverse-phase column (Applied Biosystems Inc, San Jose, USA) were employed. Mobile phase, consisting of 39% methanol and 0.5% acetic acid in glass-distilled water, at a flow rate of 0.4ml/min and 40°C, resulted in a back-pressure of 12MPa. Excitation and emission wavelengths of 220nm and 300nm were utilized, to obtain optimal detection of R- and S-sotalol and the internal standard enantiomers in extracted whole blood samples, illustrated in Figure 2.5.

A total of 10 calibration curves in whole blood were run over a period of 18 months. Inter-assay reproducibility was determined using the individual mean PHR of four consecutive curves, obtained as the ratio of the peak heights of each of R- and S-sotalol to each of R- and S-atenolol. The CV's of the slopes were 12.2% (R-sotalol / S-atenolol PHR), 12.4% (S-sotalol / R-atenolol PHR), 10.1% (R-sotalol / S-atenolol PHR), and 11.7% (S-sotalol / S-atenolol). Mean slopes were 1.16±0.07, 1.09±0.07, 1.30±0.07, and 1.22±0.07 x 10^{-3} respectively. The CV's of r were 0.01%, 0.01%, 0.08%, and 0.09%, and mean r were 0.9998±0.0001, 0.9998±0.0001, 0.9990±0.0004, and 0.9988±0.0004 respectively. The mean calculated concentration of the QC over these four assays, and of 5 replicates of spiked whole blood within one assay, the inter- and intra-assay coefficients of variation, are represented in Table 2.2. Note that in one assay, two QC standards were extracted. Assay threshold was 10ng/ml for each of the R- and S-sotalol enantiomers. Typical calibration curves in whole blood are shown in Figure 2.6.

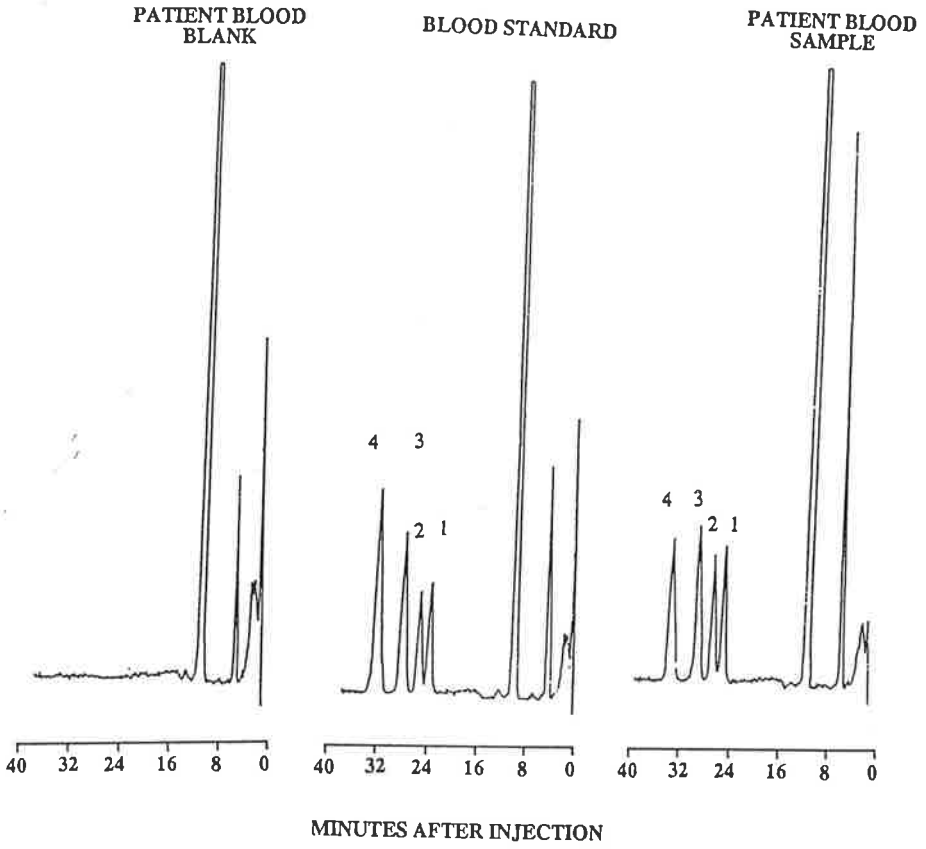


FIGURE 2.5 Typical HPLC chromatograms of patient blank blood sample, (1) R-sotalol, (2) S-sotalol, (3) R-atenolol and (4) S-atenolol in a standard containing R- and S-sotalol (500ng/ml) and R- and S-atenolol (0.5ug/ml) and a patient sample

TABLE 2.2. Coefficients of variation of the sotalol enantiomer whole blood assay

INTER-ASSAY

CONCENTRATION		250ng/ml	1000ng/ml	5000ng/ml
R-Sotalol:	Mean	261	1025	4800
R-Atenolol	n	5	5	5
	SD	8.32	47.5	298
	SE	3.72	21.2	133
	CV	3.19 %	4.63 %	6.20 %
S-Sotalol:	Mean	261	1039	4807
R-Atenolol	n	5	5	5
	SD	16.9	62.4	307
	SE	7.58	27.9	137
	CV	6.48 %	6.01 %	6.38 %
R-Sotalol:	Mean	262	1013	4855
S-Atenolol	n	5	5	5
	SD	17.3	46.5	214
	SE	7.73	20.8	95.6
	CV	6.59 %	4.59 %	4.40 %
S-Sotalol:	Mean	263	1065	5014
S-Atenolol	n	5	5	5
	SD	10.4	96.3	358
	SE	4.64	43.1	160
	CV	3.95 %	9.05 %	7.14 %

TABLE 2.2. Continued

INTRA-ASSAY

CONCENTRATION		250ng/ml	1000ng/ml	5000ng/ml
R-Sotalol:	Mean	258	1002	4984
R-Atenolol	n	5	5	5
	SD	8.54	14.4	12.8
	SE	3.82	6.45	5.73
	CV	3.31 %	1.44 %	0.26 %
S-Sotalol:	Mean	258	1018	5050
R-Atenolol	n	5	5	5
	SD	6.13	13.9	44.1
	SE	2.74	6.24	19.7
	CV	2.38 %	1.37 %	0.87 %
R-Sotalol:	Mean	265	1005	4900
S-Atenolol	n	5	5	5
	SD	14.2	16.1	88.9
	SE	6.35	7.21	39.8
	CV	5.36 %	1.60 %	1.81 %
S-Sotalol:	Mean	264	1021	4968
S-Atenolol	n	5	5	5
	SD	9.32	14.1	109
	SE	4.17	6.29	48.6
	CV	3.54 %	1.38 %	2.19 %

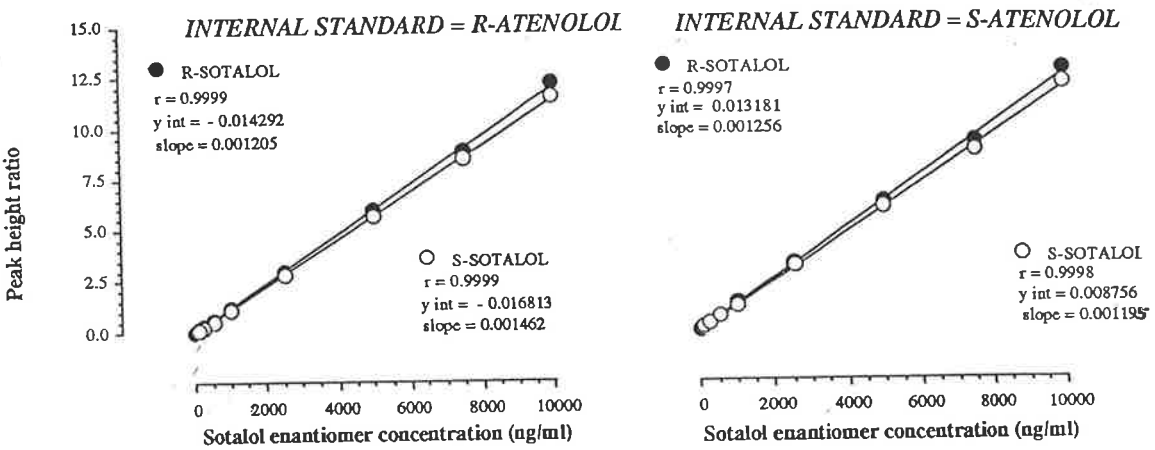


FIGURE 2.6 Typical calibration curve for R- and S-sotalol : each of R- and S-atenolol peak height ratios over 25-10 000ng/ml in whole blood

2.2.3 HPLC quantitation of milrinone in human whole blood

2.2.3.1 Analysis of milrinone in biological samples - results of previous investigations

High-performance liquid chromatographic (HPLC) analysis of milrinone has previously been reported in human plasma (Edelson *et al* 1983; Oddie *et al* 1986) and urine (Edelson *et al* 1983), and in animals in both dog (Edelson *et al* 1983) and rat plasma (Desjardins and Cauchy 1988), and body fluids and tissues from unnamed sources (Verrijk *et al* 1989). Determination of milrinone has also been reported in saline and dextrose injections (Wilson *et al* 1986; Riley 1988), but not in whole blood *per se*.

In the first published method for HPLC quantitation of milrinone, Edelson and coworkers (1983) described an extraction procedure in plasma and urine incorporating a double ethyl acetate extraction and backextraction into acid prior to injection. Simpler and more rapid sample clean-up using solid-phase extraction (disposable C_{18} columns) were later reported, both in human (Oddie *et al* 1986) and rat plasma (Desjardins and Cauchy 1988). The most recently published extraction reported sample purification utilizing a centrifuge-supported microfiltration technique, essentially a comprehensive deproteination step (Verrijk *et al* 1989). This paper claimed their technique was suitable for a variety of tissues, as well as whole blood, although no results for the latter were demonstrated.

Previously described chromatography for these methods all utilized ultraviolet (UV) detection, at 331-340nm (Verrijk *et al* 1989; Edelson *et al* 1983; Oddie *et al* 1986; Desjardins and Cauchy 1988). The exception was Wilson's group (1986), who utilized a 254nm wavelength. Columns utilized were 25cm in length, and ranged from the relatively nonspecific C_{18} (Oddie *et al* 1986), to a range of C_{18} columns with additional features, including the RP Select-B, for separation of alkaline compounds (Verrijk *et al* 1989). A methanol / ammonium acetate buffer mobile phase was employed with the RP Select-B (Verrijk *et al* 1989) and acetonitrile / phosphate buffer with the C_{18} (Oddie *et al* 1986), Supelcosil (Desjardins and Cauchy 1988)

and partisil columns (Edelson *et al* 1983), although a methanol / borate buffer was also utilized with the latter (Wilson *et al* 1986). When a 1.0ml sample was extracted, limit of detection of milrinone ranged from 4ng/ml (Edelson *et al* 1983) to 31ng/ml (Desjardins and Cauchy 1988). Because of the low doses used in the calculation of myocardial drug uptake, and samples drawn from the coronary sinus are often less than 1ml, a sensitive method for determination of milrinone in whole blood was developed (Ritchie and Horowitz 1994). Concentrations in whole blood, rather than plasma, are necessary for determination of myocardial milrinone uptake by mass balance principles.

2.2.3.2 Methodology developed for analysis of milrinone in human whole blood samples in the current investigation

Whole blood patient samples were collected into 10ml polypropylene tubes (Johns Laboratory Disposables) containing potassium oxalate anticoagulant to achieve a final concentration in whole blood of 2.88mg/ml, and stored at -20°C until time of assay. A sensitive HPLC assay was developed for determination of milrinone in these samples (Ritchie and Horowitz 1994).

All reagents, including potassium oxalate, ammonium sulphate (Ajax, Sydney, Australia), concentrated hydrochloric acid (BDH, Poole, England), sodium hydroxide, anhydrous disodium hydrogen orthophosphate and concentrated orthophosphoric acid (BDH, Kilsyth, Australia), were of analytical grade, and solvents of HPLC grade, including tetrahydrofuran, ethyl acetate (BDH, Poole, England) and acetonitrile-190nm (Ajax). Milrinone, and the internal standard amrinone, were kindly provided by Sterling Drug Inc (Rensselaer, USA). Stock solutions of both compounds were made up in 0.1N HCl, with serial dilutions in 0.01N HCl. Standard curves in drug free whole blood ranged from 1-1000ng/ml.

One ml of whole blood was aliquotted into screw-capped disposable 16x125mm borosilicate glass tubes (Kimble). To this was added 100µl of internal standard (5µg/ml amrinone) and 3ml ammonium sulphate (47% in glass-distilled water). Tubes were briefly vortex-mixed, 5ml ethyl acetate was added and samples were then vortex-mixed for 2min. Separation of aqueous and organic phases was achieved via centrifugation at 1600g for 10min at 4°C. The upper organic

phase was transferred to 12x75mm disposable borosilicate glass tubes (Kimble), and evaporated under N_2 at 50°C. Samples were then reconstituted in 250µl ethyl acetate and 100µl 0.1N HCl. Each tube was then vortex-mixed for 2min prior to transfer of contents into a clean capped 2ml disposable polypropylene Eppendorf®-type tubes (Kartell SpA Disione Labware, Milano, Italy). Samples were then centrifuged at 13500g for 10min prior to aspiration of the upper organic phase to waste. Aliquots of 75µl were then transferred into clean capped 2ml disposable polypropylene Eppendorf®-type tubes (Kartell) containing 15µl neutralizing solution (6% 10N NaOH / 94% 0.5M Na_2HPO_4). These tubes were centrifuged at 13500g for 10min to remove any particulate matter prior to injection of 20µl onto the column.

The HPLC system utilized comprised a dual piston pump, automatic injector and a tunable ultraviolet absorbance detector (Waters Assoc, Milford, USA) with a dual-pen chart recorder (Rikadenki Kyogo Co Ltd, Tokyo, Japan). A Brownlee 5µm 22cm x 4.6mm C_{18} reverse-phase column and 3cm x 4.6mm precolumn (Applied Biosystems Inc, San Jose, USA) were utilized. Mobile phase, consisting of 15 : 0.61 : 84.39 acetonitrile: tetrahydrofuran: 0.1M orthophosphoric acid (adjusted to pH 6 with concentrated sodium hydroxide), at a flow rate of 1.0ml/min and ambient temperature resulted in a back-pressure of 15MPa. A wavelength of 326nm was utilized, to obtain optimal detection of milrinone and the internal standard in extracted samples, illustrated in Figure 2.7.

A total of 13 calibration curves in whole blood were run over a period of 6 months. Inter-assay reproducibility was determined using the individual mean PHR of five consecutive curves, obtained as the ratio of the peak heights of milrinone and amrinone, and the mean calculated concentration of the QC standards over these 5 assays. The CV of r was 0.3%, and mean r was 0.998±0.003. The CV of the slope was 17.5%, and mean slope was $3.42 \pm 0.60 \times 10^{-3}$. While the slope of the calibration curve had some tendency for variability, the QC standards were consistently within 10% of the expected concentrations, and the interassay CV's of these standards were within 10%, and therefore acceptably reproducible. The mean calculated concentration of 5 replicates of spiked whole blood within one assay, the intra-assay coefficient of variation, was also determined, and is represented in Table 2.3. Assay threshold was 1ng/ml. A typical calibration curve in whole blood is shown in Figure 2.8.

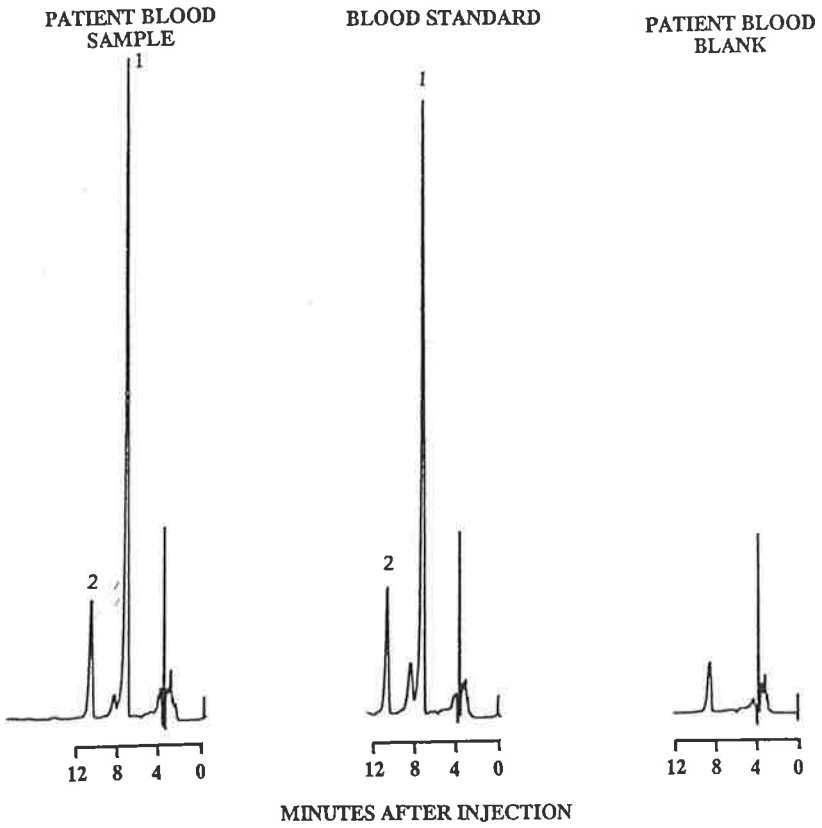


FIGURE 2.7 Typical HPLC chromatograms of patient blank blood sample, (1) amrinone and (2) milrinone in a standard containing milrinone (50ng/ml), and amrinone (0.5ug/ml) and a patient blood sample

TABLE 2.3 Coefficients of variation of the milrinone whole blood assay

INTER-ASSAY

CONCENTRATION	15ng/ml	65ng/ml	200ng/ml
Mean	14.5	64.4	214
n	5	5	5
SD	1.48	1.99	21.0
SE	0.66	0.89	9.40
CV	10.2%	3.09%	9.81%

INTRA-ASSAY

CONCENTRATION	20ng/ml	100ng/ml	500ng/ml
Mean	19.7	105	490
n	5	5	5
SD	1.29	6.97	21.7
SE	0.58	3.12	9.72
CV	6.55%	6.65%	4.44%

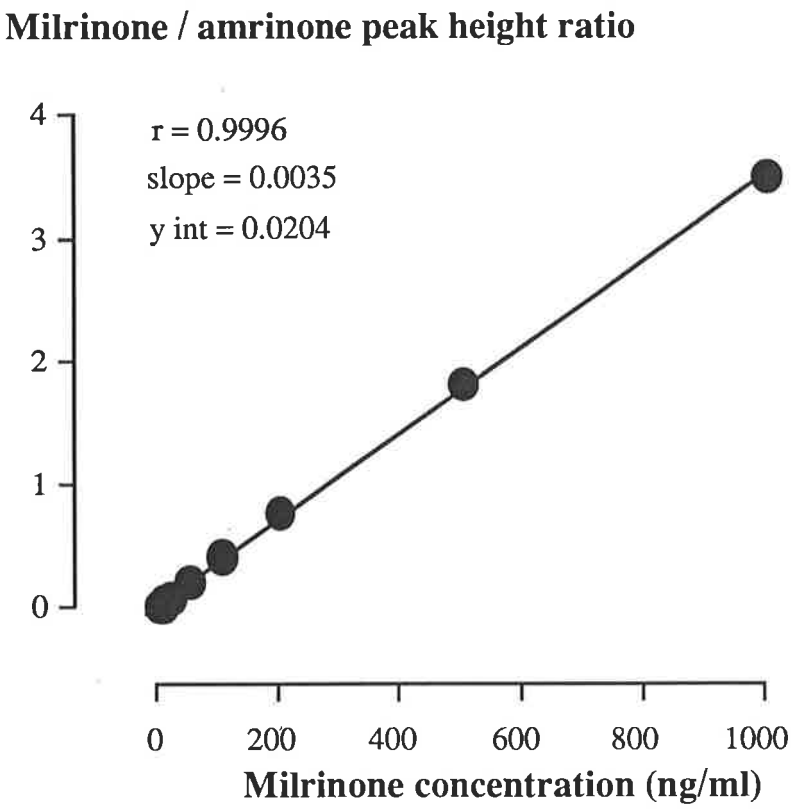


FIGURE 2.8 Typical calibration curve for milrinone : amrinone peak height ratios over 1-1000ng/ml in whole blood

2.2.4 Lowry protein assay in rat heart homogenates

Protein concentrations in rat LV myocardial homogenates was determined utilizing the Lowry protein assay. All reagents, including bovine serum albumin (Sigma Chemical Co, St Louis, USA), sodium carbonate, sodium hydroxide, trisodium citrate, cupric sulphate (BDH Chemicals, Kilsyth, Australia), and Folins reagent (Ajax Chemicals, Auburn, Australia) were of analytical grade. Bovine serum albumin (BSA) was made up in glass-distilled water. Standard curves ranged from 0.02-0.08mg protein (BSA) per 20µl.

Briefly, a 20µl aliquot of homogenate was transferred to a 12x75mm disposable borosilicate glass tube (Kimble) containing 780µl of glass-distilled water. Following vortex-mixing, a 20µl aliquot of the diluted homogenate was transferred to a fresh 12x75mm disposable borosilicate glass tube (Kimble), to which was added 2.0ml working solution (comprising 50:1 of : 2% sodium carbonate/0.10N sodium hydroxide; and 0.5% cupric sulphate/1.0% trisodium citrate v/v) and 0.98ml glass-distilled water. After vortex-mixing and a 10min incubation period at room temperature, 0.20ml of 1.0M Folins reagent was added to each tube. An additional vortex-mixing and 30min incubation period at room temperature followed. Each sample was assayed in duplicate. The absorbance of each tube at 750nm was obtained using a UV/visible light variable wavelength spectrophotometer (Varian, Australia) with a slit width of 1nm. The BSA standard curve was constructed, and the samples read off this curve. The concentration of protein in each sample was corrected to a 1.0ml aliquot of undiluted homogenate. A typical standard curve is illustrated in Figure 2.9.

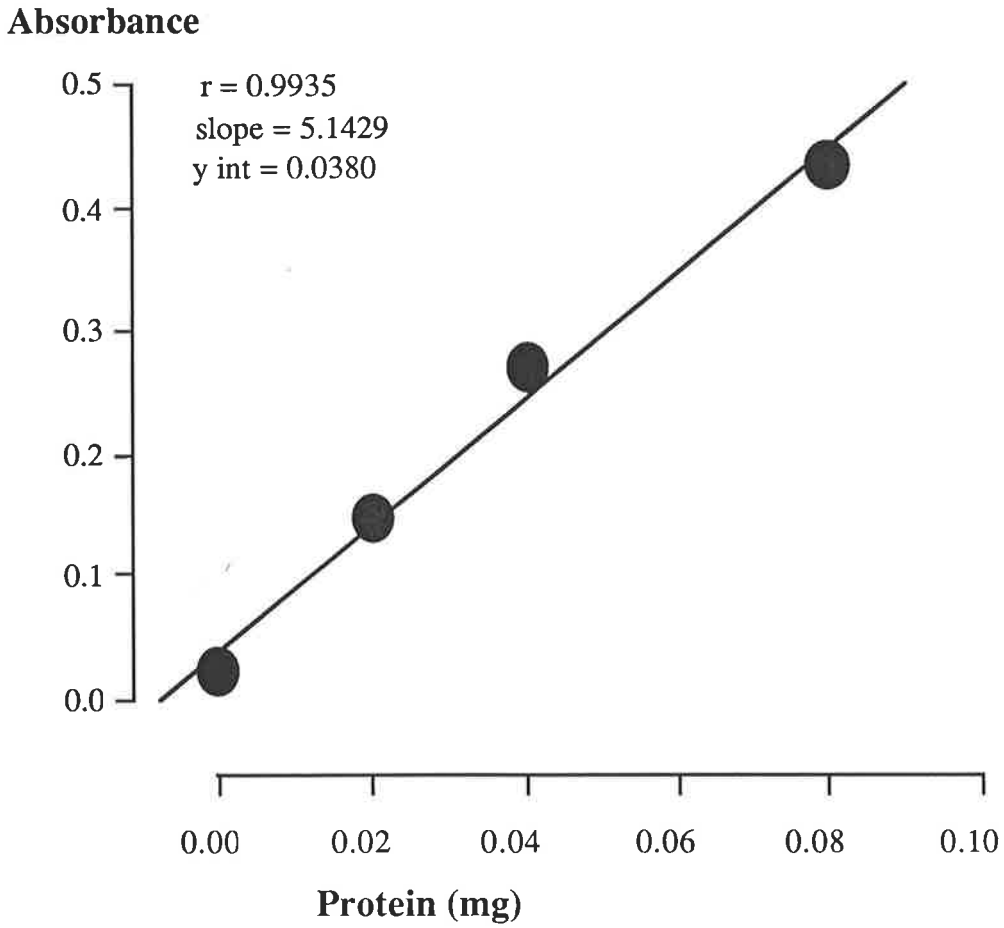


FIGURE 2.9 Typical calibration curve for the Lowry protein assay over 0.02-0.08mg

2.2.5 RIA determination of cAMP concentrations in human plasma

Whole blood patient samples were collected into ice-cold 5ml luer syringes (Becton Dickinson Medical Products, Singapore) and transferred into ice-cold 5ml screw-capped polypropylene tubes (Johns Laboratory Disposables, a division of Hardie Health Care Products, South Oakleigh, Australia) containing 3mg/ml EDTA (dipotassium salt; Sigma Chemical Co, St Louis, USA). Samples were stored on ice until centrifugation, at 4°C for 10min at 3000rpm.

Plasma was then transferred to 1.5ml screw-capped polypropylene cryotubes (Sarstaedt, Germany), snapfrozen and stored at -80°C until time of assay. A sensitive commercially available ^{125}I radioimmunoassay (RIA) research kit (Amersham, Buckinghamshire, England) was utilized for determination of cyclic adenosine-3'5'-monophosphate (cAMP) concentrations in these samples.

The kit reagents were allowed to come to room temperature. The assay buffer (final composition 0.05M acetate buffer with thimerosal, pH 5.8) was reconstituted in glass-distilled water to achieve a final volume of 500ml. Two millilitres of glass-distilled water was added to the non-acetylation cAMP standard, resulting in a final concentration of 32nM in 0.05M acetate buffer / 0.01% thimerosal. To the rabbit anti-succinyl cAMP serum (anti-serum), 11.0ml glass-distilled water was added, to obtain anti-cAMP serum in 0.05M acetate buffer / 0.01% thimerosal. The assay tracer (final composition adenosine 3'5'-cyclic phosphoric acid 2'-O-succinyl-3-[^{125}I]iodotyrosine methyl ester in 0.05M acetate buffer with 0.01% azide and thimerosal, 59kBq, 1.6μCi) was reconstituted with 11.0 assay buffer.

All procedures were performed on ice until the entire set of assay tubes had been aliquotted their sample. The standard curve for cAMP was constructed by serial dilution of the standard in assay buffer, to achieve final concentrations in the assay over the range of 25-1600fmol/tube, in 12x75mm disposable borosilicate glass tubes (Kimble). A 100μl aliquot of patient plasma was diluted in 900μl assay buffer. One hundred microlitres of sample (either sample dilution or standard dilution) was aliquotted into duplicate tapered disposable 3ml polypropylene tubes (Johns Laboratory Disposables, a division of Hardie Health Care Products, South Oakleigh,

Australia). Other tubes also included in duplicate were the total counts (TC), nonspecific binding (NSB), and the blank (B_0), comprising an empty tube, 200µl and 100µl assay buffer respectively. To this was added 100µl ^{125}I tracer (to all tubes), and 100µl antiserum (to all tubes except TC and NSB). Tubes were thoroughly vortex-mixed, covered, and incubated for 3h at 4°C. After a further 30min period allowing tubes to reach room temperature, 0.5ml second antibody (donkey anti-rabbit serum) was added to all tubes except TC. The tubes were thoroughly vortex-mixed, allowed to stand for 10min at room temperature, prior to centrifugation at 4°C for 15min at 3800rpm. A relatively fast speed of centrifugation facilitated better pellet formation in the plasma samples. The supernatant was then aspirated to waste (not TC), and the radioactivity of the residual pellet was determined by counting for sixty seconds in a LKB Multigamma counter (Wallac, Turku, Finland).

The average counts per minute, and the CV for each set of duplicate tubes was calculated. The ratio of B_0 / TC was determined as :

$$\%B_0 / TC = \frac{B_0 \text{ counts/min} \times 100}{TC \text{ counts/min}}$$

For each standard and sample, the percent bound (%B / B_0) was calculated using the equation :

$$\%B / B_0 = \frac{\text{sample counts/min} \times 100}{B_0 \text{ counts/min}}$$

The standard curve was generated by plotting %B/B_0 as a function of the log cAMP concentration per tube. All of the above calculations were facilitated with the use of a computer programme, which also fitted an algorithm to the standard curve, and the cAMP concentrations (fmol/tube) were then calculated from this equation. A typical calibration curve in blood is shown in Figure 2.10. Intraassay reproducibility was determined using 6 replicates of spiked human plasma within one assay, each in duplicate, giving a CV of 6.59% at a concentration of 11.3nM. Interassay reproducibility was not determined, as only two assays were required to analyse the samples.

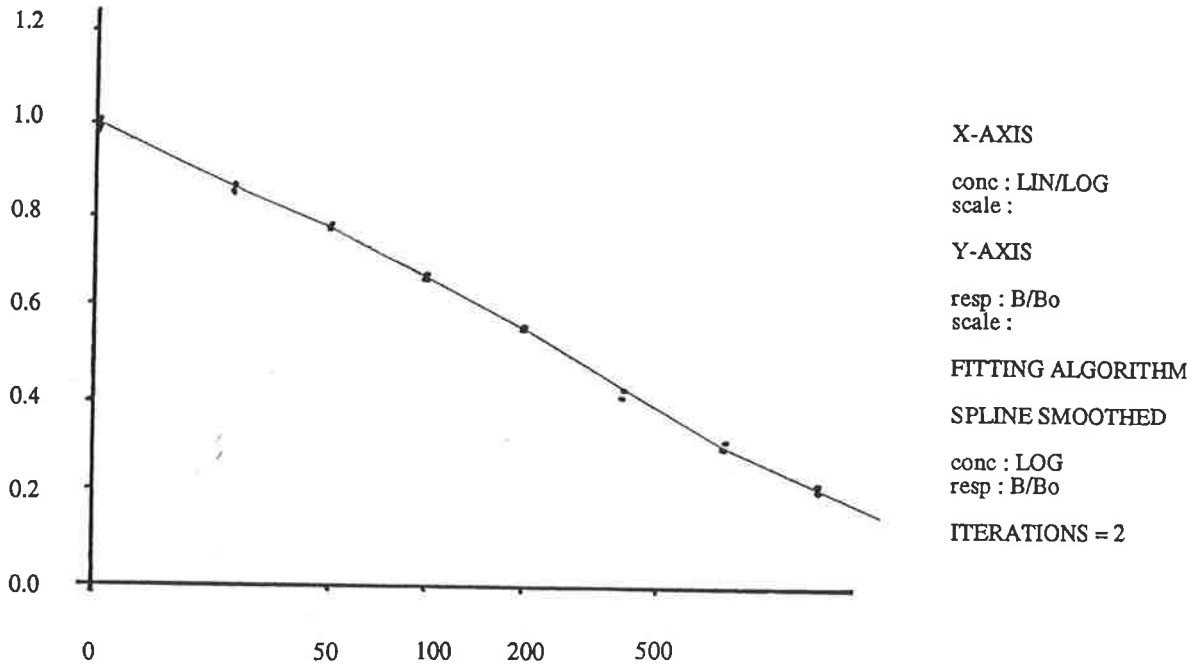


FIGURE 2.10 Typical calibration curve for cAMP via RIA over 25 - 1600fmol/tube in human plasma

2.3 Quantitation of haemodynamic, electrocardiographic and electrophysiologic effects

Cardiac index was calculated as cardiac output divided by body surface area. Coronary blood flow was determined as previously described (Ganz *et al* 1971) from chart recordings at 5mm/sec. Measurement of ECG intervals, LV pressure and +dP/dt were made from recordings at 50mm/sec, shown in Figures 2.11 and 2.12 respectively. Systemic vascular resistance was calculated as mean arterial pressure divided by cardiac output, and coronary vascular resistance as mean arterial pressure divided by coronary sinus flow.

2.4 Calculation of myocardial drug uptake

Myocardial drug uptake (MDU) was determined utilizing paired transcoronary sampling and simultaneous coronary sinus blood flow estimation as previously described (Horowitz *et al* 1986). Preliminary experiments were undertaken to ensure the validity of utilization of femoral artery levels as a surrogate for aortic concentrations. Instantaneous MDU was calculated as the product of the transcoronary drug concentration gradient and the simultaneous coronary sinus flow. Changes in myocardial drug content (MDC) were determined as the mean of the instantaneous uptakes for the beginning and the end of the current sampling period multiplied by the duration of the current sampling period, and cumulative MDC calculated as :

MDU = ∂C x CSF
where MDU = instantaneous myocardial drug uptake
∂C = transcoronary concentration gradient
= difference between femoral artery and coronary sinus whole blood
CSF = coronary sinus blood flow

$$MDC = MDC_1 + \frac{MDU_1 + MDU_2}{2} \times t$$

where MDC_1 = MDC at end of last sampling period
MDU_1 & MDU_2 = MDU at beginning and end of current sampling period
t = duration of current sampling period (min)

Myocardial drug content was then expressed relative to resting coronary sinus flow for each patient to reduce the influence of variability of coronary sinus catheter position between patients (Horowitz *et al* 1986).

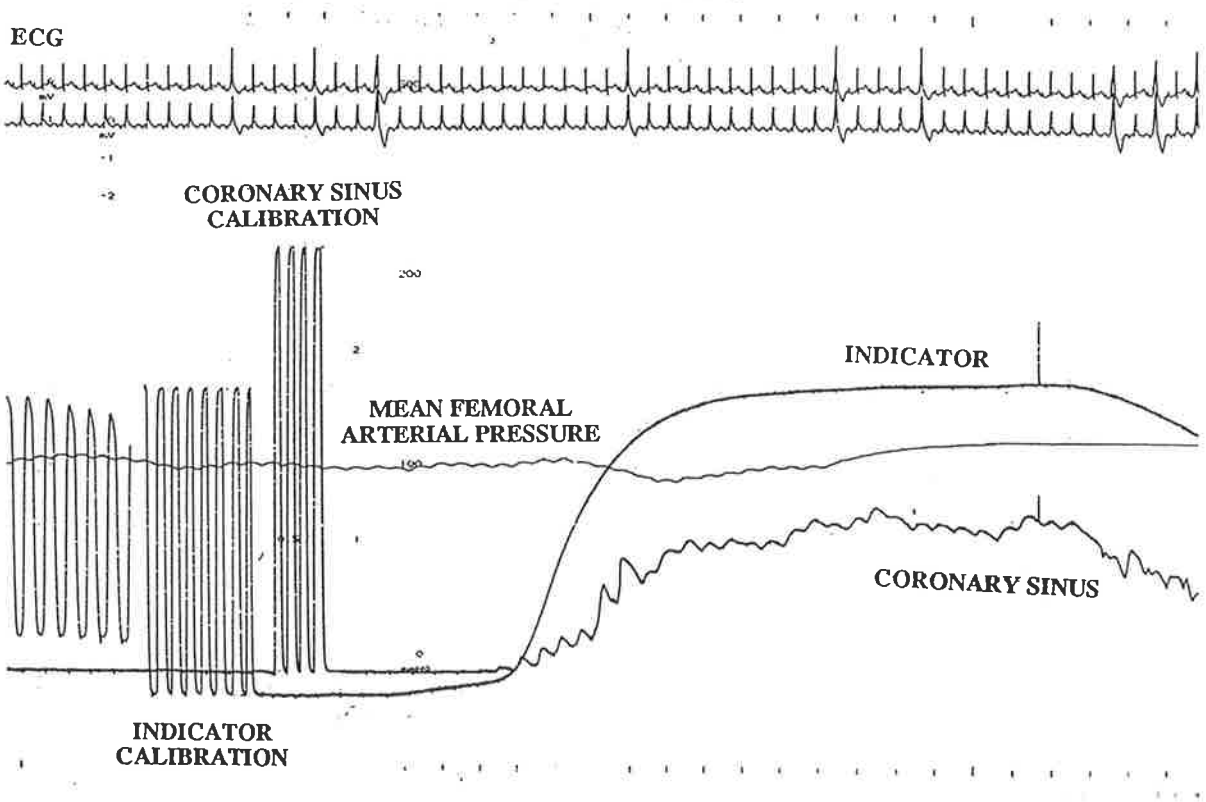


FIGURE 2.11 Chart recording of coronary sinus blood flow, ECG and mean femoral arterial pressure

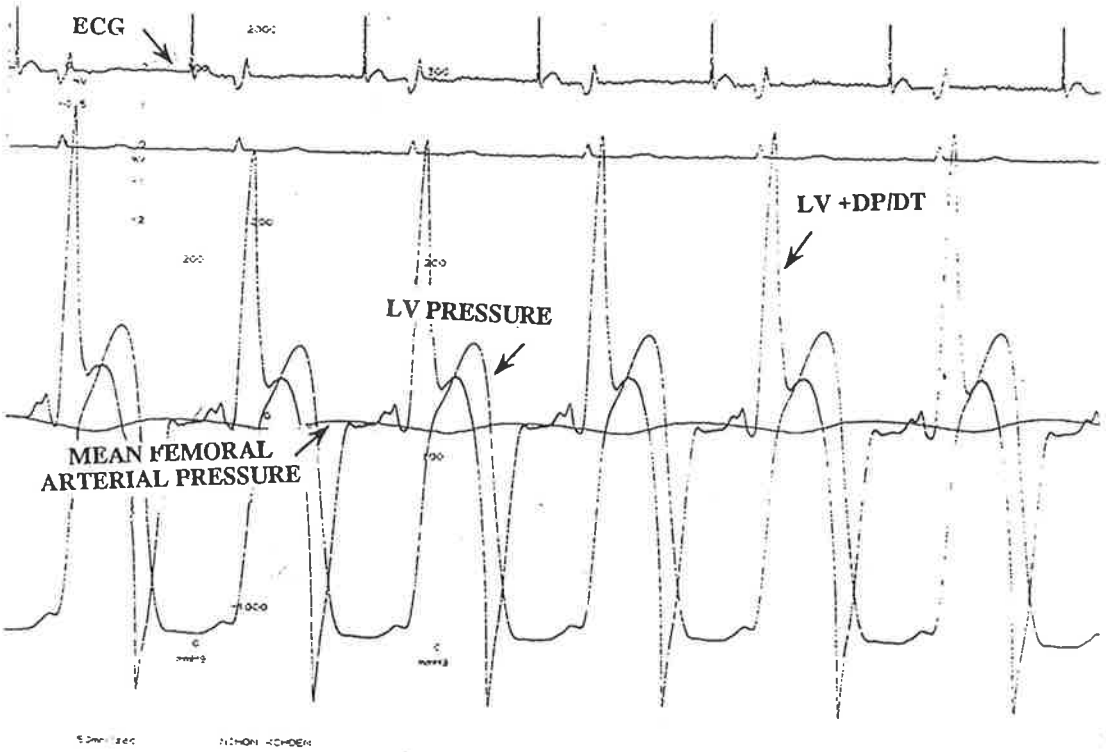


FIGURE 2.12 Chart recording of LV pressure and its first time derivative, +dP/dt, ECG and mean femoral arterial pressure

2.5 Correlation between myocardial drug content and simultaneous effects

Investigation of the relationship between MDC and acute effects of the three cardioactive drugs included a plot of effects as a function of simultaneous content. Where the time course of MDC and effects are not parallel, illustrated in Figure 2.13, an anti-clockwise hysteresis loop between the two characterizes the equilibration delay when points are connected in time order (Fuseau and Sheiner 1984; Holford and Sheiner 1981; Unadkat *et al* 1986). If the delay cannot be attributable to formation of an active metabolite, this hysteresis can be eliminated by predicting the concentration of the drug in another compartment with a pharmacokinetic / pharmacodynamic link model (Holford and Sheiner 1981). Put simply, the link model comprises input to and output from a theoretical effect compartment by first order processes, illustrated in Figures 2.13 and 2.14. Initally, the pharmacokinetic model, and hence the absorption rate constant Ka, are determined from MDC versus time. The pharmacodynamic model assumes the existence of the link, so the time course of effect-site concentration (Ce) is delayed relative to MDC, and Keo, the elimination rate constant from the effect-site, is chosen such that the hysteresis loop collapses and there is no delay between Ce and effect (Fuseau and Sheiner 1984; Holford and Sheiner 1981; Unadkat *et al* 1986; Oosterhuis and van Boxtel 1988).

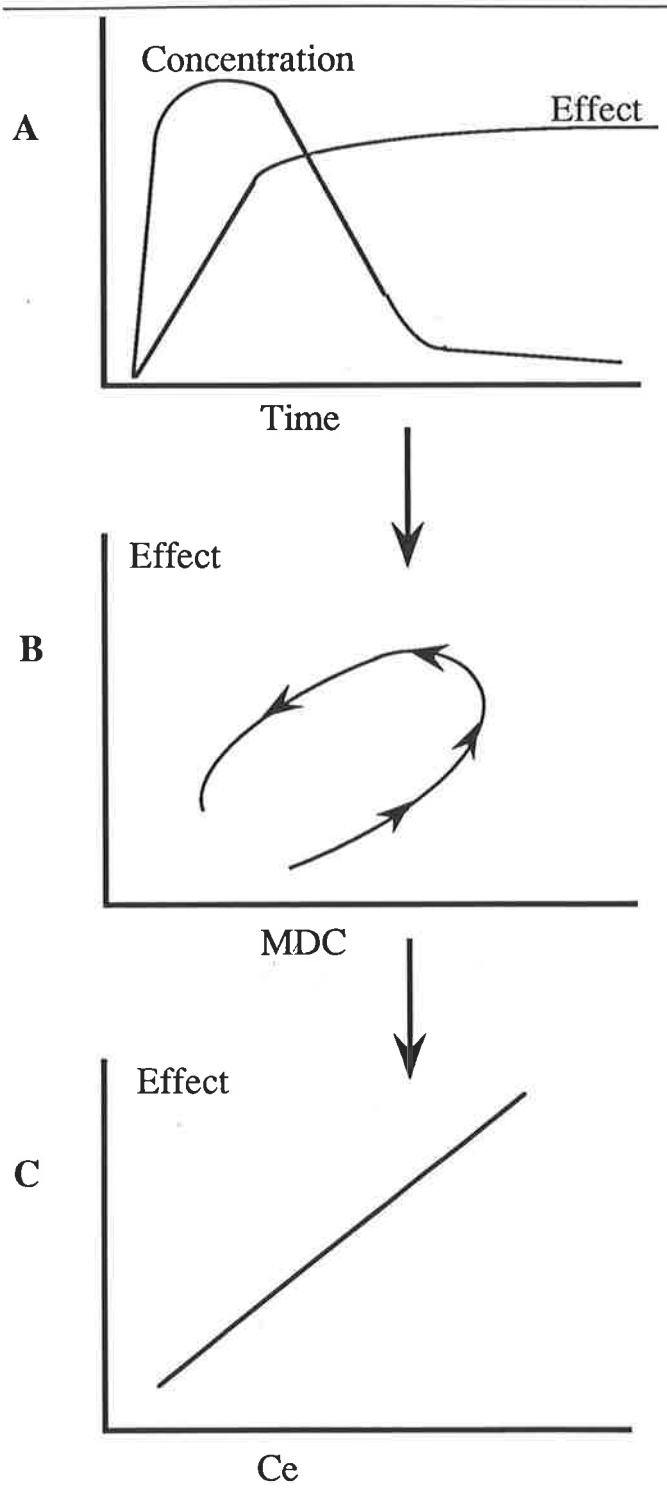


FIGURE 2.13 Explanation of the theoretical pharmacokinetic / pharmacodynamic link model. **A** : the plots of MDC (myocardial drug content) and effect versus time are not parallel; **B** : the plot of effect as a function of MDC shows an anticlockwise hysteresis loop; **C** : the link model predicts Ce (effect-site concentration) from MDC, and the plot of Ce versus effect has no hysteresis (adapted from Fuseau and Sheiner 1984)

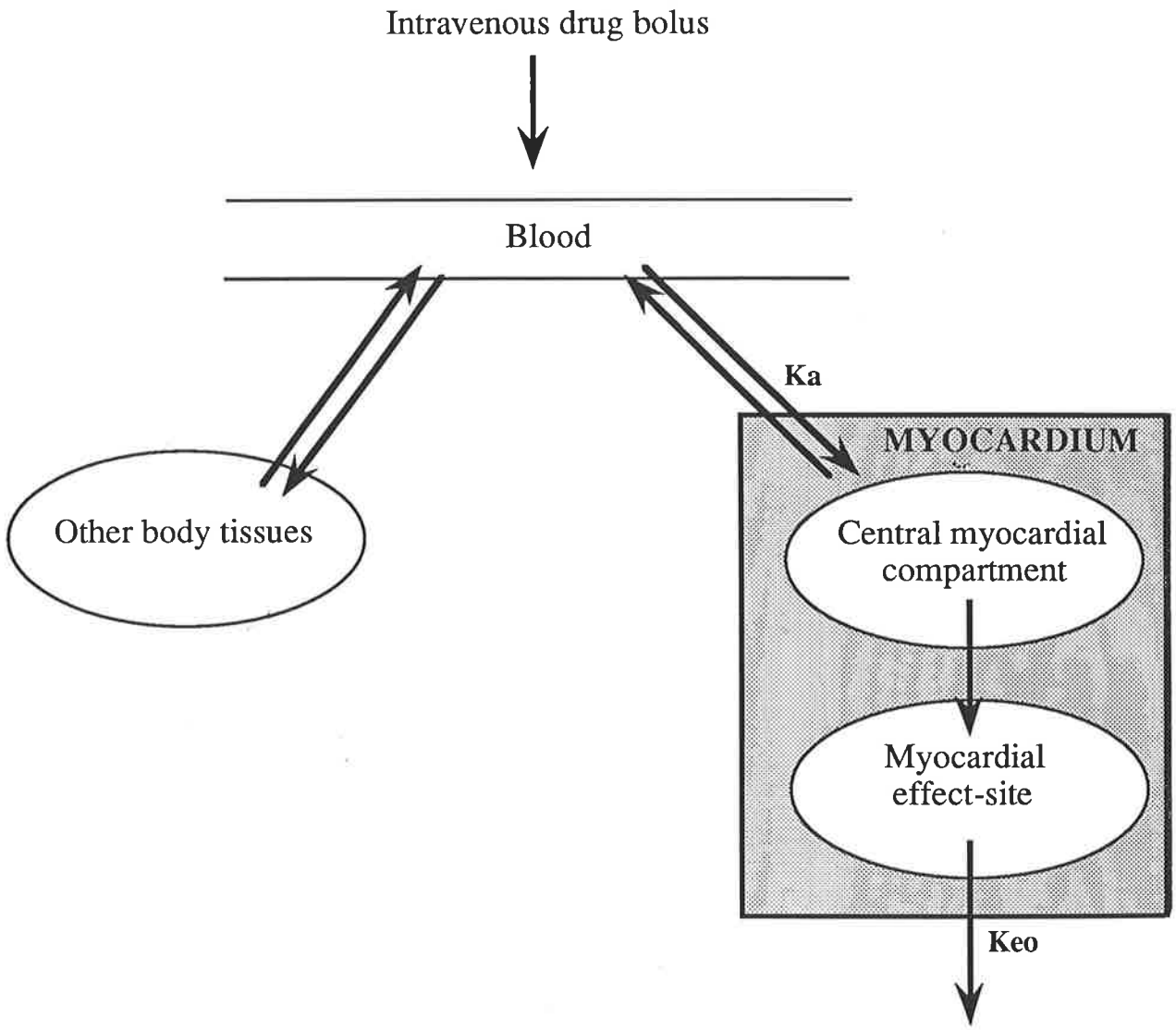


FIGURE 2.14 Schematic representation of the theoretical pharmacokinetic / pharmacodynamic link model. The drug is equilibrating between the myocardium and other body tissues. Ka describes the absorption of drug into the myocardium. Hysteresis is explained by delay required for equilibration into the hypothetical effect-site. The elimination rate constant Keo is not directed back into the central myocardial compartment (adapted from Oosterhuis and van Boxtel 1988)

2.6 Pharmacokinetic and pharmacodynamic models utilized for examining the relationship between myocardial drug content and effect

For the two ß-adrenoceptor antagonists investigated, metoprolol and sotalol, the relationship between MDC and acute effects was further investigated with pharmacokinetic / pharmacodynamic link models. The time course of MDC was fitted to either a Bateman function or a 2-compartment pharmacokinetic function, shown in Figure 2.15. Amount of drug in a theoretical effect compartment (Ce) is postulated to directly determine acute myocardial effects (Holford and Sheiner 1981; Unadkat *et al* 1986). The profile of Ce versus time was obtained by combining the linear pharmacodynamic model :

$$\text{Effect (E)} = \text{Slope (S)} * \text{Ce}$$

with the equation describing the time course of Ce, a 1- or 2-compartment first-order absorption model, ie a pharmacokinetic / pharmacodynamic link model (Holford and Sheiner 1981; Unadkat *et al* 1986).

The one-compartment first-order absorption model is described :

$$Ce = D * Ka * Keo * \left[\frac{e^{-K t}}{(Ka-K)(Keo-K)} + \frac{e^{-Ka t}}{(K-Ka)(Keo-Ka)} + \frac{e^{-Keo t}}{(K-Keo)(Ka-Keo)} \right]$$

and the two-compartment first-order absorption model is described :

$$Ce = D * Ka * Keo * \left[\frac{(K_{21}-\alpha)\, e^{-\alpha t}}{(ß-\alpha)(Ka-\alpha)(Keo-\alpha)} + \frac{(K_{21}-ß)\, e^{-ß t}}{(\alpha-ß)(Ka-ß)(Keo-ß)} + \frac{(K_{21}-KA)\, e^{-KA t}}{(\alpha-Ka)(ß-Ka)(Keo-Ka)} + \frac{(K_{21}-Keo)\, e^{-Keo t}}{(\alpha-Keo)(ß-Keo)(Ka-Keo)} \right]$$

where D is the dose of drug recieved by the heart from the bloodstream; Ka, K, K_{21}, α, and ß are rate constants related to the absorption process; and Keo is the rate constant of elimination (Holford and Sheiner 1981; Unadkat *et al* 1986). Once the value of these parameters was estimated, Ce was calculated by inserting the estimates into above equations. The plot of ∂effect as a function of Ce was then obtained, and subjected to a simple linear regression. The above pharmacokinetic / pharmacodynamic modelling was facilitated with the use of a curve-fitting programme for the Apple Macintosh computer, Multifit (Day Computing, Milton, Cambridge, UK).

Bateman function :

$$MDC = D * Ka * \frac{(e^{-Kt} - e^{-Kat})}{(Ka - K)}$$

Two-compartment model :

$$MDC = D*Ka*\left[\frac{(K_{21}-\alpha)e^{-\alpha t}}{(Ka-\alpha)(\beta-\alpha)} + \frac{(K_{21}-\beta)e^{-\beta t}}{(Ka-\beta)(\alpha-\beta)} + \frac{(K_{21}-Ka)e^{-Kat}}{(\alpha-Ka)(\beta-Ka)}\right]$$

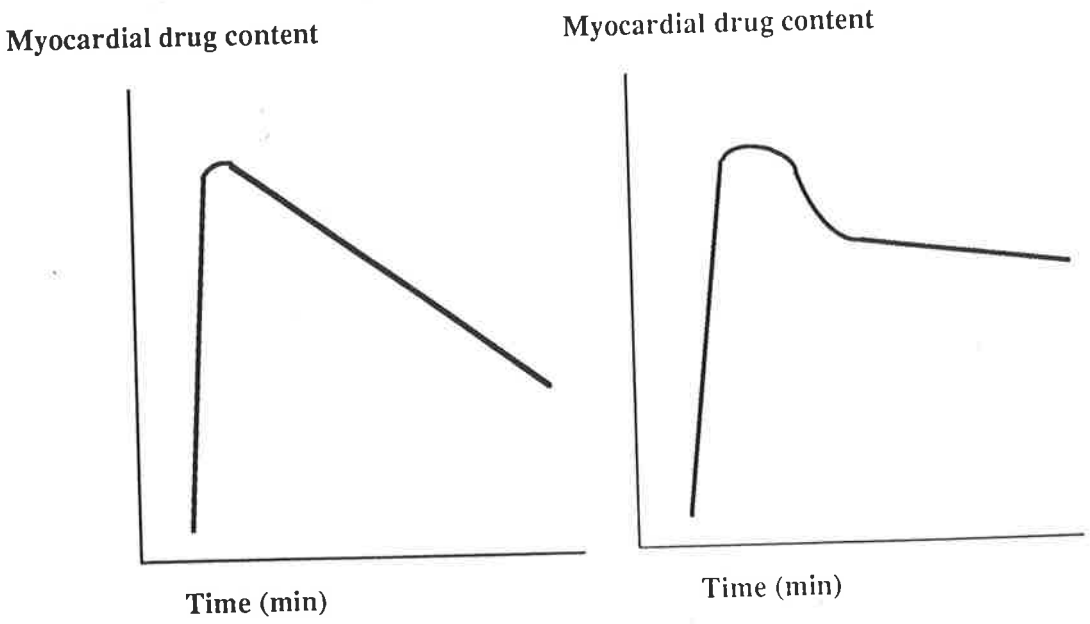


FIGURE 2.15 The equations describing the pharmacokinetic Bateman and two-compartment models for myocardial drug content as a function of time, with graphical representation of their approximate shapes

2.7 Statistical analyses utilized in this thesis

All results presented in this thesis are expressed as mean ± SEM. Where RR intervals or LV+dP/dt are discussed, they are expressed relative to values at the baseline pacing cycle length prior to drug administration, to facilitate comparisons between patients. A range of statistical methods were employed, as listed below.

(i) Residual standard deviation (SD)

Chapter 3: * goodness-of-fit of the mechanical restitution curve (a measure of the distance of each point from the fitted curve)

(ii) Ninety-five percent confidence intervals (95% CI)

Chapter 3: * parameters and goodness of fit of the parameters of the fitted hyperbolae

(iii) One group *t* -test

Chapter 4: * ∂ dP/dt_{PAC} versus 0

Chapter 5: * ∂ dP/dt_{PAC} versus 0

Chapter 6: * ∂ dP/dt_{PAC} versus 0

(iv) Two-tailed paired *t* -test

Chapter 5: * submaximal coronary venous reserve pre and post-sotalol administration

* the comparison of the area under the myocardial-content-as-a-function- of-time curve for R- and S-sotalol following an intravenous RS-sotalol bolus

Chapter 6: * cAMP concentration in femoral artery and coronary sinus prior to milrinone injection

* 0 versus 10min PESP curves after milrinone for each of the 2 individual patients in which PESP examined with a compensatory pause

* submaximal coronary venous reserve pre and post-milrinone administration

(v) Simple linear regression

Chapter 2: * correlations between peak height ratios (or absorbance) and substrate concentrations for the various calibration curves for the range of analytical techniques described

Chapter 3: * correlations between the parameters and goodness-of-fit of the model with patient characteristics (with real number values) at baseline

Chapter 4: * correlations between the characteristics of myocardial metoprolol u ptake with patient characteristics (with real number values) at baseline

* correlations between peak myocardial metoprolol content and maximum significant effects

Chapter 5: * correlations between the characteristics of myocardial sotalol uptake with patient characteristics (with real number values) at baseline

* correlations between peak myocardial sotalol content and maximum significant effects

Chapter 6: * correlations between the characteristics of myocardial milrinone uptake with patient characteristics (with real number values) at baseline

* correlations between peak myocardial milrinone content and maximum significant effects

(vi) <u>One-factor analysis of variance</u>

Chapter 3: * correlations between the parameters and goodness-of-fit of the model with patient characteristics (with integer or category values) at baseline

Chapter 4: * correlations between the characteristics of myocardial metoprolol uptake with patient characteristics (with integer or category values) at baseline

* comparison of LV myocardial metoprolol concentrations in isolated perfused Langendorff rat hearts under normoxic and hypoxic perfusion conditions at each of the time points of sacrifice

Chapter 5: * correlations between the characteristics of myocardial sotalol uptake with patient characteristics (with integer or category values) at baseline

Chapter 6: * time course of cAMP concentrations in coronary sinus plasma following milrinone injection

* correlations between the characteristics of myocardial milrinone uptake with patient characteristics (with integer or category values) at baseline

(vii) Two-factor analysis of variance with Dunnett's correction for repeated comparisons (Anova)

Chapter 4:

* time course of acute haemodynamic, and ECG effects of an intravenous metoprolol bolus dose

* time course of the ratio of ∂ significant acute effects of metoprolol and simultaneous myocardial metoprolol content

* time course of the asymptotes of the curve-fitting model, a and d, of the MRC after metoprolol administration

* time course of the parameters of rate-dependence of the curve-fitting model, c and RDI, after metoprolol administration

* the comparison of the reduction of LV+dP/dt induced by metoprolol prior to and 5 and 60s after the institution of rapid pacing

Chapter 5:

* time course of acute haemodynamic, ECG and EP effects of an intravenous sotalol bolus dose

* time course of the ratio of ∂ significant acute effects of sotalol and simultaneous myocardial sotalol content

* time course of the asymptotes of the curve-fitting model, a and d, of the MRC after sotalol administration

* time course of the parameters of rate-dependence of the curve-fitting model, c and RDI, after sotalol administration

* the comparison of the reduction of LV+dP/dt induced by sotalol prior to and 5 and 60s after the institution of rapid pacing

Chapter 6: * time course of acute haemodynamic, ECG and EP effects of an intravenous milrinone bolus dose

* time course of the ratio of ∂ significant acute effects of milrinone and simultaneous myocardial milrinone content

* time course of the asymptotes of the curve-fitting model, a and d, of the MRC after milrinone administration

* time course of the parameters of rate-dependence of the curve-fitting model, c and RDI, after milrinone administration

* the comparison of the increase of LV+dP/dt induced by milrinone prior to and 5 and 60s after the institution of rapid pacing

* the time course of plasma cAMP concentrations after an intravenous milrinone bolus dose

(viii) Area-under-the curve (AUC)

Chapter 5: * the comparison of the area under the myocardial-content-as-a-function-of-time curve for R- and S-sotalol following an intravenous RS-sotalol bolus

CHAPTER 3 :

DETERMINATION OF RATE-RELATED INOTROPIC EFFECTS IN HUMANS.

3.1 Mechanical restitution curve construction

It has been known since Bowditch's original observations (Bowditch 1871) that the contractile behaviour of cardiac muscle is influenced by changes in the rate of muscle stimulation (Edman and Johannsson 1976; Koch-Weser and Blinks 1963; Anderson *et al* 1976). The staircase or "Treppe" phenomenon observed in a train of beats of increased stimulation frequency raising muscle tension to a new plateau, illustrates this influence, probably a manifestation of increased intracellular calcium concentrations and / or catecholamine release (Bowditch 1871; Woodworth 1902; Koch-Weser and Blinks 1963; Pidgeon *et al* 1982; Seed and Walker 1988). Increased intracellular calcium concentrations (from internal stores and / or influx) and catecholamine release have been postulated as possible mechanisms for this observation (Seed and Walker 1988; Koch-Weser and Blinks 1963). The force-interval relationship has been examined as two distinct components : mechanical restitution and postextrasystolic potentiation (Braveny and Kruta 1958). Mechanical restitution describes the recovery of myocardial contractility after an extrasystolic stimulation (Cooper and Fry 1990; Johnson 1979) and postextrasystolic potentiation represents the contractile force in the beat subsequent to the non-steady-state beat (Johnson 1979; Hoffman *et al* 1956). The mechanical restitution curve (MRC) is obtained by plotting contractile strength of an extrasystole as a function of the extrasystolic interval (Cooper and Fry 1990).

A "full" MRC is obtained when the extrasystolic beats inserted at regular intervals between trains of steady-state beats range from extremely premature to several-fold greater than the steady-state intervals which would normally apply *in vivo* . This can only be studied in preparations lacking spontaneous prepotential characteristics, and has been extensively examined in a variety of preparations ranging from isolated cardiac muscle strips of animals (Edman and Johannsson 1976; Anderson *et al* 1976; Cooper and Fry 1990; Bouchard and Bose 1989; Anderson *et al* 1973; Anderson *et al* 1977; Ragnarsdottir *et al* 1982; Minelli *et al* 1985; Allen *et al* 1976; Posner and Berman 1967; Johnson and Kuohung 1968; Johnson and Shepherd 1971; Wier and Yue 1986; Bose *et al* 1988; Maylie 1982; Fry *et al* 1983; Drake-Holland *et al* 1992) and humans (Cooper and Fry 1990; Fry *et al* 1983; Cooper *et al* 1992),

to isolated animal ventricles or whole hearts (Burkhoff *et al* 1984a; Burkhoff *et al* 1984b; Sands and Winegrad *et al* 1970; Yue*et al* 1985; Pidgeon *et al* 1980).

The only *in vivo* possibility for construction of a "full" MRC would arise in pacemaker-dependent intact animals and humans, for example in dogs with complete heart block (Pidgeon *et al* 1980; Slinker 1991; Freeman and Colston 1990; Drake-Holland *et al* 1992). Full MRC have not been examined in humans *in vivo* , because of ethical implications, but a limited number of investigations have examined the "left half of the MRC", the component of the MRC which is associated with extrasystolic intervals less than the cycle length of spontaneous heart rate (Anderson *et al* 1979; Franz *et al* 1983; Pidgeon *et al* 1982). Similar studies have been reported with animals *in vivo* (Anderson *et al* 1976).

In previous studies, construction of the MRC (largely *in vitro* , as indicated above) has been utilized primarily to examine the process of restoration of contractile performance associated with abnormally prolonged interbeat intervals, and thus to obtain insight into underlying changes in intracellular calcium fluxes. If these changes were to be examined on the basis of data obtained *in vivo* , only the short cycle length component of the MRC could be utilized. However, detailed examination of this interval-strength relationship in man may also serve other, more directly clinically relevant, purposes (Stewart *et al* 1986; Sharma *et al* 1990; Rankin *et al* 1987; Buxton *et al* 1987; Switzer *et al* 1986; Gottlieb *et al* 1990; Josephson *et al* 1985; Greene *et al* 1989). For example, construction of the "short cycle length" MRC in an individual patient might contribute to prediction of the probability of LV haemodynamic deterioration development, if a tachyarrythmia occurs. An extension of this is the potential utility for acute haemodynamically-oriented studies of cardioactive drugs to ascertain which agents, while well-tolerated in sinus rhythm, might elicit haemodynamic deterioration during tachycardia.

3.2 Development of a quantitative model for the mechanical restitution curve

3.2.1 Background

Analysis of the short cycle length component of the strength-interval relationship would be markedly improved by the existence of an accurate mathematical description, incorporating a measure of susceptibilty to reduced contractile performance at short cycle lengths. This has not been attempted in previous investigations in either intact (ie nonautonomically blocked) animals or man. The only previous mathematical models incorporated the full MRC, and the equations that best described them are therefore weighted by data for extrasystoles far longer than could possibly be elicited in spontaneously beating preparations, such as the intact human circulation (Cooper and Fry 1990; Posner and Berman 1967; Johnson and Kuohung 1968; Johnson and Shepherd 1971; Wier and Yue 1986; Maylie 1982; Fry *et al* 1983; Cooper *et al* 1992; Burkhoff *et al* 1984a; Yue*et al* 1985; Slinker 1991; Freeman and Colston 1990).

The objectives in the present study were therefore :

(i) to construct the short-cycle length component of the MRC in normal subjects and patients with ischaemic heart disease;

(ii) to develop an accurate mathematical model of this component of the MRC; and (iii) to seek correlations between the susceptibilty of individual subjects to short cycle-length-induced deterioration of mechanical performance, and other indices of systolic left ventricular function.

3.2.2 Methods

Selection of patients for the current study was as described in Section 2.1.1 of this thesis. The 4F Millar micromanometer-tipped catheter was inserted via the femoral artery sheath into the left ventricle, to facilitate measurement of LV pressure and determination of its first time derivative, peak LV+dP/dt. In order to eliminate minor fluctuations in heart rate, patients were subjected to

continuous atrial pacing, 10.4±1.8% faster than the spontaneous heart rate. Once every eight beats, an isolated premature stimulus was inserted at progressively decreasing cycle lengths in 100ms steps. As the extrasystole approached the absolute refractory period of the left ventricle, 50ms reductions in cycle length were utilized, until attaining a stimulus intervalwhich failed to elicit a contraction.

3.2.3 Results

3.2.3.1 Theoretical considerations

Construction of the designated component of the MRC involves introduction of premature right atrial stimuli once every eight beats at progressively decreasing test pulse intervals, as illustrated in Figure 3.1. From this, the relationship between LV+dP/dt of the test stimulus is plotted against the extrasystolic (RR) interval. MRC were constructed in duplicate and the average of the two curves was analysed.

A non-linear curve of best fit was required to compare the shape (ie sharp or smooth) of the MRC between patients prior to drug administration, and within a patient at various times post drug injection. Simple linear regression was not appropriate as the physiological MRC is indeed a curve, with LV+dP/dt eventually abolished as the extrasystolic interval approaches zero. As discussed in more detail in section 3.2.4, a vertical asymptote was therefore desired to reflect the absolute refractory period of the ventricle. A horizontal asymptote, representing the theoretical maximal contraction strength that the myocardium can ever elicit, would also be advantageous. In summary, these three requirements were met by half of a rectangular hyperbola :

$$y = a + \frac{b}{(x - d)} \qquad (i)$$

rewritten as :

$$y = a - \frac{c\ (100 - d)\ (60 - d)}{40\ (x - d)} \qquad (ii)$$

This equation, illustrated in Figure 3.2, was then applied to the MRC obtained in each patient. The horizontal and vertical asymptotes are represented by a and d respectively, while c was chosen as the difference between the calculated values of LV+dP/dt obtained from the fitted curves, when the RR interval is 100%, and 60%, of the baseline pacing rate, on the basis that at least a 40% reduction in RR intervals could be utilized in the majority of patients examined, before reaching the refractory period of the ventricle. The most important of these is the parameter c , which represents a convenient measure of individual sensitivity of contractile performance to reductions in cycle length.

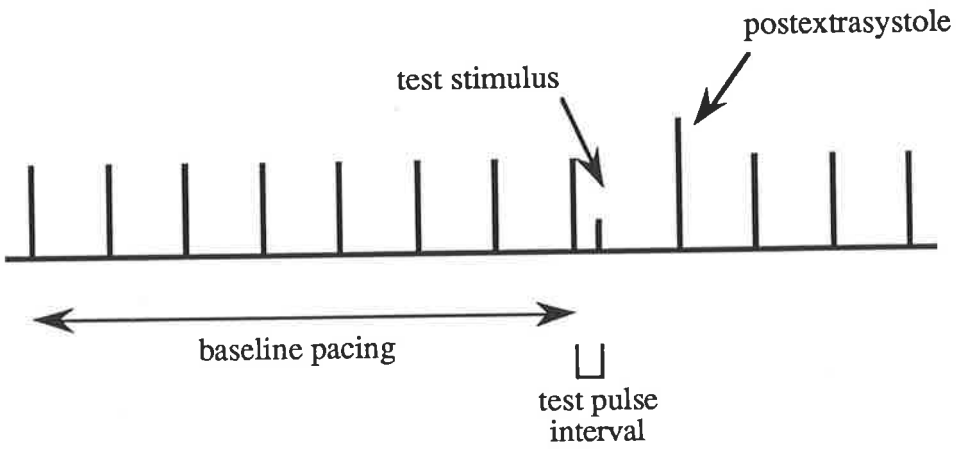


FIGURE 3.1 Protocol for obtaining mechanical restitution and postextrasystolic potentiation curves in patients with ischaemic heart disease

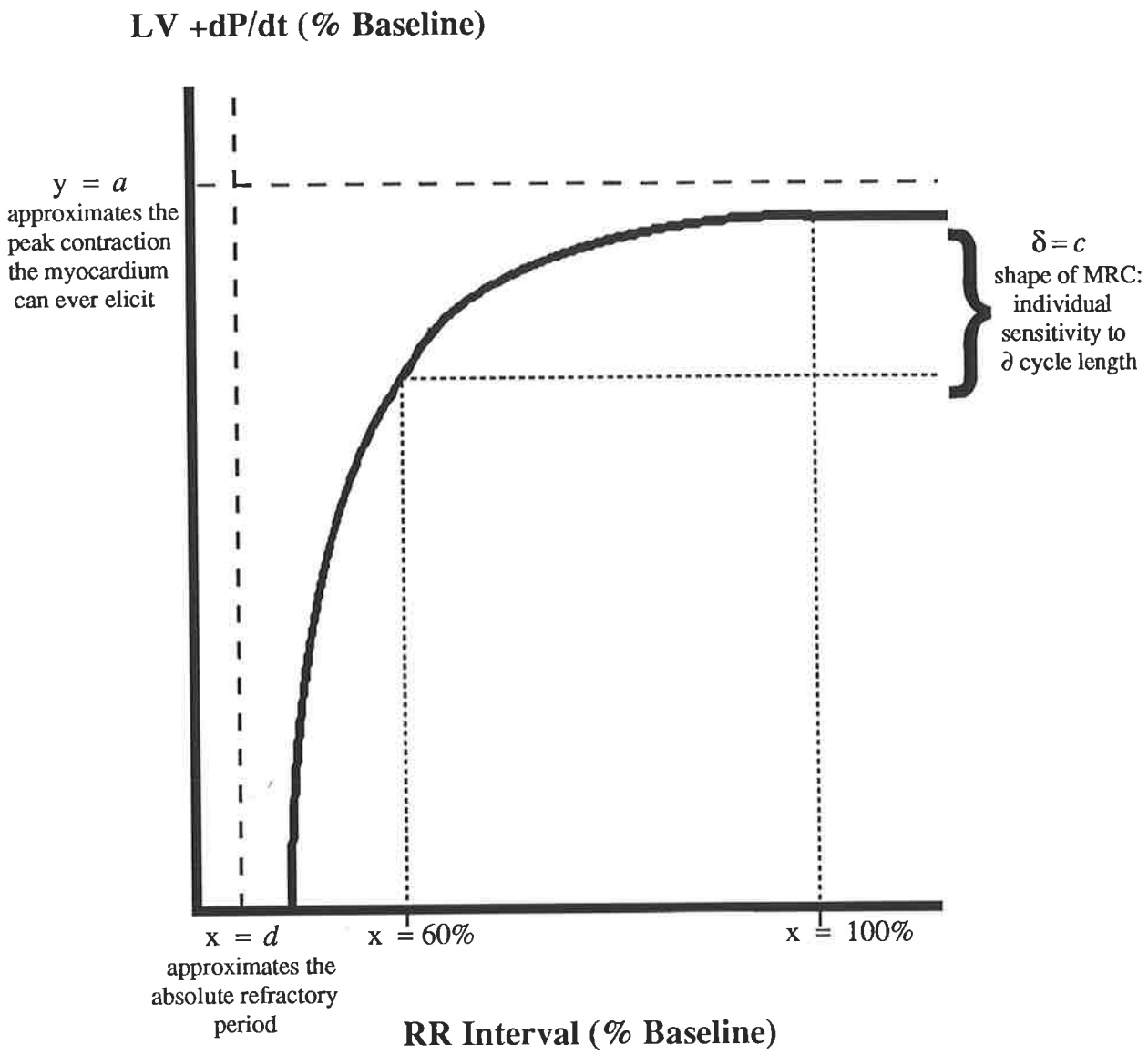


FIGURE 3.2 A graphical representation of the **theoretical** MRC, which was described by half of a rectangular hyperbola. The horizontal and vertical asymptotes were represented by a and d respectively, while c represents the reduction in LV+dP/dt when the RR interval is decreased from 100% to 60% of the baseline pacing rate

3.2.3.2 The present investigation

Twentyseven patients were studied, and their characteristics are summarized in Table 3.1. In general, baseline LV systolic function was normal, but 2 had LV ejection fractions less than 50% and 8 patients had cardiac indices lower than normal (<2.5 L/min/m^2). Nineteen of the 27 patients studied had at least one stenosis of greater than 50% within major branches of their coronary circulation. Pacing throughout the study was at a mean cycle length of 802±25msec. The procedure was well tolerated in all patients.

MRC obtained in the 27 patients investigated are depicted in Figure 3.3. As the RR interval (the interval between beats) was decreased from 100% to 51.4±1.3% of the baseline cycle length, LV+dP/dt also decreased from 100% to 56.7±6.3 % of its original value, until the absolute refractory period was reached.

A typical example of the fitted equation with original data points from one patient is shown in Figure 3.4. The 95%CI for the parameters of the MRC, as well as the residual standard deviations (SD), are listed in Table 3.2. The estimates of goodness of fit ranged from 1.2-14.2%, with 95%CI of 4.9 and 7.7. The rectangular hyperbola was fitted in 25 of the 27 patients - in the remaining two cases, the data was inappropriate for a rectangular hyperbolic function and were therefore fitted using linear regression, and c was calculated as before, from the fitted line. Consequently, there were no estimates for a and d in these two patients.

Correlations were sought between all parameters of the model and patient characteristics prior to the procedure. No significant trends between the curve parameters and any of cardiac index, left ventricular ejection fraction, wedge pressure, or patient age were observed. Extent of fixed coronary artery disease (CAD) was not significantly correlated with parameters a , c , or d , but patients with two-vessel CAD had significantly greater residual standard deviations for the model fit. Both baseline heart rate and the rate of baseline pacing were significantly correlated with c , such that patients with a shorter interval between heart beats exhibited greater estimates of c. These patients, with elevated baseline heart rates, and therefore reduced baseline cycle

lengths, also manifested a tendency for lower approximations for the horizontal asymptote, but this failed to reach significance ($p = 0.16$).

Both a and c were significantly augmented in the female patients compared with those determined in males. LV+dP/dt at baseline was positively correlated with a ($p < 0.02$), but was not related to c. Patients with poorer contractile indices also tended to display lower residual SD's ($p=0.08$) and larger estimates of the vertical asymptote ($p=0.11$).

TABLE 3.1 Patient characteristics

Pt	Age (yr)	Sex	CI ($L/min/m^2$)	HR (beats/min)	LV EF (%)	PCWP (mmHg)	CAD	LV+dP/dt (mmHg/s)	CL (ms)
1	64	M	2.78	71	61	11	0	1620	750
2	55	M	3.57	51	63	11	2	1630	1000
3	55	M	3.30	64	68	5	3	1870	900
4	40	M	3.20	75	73	10	2	1280	700
5	56	M	2.52	73	55	7	1	1430	750
6	61	M	3.70	68	81	10	2	1600	750
7	47	M	3.22	75	65	13	2	1760	700
8	46	F	3.09	68	76	18	0	2040	845
9	67	F	2.35	65	38	9	1	1530	650
10	49	F	2.78	80	59	3	0	1330	750
11	60	M	2.56	57	63	6	3	1430	1000
12	54	M	3.16	54	64	4	3	1430	1100
13	60	F	2.62	87	78	2	0	1480	650
14	59	M	1.84	69	52	3	0	1200	850
15	62	M	5.52	75	45	14	3	1650	700
16	64	F	2.40	73	69	3	1	1560	750
17	68	M	2.28	77	76	8	1	1590	750
18	68	F	2.45	64	65	5	2	1650	850
19	54	F	3.13	80	67	10	1	1560	750
20	64	M	3.27	54	55	8	3	1500	900
21	57	M	2.37	56	73	10	1	1620	900
22	45	M	2.66	80	74	10	0	1540	750
23	55	M	2.49	61	70	9	1	1230	950
24	43	M	2.71	56	64	7	0	1420	900
25	35	M	3.85	77	86	5	1	2260	700
26	50	M	2.77	64	71	7	1	1540	850
27	62	F	2.36	102	89	3	0	2660	550
Mean	56		2.92	70	67	8		1610	802
SE	2		0.14	2	2	1		60	25

CAD, fixed coronary artery disease (>50% stenosis); CI, cardiac index; CL, baseline pacing cycle length; EF, ejection fraction; F, female; HR, heart rate; LV, left ventricle; LV+dP/dt, peak rate of LV pressure rise; M, male; PCWP, pulmonary capillary wedge pressure; Pt, patient

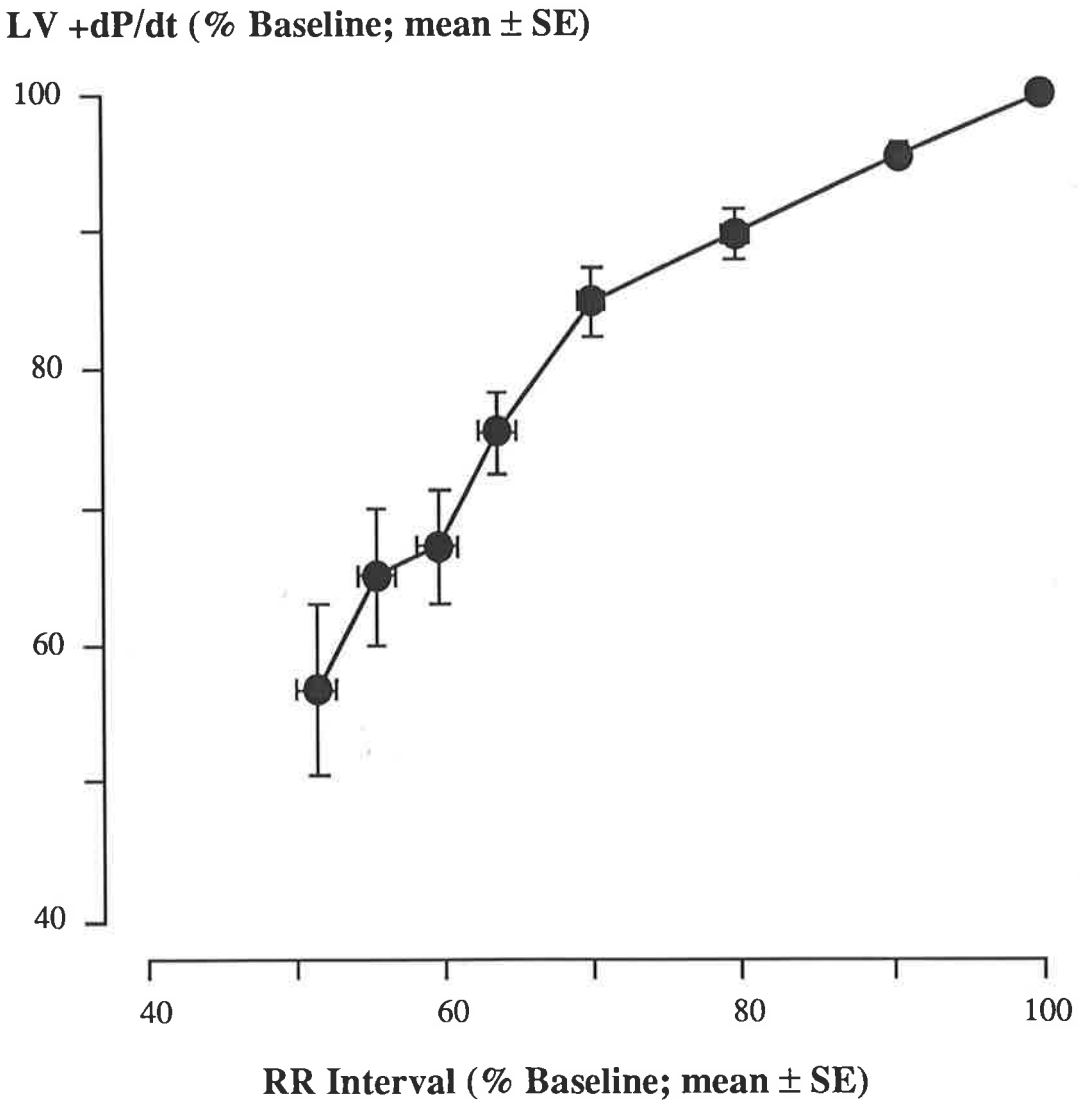


FIGURE 3.3 Mean data for baseline mechanical restitution curves obtained in 27 patients

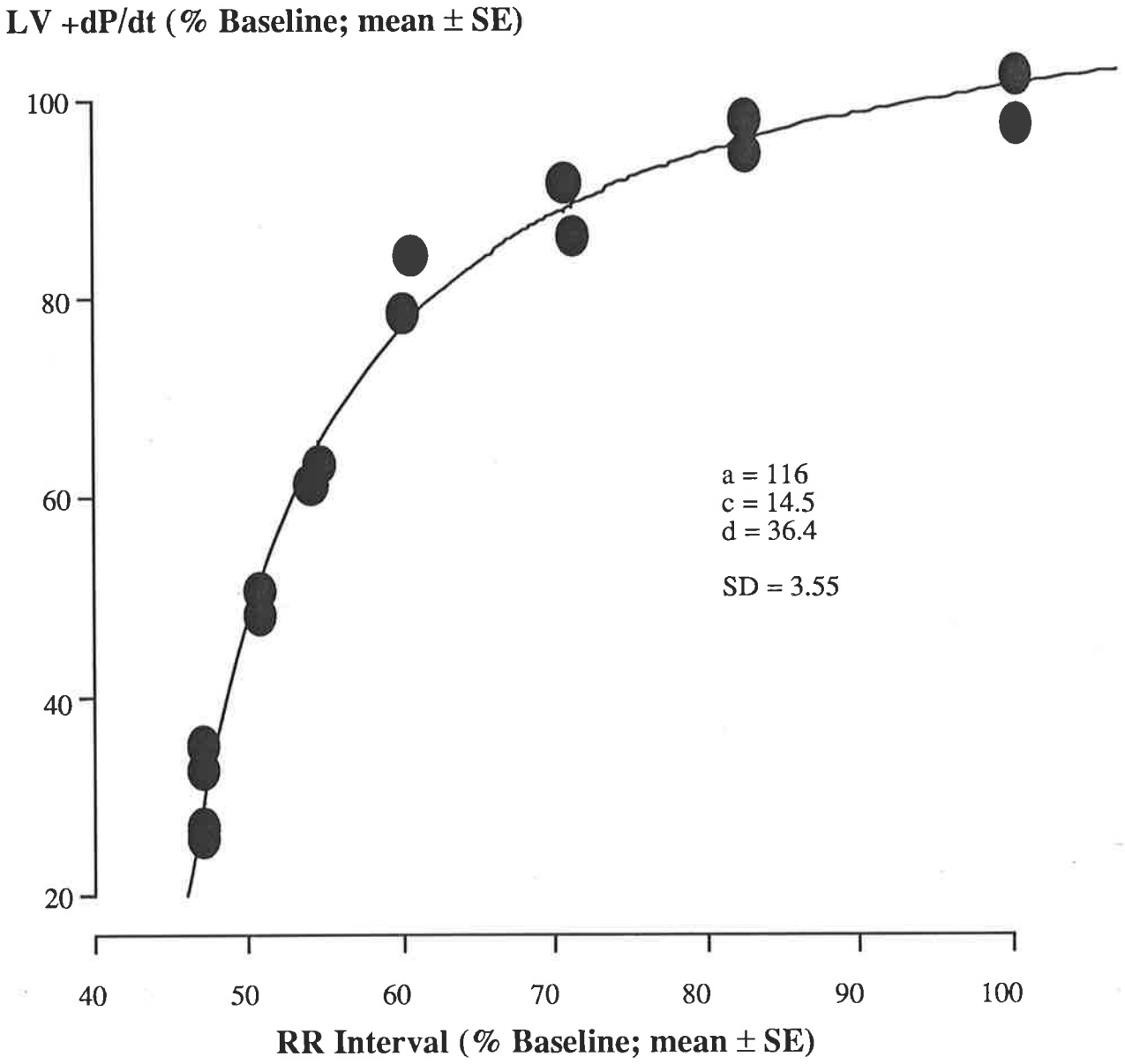


FIGURE 3.4 A typical example of the curve-fitting of the MRC model from one patient to measured baseline data points

TABLE 3.2 Parameters of the MRC curve-fitting model in all patients

Parameter	*a*	*c*	*d*	Residual SD's
Mean	177	31.9	- 9.9	6.28
Standard Error	32	4.1	25.7	0.67
Lower 95% CI	112	23.5	-63.0	4.90
Upper 95% CI	242	40.3	43.2	7.66

CI, confidence intervals

3.2.4 Discussion

The left half of the MRC, associated with extrasystolic intervals less than the cycle length of spontaneous heart rate, have previously been demonstrated in humans *in vivo* (Anderson *et al* 1979; Franz *et al* 1983; Pidgeon *et al* 1982). Quantitative descriptions of this phenomenon have only previously been attempted in isolated human (Cooper and Fry 1990; Fry *et al* 1983; Cooper *et al* 1992) and animal ventricular muscle preparations (Cooper and Fry 1990; Posner and Berman 1967; Johnson and Kuohung 1968; Johnson and Shepherd 1971; Wier and Yue 1986; Maylie 1982; Fry *et al* 1983; Cooper *et al* 1992; Burkhoff *et al* 1984a; Yue*et al* 1985) as well as in autonomically-blocked dogs *in vivo* (Slinker 1991; Freeman and Colston 1990). These descriptions were all of full MRC, the extrasystolic beats ranging from extremely premature to several-fold greater than the steady-state intervals, and were therefore weighted by data for extrasystoles far longer than could be elicited in spontaneously beating preparations, as investigated in the current study.

The present investigation determined, for the first time in intact humans, a mathematical model for the force-frequency relationship. In selecting an appropriate equation for the MRC, several factors were taken into consideration, the first of which was that only the left half of the MRC was investigated. LV+dP/dt was eventually abolished as the extrasystolic interval approached zero as the extrasystolic interval approached zero, representing the ventricular effective refractory period, was a second consideration. Thus it was necessary to incorporate a vertical asymptote into the model. An additional consideration was that LV+dP/dt increases as the stimulus interval increases. In keeping with these considerations, the arcosh curve, represented by the equation :

$$y = \text{arcosh}\left(1 + \frac{x - d}{b}\right) \qquad (iii)$$

was originally attempted as a potential mathematical model for the MRC in the present investigation, where b and d were the description of the curvature and the vertical asymptote respectively. The arcosh curve can be alternatively represented by the equation :

$$\frac{e^{y} + e^{-y}}{2} = 1 + \frac{x - d}{b} \qquad (iv)$$

This was not a very flexible model, dependent on only two parameters, with little capacity to permit a large variety in curve shape. Thus, the patient curves could not be satisfactorily fitted to this equation. The MRC model needed to incorporate two more assumptions : firstly a horizontal asymptote, representing the theoretical maximal contraction strength that the myocardium can ever elicit; and secondly the curvature between the two asymptotes. The rectangular hyperbola embodies all of the above requirements, and is given by equation *(i)* . While this provided a good fit to the data, a minor modification to the above equation was made to provide a simpler description of MRC shape. The degree of reduction of LV+dP/dt as the RR interval is also decreased, could be used to describe shape for statistical analyses of comparisons of shape between patients. The parameter c was chosen as the arbitrary drop in the y-axis when x changes from 100% to 60%, because this was the largest decrement that could be selected without excluding too many of the patients, ie :

$$c = y_{100} - y_{60} \qquad (v)$$

Equation *(i)* could then be rewritten as equation *(ii)* . This model is also advantageous because it has the capacity to statistically examine rate-related inotropic effects of cardioactive drugs. This could be accomplished by calculating "a rate-dependence index" (RDI), which would be determined as the ratio of c values obtained at a given time following drug administration and those obtained in the absence of drug.

Other investigators have also described mathematical models for the MRC, but these have predominantly been restricted to isolated ventricular muscle preparations (Cooper and Fry 1990; Posner and Berman 1967; Johnson and Kuohung 1968; Johnson and Shepherd 1971; Wier and Yue 1986; Maylie 1982; Fry *et al* 1983; Cooper *et al* 1992; Burkhoff *et al* 1984a; Yue*et al* 1985), with the exception of two studies in dogs *in vivo* (Slinker 1991; Freeman and Colston 1990). These studies have all reported equations employing one or more exponential functions to describe the full MRC. It should kowever be noted that visual examination of the results from the studies selecting exponential descriptions of the MRC restricted to the range of

beat intervals appropriate to spontaneously beating preparations, that these results could probably be equally well quantified by the rectangular hyperbolic equation *(ii)* . While this study is therefore the first to quantitatively model the MRC in the intact human circulation, it also recognizes the requirements for both vertical and horizontal asymptotes, a measure describing the decrement in LV+dP/dt as the interval between beats is reduced (c), and finally, the potential for a parameter indicating extent of modification of inotropic effects by changes in rate by pharmacologic interventions (RDI). Unlike other mathematical models of the force-interval relationship (Fry *et al* 1983; Cooper *et al* 1992), none of the parameters of the current equation were statistically correlated with conventional indices of left ventricular function.

Residual SD's of the model tended to be increased (p=0.06) in patients whose LV ejection fractions were above the average for the group, indicating that the model may, if anything, work better in patients with impaired LV function, and may therefore be particularly useful for investigating patients with heart disease. However, to date little experience has been accumulated in patients with severe systolic heart failure. Differences in sizes of the male and female populations included in this group (19 and 8 respectively), representative of the gender distribution of Australians with coronary heart disease, prevent satisfactory examination of the influence of patient sex on c and a . The significant tendency for elevated a in patients with higher baseline dP/dt could have been predicted on the basis that a represents the maximal contractile strength asymptote. The inverse relationship between c and the baseline pacing cycle length suggests that c , like LV+dP/dt, is rate-dependent. Alternatively, it follows suit that patients with a faster rate of baseline pacing must have quicker baseline heart rates, and this was indeed the case. This elevation of c may be attributable to compensated heart failure, but the lack of dependence of c on indices of LV function (cardiac index, LV ejection fraction, and LV+dP/dt) does not support this. Perhaps these patients are prone to tachycardias because of an elevated catecholamine drive, and the positive inotropic effect of these catecholamines may be less marked at shorter cycle lengths.

The force-frequency relationship described above probably reflects a fundamental cellular mechanism in the control of performance of cardiac ventricular muscle. This mechanism is probably largely explicable in terms of altered metabolism of activator calcium, which interacts

with troponin C to initiate contraction (Perreault *et al* 1990), when the rate of stimulation is manipulated (Edman and Johannsson 1976; Morad and Goldman 1973). Changes in stimulation frequency have been demonstrated to influence calcium transport across the sarcolemma, and the calcium content in the myocardial cell (Sands and Winegrad *et al* 1970). Evidence that the force-frequency effect on inotropic state is related to changes in free calcium availability within the myocardial cell, is from using the fluorescent calcium indicator aequorin to detect changes in intracellular calcium concentrations during changes in contraction frequency : a positive correlation between contraction frequency, force development and the magnitude of the calcium transient has been reported (Morgan and Blinks 1982).

The dependence of contractile force on the preceding interval is interpreted in terms of calcium fluxes into and within the cell, illustrated by the model of an intracellular calcium "store" whose contents are discharged to activate the contractile proteins on depolarization (Ragnarsdottir *et al* 1982; Pidgeon *et al* 1982), depicted in Figure 3.5. The "store" takes a finite time to fill (Ragnarsdottir *et al* 1982; Pidgeon *et al* 1982). The change in force production that occurs in response to changes in the stimulus interval can therefore be interpreted as a manifestation of the time-dependence of activator calcium cellular handling (Ragnarsdottir *et al* 1982; Wier and Yue 1986).

The calcium "store" is filled from two sources : calcium released from the contractile proteins on the previous beat, and calcium entering the cell during the depolarization of previous action potentials, enforcing a dependence on force and frequency of previous beats (Pidgeon *et al* 1982). Changes in beat strength are explained by changes in calcium release from the "store" (Langer 1973). Immediately after relaxation, calcium is not in a form in which it can be fully released to produce another full contraction (Edman and Johannsson 1976) and the model proposes the existence of separate "uptake" and "release pools" within the sarcoplasmic reticulum (Anderson *et al* 1977; Yue*et al* 1985; Edman and Johannsson 1976). Calcium is taken up by the sarcoplasmic reticulum after contraction into the uptake pool (Edman and Johannsson 1976), where it is not immediately available to the contractile apparatus, but must first be transferred to the "release pool", a time dependent process (Edman and Johannsson 1976; Seed and Walker 1988). The "store" is replenished in part by recycling calcium from the

previous beat and in part by calcium entering the cell during the previous depolarization (Seed and Walker 1988). Recovery of contractile force is dependent on the time course of calcium transfer from the "uptake" to "release" sites of the "store" (Seed and Walker 1988).

Ryanodine, a putative blocker of sarcoplasmic reticulum calcium release, nearly eliminates the increase in intracellular calcium and tension development in Purkinje fibres consequent to an action potential (Marban and Wier 1985), implying that most of the calcium which activates the contractile apparatus is from the sarcoplasmic reticulum, and the primary physiological function of transsarcolemmal calcium influx is to trigger calcium release from the sarcoplasmic reticulum (Fabiato 1983; Fabiato 1985; Fabiato and Fabiato 1978). Calcium-induced calcium release occurs through a channel across the sarcoplasmic reticulum membrane with time-dependent and calcium-dependent activation and inactivation (Fabiato 1985). Relaxation occurs as calcium is removed from the myofibrillar space, mostly into the "uptake store", but a small quantity is removed from the cell (Ragnarsdottir *et al* 1982).

The time course of mechanical recovery after a beat, and the magnitude of recirculation have been inferred from studies of LV mechanical response during pacing (Elzinga *et al* 1981). The time course of recirculation in man is approximately 800 msec (Ragnarsdottir *et al* 1982; Pidgeon *et al* 1982; Seed *et al* 1984), not significantly different from the baseline pacing rate incorporated in the current study. A particular point on the MRC reflects the amount of calcium transferred to the "release store" when the extrasystolic interval is introduced (Bose *et al* 1988; Burkhoff *et al* 1984a).

In the model, the "uptake" and "release" sites for calcium have been speculated to correlate with anatomically distinct regions of the sarcoplasmic reticulum, possibly the sarcolemmal cisternae and sarcotubular network respectively. Resequestered calcium gradually becomes more releasable during the interval between contractions (Winegrad 1979; Lewartowski and Pytkowski 1987). Depolarization in mammals results in calcium at the "release sites" entering the myoplasm to produce myofilament activation : the amount of calcium released is proportional to contractile force development for variably restituted beats (Wier and Yue 1986). This would imply that a premature stimulus which occurs before completion of the calcium

restitution process would release a reduced amount of calcium and produce a small mechanical response (Phillips *et al* 1990).

Under stable conditions, the calcium which enters the myocyte must be pumped out again during each cycle (Feher *et al* 1988) by either sarcolemmal calcium-ATPase (Caroni and Carafoli 1981) or the sodium-calcium exchange mechanism (Reeves and Sutko 1983). Similarly, calcium release by the sarcoplasmic reticulum must be reaccumulated by the sarcoplasmic reticulum calcium-ATPase to elicit relaxation and load the sarcoplasmic reticulum for the next cycle (Feher *et al* 1988).

In summary :

(i) the designated component of the mechanical restitution curve can thus be described quantitatively in the intact human circulation;

(ii) this description includes a new index of LV function, c , which represents individual sensitivity of contractile performance to changes in cycle length, and cannot be predicted on the basis of existing indices of left ventricular function;

(iii) the extent of rate-related inotropic effects of cardioactive agents can be determined and compared between different patients using this model;

(iv) this mathematical description of the MRC in man is a useful tool for investigation of the human force-interval relationship, even in patients with impaired LV systolic function.

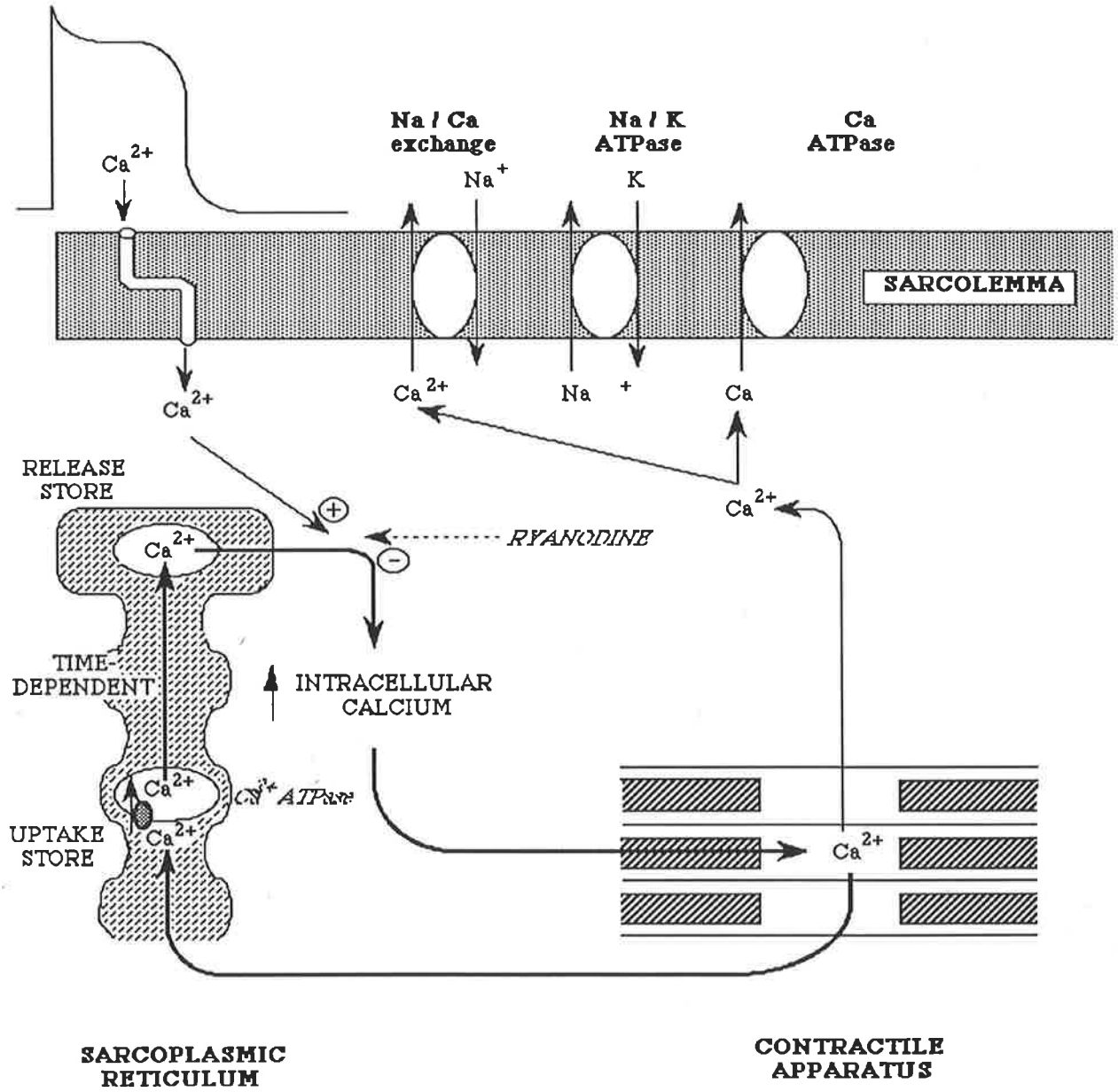


FIGURE 3.5 A hypothetical model of excitation-contraction coupling in mammalian myocardium : see text for discussion

3.3 Other methods of examining the potential for rate-dependence of drug effects on contractile state

Other methods of examining the potential for rate-related drug effects on inotropic state include :

(i) post-extrasystolic potentiation (PESP; specifically examining the recovery phase with or without a compensatory pause);

(ii) an eight beat short-cycle length MRC;

and (iii) the obvious option of sustained pacing-induced tachycardia.

The second of these methods, where the contractile force of the last beat in a train of eight premature beats is plotted as a function of cycle length, was further studied in Chapter 5 of this thesis, before and after sotalol injection, in a limited number of patients. Stability of LV+dP/dt during one minute of rapid atrial pacing before and 10-20min after cardioactive drug injection was studied for each drug investigated, but only in selected patients with mild angina. All three methods can be utilized to validate the short cycle length single beat MRC.

The phenomenon of PESP has been demonstrated in a number of preparations, including isolated ventricular preparations (Hoffman *et al* 1956; Anderson *et al* 1977; Posner and Berman 1967; Wier and Yue 1986; Bose *et al* 1988; Maylie 1982; Yue*et al* 1985; Wood *et al* 1969; Phillips *et al* 1990; Suko *et al* 1970; Drake-Holland *et al* 1992), isolated hearts (Burkhoff *et al* 1984b; Suko *et al* 1970; Hoffman *et al* 1965; Elzinga *et al* 1981), and *in vivo* in both animals (Freeman and Colston 1990; Elzinga *et al* 1981; Drake-Holland *et al* 1992) and humans (Anderson *et al* 1979; Banka *et al* 1976; Dyke *et al* 1974; Sung *et al* 1980; Kuijer*et al* 1990; van der Werf *et al* 1976; Seed *et al* 1984).

Studies in humans *in vivo* examining the beat following an extrasystole commonly utilize an extrasystolic interval shorter than, and a postextrasystolic interval longer than, the steady-state cycle length (Anderson *et al* 1979; Banka *et al* 1976; Dyke *et al* 1974; Hamby *et al* 1975; Beck *et al* 1971; Sung *et al* 1980). Other investigations of PESP (Hoffman *et al* 1956; Hoffman *et al* 1965), particularly in humans *in vivo* (Kuijer*et al* 1990; van der Werf *et al* 1976; Seed *et al* 1984), have utilized an extrasystole shorter than the steady-state cycle length,

and a postextrasystole at a cycle length equal to that at steady-state, ie in the absence of a compensatory pause. In the current study, PESP curves were constructed from LV+dP/dt of the beat following the test stimulus plotted as a function of the test pulse interval, and was examined without a compensatory pause (refer Figure 3.1) unless otherwise stated. PESP curves were constructed in duplicate, and the average of the two curves was presented.

PESP has not been examined quantitatively to quite the same extent as mechanical restitution, but has been demonstrated in humans *in vivo* (Banka *et al* 1976; Dyke *et al* 1974; Hamby *et al* 1975; Beck *et al* 1971; Sung *et al* 1980; Kuijer*et al* 1990; van der Werf *et al* 1976; Seed *et al* 1984; Schmidt *et al* 1973), usually as a diagnostic tool to determine the extent of residual contractile reserve after myocardial infarction (Banka *et al* 1976; Dyke *et al* 1974; Hamby *et al* 1975; Beck *et al* 1971; Sung *et al* 1980; Kuijer*et al* 1990). Other investigations in isolated human (Phillips *et al* 1990) and animal (Wier and Yue 1986; Bose *et al* 1988; Maylie 1982; Yue*et al* 1985; Suko *et al* 1970; Hoffman *et al* 1965) ventricular myocardial preparations, as well as in animals *in vivo* (Freeman and Colston 1990), have also been reported.

Quantitative descriptions have also been attempted for PESP in a limited number of investigations, which are also predominantly exponential functions (Wier and Yue 1986; Maylie 1982; Yue*et al* 1985; Freeman and Colston 1990). MRC and PESP curves can both be described by similar functions, suggesting a simple mechanism might underly both phenomena (Yue*et al* 1985; Wier and Yue 1986; Maylie 1982; Freeman and Colston 1990). No attempt to mathematically describe PESP in this way was made in the present study, because there was no compensatory pause following the premature stimulus, and hence the postextrasystolic interval was held constant, and equal to the basic pacing cycle length.

PESP has often been attributed to :

(i) augmented myocardial contractility, related to increased Ca^{2+} influx (Wood *et al* 1969; Suko *et al* 1970) improving contractile reserve (Kuijer*et al* 1990);

(ii) reduced systemic resistance during the compensatory pause (Beck *et al* 1971); and (iii) increased filling during the compensatory pause after the extrasystole (Banka *et al* 1976; Dyke *et al* 1974; Banka *et al* 1976; Dyke *et al* 1974; Hamby *et al* 1975). However, it has been demonstrated that augmented filling and Starling's Law do not

significantly contribute to PESP (Sung *et al* 1980; Kuijer*et al* 1990). The PESP data obtained here are in the absence of a prolonged postextrasystolic interval, hence any potentiation observed here can only be attributable to the augmented contractility.

As illustrated in Figure 3.5, during the extrasystole, Ca^{2+} entering the myoplasm becomes available to the "uptake sites" with subsequent translocation to the "release sites", suggesting if the diastolic restitution period was sufficiently long, it would increase the amount of Ca^{2+} available for release, resulting in PESP (Phillips *et al* 1990). PESP occurs via Ca^{2+} influx directly activating the myofilaments, or Ca^{2+}-induced Ca^{2+} release (Solaro *et al* 1974; Seed and Walker 1988). Changes in the slow Ca^{2+} current are not involved (Lukas and Bose 1986). Because the magnitude of a beat is dependent on the amount of Ca^{2+} released during the excitation-contraction coupling process (Phillips *et al* 1990), PESP is therefore equated to the presence of an extra "bolus" of activator Ca^{2+} made available by the extrasystole (Seed *et al* 1984), supported by peak (L_1) Ca^{2+} transients to PESP curves paralleling the contractile response (Wier and Yue 1986; Gwathmey *et al* 1990).

In human ventricular myocardium, PESP results from increased availability of activator Ca^{2+}, arising predominantly from the sarcoplasmic reticulum (Gwathmey *et al* 1990). Activator Ca^{2+} entering during the extrasystole may recirculate back to the sarcoplasmic reticulum "release site", to produce potentiation of the postextrasystole (Bose *et al* 1988). As the extrasystole becomes less premature, the amount of Ca^{2+} released during the extrasystole increases, and the amount of Ca^{2+} that can then be potentially lost during the ensuing postextrasystole is reduced, reducing the degree of potentiation. The decline in potentiation of a fully restituted postextrasystole would therefore parallel the time course by which Ca^{2+} is made available for release for the extrasystole (Wier and Yue 1986).

In conclusion, the MRC can be quantitatively described in the intact human circulation. This description includes a new index of LV function, representing individual sensitivity of contractile performance to changes in cycle length. Addtionally, the extent of rate-related inotropic effects of cardioactive agents can potentially be determined and compared between different patients using this model. PESP, rapid atrial pacing, and eight beat short-cycle length MRC's can also be employed when investigating drug effects on the force-interval relationship.

CHAPTER 4 :

ACUTE MYOCARDIAL METOPROLOL UPTAKE : CORRELATION WITH ACUTE EFFECTS AND INFLUENCE OF HYPOXIA.

4.1 Backgrounds and aims

Metoprolol is a selective $ß_1$-adrenoceptor antagonist in frequent clinical use in the management of ischaemic and tachyarrhythmic syndromes in man. Although no information regarding its myocardial kinetics following intravenous bolus injection in humans is available, significant myocardial concentrations of metoprolol have been detected in animal studies (Bodin *et al* 1975; Ablad *et al* 1987; Ryden *et al* 1990, 1991; Hatori *et al* 1991). Conventional pharmacokinetic studies in healthy individuals describe an elimination phase half-life of 3-4h and apparent volume of distribution 3-6L/kg (Regardh *et al* 1974, 1975, 1980, 1981a, 1981b; Johnsson *et al* 1975; Williams *et al* 1976; Jordo *et al* 1980; Kendall *et al* 1977, 1980; Melander *et al* 1977; Jack *et al* 1982; Quarterman *et al* 1981; Briant *et al* 1983; Godbillon *et al* 1983). The predominant acute haemodynamic and ECG effects of the drug following intravenous bolus administration have been described as reduced resting heart rate and +dP/dt, and prolongation of PR intervals (Silke *et al* 1986; Reale *et al* 1979; Marchlinski *et al* 1984; Camm *et al* 1982; Edvardsson *et al* 1984; Davila *et al* 1990; Dell'Italia and Walsh 1989).

The aims of this chapter were :

(i) to specifically examine the process of acute myocardial metoprolol uptake following intravenous bolus administration in humans;

(ii) to investigate the influence of hypoxia on myocardial metoprolol uptake and efflux in an *in vitro* preparation;

(iii) to determine the acute haemodynamic and ECG effects of the drug in man;

(iv) to correlate myocardial content with these effects, in order to test the hypothesis: **"Myocardial metoprolol content is a direct determinant of acute effect"**;

(v) to examine the potential dependence of the negative inotropic effects of metoprolol on beat interval, utilizing the short-cycle-length component of the mechanical restitution curve (MRC) in humans *in vivo*, and the mathematical model described in Chapter 3 of this thesis, Determination of rate-related inotropic effects in humans

(vi) to examine the influence of metoprolol on post-extrasystolic potentiation (PESP), where the post-extrasystolic interval is equal to the baseline cycle length.

4.2 Methods

Nineteen patients undergoing diagnostic cardiac catheterization and coronary arteriography for the investigation of chest pain were selected. The research procedure commenced at the end of the routine cardiac catheterization. The protocol for the research procedure was essentially as described in section 2.1.1 of this thesis : Protocol for cardiac catheterization for determination of myocardial drug uptake and measurement of haemodynamic effects in humans, with a few minor modification - no EP parameters were examined; at the end of the research procedure additional blood samples were collected from a peripheral vein for determination of metoprolol concentrations; and the blood / plasma metoprolol concentration ratio was examined. The influence of a period of hypoxia on the myocardial uptake profile of metoprolol was investigated, as described in section 2.1.2 of this thesis : Protocol for isolated perfused rat hearts, which utilized a total of 38 rat hearts (including two controls).

Myocardial metoprolol uptake in this group of patients was determined, by determining the concentrations of metoprolol in whole blood from femoral artery and coronary sinus, as described in section 2.2.1 of this thesis : HPLC quantitation of metoprolol in human whole blood and rat heart homogenates. This assay was also utilized to determine metoprolol concentrations in femoral / peripheral vein whole blood, and rat LV myocardium following the perfusion protocol, and concentrations of 4-hydroxy-metoprolol in whole blood. Submaximal coronary vasodilator reserve and electropysiological effects were not determined.

4.3 Results

4.3.1 Patient characteristics

The clinical characteristics of the 19 patients selected for study, obtained in the routine cardiac catheterization, are summarized in Table 4.1. These patients were predominantly male near 50 years of age, with normal LV systolic function, some degree of haemodynamically significant coronary artery disease, and previous therapy with at least one form of antianginal therapy. However, 3 patients had a cardiac index below 2.5L/min, two had LV ejection fractions below 50%, and PCWP was elevated in one patient. Myocardial metoprolol uptake (MMU) was determined in 12 patients, and the potential for rate-related effects on inotropic state was explored in 15 patients in some form. Baseline pacing rate throughout the study was at a mean cycle length of 813±19msec. The procedure was well tolerated and no patient developed adverse effects due to metoprolol.

Of the 38 rats utilized in the isolated perfused heart studies, 2 were controls, and the remainder divided into groups of 6, examined under either normoxic or hypoxic conditions. Average dry rat heart weight was 1.53±0.03g.

TABLE 4.1. Patient characteristics prior to metoprolol administration

Pt	Age (yr)	Sex	CI (L/min/m^2)	HR (beats/min)	LV EF	PCWP %	MAP (mmHg)	CAD (mmHg)	Prior Therapy
1	56	M	2.52	73	55	7	93	CIRC	-
2	47	M	2.62	75	65	13	109	LAD, RCA	B, N
3	46	F	3.09	68	76	18	135	-	B, N
4	61	M	3.70	68	81	10	122	CIRC,RCA	N
5	55	M	3.57	51	63	11	121	LAD,CIRC	B
6	65	M	3.39	73	63	3	95	LAD	B, N
7	44	M	3.78	75	71	9	107	-	N
8	41	M	3.20	61	64	6	88	-	-
9	55	M	3.30	64	68	5	107	LAD,CIRC,RCA	C
10	40	M	3.20	75	73	10	87	LAD,CIRC	-
11	64	M	2.78	71	61	11	93	-	-
12	55	M	2.20	61	62	12	117	-	B
13	67	F	2.35	65	38	9	94	LAD	C, N
14	49	F	2.78	80	59	3	115	-	B, N
15	60	M	2.55	57	63	6	92	LAD,CIRC,RCA	B, N
16	54	M	2.69	54	64	4	89	LAD,CIRC,RCA	C
17	60	F	2.61	87	78	2	94	-	-
18	59	M	1.73	69	52	3	83	-	N
19	62	M	2.72	75	45	14	90	LAD,CIRC,RCA	B, C, N
Mean	55		2.88	69	63	8	102		
SE	2		0.12	2	2	1	3		

B, ß-adrenoceptor antagonist; C, calcium channel antagonist; CAD, fixed coronary artery disease (>50% stenosis); CI, cardiac index; CIRC, left circumflex coronary artery; EF, ejection fraction; F, female; HR, heart rate; LAD, left anterior descending coronary artery; LV, left ventricle; M, male; MAP, mean arterial pressure; N, nitrate; PCWP, pulmonary capilary wedge pressure; Pt, patient; RCA, right coronary artery

4.3.2 Acute haemodynamic effects of metoprolol in humans

Various haemodynamic indices were monitored following metoprolol injection in these patients, and their effects are summarized in Table 4.2. Figures 4.1 through 4.4 illustrate the time course of these effects. Metoprolol effects on LV systolic (LV SBP) and end-diastolic (LV EDP) pressures, and on mean arterial pressure (MAP) are portrayed in Figure 4.1. Only LV EDP was significantly reduced by ß-adrenoceptor administration, 20mins post metoprolol injection ($p<0.05$). The time course of both cardiac index (CI) and coronary sinus blood flow (CSF) following metoprolol administration is illustrated in Figure 4.2. Metoprolol failed to influence either parameter in the present investigation. Consequently, there was no trend for changes in either systemic or coronary vascular resistance, illustrated in Figure 4.3. Conversely, as depicted in Figure 4.4, ß-adrenoceptor antagonism exerted significant marked reductions in both spontaneous heart rate (HR) and LV+dP/dt (at fixed heart rate). Maximal negative chronotropic and inotropic effects were observed 7.32±1.27 and 12.3±1.4mins after metoprolol administration respectively.

4.3.3 Acute electrocardiographic effects of metoprolol in humans

Both PR and QT intervals were serially measured during the study, at both constant and spontaneous heart rate. These results are also summarized in Table 4.2. As portrayed in Figure 4.5, metoprolol induced significant prolongation of PR intervals at constant heart rate, maximal 7.00±1.40min after administration ($p<0.0001$), without changing QT interval duration. The influence of ß-adrenoceptor antagonist administration on ECG intervals at spontaneous heart rate are illustrated in Figure 4.6. No significant effect of metoprolol on either interval was observed, although a nonsignificant trend for PR interval prolongation at spontaneous HR was observed ($p=0.08$).

TABLE 4.2. Haemodynamic and ECG effects of metoprolol in humans

Parameter (mean±SE)	Baseline value	Maximum change after metoprolol	*p* value	Time (mins) of maximal effect
MAP (mmHg)	102±3	+2±2	NS	9.86±1.44
CI (L/min/m^2)	2.79±0.18	+0.03±0.16	NS	13.3±1.5
SVR (dynes.s.cm^{-5})	1630±100	+60±100	NS	12.3±1.5
LV+dP/dt ($mmHgs^{-1}$)	1530±50	-250±40	0.0001	12.3±1.4
LV SBP (mmHg)	148±6	+4±6	NS	11.4±1.6
LV EDP (mmHg)	20±2	+2±3	0.0405	11.3±1.6
CS flow (ml/min)	138±25	+9±15	NS	10.1±1.7
CVR (mmHg/ml/min)	0.95±0.14	+0.13±0.19	NS	11.6±1.7
Spontaneous HR (beats/min)	68.7±2.3	-8.5±0.9	0.0002	7.32±1.27
Paced PR interval (msec)	182±6	+18±5	0.0001	7.00±1.40
Paced QT interval (msec)	364±9	+7±3	NS	6.50±1.66
Spontaneous PR interval (msec)	198±8	+10±5	NS	8.56±1.41
Spontaneous QT interval (msec)	369±8	-19±24	NS	5.79±1.27

CI, cardiac index; CS, coronary sinus; CVR, coronary vascular resistance; HR, heart rate; LV+dP/dt, peak rate of rise of left ventricular pressure; LV EDP, left ventricular end-diastolic pressure; LV SBP, left ventricular systolic blood pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance

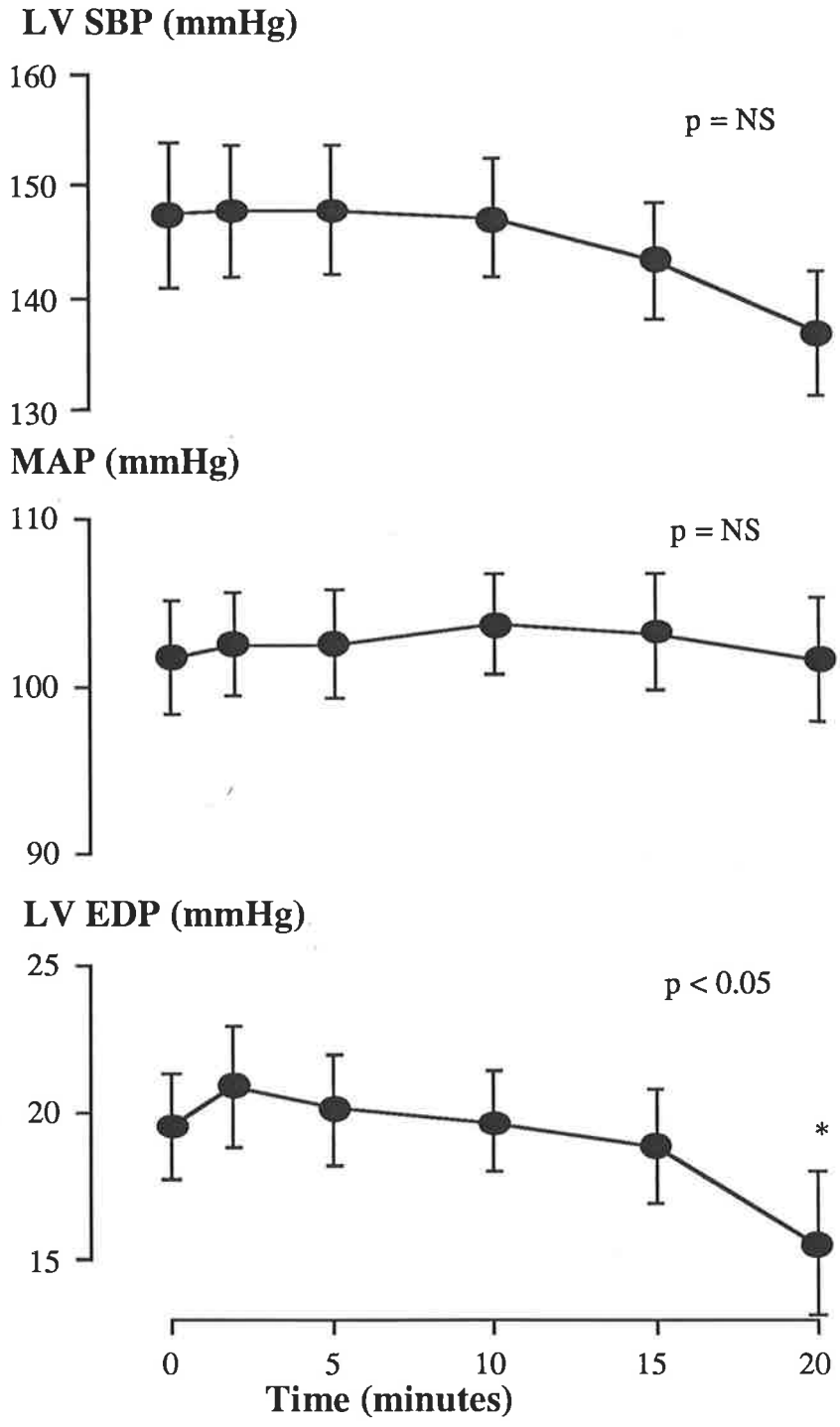


FIGURE 4.1 Time course of metoprolol effects on left ventricular systolic (LV SBP), mean arterial (MAP) and left ventricular end-diastolic (LV EDP) pressures in patients

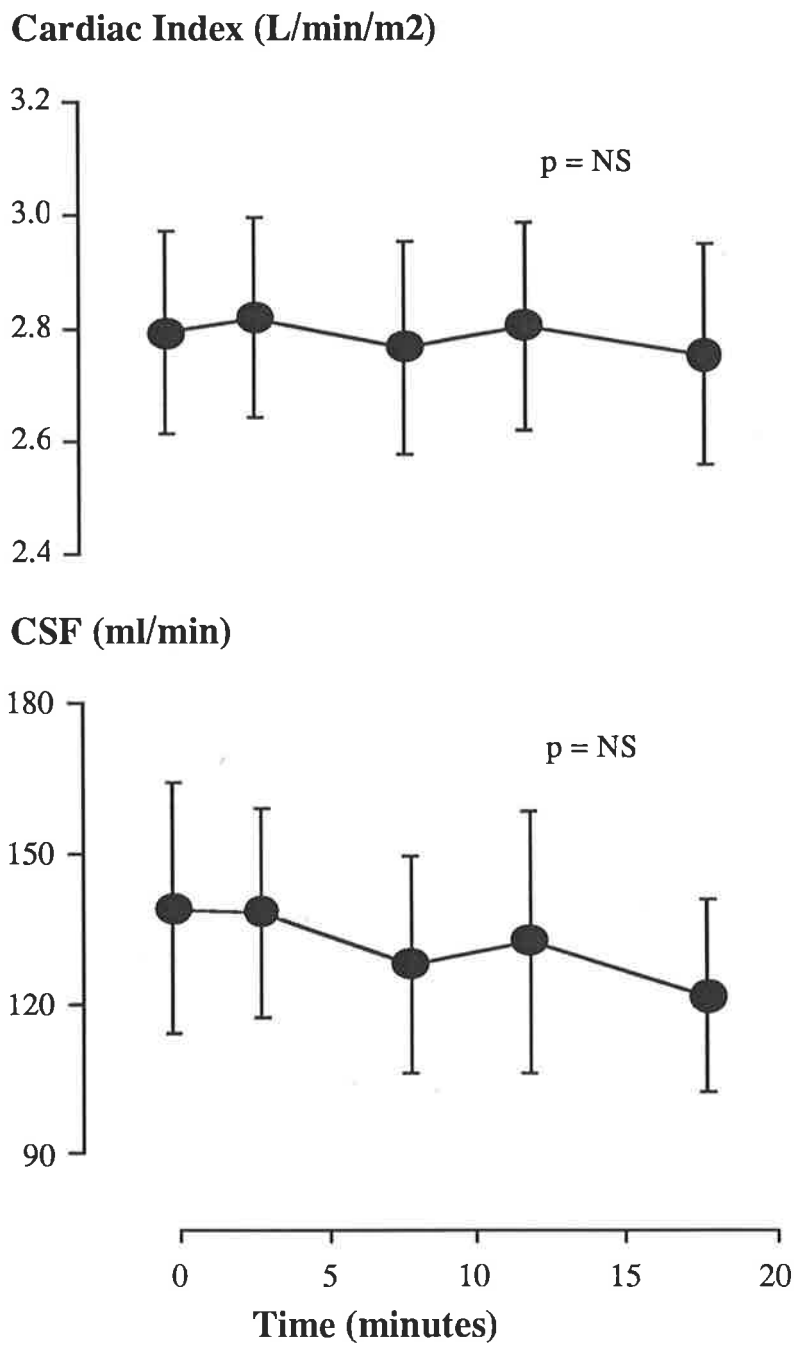


FIGURE 4.2 Time course of changes in cardiac index and coronary sinus flow (CSF) in humans for 20 minutes after metoprolol injection

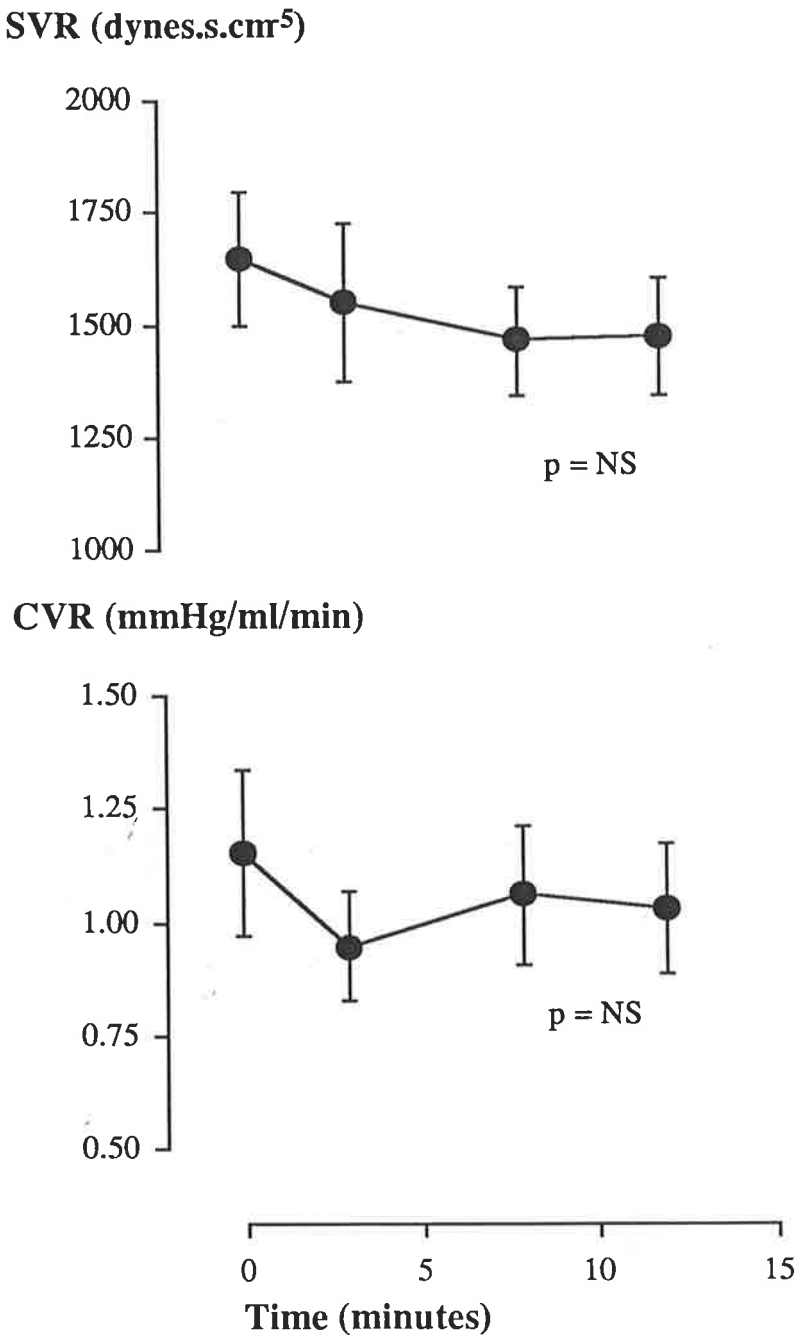


FIGURE 4.3 Time course of changes in systemic (SVR) and coronary (CVR) vascular resistances in patients for 20 minutes after metoprolol injection

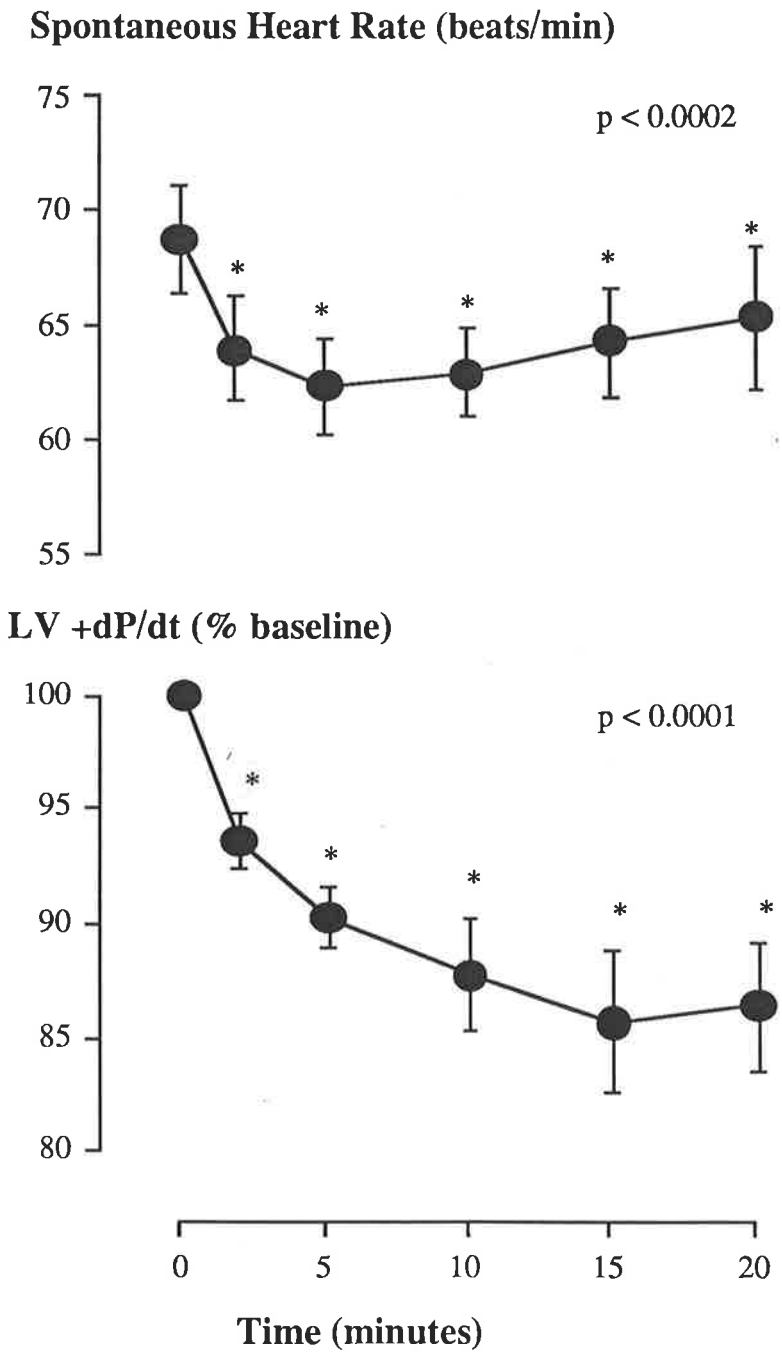


FIGURE 4.4 Time course of changes in spontaneous heart rate and peak rate of rise of LV pressure (LV +dP/dt) in humans 20min after metoprolol injection

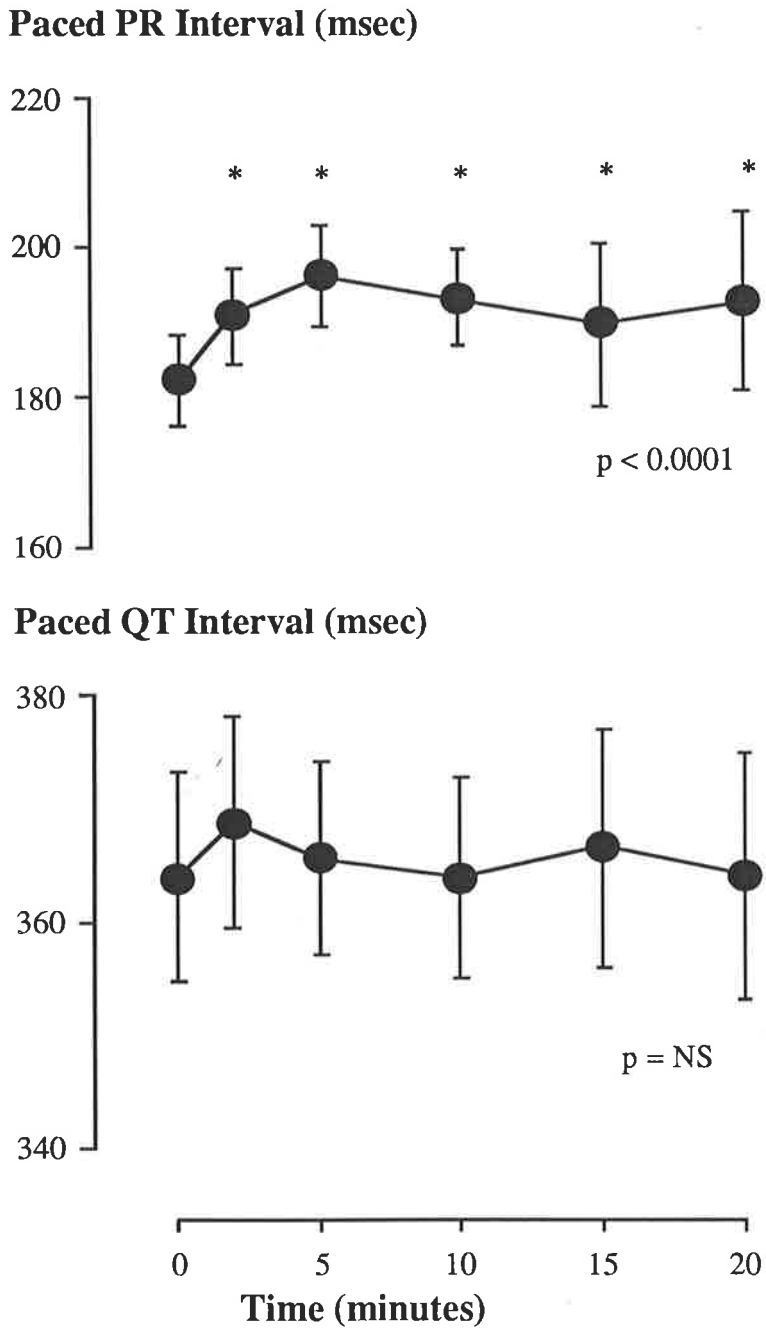


FIGURE 4.5 Time course of changes in ECG intervals at fixed heart rate in patients for 20mins after metoprolol injection

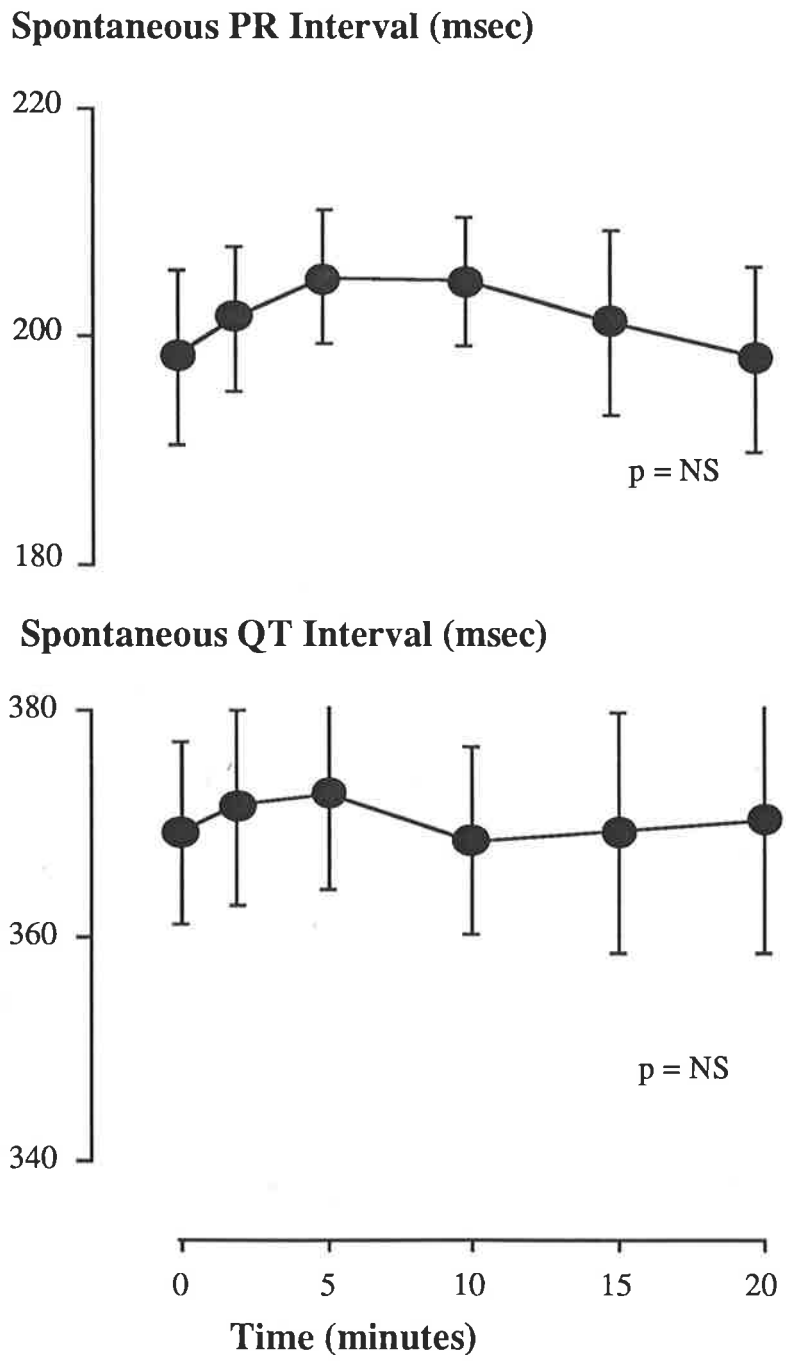


FIGURE 4.6 Time course of changes in ECG intervals at spontaneous HR in humans for 20mins after metoprolol injection

4.3.4 Validity of utilizing femoral arterial metoprolol concentrations as a surrogate for those in the aorta

The validity of utilizing femoral arterial metoprolol concentrations as a surrogate for those in the aorta was assessed in one patient following intravenous bolus administration. As demonstrated in Figure 4.7, whole blood metoprolol concentrations in the femoral artery and the aorta did not differ significantly (mean difference 2.75±0.86%, p=NS).

4.3.5 Acute myocardial metoprolol uptake in humans

The time course of metoprolol concentrations in femoral artery (FA) and coronary sinus (CS) whole blood are illustrated in the upper panel of Figure 4.8. Metoprolol concentrations in FA were 1.25±0.15μg/ml at 0.29±0.09mins after injection, before rapidly declining to 26.5±3.8ng/ml after 20.1±0.1mins. Conversely, initial CS metoprolol concentrations of 69.2±9.8ng/ml at 0.57±0.02mins, increased to a peak of 81.9±10.9ng/ml at 2.00±0.04mins, before also decreasing to 37.2±4.6ng/ml at 20.2±0.1mins.

The time course of myocardial metoprolol content (MMC) is illustrated in the lower panel of Figure 4.8. Rapid myocardial metoprolol uptake (MMU) initially was a consequence of the initially large metoprolol concentration gradient between FA and CS. The point in time at which whole blood metoprolol concentrations in FA and CS were equal, corresponding to the timepoint of maximal MMC, was achieved 2.71±0.36mins post injection. This represented 578±74ng per ml/min (when corrected for resting CSF), 1.89±0.40% of the total injected dose. Efflux of metoprolol from the human myocardium then proceeded, as indicated by the negative transcoronary concentration gradient. Residual MMC at 17.5min was 323±79ng per ml/min, or 49.1±8.7% of maximal MMC.

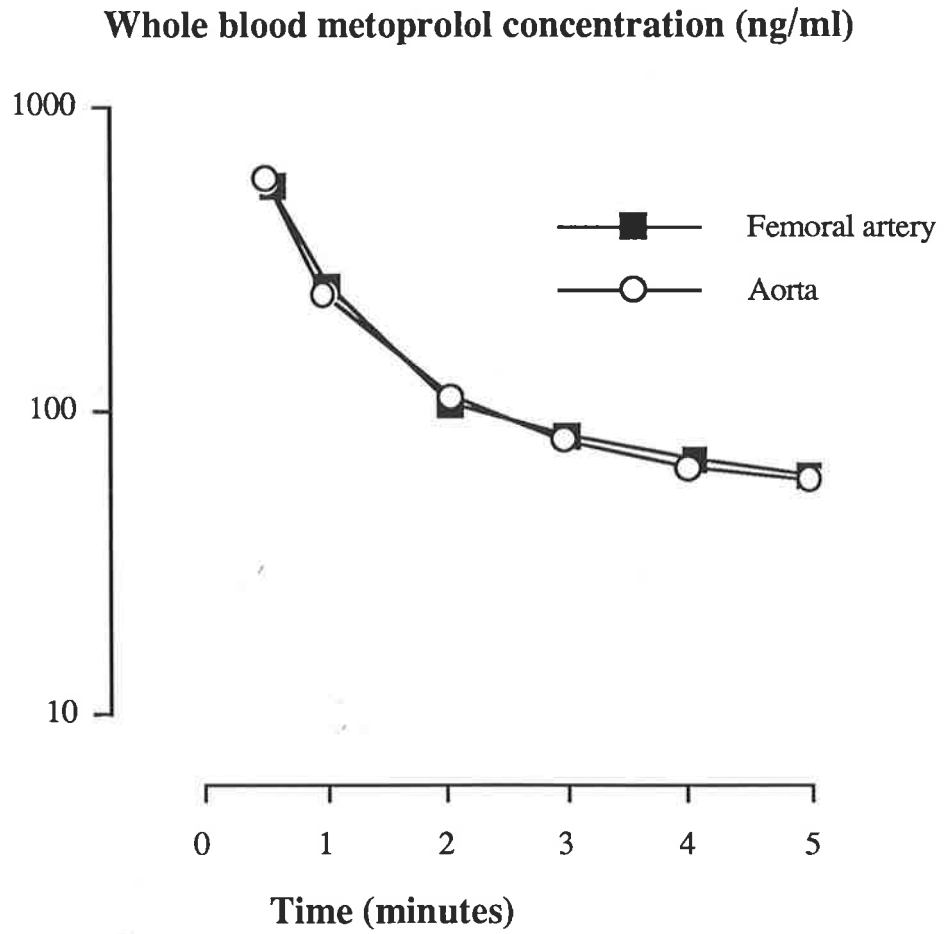


FIGURE 4.7 Time course of metoprolol concentrations in human femoral arterial and aortic whole blood in one patient

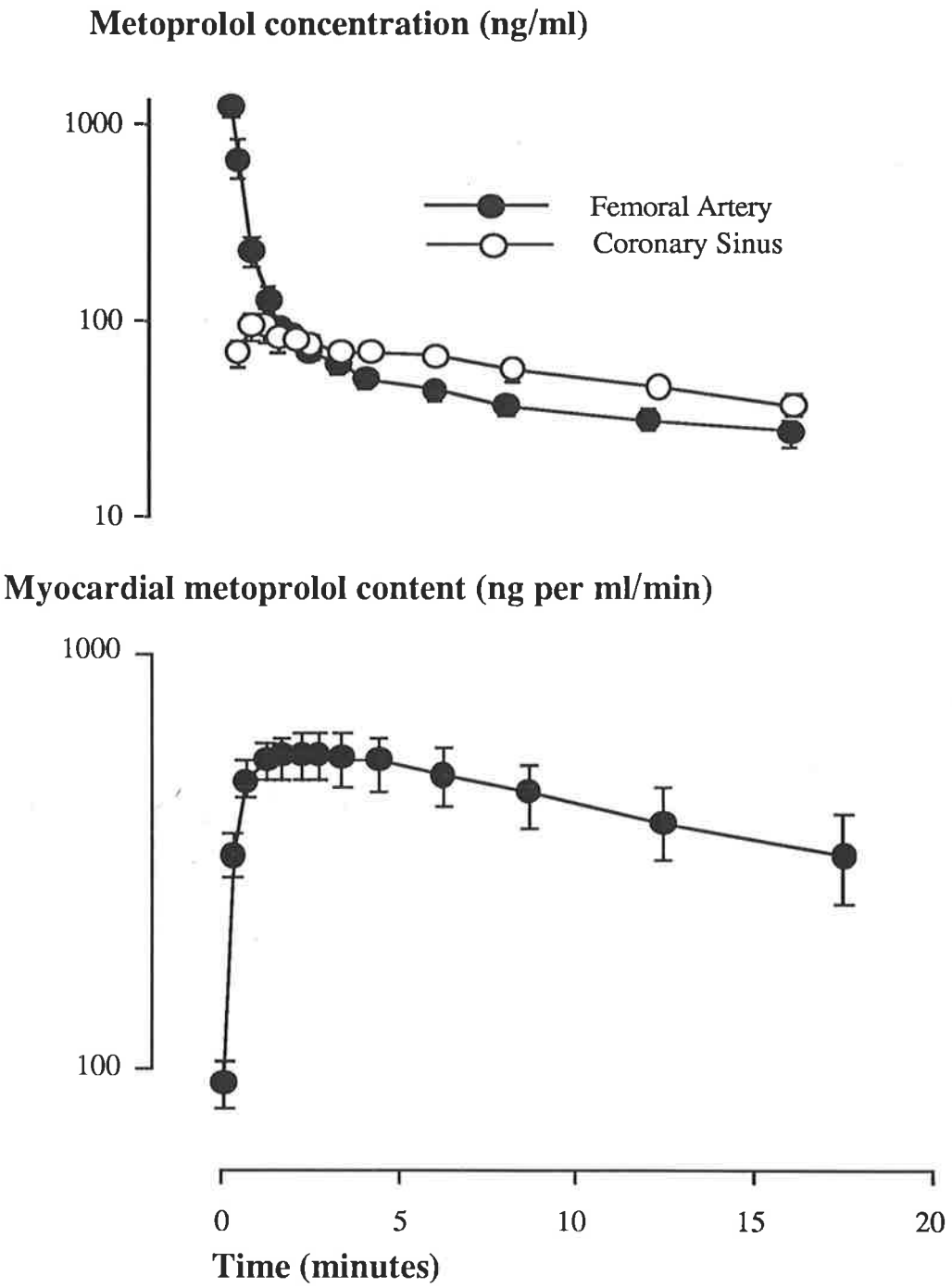


FIGURE 4.8 On the upper panel, the time course of changes in metoprolol concentrations in whole blood sampled from FA and CS 20 mins after injection, and on the lower panel, the time course of myocardial metoprolol uptake in humans, expressed relative to resting coronary sinus blood flow

As summarized in Table 4.3, the extent of maximal MMC was not predicted from patient characteristics at baseline, including resting CI, PCWP, or extent of left coronary artery disease. However, significantly delayed time of maximal MMC in extensive left coronary artery disease (involving left anterior descending and circumflex branches; $p<0.0025$) was seen.

4.3.6 Acute myocardial metoprolol uptake by isolated Langendorff-perfused rat hearts : influence of hypoxia

The time course of metoprolol uptake by both normoxic and hypoxic isolated perfused Langendorff rat hearts is illustrated in Figure 4.9. Under normoxic perfusion conditions, MMC was 3.83±1.03, 0.78±0.21, and 0.26±0.09μg/gprotein at 2, 5, and 10mins post bolus metoprolol injection, respectively. Conversely, when hypoxia was initiated 2mins prior to metoprolol, concentrations of 2.67±0.43, 0.68 ±0.15, and 1.00±0.14μg/gprotein respectively were observed. Only the 10min timepoints were significantly different ($p<0.001$). However, in some of the hypoxic hearts, the spontaneous beating activity had become very irregular.

4.3.7 Correlation between myocardial metoprolol content and acute effects in humans

As described in section 2.5 of this thesis, correlations were sought between :

(i) extent of maximum MMC and extent of maximum effects;

and (ii) the time course of MMC with that of the acute effects of the drug.

Although metoprolol induced significant negative inotropic and chronotropic effects and prolonged PR intervals at fixed heart rates, the maximum change in these parameters was not significantly related to peak MMC, as illustrated in Figure 4.10. The time course of these changes relative to simultaneous MMC is depicted in Figure 4.11. The relation between the ratio of LV+dP/dt reduction and MMC significantly increased with time ($p<0.0001$). This trend was also apparent for spontaneous HR reduction and paced PR interval prolongation, but failed to attain statistical significance ($p=0.09$ and $p=0.26$ respectively). An anticlockwise hysteresis loop was demonstrable for the relation between each of these parameters and MMC, illustrated in Figure 4.12.

TABLE 4.3. Comparison of the individual patient characteristics of myocardial metoprolol uptake with corresponding clinical characteristics at baseline

Pt	t_{max}	C_{max}	C_{max}%	CAD	Age	Sex	CI	HR	LV EF	PCWP	MAP
1	1.75	621	6.07	1	47	M	2.62	75	65	13	109
2	1.25	359	0.87	0	46	F	3.09	68	76	18	135
3	2.75	315	1.61	1	65	M	3.39	73	63	3	95
4	1.75	405	1.24	0	44	M	3.78	75	71	9	107
5	1.25	401	1.20	0	41	M	3.20	61	64	6	88
6	2.75	516	2.20	0	55	M	2.20	61	62	12	117
7	1.75	427	2.28	1	67	F	2.35	65	38	9	94
8	3.50	1190	1.55	0	49	F	2.78	80	59	3	115
9	4.50	660	1.45	2	60	M	2.55	57	63	6	92
10	4.50	887	1.62	2	54	M	2.69	54	64	4	89
11	2.25	713	1.46	0	59	M	1.73	69	52	3	83
12	4.50	436	1.09	2	62	M	2.72	75	45	14	90
Mean	2.71	578	1.89%		54		2.76	68	60%	8	101
SE	0.36	74	0.40%		2		0.16	2	3%	1	4

CAD, extent of fixed coronary artery disease (>50% stenosis) in major branches of the left coronary artery; CI, cardiac index (L/min/m^2); C_{max}, peak myocardial metoprolol content (ng per ml/min); C_{max}%, peak myocardial metoprolol content (% total injected dose); EF, ejection fraction (%); F, female; HR, heart rate (beats/min); LV, left ventricle; M, male; MAP, mean arterial pressure (mmHg); PCWP, mean pulmonary capillary wedge pressure (mmHg); Pt, patient; tmax, time of peak myocardial metoprolol content (mins)

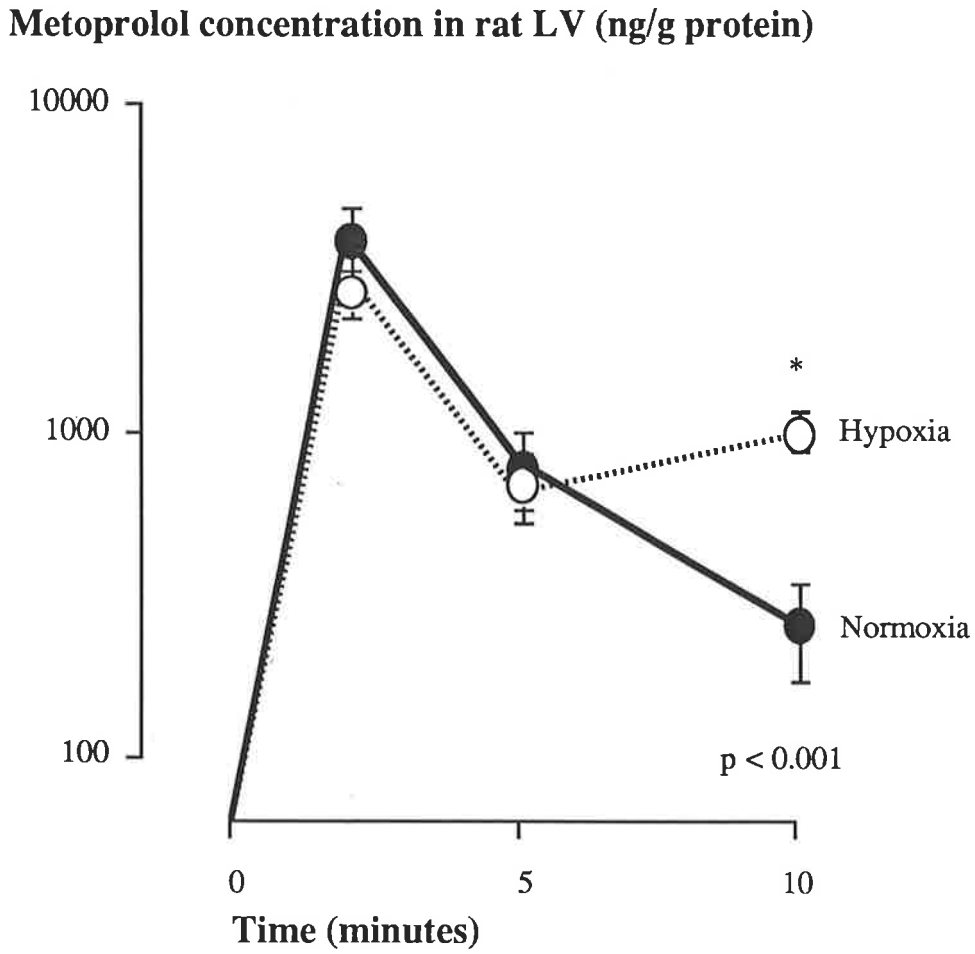


FIGURE 4.9 The time course of metoprolol uptake by both normoxic and hypoxic isolated perfused Langendorff rat hearts : each point represents n=6 hearts

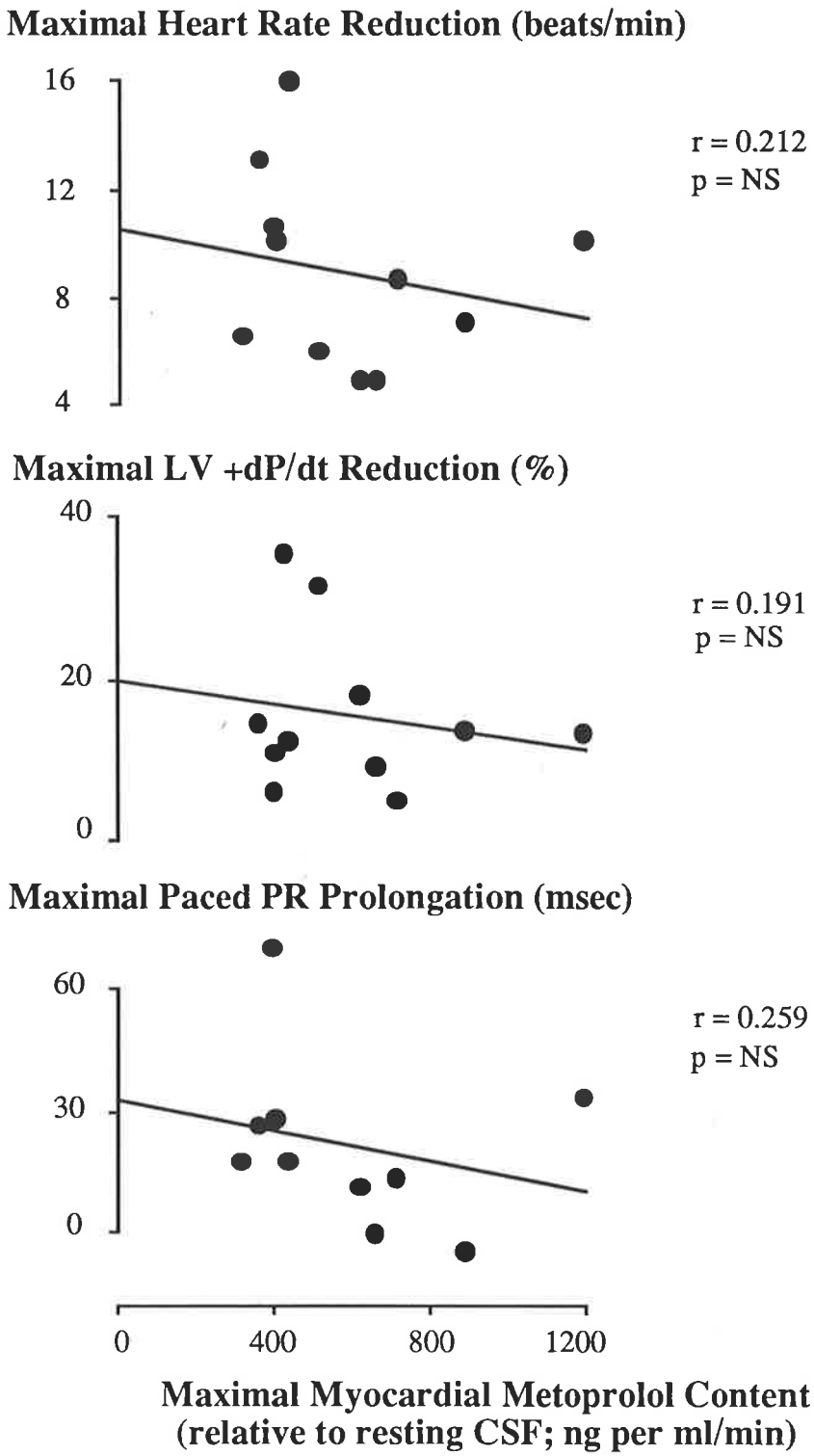


FIGURE 4.10 Individual maximal metoprolol effects on heart rate, LV+dP/dt and paced PR interval as a function of individual peak MMC (relative to resting CSF)

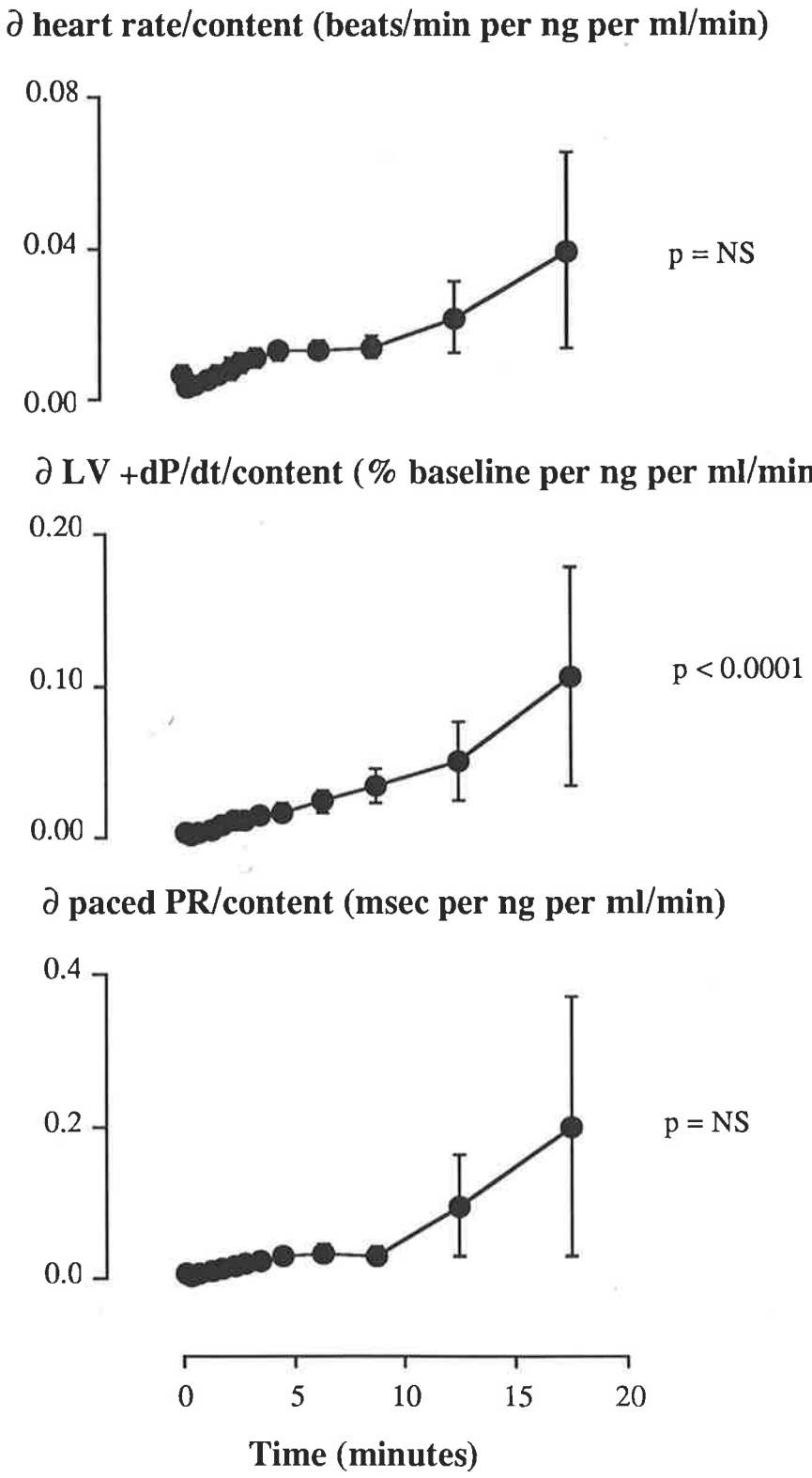


FIGURE 4.11 Time course of the ratio of effect and MMC (relative to resting CSF)

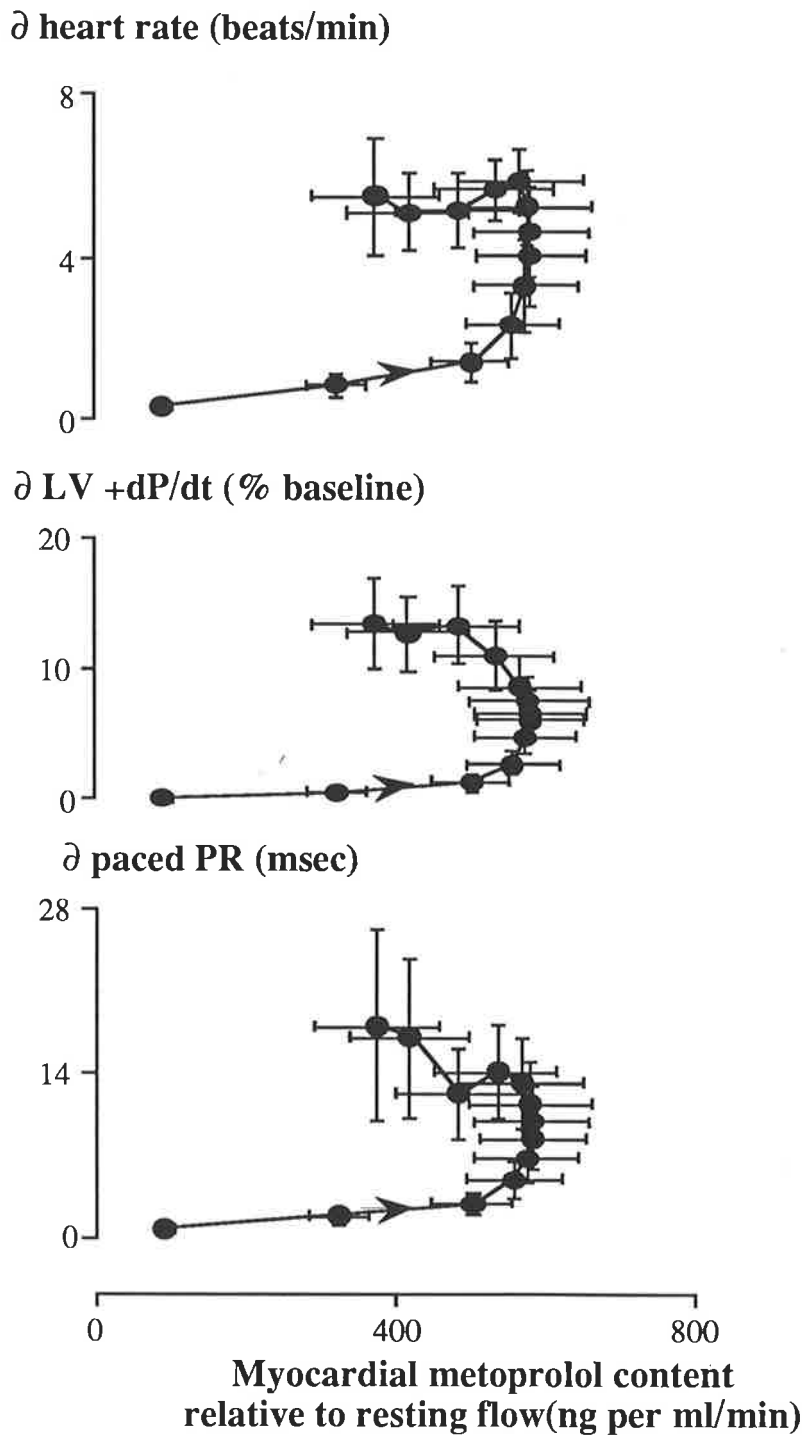


FIGURE 4.12 Relationship between metoprolol-induced changes in spontaneous HR, LV+dP/dt and paced PR interval with MMC (relative to resting CSF)

4.3.8 Pharmacokinetic - pharmacodynamic link models between myocardial metoprolol content and effect in humans

As described in section 2.6, pharmacokinetic / pharmacodynamic link models were employed to model the hysteresis between MMC and the three predominant significant effects of metoprolol: spontaneous HR reduction, reduction of LV+dP/dt, and paced PR interval prolongation. The estimates of the parameters of the myocardial uptake pharmacokinetic model for each of the 12 patients in whom MMU was determined (using the Bateman function) are listed in Table 4.4, and the estimates of the parameters of the pharmacokinetic / pharmacodynamic link models for each of the 3 effects are summarized in Table 4.5. The calculated dose received by the heart , D, was 644±73ng per ml/min, the absorption rate constant , Ka, was 1.85±0.13, and the arbitrary constant, K, was $6.11\pm1.61\times10^{-3}$. The relation between reduction in both spontaneous HR and LV+dP/dt with the theoretical effect-site metoprolol concentration (Ce) for every patient was linear, as it was for prolongation of the PR interval at fixed HR in all except 2 patients, one of whom had no change in this interval whatsoever. Typical results in one patient of this link model are illustrated in Figure 4.13, with reduction in LV+dP/dt as the example effect.

4.3.9 Metoprolol redistribution into other vascular beds in humans

The time course of metoprolol redistribution into vascular beds other than the myocardium in humans is represented by the profile of whole blood metoprolol concentrations in the FA and the femoral vein (FV) for 20mins following injection, portrayed in the upper panel of Figure 4.14. Metoprolol FV concentrations declined from 84.9±42.9ng/ml at 2.10±0.05mins to 21.7±2.0ng/ml at 20.2±0.1mins post administration. A consistent arteriovenous metoprolol concentration gradient in the femoral bed was apparent from 4min onwards, indicating longer metoprolol uptake by the lower limbs than by the myocardium in humans. The time course of metoprolol concentrations in a peripheral vein (PV) was also examined for up to 12h following intravenous bolus injection. As illustrated in the lower panel of Figure 4.14, PV metoprolol concentrations declined biexponentially to 4.05±0.98ng/ml 10.9±0.4h after injection.

TABLE 4.4 Estimates of the constants of the pharmacokinetic model of myocardial metoprolol uptake in humans, utilizing the Bateman function

Patient	D	Ka	K
1	469.9490	1.5486	0.0608
2	362.9834	1.5057	0.0512
3	667.6273	2.0271	0.0337
4	453.0481	2.3799	0.1885
5	494.7777	2.0451	0.1264
6	532.0118	2.3814	0.0156
7	558.5442	1.4100	0.1234
8	1249.7552	1.3871	0.0194
9	795.7428	1.0853	0.0521
10	927.8218	1.7960	0.0183
11	758.8573	2.0527	0.0267
12	455.4926	2.5727	0.0174
Mean	643.8843	1.8493	0.0611
SE	73.2184	0.1349	0.0161

D, calculated dose received by heart; Ka, absorption rate constant for myocardial metoprolol content; K, an arbitrary constant

TABLE 4.5 Estimates of the constants of pharmacokinetic-pharmacodynamic link model for myocardial metoprolol content and acute effects

Effect	Keo	S	r	slope	y int	p
Heart rate	1.30±0.61	0.0272±0.0086	0.874±0.031	66.7±15.2	66.9±21.9	0.00117±0.00075
Paced PR	0.30±0.08	0.109±0.053	0.748±0.132	20.3±6.8	-6.38±7.67	0.283±0.208
LV +dP/dt	0.29±0.15	0.087±0.032	0.911±0.033	26.0±7.8	21.8±12.9	0.00193±0.00191

K_{eo}, elimination rate constant from the effect-site for extrapolated ∂ effect versus time; S, slope factor of extrapolated ∂ effect versus time; slope, slope of extrapolated ∂ effect versus effect-site concentration; y int, y axis intercept of extrapolated ∂ effect versus effect-site concentration; r, regression coefficient of extrapolated ∂ effect versus effect-site concentration

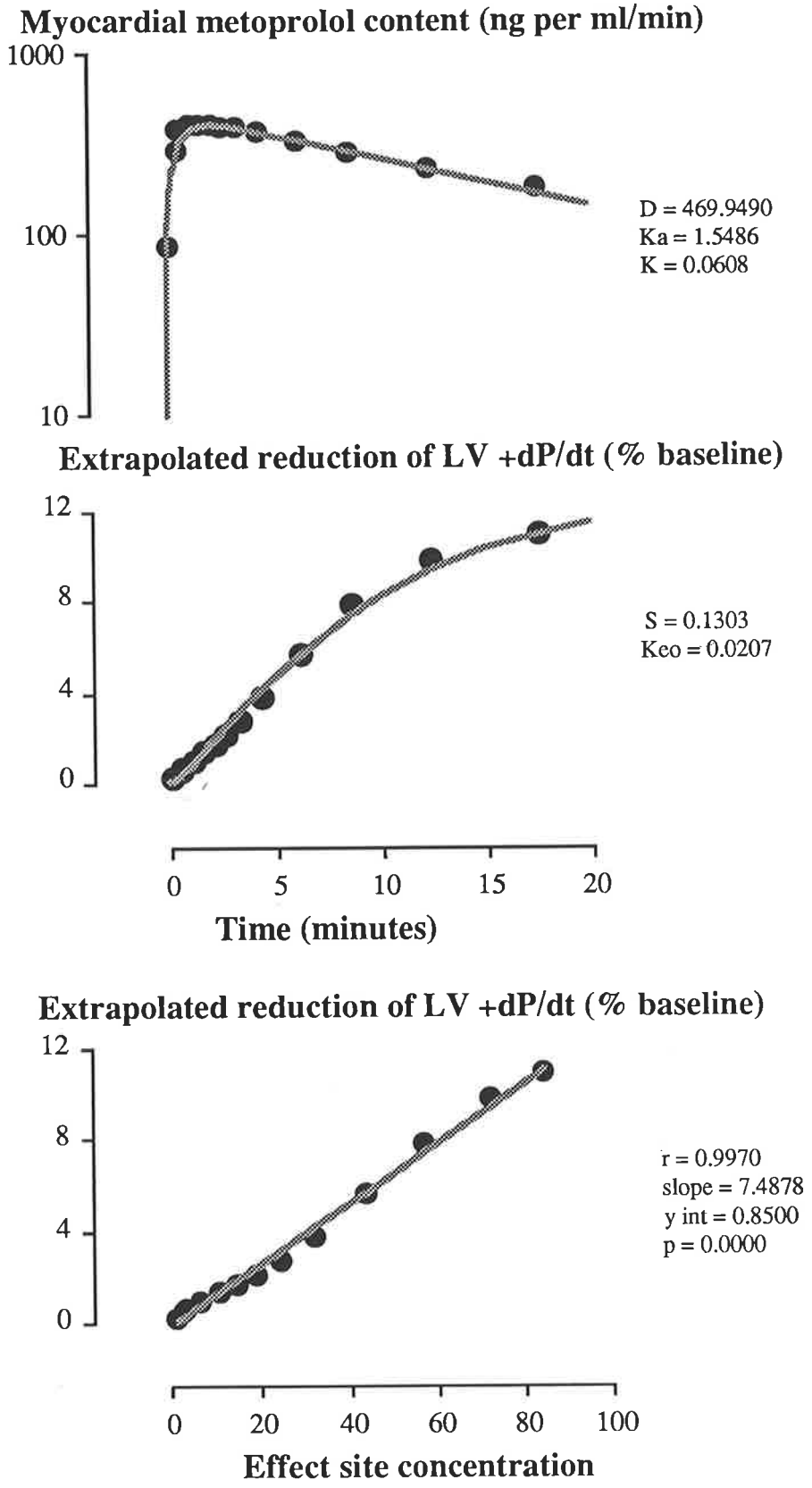


FIGURE 4.13 Typical results for pharmacokinetic/pharmacodynamic modelling for MMC and effects: MMC as a function of time; effect (LV+dP/dt reduction) as a function of time; and the link model for effect-site metoprolol concentration and effect

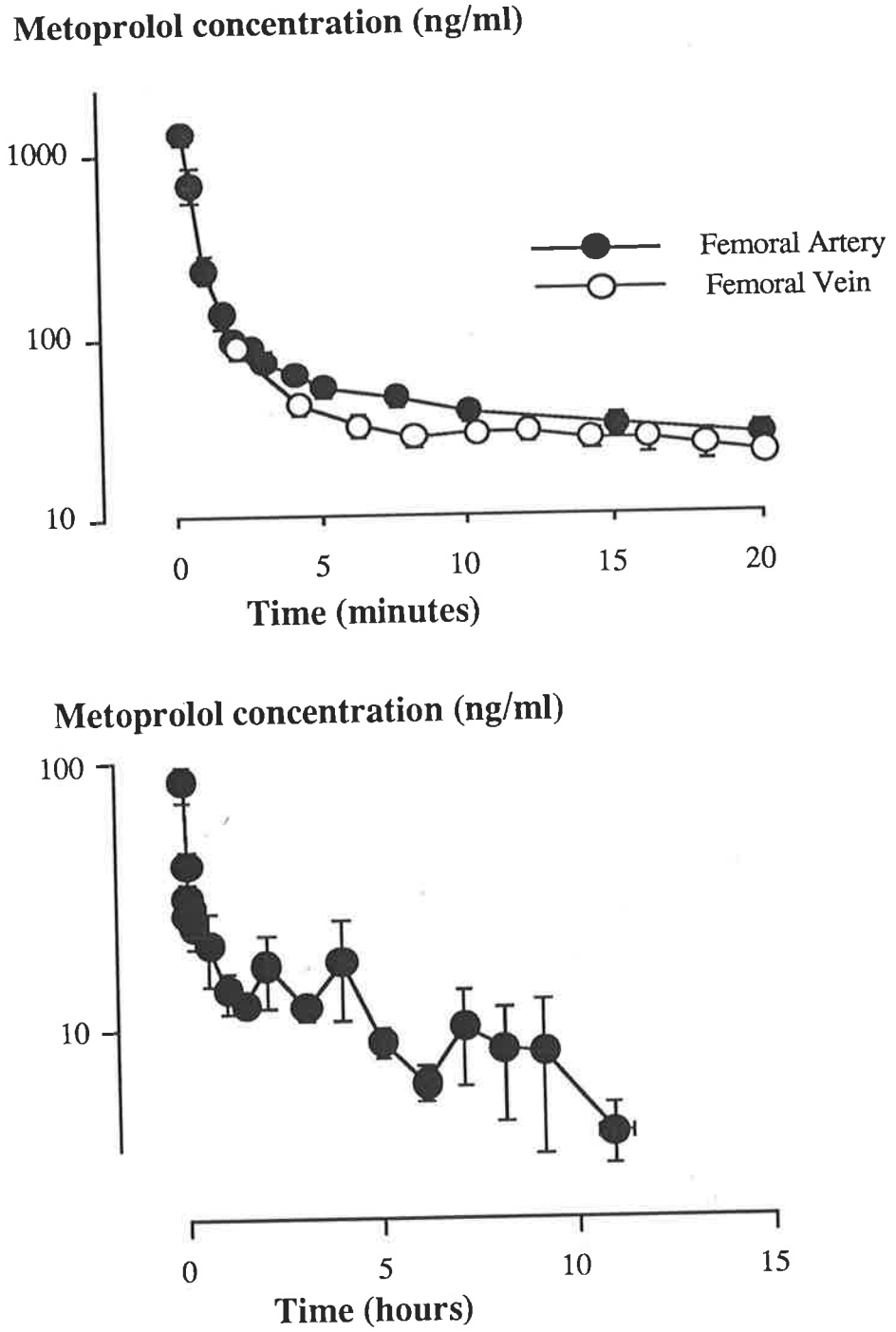


FIGURE 4.14 On the upper panel, the time course of the metoprolol concentration gradient across the femoral vascular bed in patients, and on the lower panel, the time course of metoprolol concentrations in peripheral vein whole blood up to 12h post injection in humans

4.3.10 Formation of active metoprolol metabolites in humans

The time course of 4-hydroxy-metoprolol (4-OH-M) concentrations in FV and PV over 20min and 12h respectively are shown in the upper and lower panels of Figure 4.15. Significant concentrations of 4-OH-M (>10ng/ml) were not apparent in whole blood at 20min. Peak PV 4-OH-M was 12±6ng/ml at 2.16±0.06h, before declining to 6±3ng/ml 10.9±0.4h after injection.

4.3.11 Concentration of metoprolol by red blood cells in humans

The ratio of metoprolol concentrations in whole blood : plasma was determined in 10 patients, shown in Table 4.6. Blood metoprolol concentrations were roughly 20-25% greater than plasma, emphasizing the necessity for use of whole blood rather than plasma for determination of MMU in man.

4.3.12 Serial mechanical restitution curve construction in humans.

Serial construction of the *short-cycle length single beat* mechanical restitution curve (MRC) was employed to investigate the potential for the negative inotropic effects of metoprolol, demonstrated in Figure 4.4, to be influenced by reductions in cycle length. Serial MRC are shown in Figure 4.16. Both cycle length and the contractile index are expressed as a percent of the baseline pre-metoprolol values. Prior to ß-adrenoceptor blockade, LV+dP/dt was progressively diminished from 100% to 59.3±5.6%, with progressive prematurity of the early beat from 100% to 57.2±1.7%. As demonstrated in Figure 4.4, at a cycle length of 100%, metoprolol induced a 11.7±2.7% reduction of LV+dP/dt 10min after injection. However, as the interval between beats was decreased, this reduction appeared to become progressively less marked, to the extent that it may have been completely abolished when the cycle length of the early beat was reduced by 40% (LV+dP/dt was 61.3±4.8% at an RR interval of 56.8±1.8% of baseline).

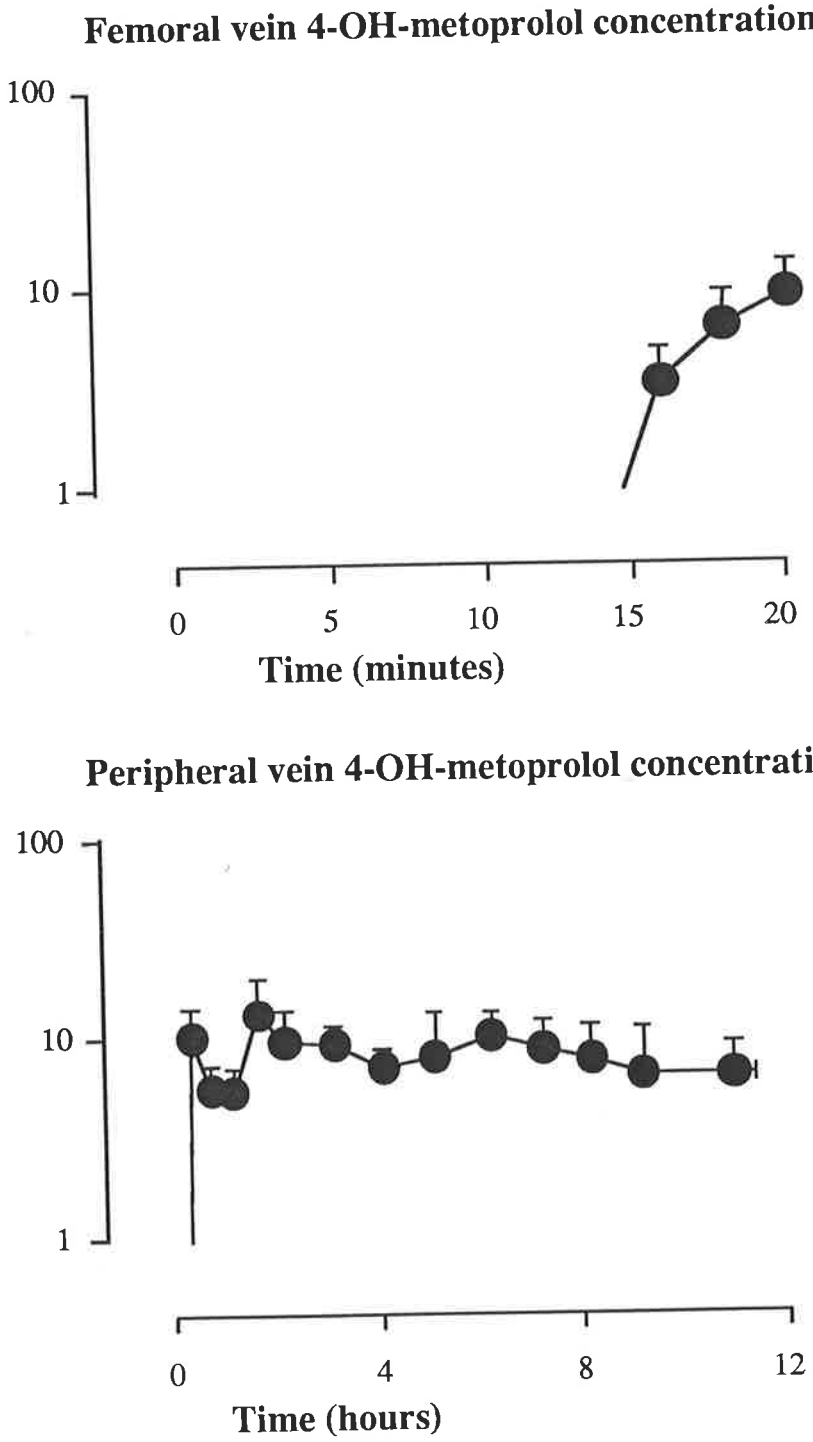


FIGURE 4.15 Time course of 4-hydroxy-metoprolol concentrations in FV and PV whole blood in patients up to 20min and 12h, on the upper and lower panels respectively

TABLE 4.6 Ratio of metoprolol concentrations in whole blood and plasma sampled from FA and CS in humans

Patient	Femoral artery	Coronary sinus
1	1.32±0.03	1.34±0.04
2	1.24±0.11	1.23±0.05
3	1.06±0.01	NA
4	1.51±0.11	NA
5	1.15±0.03	1.13±0.02
6	1.27±0.06	1.20±0.03
7	1.13±0.04	1.08±0.02
8	1.43±0.13	NA
9	1.37±0.03	1.38±0.02
10	1.20±0.07	1.25±0.10

NA, not available because of small coronary sinus sample size

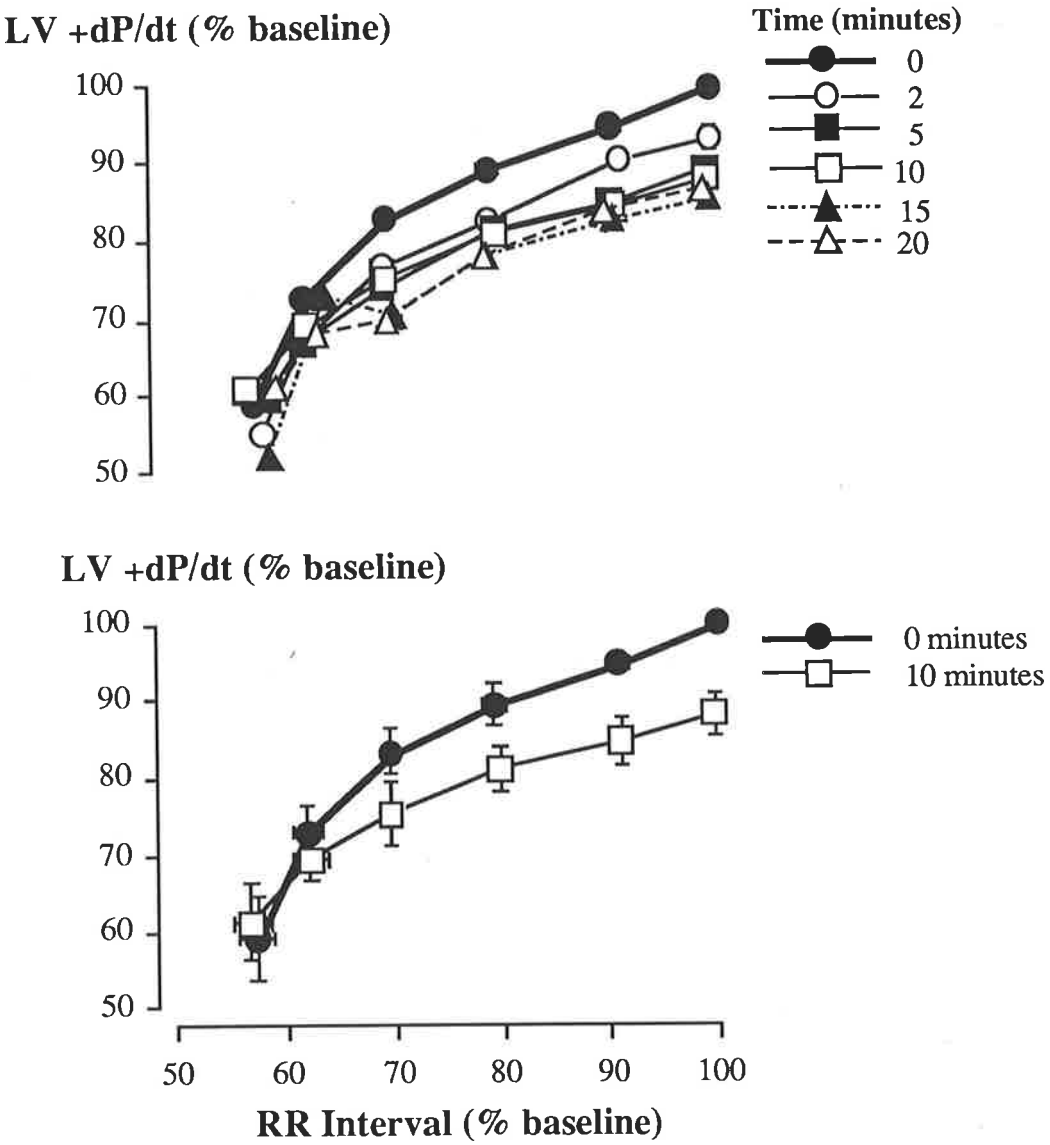


FIGURE 4.16 On the upper panel, serial MRC obtained at all time points in patients prior to and after injection, and on the lower panel, the pre- and 10mins post-metoprolol MRC : for ease of presentation, SE's not shown on upper panel

4.3.13 Post-extrasystolic potentiation without a compensatory pause in humans

Examination of the degree of potentiation of the beat following the extrasystolic interval at each point of the MRC was also investigated before and up to 20min following metoprolol administration. Termed post-extrasystolic potentiation (PESP), this phenomenon was examined utilizing a post-extrasystolic interval of 100%, ie in the absence of a compensatory pause. Despite the lack of this pause, the post-extrasystole was still potentiated. As portrayed in Figure 4.17, LV+dP/dt was progressively augmented from 6.2±1.5% to 21.9±4.5% above baseline, as the extrasystolic interval was decreased from 90.5±0.9% to 57.4±1.9% of baseline cycle length, prior to drug administration. As represented in the lower panel of Figure 4.17, 10min after metoprolol administration, this relation between extrasystolic interval and potentiation of the postextrasystolic beat did not appear to have altered, with a similar augmentation of LV+dP/dt from 4.3±0.9% to19.0±3.8% as the extrasystolic interval was decreased from 90.9±0.9% to 56.8±1.8% of baseline cycle length.

4.3.14 Application of the curve-fitting model to mechanical restitution curves obtained post-metoprolol administration in humans

Values of c and the rate-dependence index (RDI; the ratio of c values obtained after and before metoprolol administration) were determined utilizing the curve-fitting model of the MRC, described in Chapter 3 of this thesis, to determine the influence of extrasystolic interval on the negative inotropic effects of metoprolol. The time course of changes in these two parameters is illustrated in Figure 4.18. In 2 patients, the MRC model provided negative values for c , and was therefore not apprpriate (because the lowest extrasystolic interval obtainable in these patients was greater than 60% of baseline cycle length). In the remaining 13 patients, c was significantly reduced from 28.4±4.0% to 21.9±4.9% 20min after administration ($p<0.0002$). Consequently, RDI decreased from 1 to 0.683±0.079 over the same time period ($p<0.002$).

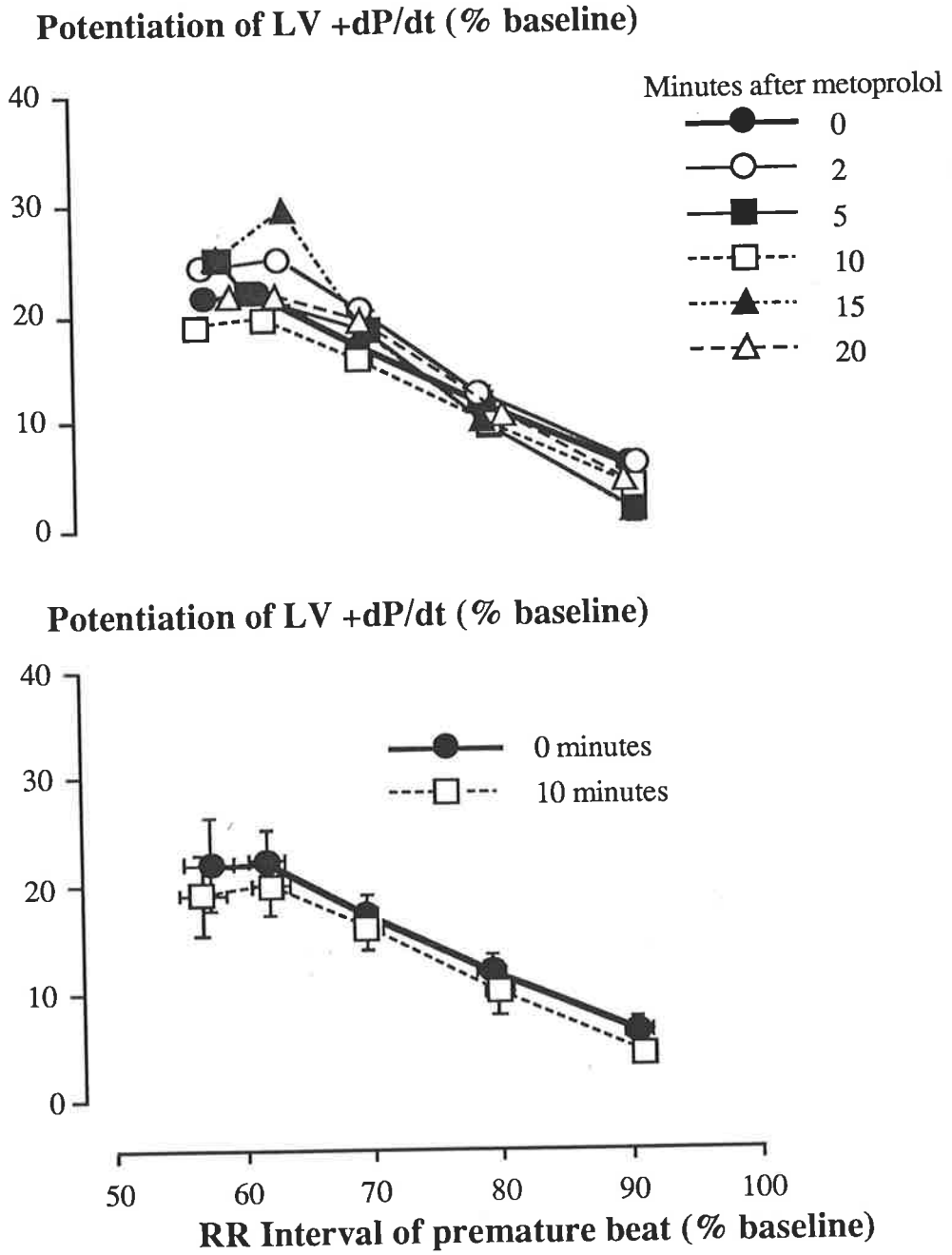


FIGURE 4.17 On the upper panel, the PESP curves obtained at all time points prior to and after injection in man, and the lower panel, the pre- and 10mins post-metoprolol curves : for ease of presentation, SE's not shown on upper panel

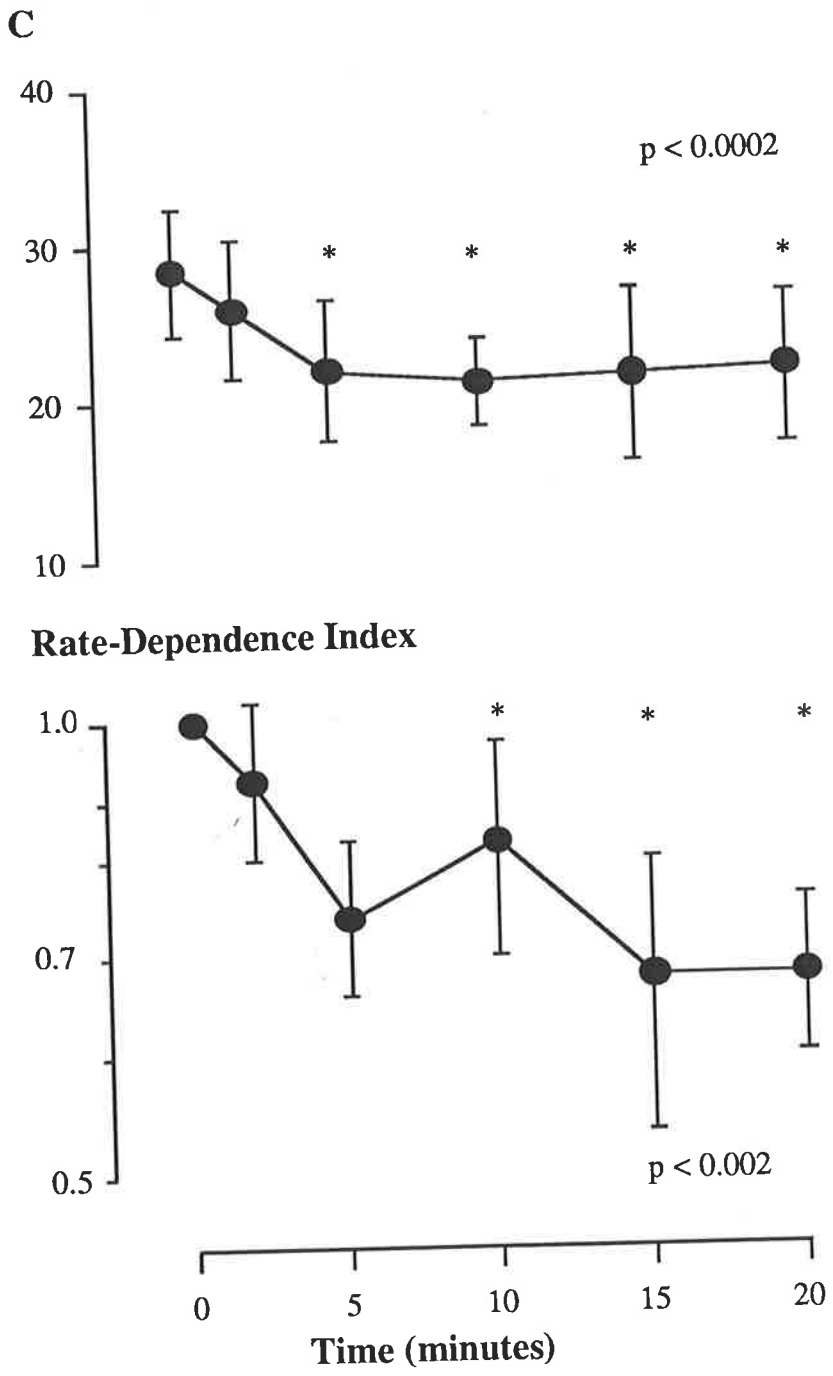


FIGURE 4.18 Time course of changes in c and the rate-dependence index (RDI) after metoprolol injection in patients

Thus, the pre-and post-metoprolol MRC tended to significantly converge towards the left hand-side, demonstrating attenuation of negative inotropic effects of the drug with decreasing cycle length in this model, ie a reverse use-dependent effect. As represented in Table 4.7, metoprolol altered neither the horizontal or the vertical asymptotes, a and d respectively, up to 20min post administration. Residual SD's of the model were 4.67 (95%CI 3.57, 5.97), the measure of goodness-of-fit of the model.

4.3.15 Examination of hysteresis between myocardial metoprolol content and the rate-dependence index following metoprolol administration in humans

Having established a significant metoprolol-induced reduction of the RDI, the potential for a delay between MMC and RDI reduction was examined in the 6 patients in whom both MMU and MRC curve-fitting was investigated. As represented in Figure 4.19, there was no correlation between peak MMC and peak RDI reduction, there was a significant increase in RDI reduction relative to simultaneous MMC up to 20min after administration ($p<0.0001$), and the relation between RDI reduction and MMC revealed an anticlockwise hysteresis loop. Thus, as for the other significant effects of metoprolol, the time course of the reverse use-dependence of the negative inotropic effects of metoprolol was also not parallel with MMC.

Pharmacokinetic / pharmacodynamic link models were employed to model the hysteresis between MMC and rate-dependence index (RDI). The relation between reduction in RDI with the theoretical effect-site metoprolol concentration (Ce) for every patient was also linear. The parameters of this link model are summarized for these 6 patients in Table 4.8, and typical results in one patient are portrayed in Figure 4.19.

TABLE 4.7 Time course of the asymptotes of the MRC curve-fitting model in patients

Minutes post metoprolol	0	2	5	10	15	20	p
a	127±8	148±25	111±7	122±18	134±40	107±9	NS
d	30±6	-8±31	37±5	18±17	17±31	26±12	NS

a , horizontal asymptote; *d* , vertical asymptote

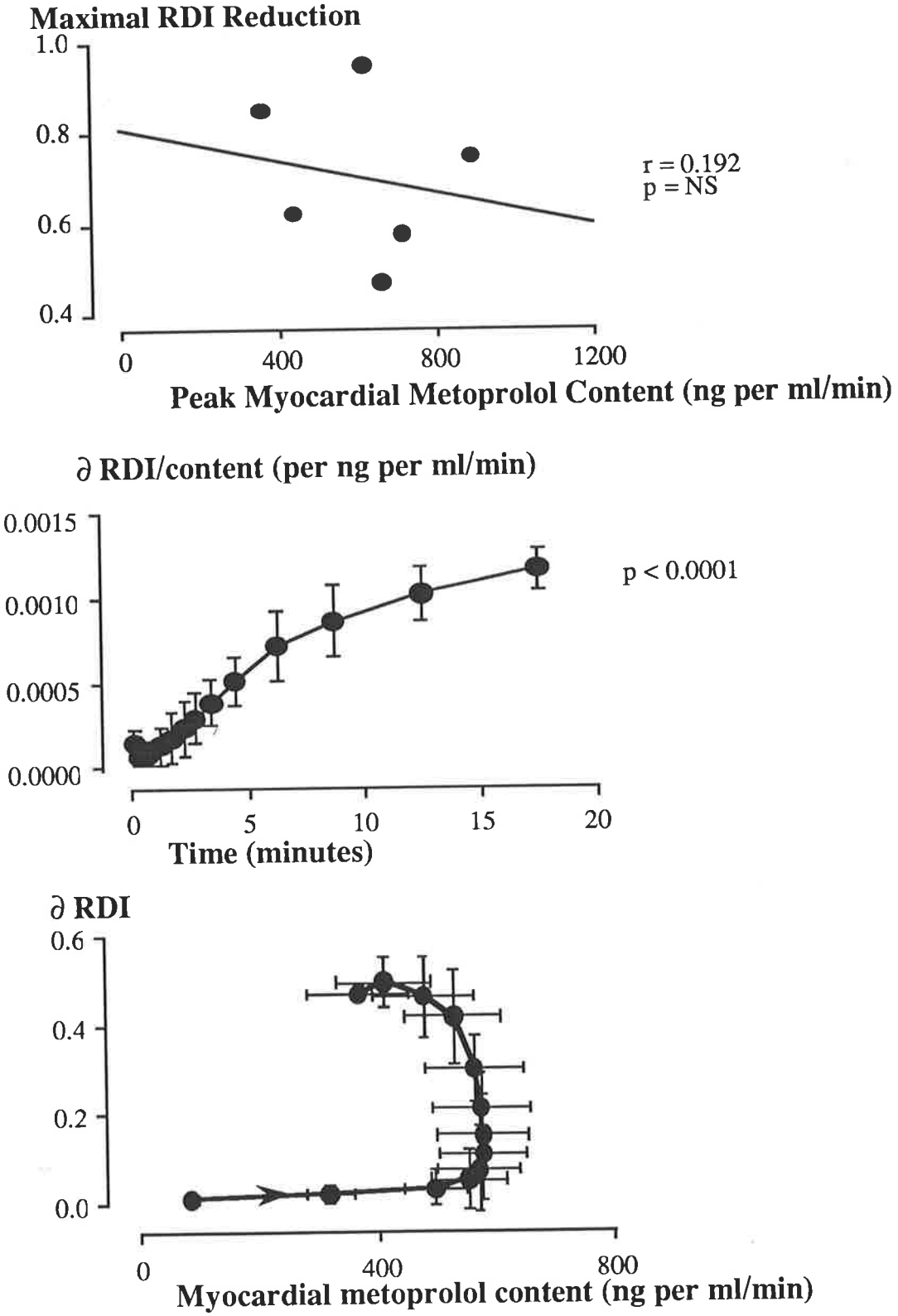


FIGURE 4.19 The relation between peak content and peak RDI reduction, the time course of the ratio of RDI reduction and MMC, and anticlockwise hysteresis loop for the relation between MMC and RDI reduction in humans

TABLE 4.8 Estimates of the constants of the pharmacokinetic-pharmacodynamic link model between myocardial content and reduction in the rate-dependence index

Patient	Keo	S	r	slope	y int	p
1	0.30832	0.001292	0.9558	681.4741	37.7713	0.00000
2	1.44302	-0.001444	-0.4473	-187.2854	125.5167	0.10877
3	0.02081	0.002332	0.9771	425.1108	-4.1701	0.00000
4	0.02924	0.001675	0.8885	292.5113	73.8982	0.00002
5	0.15534	0.001044	0.9951	944.3903	2.3216	0.00000
6	0.22067	0.001309	0.9426	794.8186	-20.8289	0.00000
Mean	0.36290	0.001035	0.7186	491.8366	35.7515	0.01813
SE	0.22070	0.000528	0.2337	167.1282	22.6787	0.01813

K_{eo}, elimination rate constant from the effect-site for extrapolated ∂ effect versus time; r, regression coefficient of extrapolated ∂ effect versus effect-site concentration; S, slope factor of extrapolated ∂ effect versus time; slope, slope of extrapolated ∂ effect versus effect-site concentration; y int, y axis intercept of extrapolated ∂ effect versus effect-site concentration

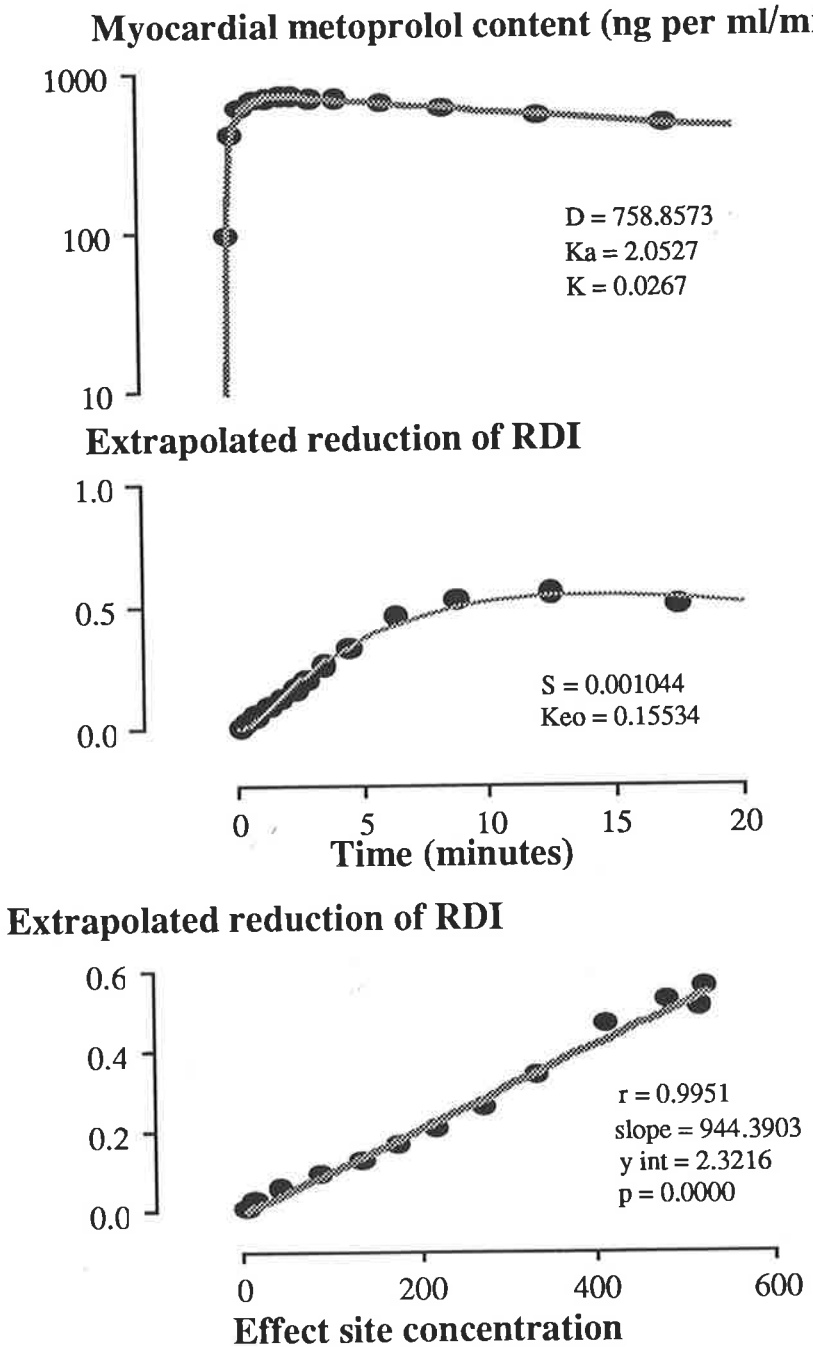


FIGURE 4.20 Typical results in one patient of pharmacokinetic / pharmacodynamic modelling for MMC and RDI reduction: MMC as a function of time; RDI reduction as a function of time; and the link model

4.3.16 Rapid atrial pacing before and after metoprolol injection

An additional method of examining the force-interval relationship following ß-adrenoceptor antagonist administration was investigation of the effect of 1min of pacing-induced tachycardia on LV+dP/dt before and after injection. In 10 patients suffering mild angina only, this was studied before and 10-20min post injection. Heart rate was increased from 77±3 to 107±2beats/min, by reducing pacing cycle length from 800±41 to 562±13msec. The influence of this 1min rapid pacing is shown on the upper panel of Figure 4.21. Prior to metoprolol, a 12.4±2.3% increase in LV+dP/dt was induced by the onset of pacing, which persisted for 1min : LV+dP/dt was 12.5±2.7% above the baseline value at 60s. Similarly, after metoprolol injection, a 15.4±4.6% increase in LV+dP/dt was induced by the onset of pacing, and again presisted for 1min : LV+dP/dt was 11.2±2.6% above the baseline value at 60s. As illustrated in the lower panel of Figure 4.21, metoprolol thus elicited an 11.8±3.5% negative inotropic effect at baseline pacing, which was not significantly different from that observed after both 5 and 60s of rapid atrial pacing in humans : 14.5±4.3% and 13.1±3.2% respectively (p=NS), demonstrating no evidence for haemodynamic deterioration during pacing-induced tachycardia after metoprolol injection.

The extent of metoprolol-induced deterioration of dP/dt occurring with 60s of rapid atrial pacing, $\partial dP/dt_{PAC}$, was further examined by calculating the difference in metoprolol-induced negative inotropic effects at both 5 and 60s into the rapid pacing protocol thus :

$$\partial dP/dt_{PAC} = (control_{\partial 5} - metoprolol_{\partial 5}) - (control_{\partial 60} - metoprolol_{\partial 60})$$

where $control_{\partial 5}$ = increase in dP/dt at 5 seconds of pacing, prior to injection
$metoprolol_{\partial 5}$ = increase in dP/dt at 5 seconds of pacing, post-metoprolol
$control_{\partial 60}$ = increase in dP/dt at 60 seconds of pacing, prior to injection
$metoprolol_{\partial 60}$ = increase in dP/dt at 60 seconds of pacing, post-metoprolol.

Metoprolol-induced deterioration in dP/dt with rapid atrial pacing, $\partial dP/dt_{PAC}$, was determined as -4.4±5.6% (range -48.6 to 15.1%), which was not significantly different from zero, statistically demonstrating no deterioration in LV+dP/dt had occurred.

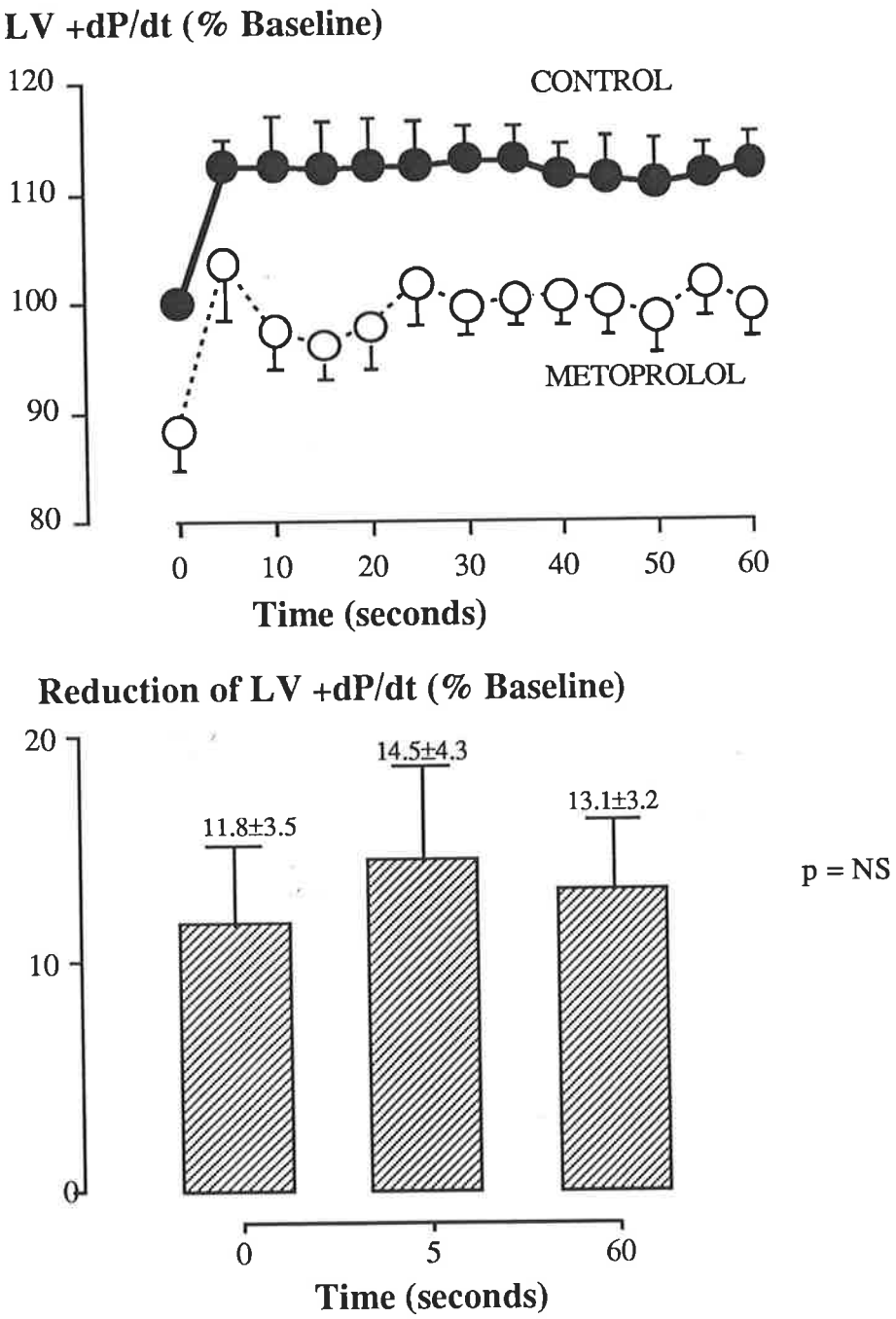


FIGURE 4.21 Influence of 60s rapid atrial pacing on LV+dP/dt before and after metoprolol injection : on the upper panel, the time course of LV+dP/dt for the duration of pacing, and on the lower panel, depression of LV+dP/dt before, and after 5 and 60s of rapid atrial pacing

4.3.17 Summary of results

The major findings following an acute intravenous metoprolol injection in the present investigation were :

(i) a reduction of LV+dP/dt at baseline heart rate maximal at 12min;

(ii) a reduction of spontaneous heart rate maximal at 7min;

(iii) a prolongation of PR intervals maxiaml at 7min;

(iv) myocardial metoprolol uptake of 1.9% of the dose peaking at 2.7min;

(v) residual myocardial metoprolol content at 17.5min 50% of maximal content;

(vi) a delayed time to peak myocardial metoprolol content in patients with extensive left coronary artery disease;

(vii) impaired myocardial metoprolol efflux during hypoxia;

(viii) the extent of peak cardiac effects was not related to the extent of peak myocardial content;

(ix) the time course of myocardial metoprolol content and acute effects were not parallel;

(x) the delay between content and effect was eliminated by predicting the amount of drug in a theoretical effect-site, using a pharmacokinetic/pharmacodynamic link model;

(xi) during the period of nett metoprolol efflux from the myocardium, the drug redistributes to other vascular beds, prior to bioexponential decay of venous levels;

(xii) significant concentrations of the active 4-hydroxy-metabolite were not apparent in the acute phase of the experiment;

(xiii) metoprolol was actively concentrated by red blood cells;

(xiv) the negative inotropic effects of metoprolol were abolished at short extrasystolic intervals;

(xv) metoprolol did not influence postextrasystolic potentiation studied in the absence of a compensatory pause;

(xvi) the negative inotropic effects of metoprolol were not influenced during pacing-induced tachycardia in patients with only mild angina.

4.4 Discussion

In a number of previous studies, the disposition of cardioactive drugs, including propafenone, quinidine, verapamil, bretylium, lignocaine, and mexiletine, within the myocardium has been identified as a major determinant of the acute effects of the agent under investigation, in isolated tissues, in *in vivo* studies in animals, and in humans (Gillis and Kates 1986; Gillis and Keashly 1991; Anderson *et al* 1980a,1980b; Keefe and Kates 1982; Upton *et al* 1988; Horowitz *et al* 1986; Powell *et al* 1990b). As a result of the present investigation, this list of drugs now includes the acute myocardial disposition of metoprolol after an intravenous bolus in humans, and its relationship to the acute effects of the drug, not previously described. Further studies *in vitro* examined the influence of hypoxia on these myocardial kinetics. For the first time in any preparation, the influence of systolic interval on the negative inotropic effects of metoprolol was also investigated.

The biexponential decay of metoprolol concentrations in peripheral venous blood up to 12h after the intravenous bolus are in agreement with previous results in animal and human studies after either oral or intravenous metoprolol administration (Borg *et al* 1975a; Johnsson *et al* 1975; Jordo *et al* 1980; Regardh *et al* 1980, 1981). With respect to the less conventional myocardial disposition of the drug, approximately 2% of the total injected dose of metoprolol represented peak MMC, which was attained 2.7min post injection. Subsequent nett efflux from the myocardium was observed, with residual MMC approximately 50% of the maximum after 17.5min. One site of redistribution during this time period was the femoral vascular bed, somewhat slower than the distribution to the myocardium. The acute myocardial uptake of lignocaine, mexiletine, verapamil and digoxin have previously been examined utilizing this technique in man (Horowitz *et al* 1986; Powell *et al* 1990a, 1990b), and the times to peak myocardial drug content have varied between agents, with metoprolol uptake comparable in rate to that of lignocaine (Horowitz *et al* 1986), and more rapid than the other agents examined. In contrast to the results of *in vitro* studies (Horowitz and Powell 1986), the results of the current investigation, and of previous studies of myocardial drug uptake in man (Horowitz *et al* 1986;

Powell *et al* 1990a, 1990b), demonstrates a lack of association between lipid solubility (from octanol:water partition coefficients) and the time course and extent of myocardial accumulation.

The potential for variability in myocardial drug uptake theoretically imposed by the presence of ischaemia / hypoxia was also investigated in the case of metoprolol, utilizing the isolated perfused Langendorff rat heart. In the current investigation, metoprolol was easily detected in all rat hearts regardless of length of perfusion period, or perfusion conditions. Of the 3 time points examined, 2mins of perfusion represented the highest concentration of metoprolol in the rat LV, not dissimilar from the time of peak MMC recorded here in humans. MMC then progressively decreased up to 10min post bolus injection : recent studies of the disposition of metoprolol in rat brain *in vivo* following intravenous bolus injection demonstrated a slower uptake phase (Nakazono *et al* 1992) than observed here *in vitro* . The period of hypoxia utilized in this study failed to impair MMU, as demonstrated by comparable results at 2min under normoxic and hypoxic conditions. However, hypoxia significantly impeded myocardial metoprolol efflux from the rat LV, predominantly in the latter part of the protocol, as demonstrated by the marked difference in residual MMC at 10min between hypoxia and normoxia. Notably, MMC did not decrease after 5min under hypoxic perfusion conditions, implying impaired efflux.

These results were in contrast to previous findings of reduced myocardial cardioactive drug concentrations in regions of impaired blood flow for metoprolol, atenolol, procainamide, digoxin and lignocaine in animal models of myocardial ischaemia / infarction (Ablad *et al* 1987; Avitall *et al* 1990; Wenger *et al* 1978, 1980; Ku 1983; Patterson *et al* 1982; Ryden *et al* 1990, 1991). While this contrast may reflect some basic fundamental difference between ischaemia and hypoxia, it should be noted that hypoxia has also been reported to reduce maximal myocardial concentrations (Gillis and Keashly 1991). An alternative explanation may be related to the severity of the hypoxia utilized here, up to 12min of perfusion in the complete absence of oxygen, possibly inducing cell death. Because of its lipophilicity, the accumulation of metoprolol by regions of hypoxic myocardium may be more extensive (Ablad *et al* 1987) than for more hydrophilic ß-adrenoceptor antagonists, an example of which is atenolol. In the present investigation, the spontaneous beating activity of some of the rat hearts was irregular at

10min. Thus, the "squeezing" effects on the extracellular myocardial compartments were probably minimal, and the ability of metoprolol to diffuse away from the site of uptake was therefore limited

The distribution of metoprolol to the various tissues of the body has been described in a range of animal preparations (Bodin *et al* 1975; Ablad *et al* 1987; Ryden *et al* 1990, 1991; Hatori *et al* 1991; Nakazono *et al* 1992). Significant myocardial concentrations of ^{3}H-metoprolol 5min after either oral or intravenous administration, progressing to nondetectable levels at 8h have been reported in mice and rats (Bodin *et al* 1975). Metoprolol distribution to the myocardium with intravenous administration has also been examined in dogs (Ablad *et al* 1987) and pigs (Ablad *et al* 1987; Ryden *et al* 1990, 1991; Hatori *et al* 1991). These studies included investigations of concentration gradients between ischaemic and nonischaemic myocardial zones : metoprolol concentrations were markedly lower in the ischaemic myocardium (Ablad *et al* 1987; Ryden *et al* 1990, 1991). Gradients were also demonstrated across endo-, mid-, and epimyocardial zones (Ryden *et al* 1990, 1991).

Studies utilizing coronary venous retroinfusion indicated substantial accumulation of metoprolol in the ischaemic myocardium, which was significantly greater in all regions of ischaemic than nonischaemic myocardium (Ryden *et al* 1990, 1991; Hatori *et al* 1991). The same factors probably govern myocardial metoprolol uptake and efflux. Therefore, the same reasons limiting metoprolol accumulation by ischaemic myocardium following conventional intravenous injection, account for the elevated accumulation of the drug in these regions after coronary venous retroinfusion. More simply, uptake is slow during ischaemia, and the same applies for efflux.

Maximal MMU was not significantly related to the extent of fixed coronary artery disease or underlying LV systolic dysfunction in the present investigation. Although the time to peak content was not correlated with initial systolic function, it was prolonged in the patients with extensive coronary disease. The mechanism of this difference, which was not observed in previous analogous studies with verapamil (Powell *et al* 1990b) and digoxin (Powell *et al* 1990a), is uncertain, but cannot be explained by overall changes in coronary blood flow within the region drained by the coronary sinus, because metoprolol failed to demonstrate significant

effects on coronary sinus flow in the present study. The difference may reflect induction of subclinical ischaemia, which has been shown to impede the process of metoprolol uptake in animal models (Ablad *et al* 1987; Ryden *et al* 1990, 1991). The finding that peak metoprolol uptake is not affected by extent of fixed coronary artery disease, is in contrast with results obtained with verapamil, where peak myocardial content was inversely proportional to the extent of disease (Powell *et al* 1990b).

The major acute haemodynamic and ECG effects of a 4mg intravenous bolus of metoprolol in the present investigation were :

(i) a progressive reduction of LV+dP/dt, peaking 10 minutes after injection;

(ii) significant diminution of spontaneous heart rate, maximal at 5 minutes;

and (iii) prolongation of PR interval, maximal at 20 minutes.

All effects persisted for the duration of the study. These findings were essentially consistent with those reported by previous investigators : after either a single intravenous dose or a series of injections (total dose 5-20 mg), metoprolol induced substantial reductions in spontaneous heart rate (6-16 beats/min), with minimal changes in mean arterial pressure, cardiac index and systemic vascular resistance (The MIAMI Trial Research Group 1985; Halinen *et al.* 1989; Murray *et al* 1987; Dell-Italia and Walsh 1989; Silke *et al* 1986; Silke *et al* 1985; Edvardsson *et al* 1984; Sannerstedt and Wasir 1977; Bourdillon *et al* 1979; Reale *et al* 1979; Johnsson *et al* 1975; Astrom and Jonsson 1977; Kronenberg *et al* 1990; Waagstein *et al* 1989) in both normal subjects and patients with coronary artery disease. Similar doses also resulted in significant negative inotropic effects, as demonstrated by reductions in LV+dP/dt (Dell-Italia and Walsh 1989; Bourdillon *et al* 1979; Reale *et al* 1979) or LV ejection fraction (Dell-Italia and Walsh 1989; Silke *et al* 1986; Kronenberg *et al* 1990).

This negative inotropic effect of metoprolol at baseline cycle length was progressively lessoned as the extrasystolic interval of the MRC was decreased, as demonstrated in Figure 4.16. Consequently, metoprolol induced significant reductions in both c and RDI, statistically confirming that the negative inotropic effects of the β_1-adrenoceptor antagonist were actually reverse use-dependent. As discussed in Chapter 3, despite the lack of a compensatory pause, the contractile force of the post-extrasystolic beat was potentiated (Figure 4.17), with the extent

of augmentation inversely related to extrasystole cycle length (Hoffman *et al* 1956, 1965; Kuijer *et al* 1990; van der Werf *et al* 1976; Seed *et al* 1984). Despite the reverse use-dependence of the negative inotropic effects of metoprolol demonstrable with the MRC, the drug did not influence PESP in any way. Additionally, the negative inotropic effects of metoprolol during one minute of pacing-induced tachycardia were not influenced. Reverse use-dependent effects have not been extensively reported for negatively inotropic agents. The only example is the electrophysiological effects of the class III antiarrhythmic agents, including sotalol (Schmitt *et al* 1991, 1992; Lathrop *et al* 1989). In the case of sotalol, ß-adrenoceptor blockade are probably not involved in these effects, as they persist when only d-sotalol is administered (Huikuri and Yli-Mayry 1992).

Only one previous study on the rate-dependence of the negative inotropic effects of metoprolol in humans *in vivo* has been described. In that study, the reduction of LV+dP/dt by metoprolol was increased from 10% at rest, to 43% during one minute of rapid atrial pacing in patients with coronary artery disease (Bourdillon *et al* 1979). When these results where expressed in absolute rather than relative values, metoprolol induced a 158mmHg/s reduction in LV+dP/dt compared with 197mmHg/s during pacing, which was probably not a significant difference : metoprolol thus exhibited rate-independent negative inotropic effects during 1min pacing in this patient group (Bourdillon *et al* 1979), similar to the findings of the present investigation.

In the present study, MMC was not a direct determinant of the ensuing acute haemodynamic or ECG effects (comprising changes in PR intervals, spontaneous heart rate, and LV+dP/dt), in contrast with the results of previous studies with verapamil (Powell *et al* 1990b). The implication of this negative finding for one of the major hypotheses is that responsiveness to acute ß-adrenoceptor blockade in man may be modulated extensively by, for example, ß-adrenoceptor density and / or affinity, or fluctuations in autonomic tone (Waagstein *et al* 1989; Colucci *et al* 1981; Fowler *et al* 1986). The influence of such modulating factors, which vary markedly between individuals, appears to have persisted despite prior withdrawal of ß-adrenoceptor antagonist therapy and attempted minimization of stress during the procedure.

The peak haemodynamic and ECG effects occurred considerably later than the time of peak MMC, representing a hysteresis. This time delay prior to optimal efficacy on spontaneous heart

rate (Figures 4.11 and 4.12) might be explicable in terms of a slower rate of onset of metoprolol on central sympathetic mechanisms and / or altered sinus node metoprolol kinetics. Either is unlikely, as the effects of metoprolol on both atrioventricular nodal conduction and inotropic state also tended to exhibit marked temporal fluctuations (Figures 4.11 and 4.12). The anticlockwise hysteresis loops illustrating the association between each of PR interval, spontaneous heart rate, and LV+dP/dt with MMC suggest a distinct period of time must pass prior to MMC inducing pharmacodynamic effects. This time interval could not be explained in terms of a mediation of effects by a progressively generated metabolite, because the only stable metabolite of metoprolol which has been shown to exert any ß-adrenoceptor antagonistic activity, 4-hydroxy-metoprolol (Regardh *et al* 1981), was not detectable in whole blood until well after the initial 20min sampling period. The delay in the present study was also not attributable to stereoselective myocardial kinetics of metoprolol, as no significant difference in the time course of MMC was observed between the R- and S-metoprolol enantiomers (Stafford *et al* 1991, 1994), in contrast to the plasma disposition reported previously (Lennard *et al* 1983). Additionally, the time course of interaction between a range of other inotropic agents and cellular effector mechanisms has previously been shown to persist in the absence of diffusion barriers within myocardial cells, implicating a role for a "biochemical lag phase" (Horowitz and Powell 1986; Barry *et al* 1985; Horowitz *et al* 1982). The relationship between MMC and acute effects was analysed with the use of pharmacokinetic / pharmaco-dynamic link models, in which the amount of drug in a hypothetical effect compartment (Ce), postulated to directly determine acute myocardial effects (Holford and Sheiner 1981; Unadkat *et al* 1986). Utilization of these techniques in the current study demonstrated the plot of the two was well-fitted by a simple linear regression.

No information regarding myocardial drug uptake in patients during tachycardia was sought in the present study, because content was only examined at fixed heart rates (73beats/min). Studies with both mexiletine and lignocaine (Horowitz*et al* 1986) indicated greater uptake of these agents by the human myocardium during periods of increased heart rate. This finding suggests that myocardial uptake of metoprolol may also be enhanced in patients with tachycardia.

In conclusion, MMC is not a major determinant of acute effect in individual patients, implying the existence of marked end-organ variability in sensitivity to ß-adrenoceptor blockade. The efficacy of metoprolol relative to content increases progressively during the first 10-15 minutes after metoprolol injection, confirming there is a marked "lag phase" . This may possibly be imposed by the kinetics of drug / receptor interactions, or the biochemical changes which are initiated by these interactions (Horowitz and Powell 1986). The negative inotropic effects of metoprolol were not modified during sustained tachycardia, but were reduced at single beats of short cycle length, and this may imply low probability of acute haemodynamic deterioration associated with metoprolol administration in patients prone to tachyarrhythmias.

CHAPTER 5 :

ACUTE MYOCARDIAL UPTAKE OF D - AND L - SOTALOL : CORRELATION WITH ACUTE EFFECTS IN HUMANS.

5.1 Backgrounds and aims

Sotalol is a nonselective ß-adrenoceptor antagonist which also possesses important class III antiarrhythmic properties. However, virtually no information is available regarding its myocardial kinetics. Conventional pharmacokinetic studies in normal individuals describe an elimination phase half-life between 5-17h, and anapparent volume of distribution 1 - 3L/kg, whether orally or intravenously administered (Anttila *et al* 1976; Tjandramaga *et al* 1976; Kahela *et al* 1979; Ishizaki *et al* 1980; Blair *et al* 1981; Poirier *et al* 1981). This is a longer half-life, and smaller volume of distribution, than recorded for metoprolol (Regardh and Johnson 1980; Benfield *et al* 1986; Regardh *et al* 1974, 1975), implying slower elimination kinetics and reduced lipophilicity of sotalol.

The aims of the studies described in this chapter were :

(i) to specifically examine the process of acute myocardial sotalol uptake following intravenous bolus administration, and the potential for this uptake process to be enantioselective for either the d- or l-sotalol isomers;

(ii) to determine the acute haemodynamic, electrocardiographic (ECG) and electrophysiological (EP) effects of the drug in man;

(iii) to correlate myocardial content with these effects, in order to test the hypothesis: **"Myocardial sotalol content is a direct determinant of acute effect;**

(iv) to examine the potential dependence of the negative inotropic effects of sotalol on beat interval, utilizing the short-cycle-length component of the mechanical restitution curve (MRC) in humans *in vivo* , and the mathematical model described in Chapter 3 of this thesis;

(v) to examine the influence of sotalol on the force-interval relationship in terms of an eight beat MRC, where the contractile force of the last in a train of 8 short beats was plotted as a function of cycle length;

(vi) to examine the influence of this novel ß-adrenoceptor antagonist on post-extrasystolic potentiation (PESP), where the post-extrasystolic interval is equal to the baseline cycle length.

5.2 Methods

Fifteen patients undergoing diagnostic cardiac catheterization and coronary arteriography for the investigation of chest pain were selected. The research procedure commenced at the end of the routine cardiac catheterization. The protocol for the research procedure was essentially as described in section 2.1.1 of this thesis : Protocol for cardiac catheterization for determination of myocardial drug uptake and measurement of haemodynamic effects in humans.

Myocardial sotalol uptake in this group of patients was determined for the individual d- and l-sotalol enantiomers, by determining the concentrations of each isomer in whole blood from femoral artery and coronary sinus, as described in section 2.2.2 of this thesis : HPLC quantitation of sotalol enantiomers in human whole blood. Racemic sotalol concentrations were calculated as the sum of the concentrations of the two individual enantiomers. The influence of hypoxia on the myocardial uptake profile of sotalol was not examined.

The force-interval was further examined in this chapter, utilizing an eight beat model, where the contractile force of the last beat in a train of eight premature beats was plotted as a function of cycle length. This *short cycle length eight beat* mechanical restitution curve was examined prior to and 20mins after sotalol injection. Coronary vasodilator reserve, determined as the ratio of coronary sinus flow (CSF) at baseline and at submaximal pacing, was evaluated prior to and 20mins after sotalol injection also.

5.3 Results

5.3.1 Patient characteristics

A total of 15 patients were studied, and their characterstics are summarized in Table 5.1. Of these, 10 patients were male and 5 were female. No patient had LV systolic dysfunction, with ventriculography-derived ejection fraction consistently greater than fifty percent. Cardiac indices and baseline pulmonary capillary wedge pressure (PCWP) were all in the normal range, although 7 patients had cardiac indices below 2.50L/min/m^2. Haemodynamically significant coronary artery disease, defined by at least a fifty percent stenosis of one or more coronary arteries, was present in 11 patients. Baseline pacing rate throughout the study was at a mean cycle length of 776±33msec.

Twelve patients had some history of antianginal therapy prior to the investigation, including 5 with a ß-adrenoceptor antagonist, and 7 patients with a calcium channel antagonist, which were stopped at least five half-lives prior to the study. Previous exposure to nitrates was documented in 12 patients.

The procedure was well tolerated and no patient developed adverse effects due to sotalol. Myocardial sotalol uptake was determined in 12 patients, while the potential for rate-related negative inotropic effects was explored in all patients in some form.

TABLE 5.1. Patient characteristics prior to sotalol administration

Pt	Age (yr)	Sex	CI ($L/min/m^2$)	HR (beats/min)	LV EF %	PCWP (mmHg)	MAP (mmHg)	CAD	Prior Therapy
1	59	F	3.72	71	71	2	99	CIRC	C, N
2	68	M	2.36	77	76	8	96	LAD	B, N
3	68	F	2.45	64	65	5	120	LAD,CIRC	B, C, N
4	54	F	2.34	80	67	10	105	LAD	B, N
5	64	M	3.27	54	55	8	115	LAD,CIRC,RCA	C, N
6	57	M	2.37	56	73	10	124	LAD	C, N
7	45	M	2.51	80	74	10	119	-	N
8	55	M	2.49	61	70	9	113	RCA	B, C, N
9	43	M	2.70	56	64	7	94	-	-
10	35	M	3.85	77	86	5	98	CIRC	C, N
11	50	M	2.77	64	71	7	111	RCA	C, N
12	62	F	2.33	102	89	3	131	-	N
13	49	M	3.02	92	61	8	97	LAD,CIRC,RCA	B, N
14	56	M	2.09	94	85	3	114	LAD	-
15	44	F	2.80	63	66	9	99	-	-
Mean	54		2.74	73	72	7	109		
SE	2		0.13	4	2	1	3		

B, ß-adrenoceptor antagonist; C, calcium channel antagonist; CAD, fixed coronary artery disease (>50% stenosis); CI, cardiac index; CIRC, left circumflex coronary artery; EF, ejection fraction; F, fenale; HR, heart rate; LAD, left anterior descending coronary artery; LV, left ventricular; M, male; MAP, mean arterial pressure; N, nitrate; PCWP, pulmonary capillary wedge pressure; Pt, patient; RCA, right coronary artery

5.3.2 Acute haemodynamic effects of sotalol

The effects of sotalol on the various haemodynamic indices monitored after injection are summarized in Table 5.2, with the time course of these effects illustrated in Figures 5.1 - 5.6. The influence of sotalol on mean arterial, LV systolic and end-diastolic pressures are illustrated in Figure 5.1 : only LV systolic pressure tended to be reduced by sotalol, with maximal reduction at 11.5±1.9mins post administration, although this effect was not significant ($p=0.09$). Figure 5.2 shows cardiac index and coronary sinus flow following sotalol intravenous bolus injection. Cardiac index was not monitored during the research procedure in two patients. In the remaining patients, sotalol did not influence cardiac index. Coronary sinus flow was elevated by the sotalol, but this also barely escaped attaining statistical significance. Peak coronary sinus flow was observed 10.6±1.8mins after injection.

The influence of sotalol on both systemic (SVR) and coronary vascular resistances (CVR) are illustrated in Figure 5.3. Although neither were significantly changed by sotalol, a nonsignificant trend for reduced CVR was seen ($p<0.12$), which peaked 10.0±1.9mins post administration. Figure 5.4 depicts the profile of spontaneous heart rate (HR) and LV+dP/dt (at fixed heart rate) following sotalol administration. Both spontaneous HR and LV+dP/dt were significantly and markedly reduced, with maximal effects at 9.97±1.7 ($p<0.0001$) and 11.1±1.5mins ($p<0.0002$).

5.3.3 Acute electrocardiographic effects of sotalol

The influence of sotalol on electrocardiographic intervals (ECG) at either fixed or spontaneous heart rate are summarized in Table 5.2, and shown in Figures 5.5 and 5.6. Sotalol significantly prolonged PR intervals at both fixed ($p<0.05$) and spontaneous heart rates ($p<0.05$), inducing maximal prolongation at 8.61±1.71 and 8.23±1.34mins post administration. While no significant effects on the QT interval in either circumstance were observed, sotalol exerted nonsignificant prolongation of both paced and spontaneous QT intervals ($p=0.13$ and $p=0.06$ respectively), also achieving maximal effects at 8.73±1.47 and 9.00±1.71mins after injection.

TABLE 5.2 Haemodynamic and electrocardiographic effects of sotalol

Parameter (mean±SE)	Baseline value	Maximum change after sotalol	*p* value	Time (mins) of maximal effect
MAP (mmHg)	109±3	+3±3	NS	9.67±1.82
CI ($L/min/m^2$)	2.70±0.15	-0.36±0.13	NS	8.64±1.17
SVR ($dynes.s.cm^{-5}$)	1700±120	+310±100	NS	9.62±1.40
LV+dP/dt ($mmHgs^{-1}$)	1630±100	-280±70	0.0002	11.0±1.6
LV SBP (mmHg)	162±5	-11±6	NS	11.5±1.9
LV EDP (mmHg)	16±3	+3±2	NS	9.58±1.68
CS flow (ml/min)	118±15	+27±16	NS	10.6±1.8
CVR (mmHg/ml/min)	1.08±0.15	-0.07±0.12	NS	10.0±1.9
Spontaneous HR (beats/min)	72.6±3.8	-10.6±2.1	0.0001	9.97±1.71
Paced PR interval (msec)	193±9	+13±4	0.0103	8.61±1.71
Paced QT interval (msec)	379±8	+8±5	NS	8.73±1.47
Spontaneous PR interval (msec)	210±8	+14±5	0.0475	8.23±1.24
Spontaneous QT interval (msec)	375±5	+26±5	NS	9.00±1.71

CI, cardiac index; CS, coronary sinus; CVR, coronary vascular resistance; HR, heart rate; LV+dP/dt, peak rate of rise of left ventricular pressure; LV EDP, left ventricular end-diastolic pressure; LV SBP, left ventricular systolic blood pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance

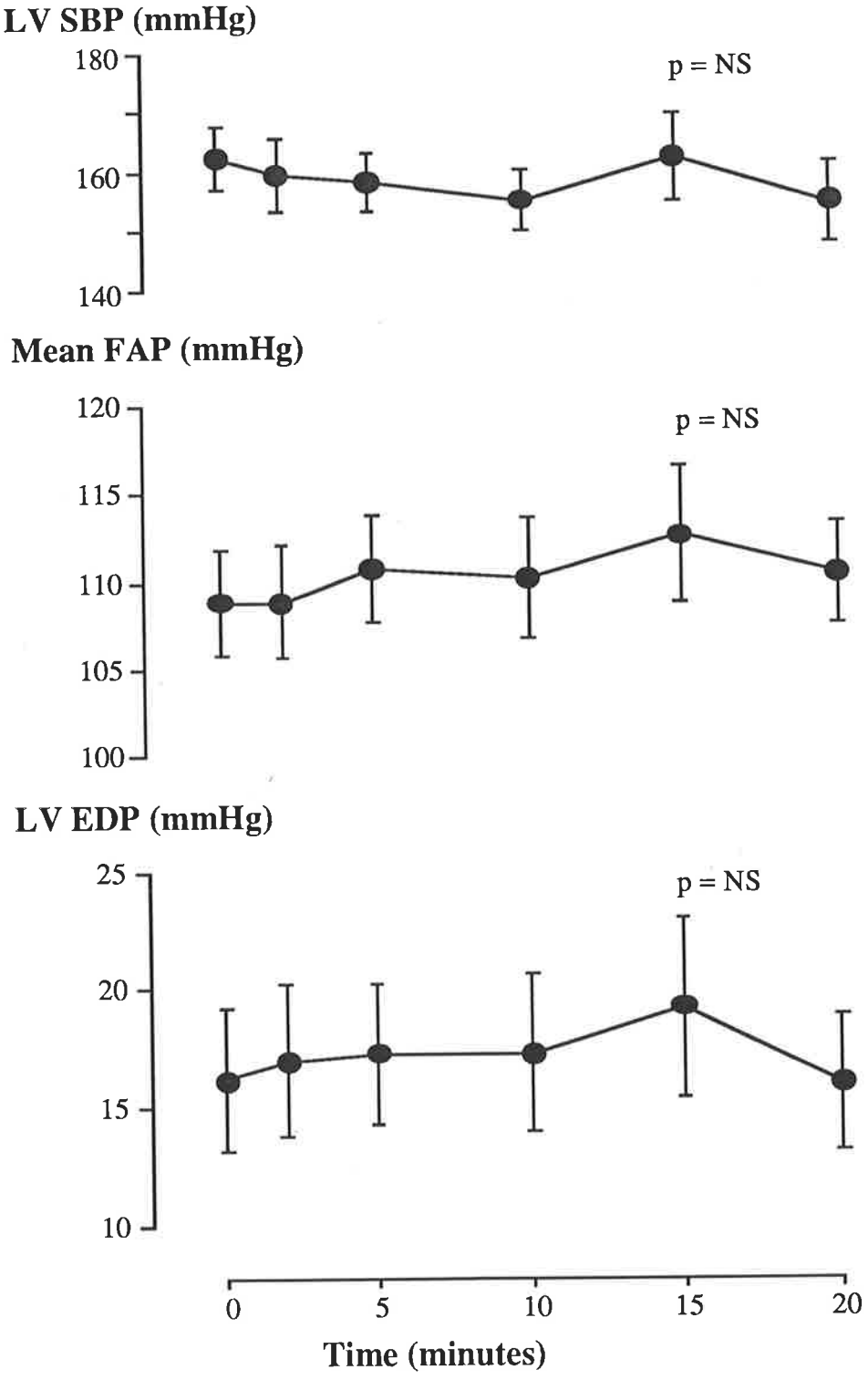


FIGURE 5.1 Time course of changes in left ventricular systolic (LV SBP), mean femoral arterial (mean FAP) and left ventricular end-diastolic (LV EDP) pressures for 20min after sotalol injection

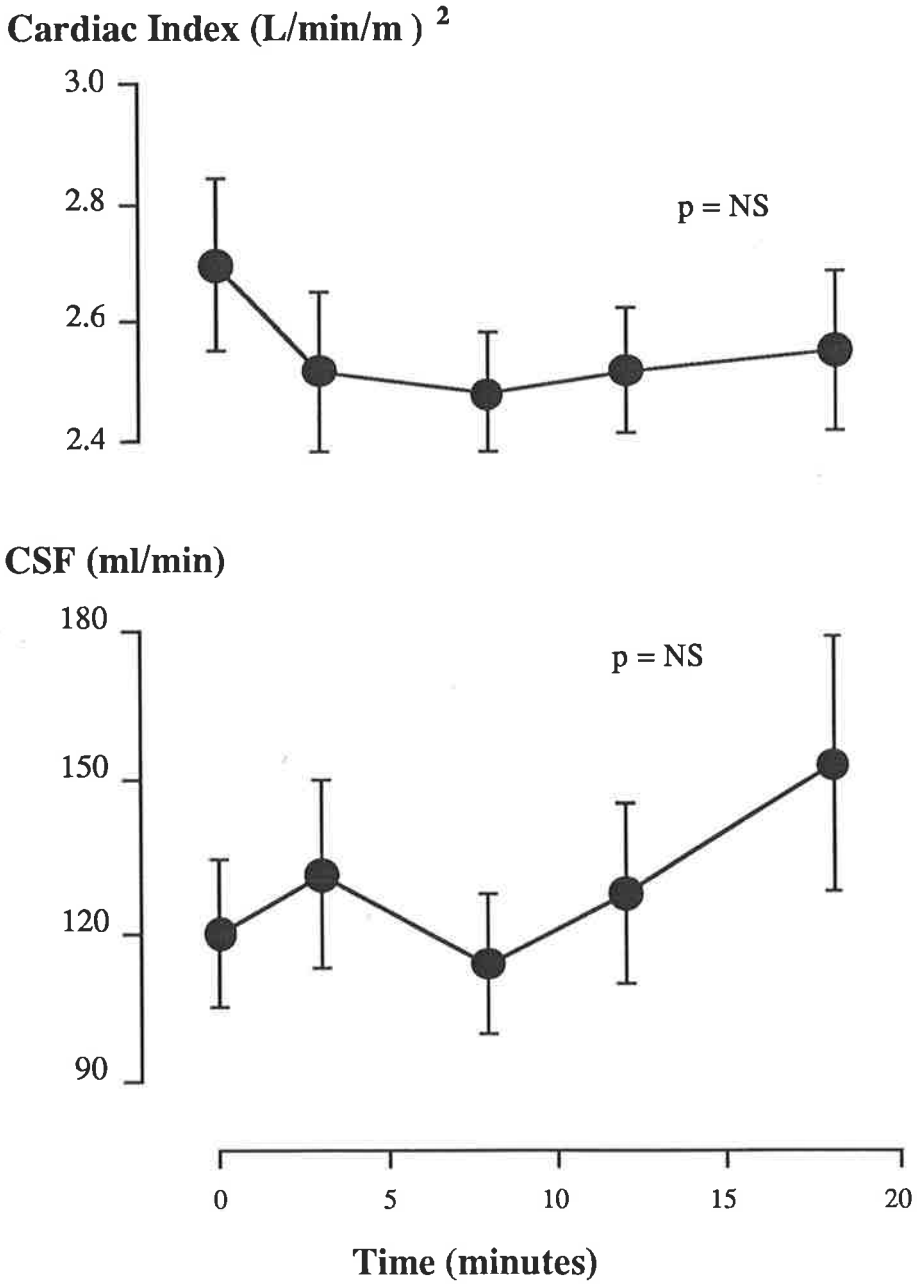


FIGURE 5.2 Time course of changes in cardiac index and coronary sinus flow (CSF) for 20min after sotalol injection

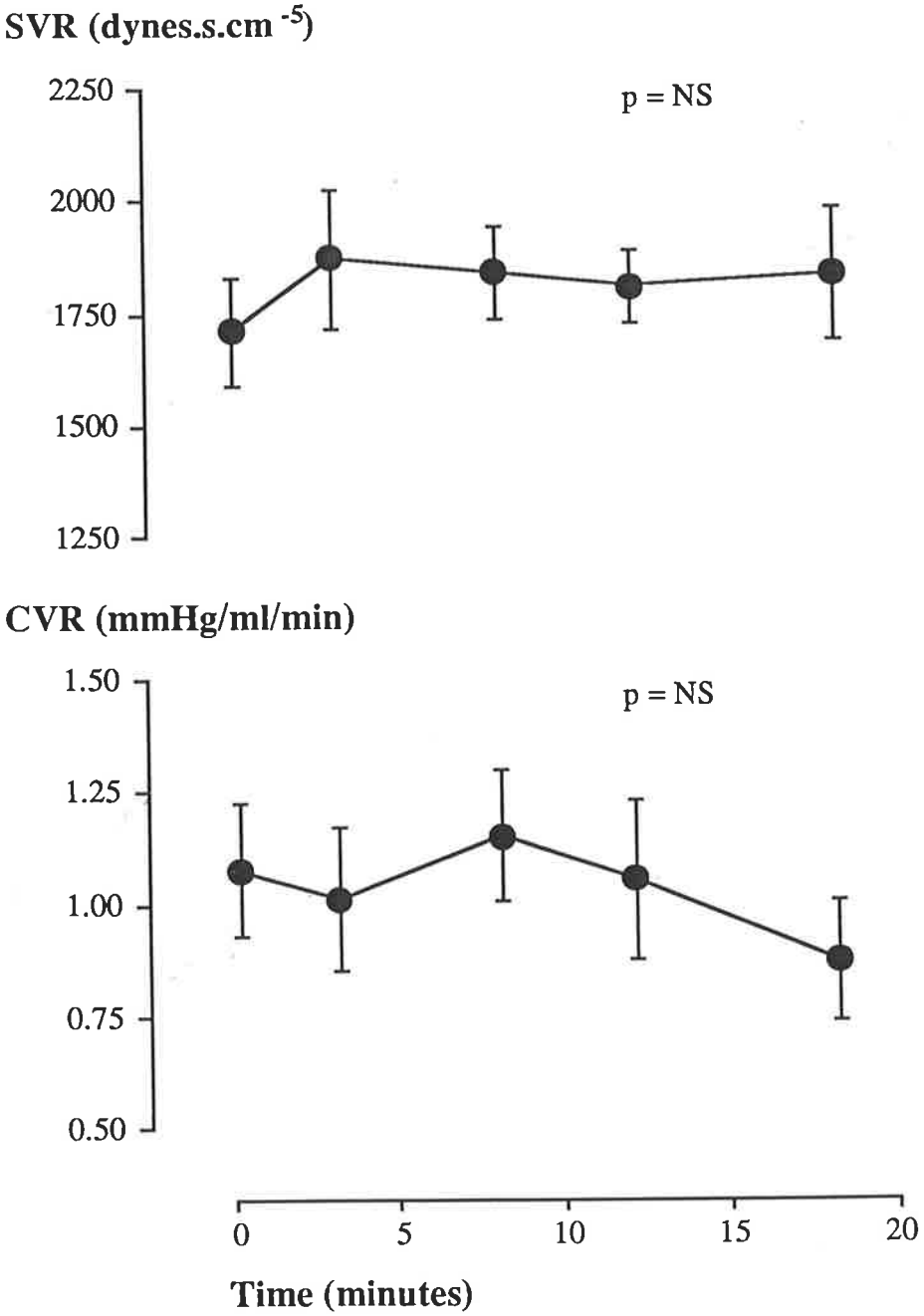


FIGURE 5.3 Time course of changes in systemic (SVR) and coronary (CVR) vascular resistances for 20min after sotalol injection

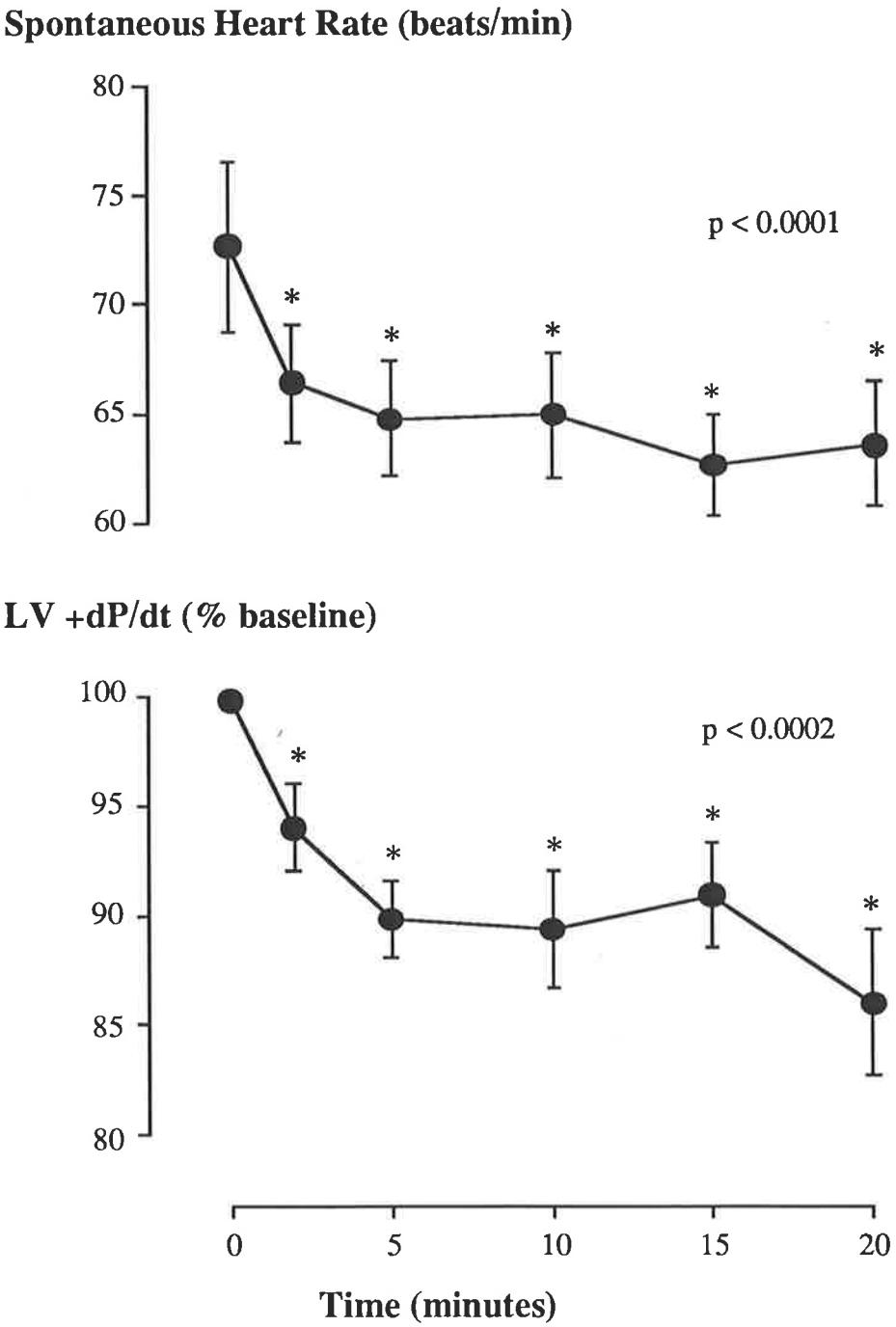


FIGURE 5.4 Time course of changes in spontaneous heart rate and LV +dP/dt for 20min after sotalol injection

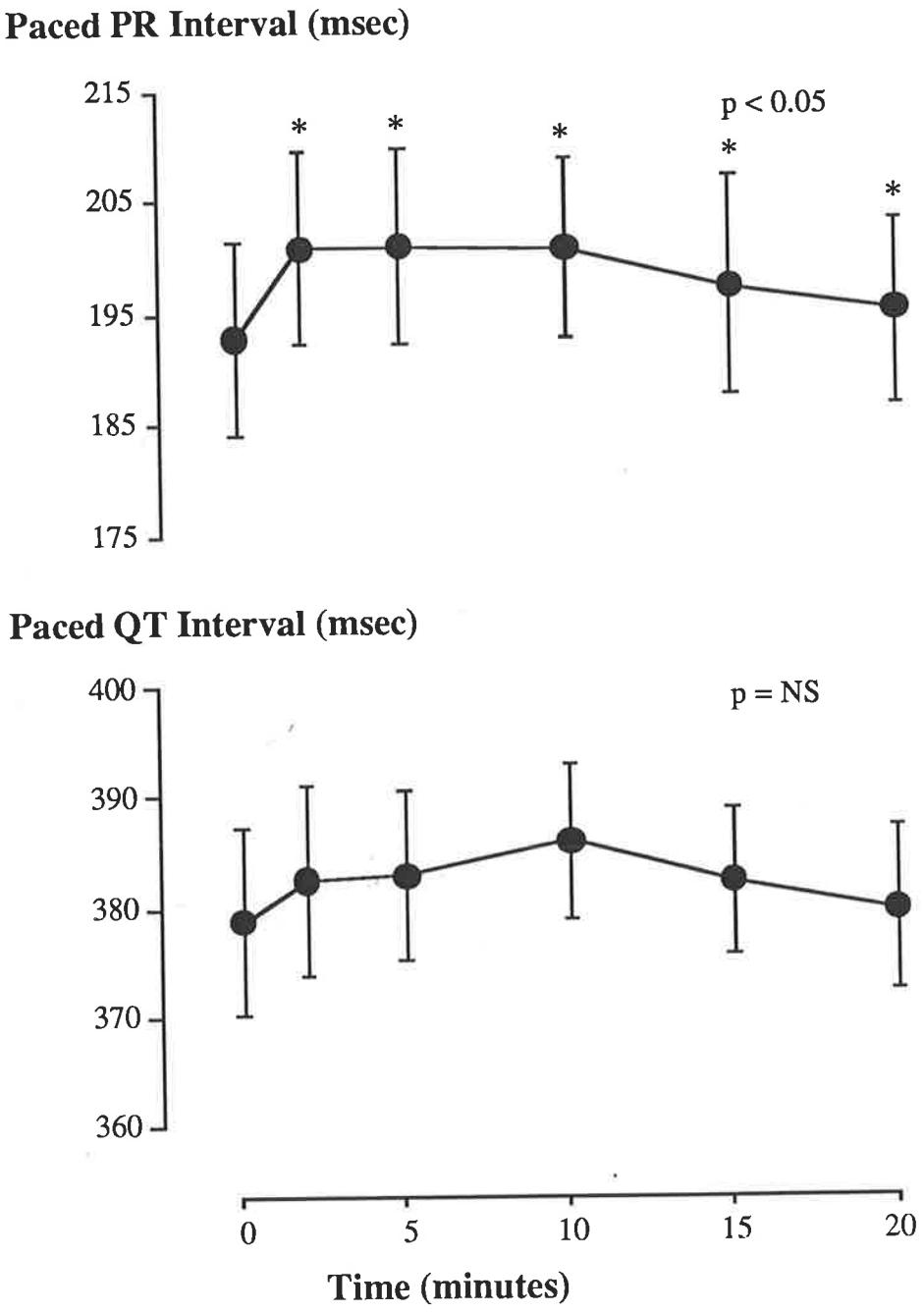


FIGURE 5.5 Time course of changes in electrocardiographic intervals at fixed heart rate for 20min after sotalol injection

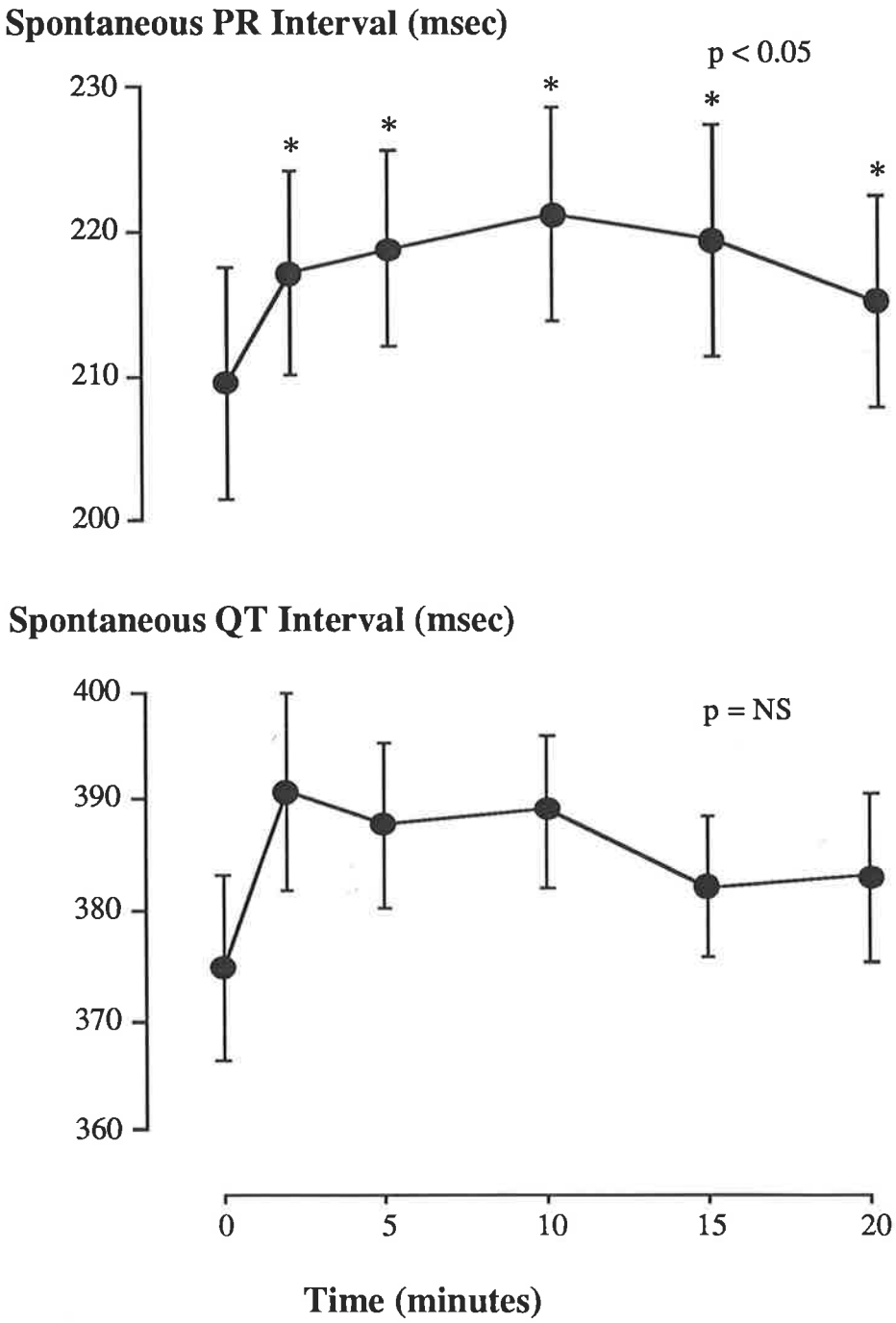


FIGURE 5.6. Time course of changes in electrocardiographic intervals at spontaneous heart rate for 20min after sotalol injection

5.3.4 Acute electrophysiologic effects of sotalol

The influence of sotalol on electrophysiologic intervals are described in Table 5.3. There was significant prolongation of both the AH interval and the atrioventricular nodal effective refractory period (AVNERP), from 93±11 to 109±9ms and from 290±22 ($p<0.01$)to 344±14ms ($p<0.001$), with maximal mean effects on both parameters at 10min. No significant effect of sotalol on the HV interval was observed.

5.3.5 Validity of utilizing femoral arterial sotalol concentrations as a surrogate for those in the aorta

The validity of using femoral arterial (FA) sotalol concentrations as a surrogate for those in the aorta (Ao) was assessed in one patient. As shown in the upper panel of Figure 5.7, RS-sotalol concentrations in FA and Ao did not differ significantly (mean difference 1.19±7.62%, p=NS). The discrepancy between FA and Ao concentrations in the first sample (approximately 50% different) is difficult to explain - if the FA was not a valid surrogate, one would expect Ao contractions to exceed FA. However, the opposite was observed, and may be related to void volume of the sampling catheter positioned in the Ao in this patient.

5.3.6 Acute myocardial sotalol uptake

Concentrations of the sotalol racemate in 12 patients in FA and CS whole blood are shown in the lower panel of Figure 5.7, and of the individual enantiomers in Figure 5.8. In FA, RS-sotalol concentrations were initially high, 12.5±2.0μg/ml at 0.26±0.02mins. FA levels then rapidly declined to 418±32ng/ml at 19.9±0.04mins. Conversely, racemic sotalol CS concentrations were lower initially, 2.51±0.58μg/ml at 0.61±0.06mins, rapidly reaching a peak of 2.88±0.36μg/ml at 1.20±0.11mins, before also declining, to 421±38ng/ml at 20.1±0.1mins. From visual analysis of Figure 5.8, it is apparent that no difference between concentrations of R- or S-sotalol was observed, in either the arterial or venous sampling sites.

TABLE 5.3 Electrophysiologic effects of sotalol up to 20 minutes after administration

Parameter (mean±SE)	Baseline value	2	5	10	15	20	*p* value
		---------- Minutes after sotalol ----------					
AH interval (msec)	93±11	104±11	106±11	109±9	102±13	97±16	0.008
HV interval (msec)	51±4	49±3	47±4	49±4	45±5	42±3	NS
AVN ERP (msec)	290±22	329±15	342±14	344±14	343±22	340±17	0.001

AVN ERP, atrioventricular nodal effective refractory period

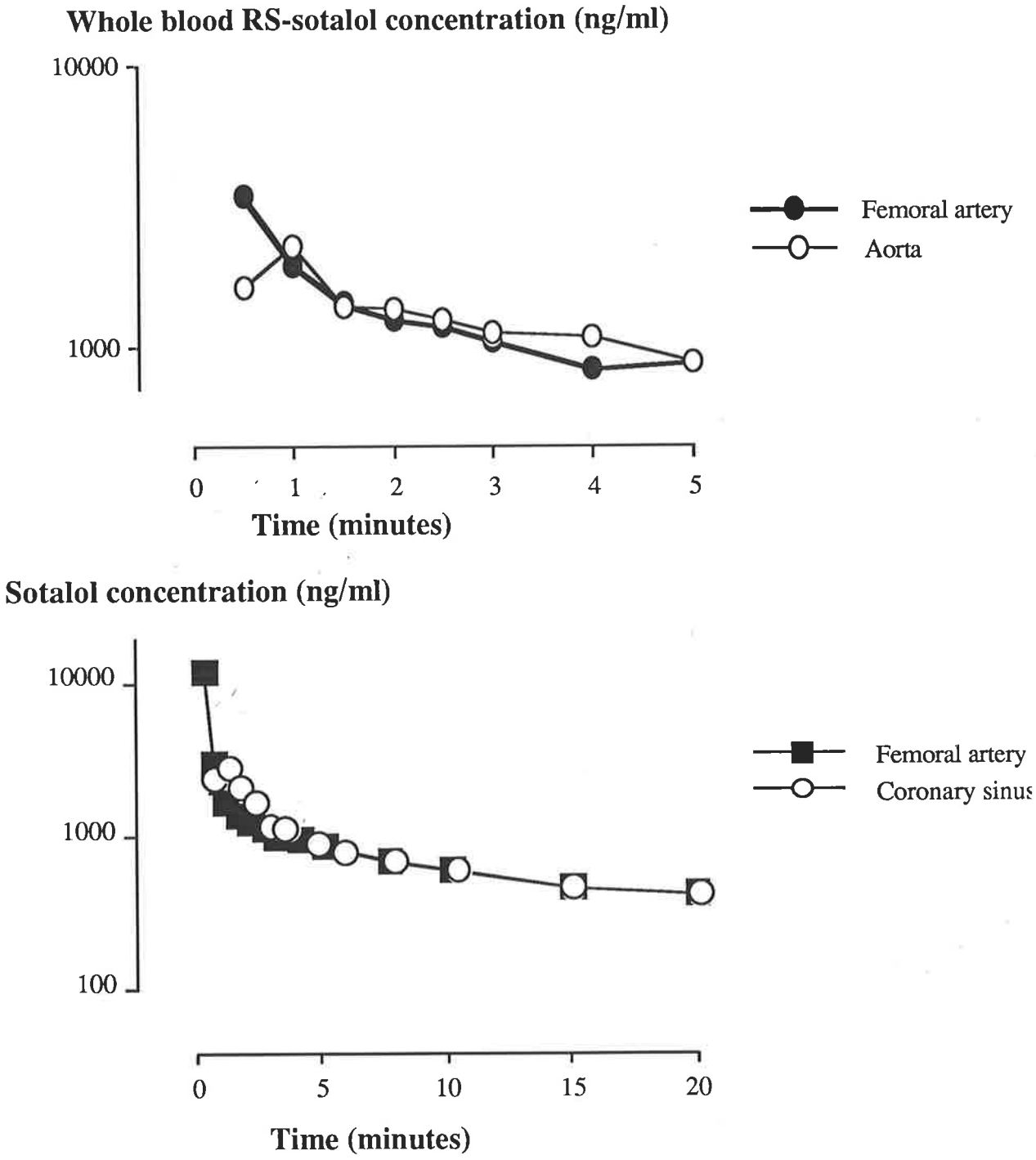


FIGURE 5.7 On the upper panel, the time course of changes in R,S-sotalol concentrations in femoral artery (FA) and aorta for 5min in one patient, and on the lower panel, the time course of RS-sotalol concentrations in FA and coronary sinus (CS) for 20min after sotalol injection in 12 patients

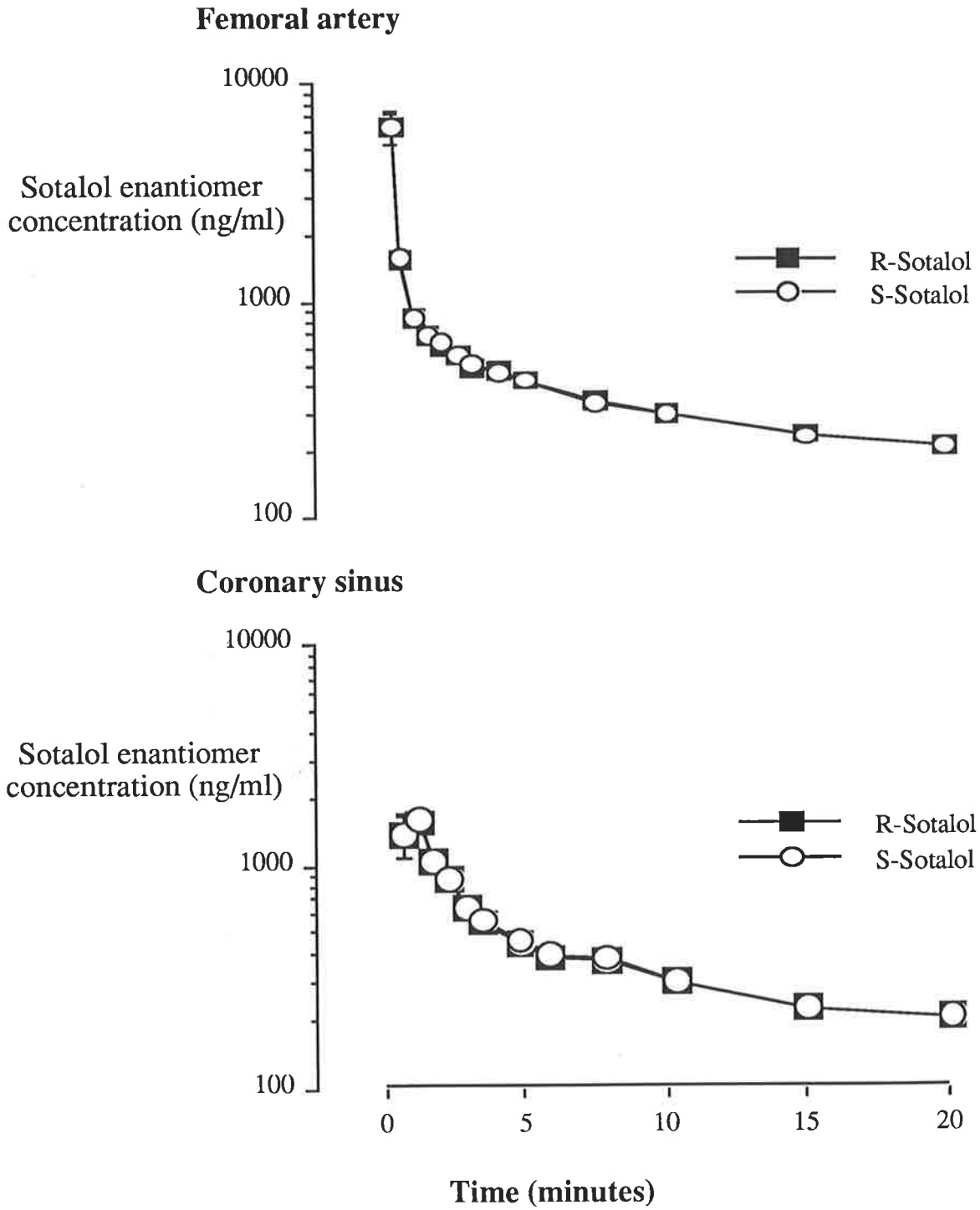


FIGURE 5.8 Time course of R- and S-sotalol concentrations in FA and CS for 20min after sotalol injection

The time course of both enantiomeric and racemic myocardial sotalol uptake is illustrated in Figure 5.9. The initially large difference between FA and CS RS-sotalol concentrations corresponded to rapid nett uptake of the ß-adrenoceptor antagonist by the myocardium. However, peak myocardial sotalol content, the timepoint at which whole blood RS-sotalol concentrations in FA and CS were equal, was rapidly achieved, 0.74±0.10mins post administration.

Corrected for resting coronary sinus flow, peak myocardial racemic sotalol content was 3.34±0.38µg per ml/min, 2.05±0.45% of the total injected dose. The extent of peak myocardial sotalol content, or the time taken to achieve peak content, were not significantly dependent on individual patient characteristics at baseline, including resting cardiac index, ejection fraction, or the extent of fixed coronary artery disease in major branches of the left coronary artery. Subsequently, the transcoronary concentration gradient quickly became quite negative, indicative of a rapid nett sotalol efflux phase from the myocardium. During the latter phase of the experiment, RS-sotalol concentrations in the femoral artery and coronary sinus remained similar, revealing a plateau phase of myocardial sotalol content. Thus, myocardial sotalol efflux was biphasic, with residual RS-sotalol 1.31±0.44µg per ml/min, or 31.3±18.6% of maximal content, still present at 17.5±0.0mins post administration. The characteristics of myocardial sotalol uptake in the individual patients, and their corresponding clinical characteristics at baseline, are listed in Table 5.4.

From the upper panel of Figure 5.9, it is apparent that little difference between levels of R- or S-sotalol in the human myocardium was achieved. This lack of stereoselectivity of myocardial sotalol uptake (or indeed efflux) was further confirmed with analysis of the area under the time course of myocardial content of each enantiomer, as demonstrated in Table 5.5. For the remainder of this chapter I will therefore only discuss RS-sotalol.

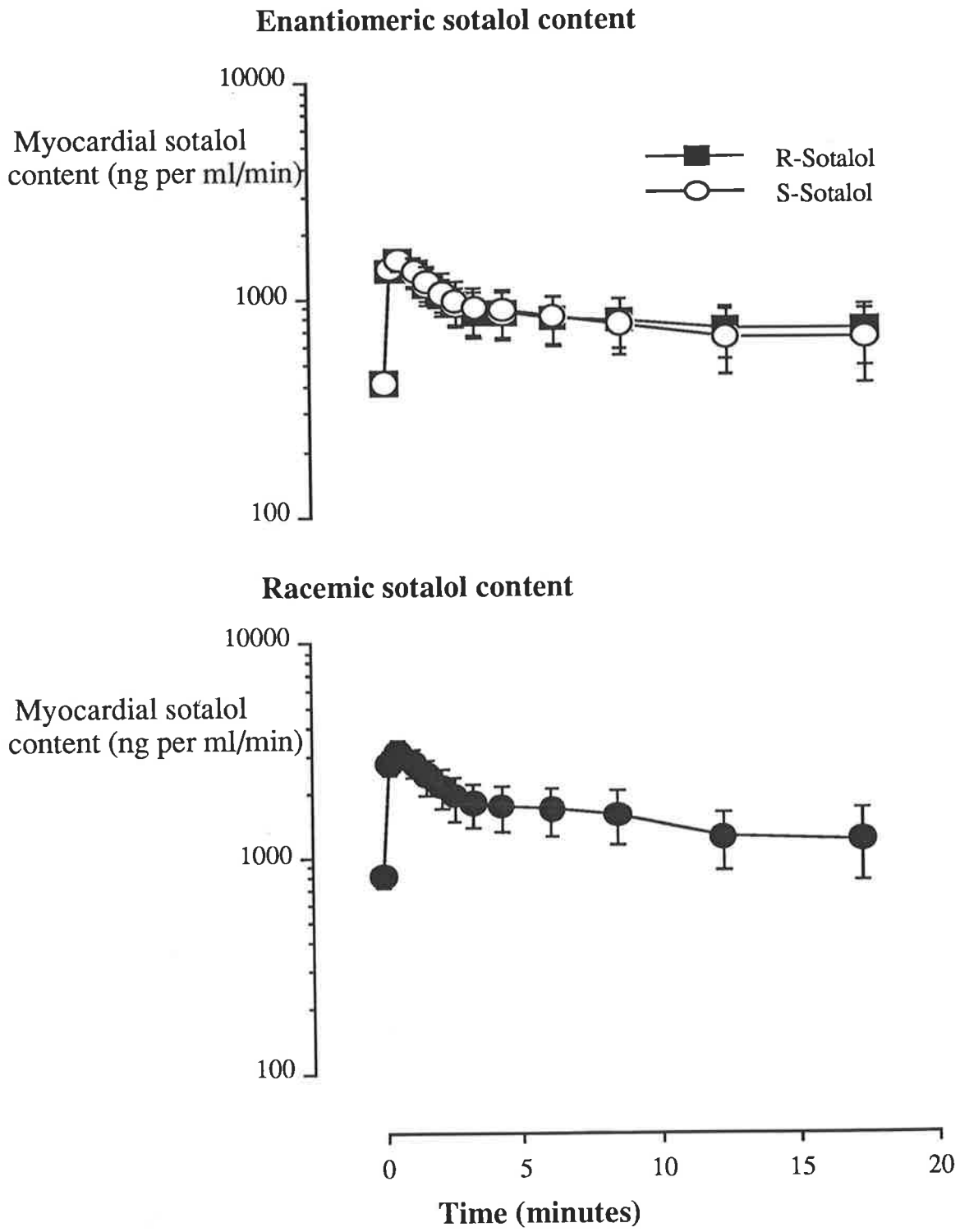


FIGURE 5.9 Time course of myocardial sotalol uptake, expressed relative to resting CSF : the upper panel shows myocardial uptake of R- and S-sotalol, while racemic sotalol is shown on the lower panel

TABLE 5.4 Comparison of the individual patient characteristics of myocardial RS-sotalol uptake with corresponding clinical characteristics at baseline

Pt	t_{max}	C_{max}	C_{max}%	CAD	Age	Sex	CI	HR	LV EF	PCWP	MAP
1	1.25	4.24	1.18%	1	59	F	3.72	71	71%	2	99
2	0.75	2.44	1.92%	0	55	M	2.49	61	70%	9	113
3	0.75	4.37	4.35%	1	68	M	2.36	77	76%	8	96
4	0.36	1.32	1.07%	2	68	F	2.45	64	65%	5	120
5	0.75	2.79	1.69%	1	57	M	2.37	56	73%	10	124
6	1.25	2.00	0.54%	0	45	M	2.51	80	74%	10	119
7	1.25	5.88	5.95%	1	54	F	2.34	80	67%	10	105
8	0.35	2.27	1.05%	0	43	M	2.70	56	64%	7	94
9	0.75	4.13	1.90%	1	35	M	3.85	77	86%	5	98
10	0.35	4.52	1.66%	0	50	M	2.77	64	71%	7	111
11	0.75	3.20	1.45%	2	49	M	3.02	92	61%	8	97
12	0.38	2.98	1.82%	0	44	F	2.80	63	66%	9	99
Mean	0.74	3.34	2.05%		52		2.78	70	70%	8	106
SE	0.10	0.38	0.45%		3		0.15	3	2%	1	3

CAD, fixed coronary artery disease in major branches of the left coronary artery (>50% stenosis); CI, cardiac index; C_{max}, peak myocardial sotalol content; C_{max}%, peak myocardial sotalol content as a percent of the total injected dose; EF, ejection fraction; F, female; HR, heart rate; LV, left ventricle; M, male; MAP, mean arterial pressure; PCWP, mean pulmonary capillary wedge pressure; Pt, patient; tmax, time of peak myocardial sotalol content

TABLE 5.5 Comparison of the AUC for myocardial content of the sotalol enantiomers

Patient	R-Sotalol AUC	S-Sotalol AUC
1	0.70	0.62
2	0.50	0.27
3	16.4	6.13
4	0.01	0.01
5	0.30	0.24
6	0.03	0.03
7	1.49	1.51
8	20.0	1.79
9	1.12	1.49
10	0.74	0.80
11	0.10	0.11
12	0.96	0.42
Mean	3.53	1.12
SE	2.00	0.49
p = 0.NS		

AUC, area under the time course of myocardial content

5.3.7 Correlation between myocardial sotalol content and acute effects

As described in section 2.5 of this thesis, correlations were sought between :

(i) extent of maximum MSC and extent of maximum effects;

and (ii) the time course of MSC with that of the acute effects of the drug.

Sotalol significantly prolonged PR intervals (at both fixed and spontaneous heart rates) and induced negative inotropic and chronotropic effects. However, the maximum change in these parameters was not significantly correlated with peak myocardial sotalol content relative to resting coronary sinus flow, illustrated in Figure 5.10. Peak haemodynamic and electrocardiographic effects were attained much later than peak myocardial content, the latter being achieved quite rapidly after injection (0.74±0.10mins). Figure 5.11 portrays the time course of these changes relative to simultaneous myocardial sotalol content, with significant fluctuations in the relationship between effect and myocardial content for the ratio of each of reduction in spontaneous heart rate ($p<0.001$) and prolongation of PR intervals at fixed HR ($p<0.01$) and simultaneous myocardial sotalol content. The ratio of reduction of LV+dP/dt with corresponding myocardial content also tended to vary with time after injection, but failed to attain statistical significance ($p=0.10$).

For all parameters examined, the ratio of effect : content tended to increase over the first 6min of the study, before declining up to 12.5mins. The ratio at 17.5mins was also elevated, such that the maximal changes in all three parameters relative to myocardial sotalol content (expressed relative to resting coronary sinus flow) occurred at 17.5mins post administration. This may have been influenced by incomplete data for some patients at the last time point of uptake. The relationship between change in haemodynamic and electrocardiographic effects and the corresponding myocardial sotalol level (relative to resting coronary sinus flow) for 20mins following sotalol administration is shown in Figure 5.12. This allows visual examination of the potential for hysteresis between peak content and effects, as suggested by the anticlockwise loops, perhaps representing a lag time for the onset and offset of effect due to drug-receptor interactions.

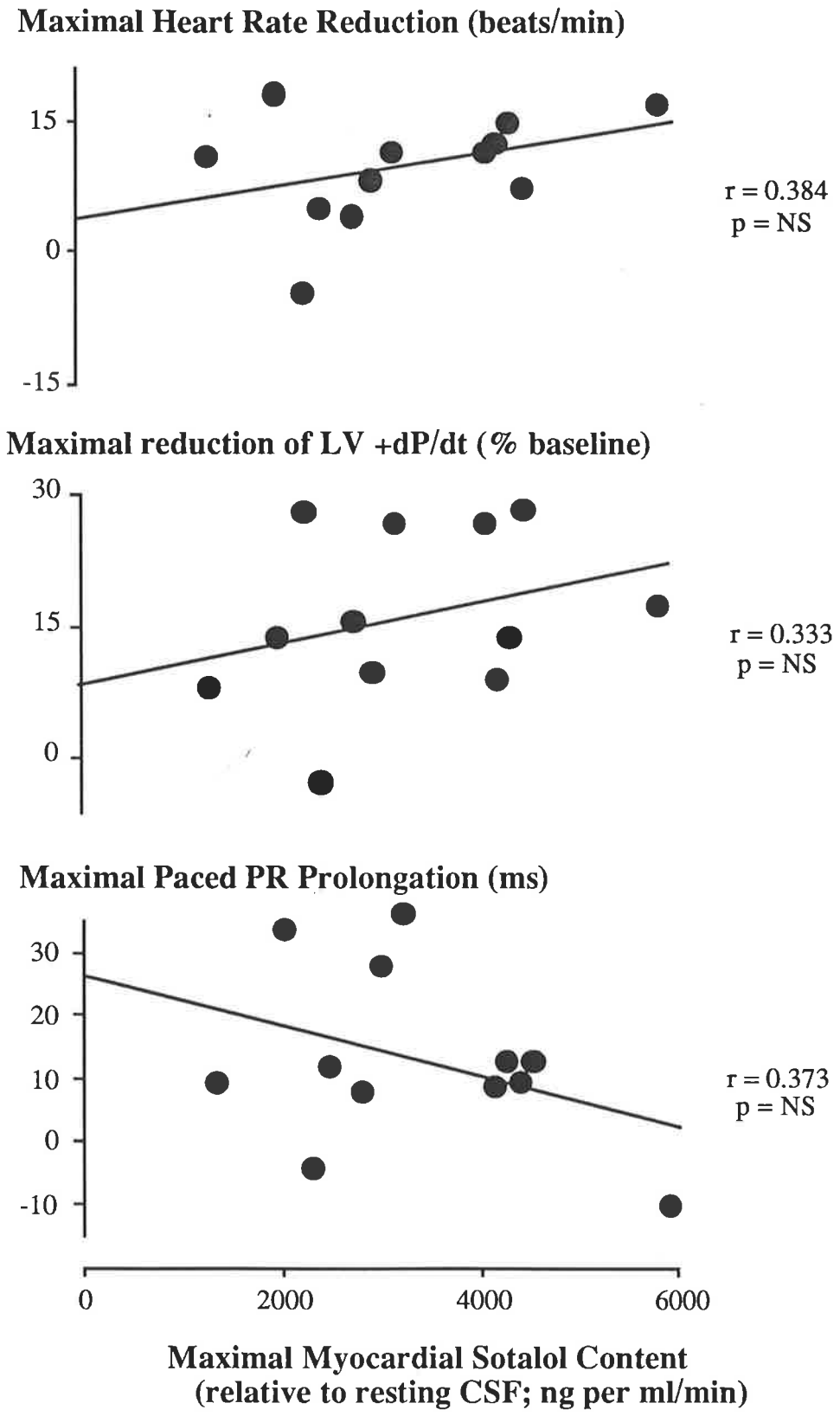


FIGURE 5.10 Individual maximal sotalol effects on heart rate, LV+dP/dt and paced PR interval as a function of peak myocardial sotalol content (relative to resting CSF)

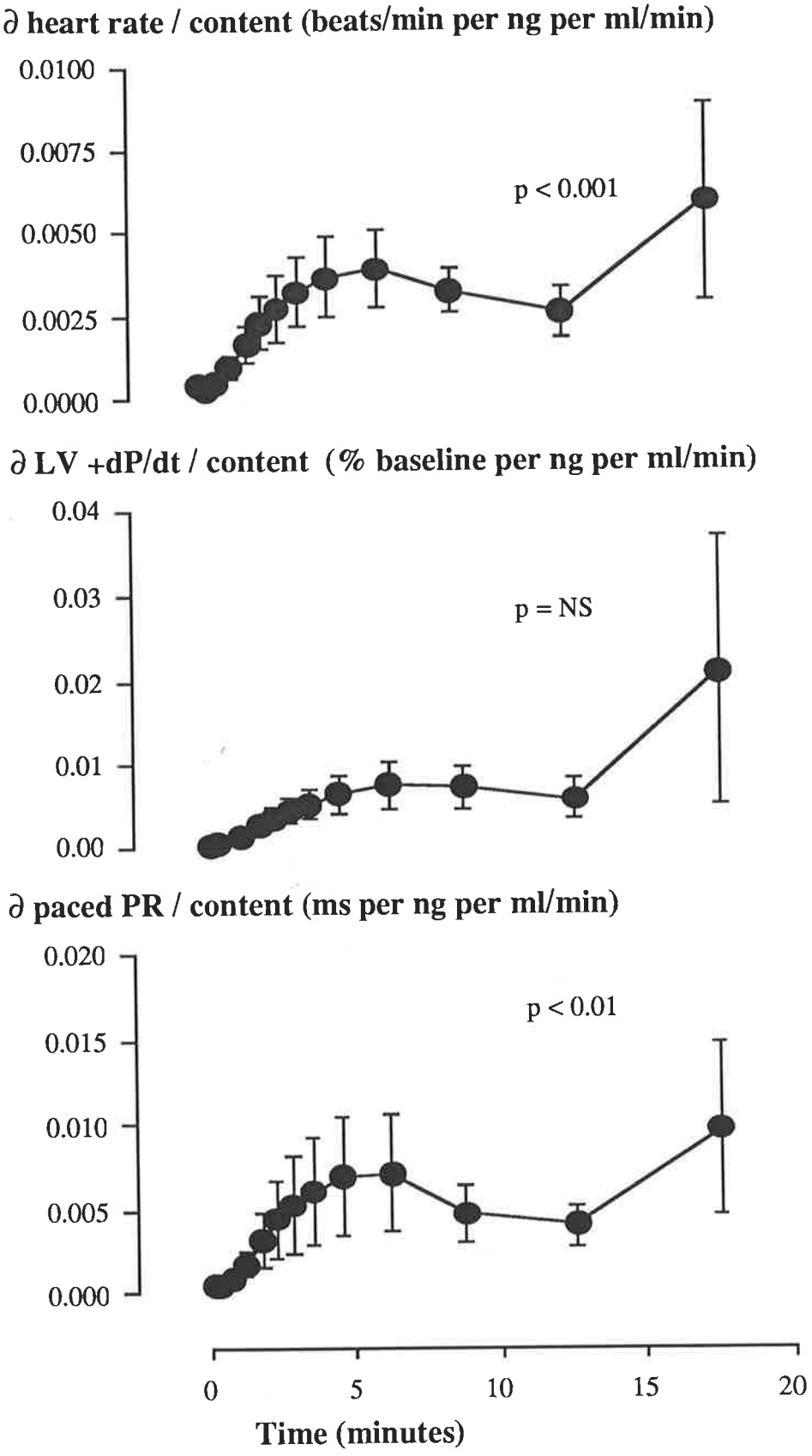


FIGURE 5.11 Time course of the ratio of effect and myocardial sotalol content (relative to resting CSF)

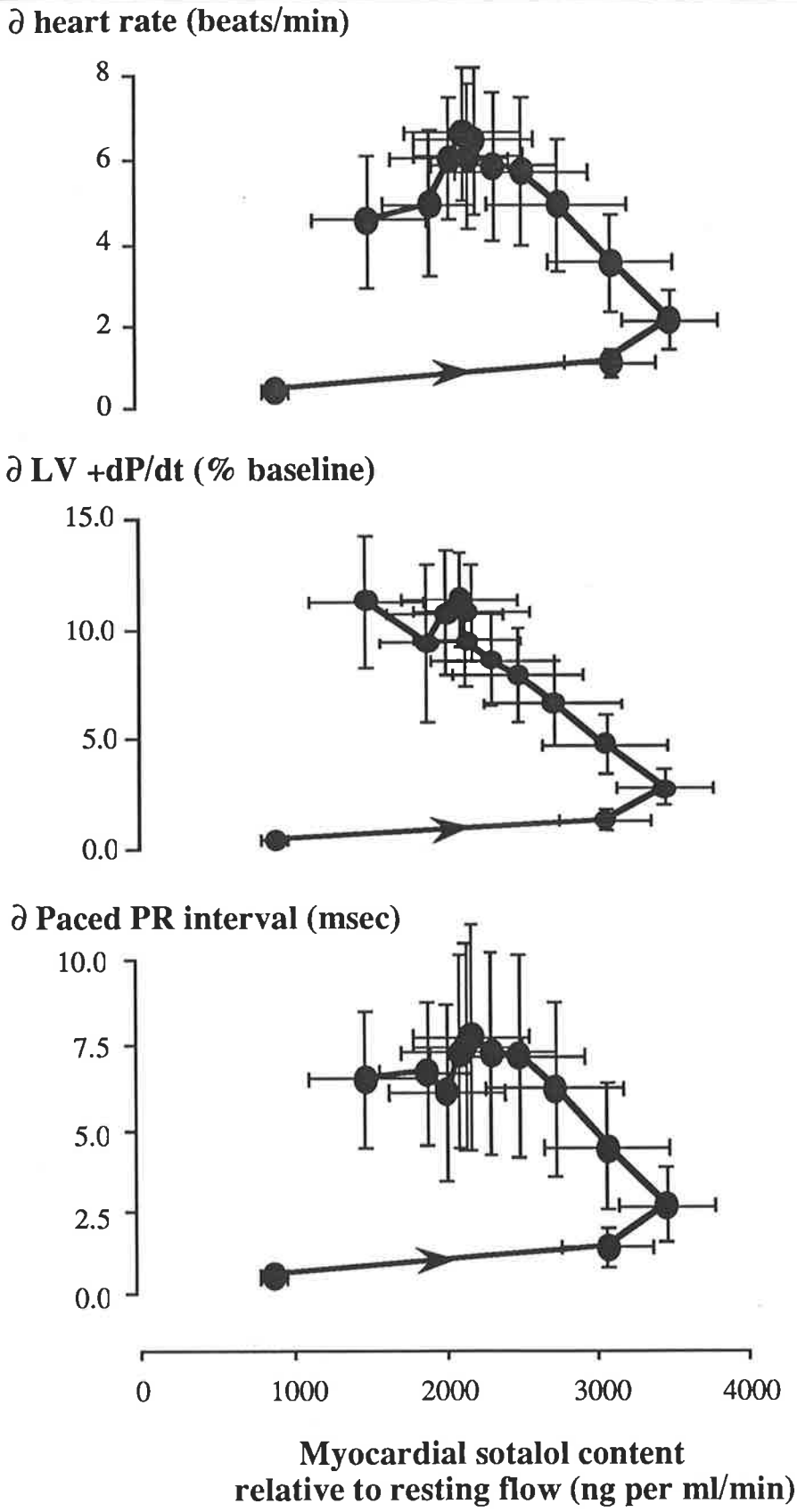


FIGURE 5.12 Relationship between sotalol-induced changes in spontaneous heart rate, LV+dP/dt and paced PR interval with myocardial sotalol content (relative to resting CSF)

5.3.8 Pharmacokinetic - pharmacodynamic link models between myocardial sotalol content and effect

As described in section 2.6, pharmacokinetic / pharmacodynamic link models were employed to model the hysteresis between MSC and significant effects : PR interval prolongation, and heart rate and LV +dP/dt reduction. The estimates of the constants of the myocardial uptake two-compartment pharmacokinetic model for 10 patients are listed in Table 5.6, and the estimates of the constants of the pharmacokinetic / pharmacodynamic link models for each of the three significant effects are summarized in Table 5.7. The calculated dose, D, received by the heart was 7.28±0.58µg per ml/min, the absorption rate constant, Ka, was 1.98±0.17, and the arbitrary constants K_{21}, α, and ß, were 0.48±0.09, 1.75±0.20, and $0.45\pm6.76\times10^{-3}$ respectively. The relation between reduction in spontaneous HR with the theoretical effect-site metoprolol concentration (Ce) for every patient was linear, while prolongation of the PR interval and reduction in LV+dP/dt at fixed HR were linearly related to Ce also, in all except 1 and 2 patients respectively, whom only displayed minor fluctuations in these parameters with sotalol administration. Typical results in one patient of this pharmacokinetic / pharmacodynamic link model are shown in Figure 5.13, with reduction in spontaneous HR as the example effect.

5.3.9 Sotalol redistribution into other vascular beds

The profile of sotalol concentrations in FA and femoral vein (FV), and that of the R- and S-sotalol enantiomers in FV for 20mins following intravenous bolus administration is shown in Figure 5.14. Racemic sotalol FV were 1.36±0.11µg/ml initially (2.03±0.10mins). These levels then progressively declined, reaching 388±17ng/ml at 20.0±0mins post injection. Again, visual examination of FV R- and S-sotalol concentrations suggest nonenantioselective behaviour of sotalol in humans. A consistent but small arteriovenous RS-sotalol concentration gradient was apparent from 4min onwards, with FA concentrations exceeding FV, representing nett uptake of the ß-adrenoceptor antagonist into the femoral bed. Uptake of sotalol into the lower limbs therefore continues for a considerably longer period than uptake into the myocardium.

TABLE 5.6 Estimates of the constants of the myocardial sotalol uptake two-compartment pharmacokinetic model

Patient	D	Ka	K_{21}	α	ß
1	6497.5649	2.257460	0.422948	2.238201	-0.009410
2	9005.8785	1.001609	0.213948	0.989302	-0.002131
3	5522.9461	1.636319	0.144449	1.636161	-0.032896
4	10040.9988	2.297433	0.592301	1.879647	-0.013985
5	5605.4817	1.833925	0.580164	1.831780	0.046836
6	7355.0107	2.468025	0.337489	0.576187	-0.008061
7	4448.5828	2.698764	1.016029	2.694059	-0.006050
8	7711.3330	1.684631	0.633776	1.684623	0.000470
9	9597.4038	2.395603	0.680522	2.395393	0.016002
10	6980.5446	1.563702	0.192623	1.563675	0.013711
Mean	7276.5744	1.983747	0.481425	1.748903	0.000449
SE	584.7482	0.165408	0.085851	0.199389	0.006756

α, an arbitrary constant for myocardial content versus time; ß, an arbitrary constant for myocardial content versus time; D, calculated dose received by heart; Ka, absorption rate constant for myocardial sotalol content versus time; K_{21}, an arbitrary constant for myocardial content versus time

TABLE 5.7 Estimates of the constants of the pharmacokinetic-pharmacodynamic link model

Parameter	Keo	S	r	slope	y int	p
Heart rate	0.664±0.189	0.0036±0.0010	0.811±0.032	148±67	459±132	0.00178±0.00073
Paced PR	0.598±0.192	0.0066±0.0031	0.638±0.082	29±78	663±274	0.03056±0.02054
LV+dP/dt	0.651±0.184	0.0065±0.0023	0.742±0.074	154±47	480±216	0.05901±0.04718

K_{eo}, elimination rate constant from the effect-site for extrapolated ∂ effect versus time; r, regression coefficient of extrapolated ∂ effect versus effect-site concentration; S, slope factor of extrapolated ∂ effect versus time; slope, slope of extrapolated ∂ effect versus effect-site concentration; y int, y axis intercept of extrapolated ∂ effect versus effect-site concentration

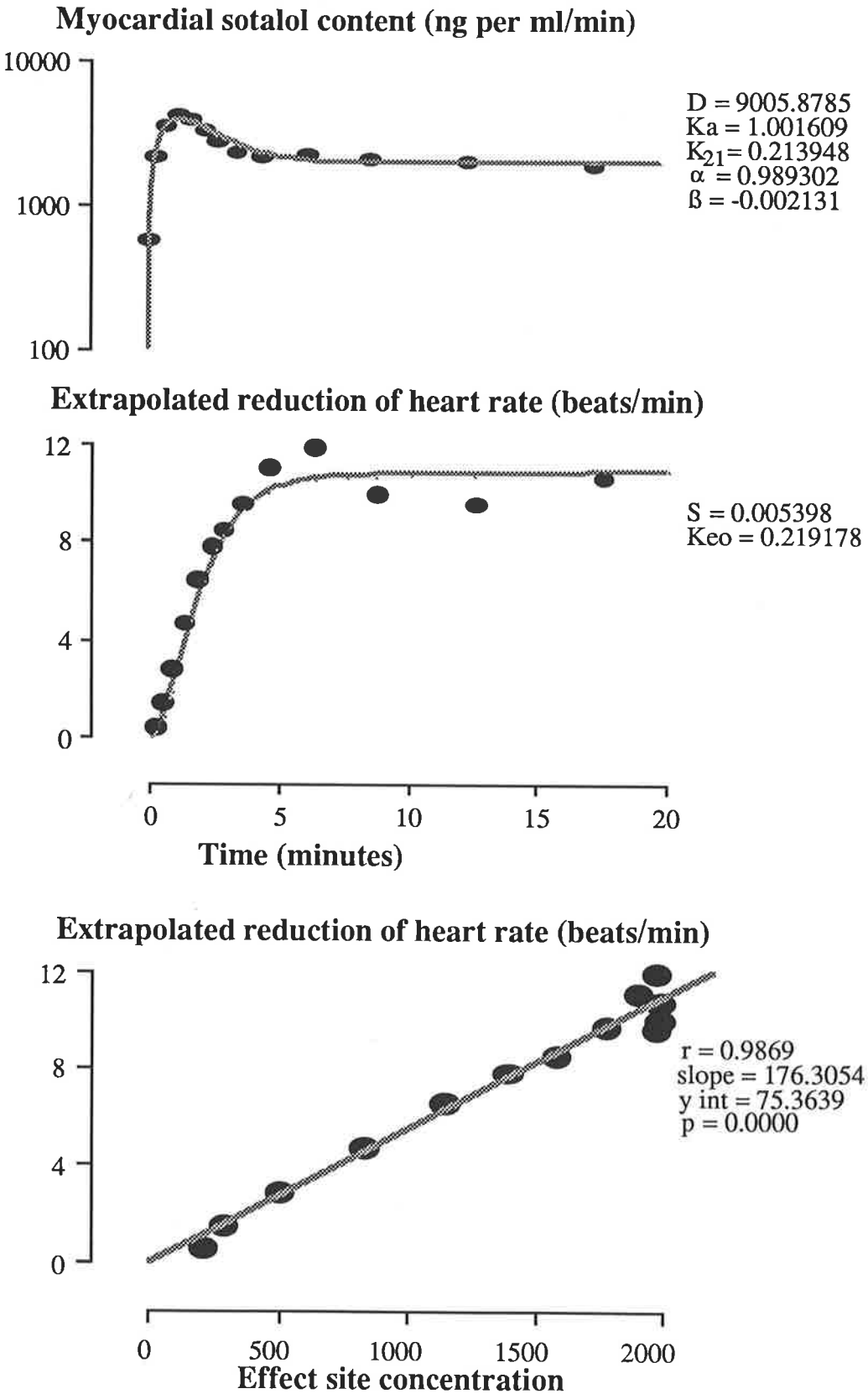


FIGURE 5.13 Typical results for pharmacokinetic/pharmacodynamic modelling for MSC and effects: MSC as a function of time; effect (spontaneous HR reduction) as a function of time; the link model for effect-site sotalol concentration and effect

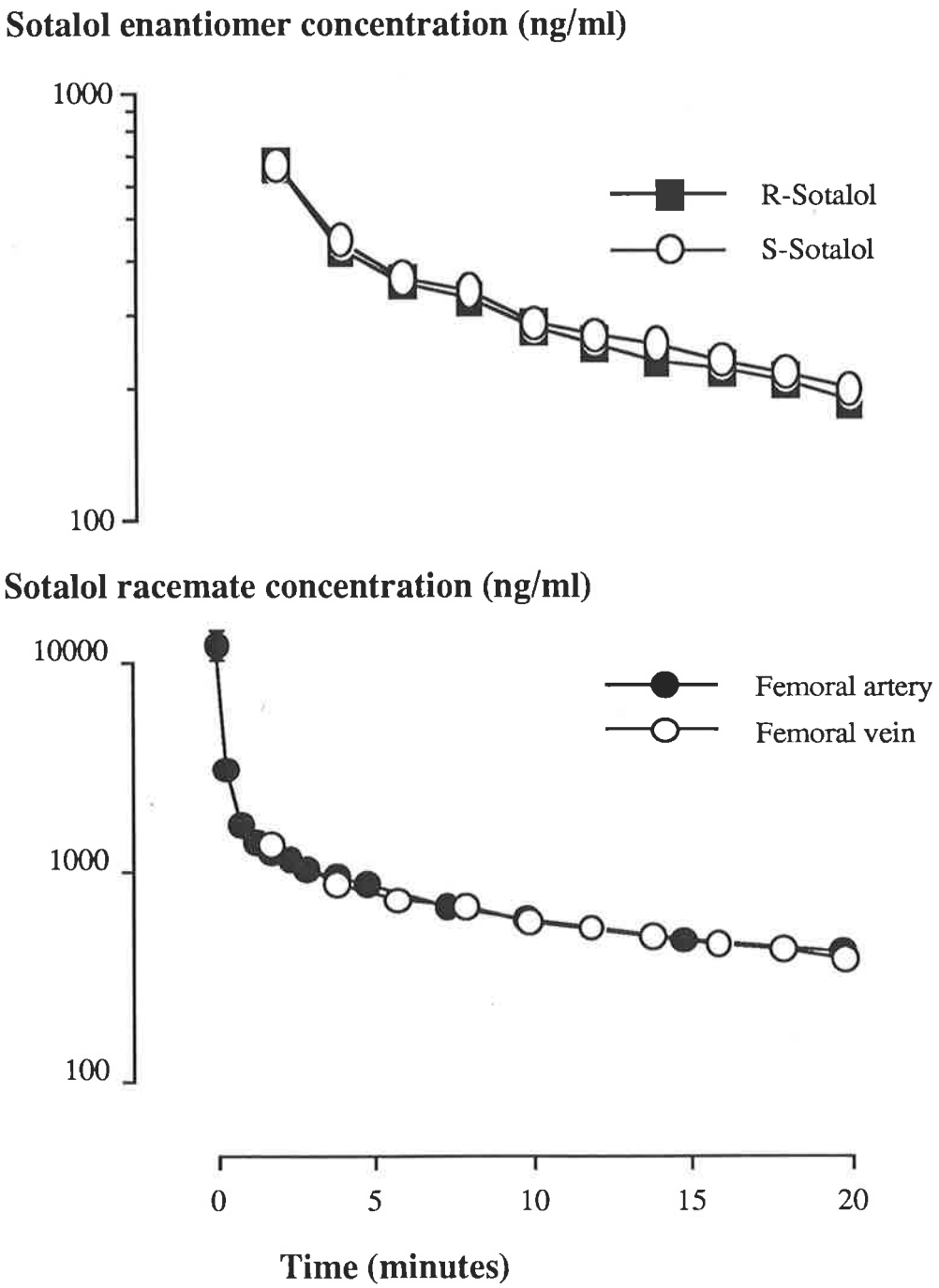


FIGURE 5.14 On the upper panel, the time course of R- and S-sotalol concentrations in femoral vein whole blood, and on the lower panel, the timecourse of the transvascular RS-sotalol concentration gradient in the femoral bed

5.3.10 Serial mechanical restitution curve construction

The potential for the negative inotropic effects of sotalol to be dependent on cycle length were examined utilizing serial construction of the *short-cycle length single-beat* mechanical restitution curve (MRC) before and at regular intervals after sotalol injection in 14 patients, shown in Figure 5.15. Prior to injection, LV+dP/dt (percent of the baseline pre-sotalol value) diminished with decreasing extrasystolic intervals (percent of the baseline pre-sotalol value), from 100% at a cycle length of 100%, to 73.9±6.3% at a cycle length of 62.0±1.7%. The negative inotropic effects of sotalol at baseline cycle length have already been established, with LV+dP/dt reduced to 89.2±3.0% of baseline at a cycle length of 100±0%, 10mins post administration (Figure 5.4). As the extrasystolic interval decreased, this negative inotropic effect was maintained, attaining contractile force of 70.2±5.9% at a cycle length of 63.0±2.2%. These results suggest that the negative inotropic effects of sotalol were constant at all RR intervals studied, and the curves before and after injection were approximately parallel. The effect of sotalol on this *short-cycle length single-beat* MRC appeared to be maximal at approximately 20mins after injection.

In 5 patients, the MRC was also examined in terms of an eight beat model, where the contractile force of the last beat in a train of eight beats of short duration, was plotted as a function of cycle length. This *short cycle length eight beat* MRC, obtained prior to and 20mins after injection, was subjected to a great degree of interindividual variation, to the extent that pooled results could not be obtained. These curves were either one of two essential shapes, with typical examples for each shape shown in Figure 5.16 in two indivdual patients. LV+dP/dt either decreased with decreasing RR intervals of the beats, producing a similar shape to that achieved with the single beat MRC (lower panel of Figure 5.16), or it increased with increasing RR intervals of the beats, similar to that seen in tachycardia (upper panel of Figure 5.16). Regardless of shape, the curve obtained following sotalol administration was always parallel and slightly below that of the pre-drug state for all 5 patients.

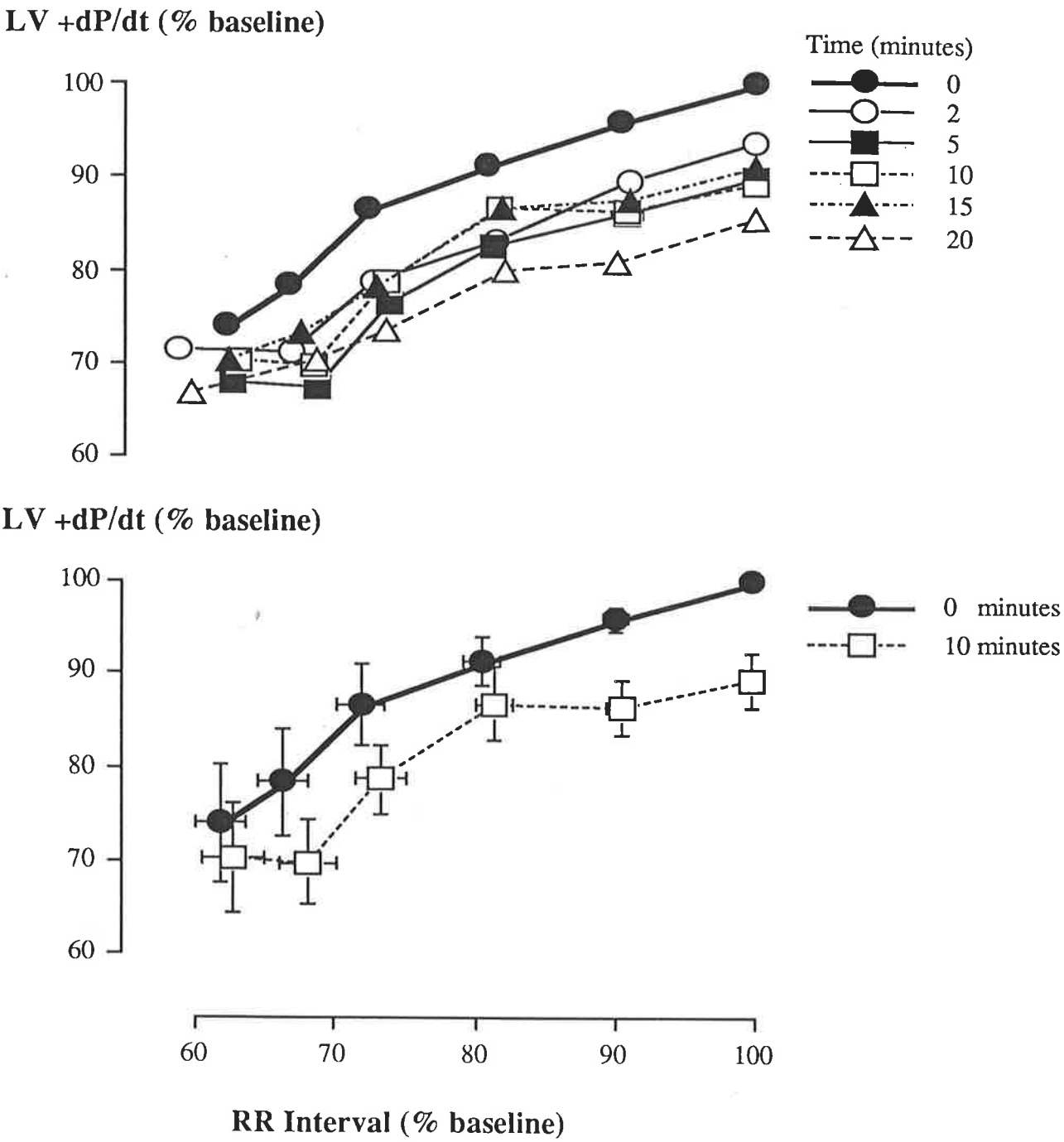


FIGURE 5.15 On the upper panel, the serial mechanical restitution curves obtained at all times prior to and after administration, and on the lower panel, the pre- and 10mins post-sotalol curves : for ease of presentation, SE's not shown on upper panel

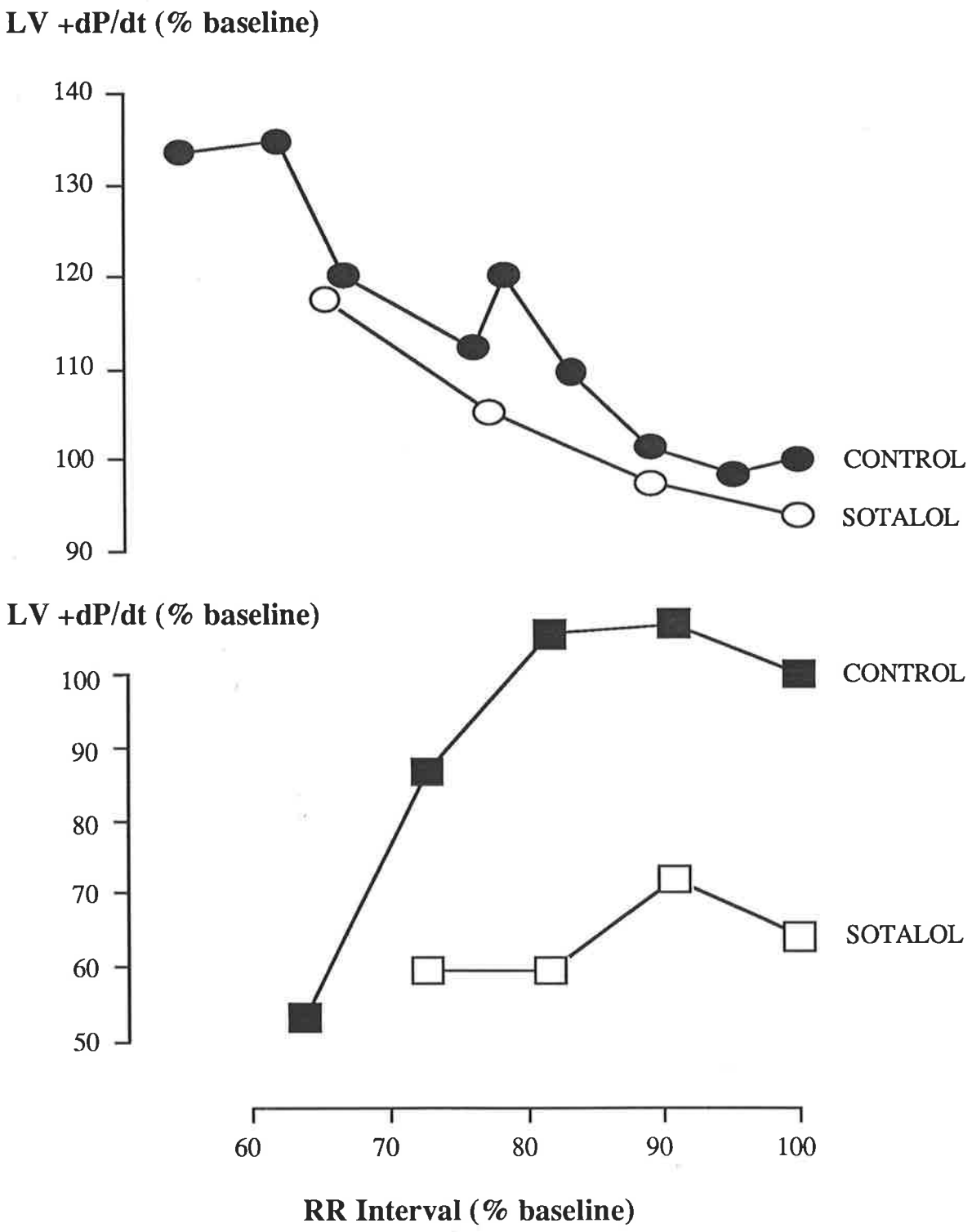


FIGURE 5.16 Typical examples of the two shapes of *short cycle length eight beat* mechanical restitution curves obtained at pre- and 20mins post-sotalol curves

In one of these patients, the*short-cycle length single-beat* mechanical restitution curve before sotalol injection was also examined at a faster pacing rate (cycle length 83% of baseline), described in Figure 5.17. At this cycle length, LV+dP/dt was 127%, gradually decreasing to 109% at 65% of baseline cycle length.

5.3.11 Post-extrasystolic potentiation without a compensatory pause

Examination of the degree of potentiation of the beat following the extrasystolic interval at each point of the MRC was also investigated before and up to 20min following sotalol administration. Termed post-extrasystolic potentiation (PESP), this phenomenon was examined utilizing a post-extrasystolic interval of 100%, ie in the absence of a compensatory pause. Despite the lack of a compensatory pause, the postextrasystolic beat was still potentiated. The influence of sotalol on PESP is illustrated in Figure 5.18. Prior to injection, LV+dP/dt (percent of the baseline pre-sotalol value) of the postextrasystolic beat was progressively augmented from 5.8±1.3% to 18.6±3.2% greater than baseline, as the extrasystolic intervalwas decreased from 90.3±0.9% to 62.3±2.0% of the baseline pacing cycle length.

The PESP curve obtained in the absence of sotalol appears to be a mirror image of the impairment of contractile performance of the extrasystolic beat, illustrated in Figure 5.15. Ten minutes after sotalol injection, LV+dP/dt of the postextrasystolic beat was again progressively augmented from 2.7±1.5% to 19.1±2.8% greater than baseline, as the extrasystolic interval was decreased from 91.3±0.8% to 61.2±1.7% of the baseline pacing cycle length. As portrayed in Figure 5.18, sotalol did not appear to influence postextrasystolic potentiation.

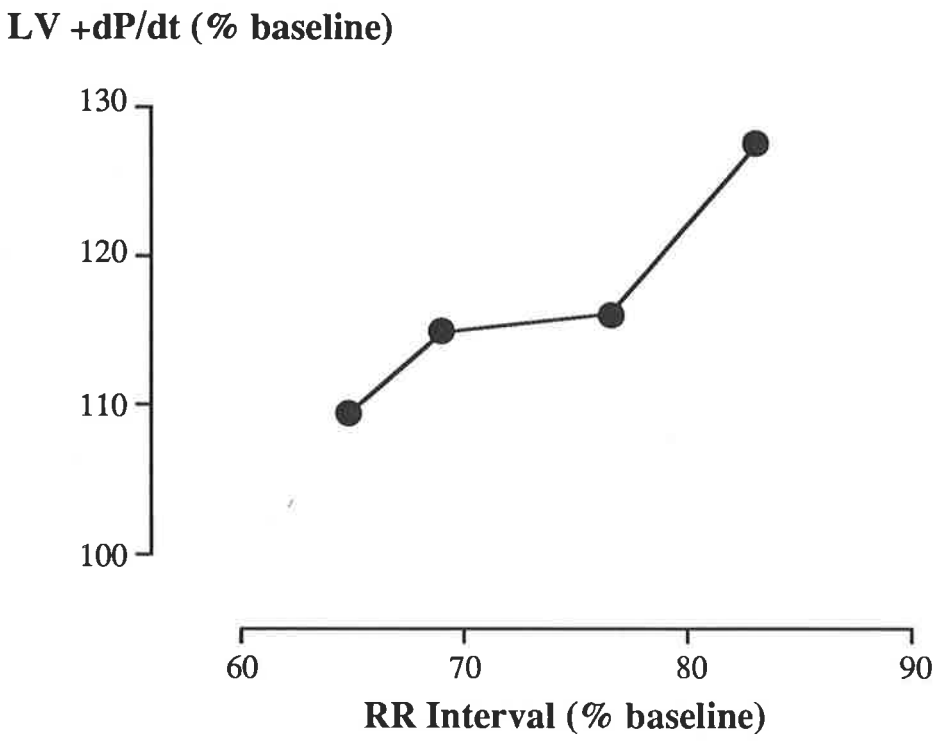


FIGURE 5.17 The mechanical restitution curve obtained at a cycle length of 83.3% of baseline in one patient in the absence of drug

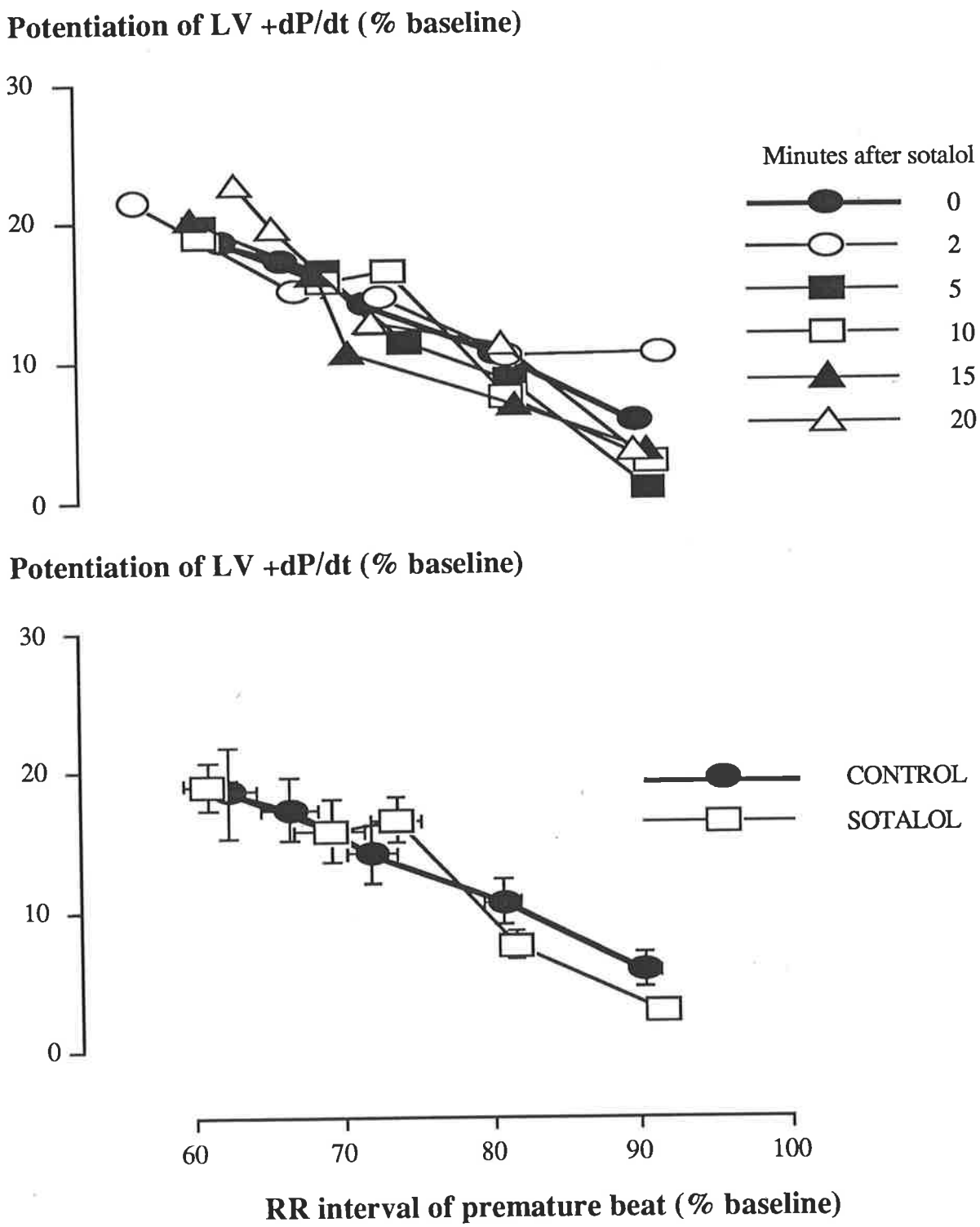


FIGURE 5.18 On the upper panel, the postextrasystolic potentiation curves obtained at all times prior to and after administration, and on the lower panel, the pre- and 10mins post-sotalol curves : for ease of presentation, SE's not shown on upper panel

5.3.12 Application of the curve-fitting model to mechanical restitution curves obtained post-sotalol injection

Values of c and the rate-dependence index (RDI; the ratio of c values obtained after and before sotalol administration) were determined utilizing the curve-fitting model of the MRC, described in Chapter 3 of this thesis, to determine the influence of extrasystolic interval on the negative inotropic effects of the drug. The time course of changes in these two parameters is illustrated in Figure 5.19. In 4 patients, the model failed to provide positive values for c , as the shortest extrasystolic interval obtainable in these patients was consistently >60% of the baseline cycle length: results are from the remaining 10 patients. Sotalol tended to increase both c and RDI, from 22.3±5.0% to 32.4±7.2% (p=NS), and 1 to 1.52±0.27 (p=NS) at 10min, the time of mean maximal effects. Sotalol failed to influence the asymptotes of the model, represented in Table 5.7. Goodness-of-fit of the quantitative model, the residual SD's, were 8.1 (5.7, 10.5 95% confidence intervals, n=10).

5.3.13 Rapid atrial pacing before and after sotalol injection

In an alternative method of assessing the potential for rate-related negative inotropic effects, in 11 selected patients with only mild angina, the effect of 60s of rapid atrial pacing on LV+dP/dt before and 10-20min after sotalol administration was examined. In this group of patients, the baseline cycle length was reduced from 799±31 to 517±18ms, representing an increased heart rate of 45±2beats/min. These results are shown on the upper panel of Figure 5.20. Prior to drug administration, onset of pacing produced a 16.8±5.9% increase in LV+dP/dt, which persisted for the duration of pacing : at 60s, LV+dP/dt was 20.0±4.1% of that prior to onset of pacing. After sotalol administration, pacing induced a 20.8±7.0% positive inotropic effect, which was not different to the increases in LV+dP/dt obtained prior to injection. Following 60s of rapid atrial pacing, LV+dP/dt gradually but nonsignificantly declined to 12.2±4.6% above that obtained prior to the onset of pacing, implying that perhaps LV+dP/dt was deteriorating as pacing progressed.

TABLE 5.7 Time course of the asymptotes of the MRC curve-fitting model

Minutes post sotalol	0	2	5	10	15	20	p
a	146±29	120±8	116±7	122±12	103±4	119±19	NS
d	-6±44	28±7	26±9	8±30	42±5	-1±28	NS

a, horizontal asymptote; d, vertical asymptote

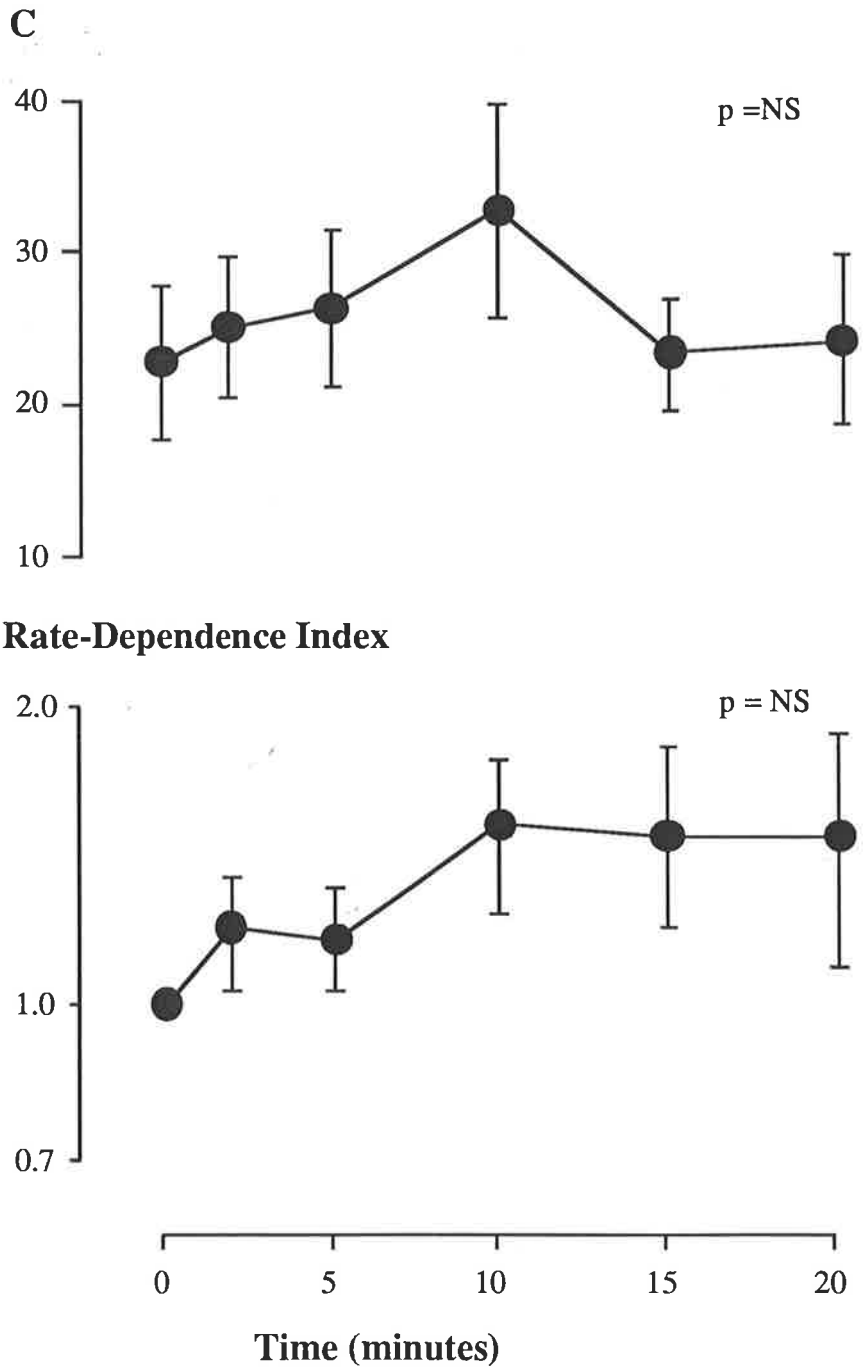


FIGURE 5.19 Time course of changes in *c* and the rate-dependence index (RDI) after sotalol

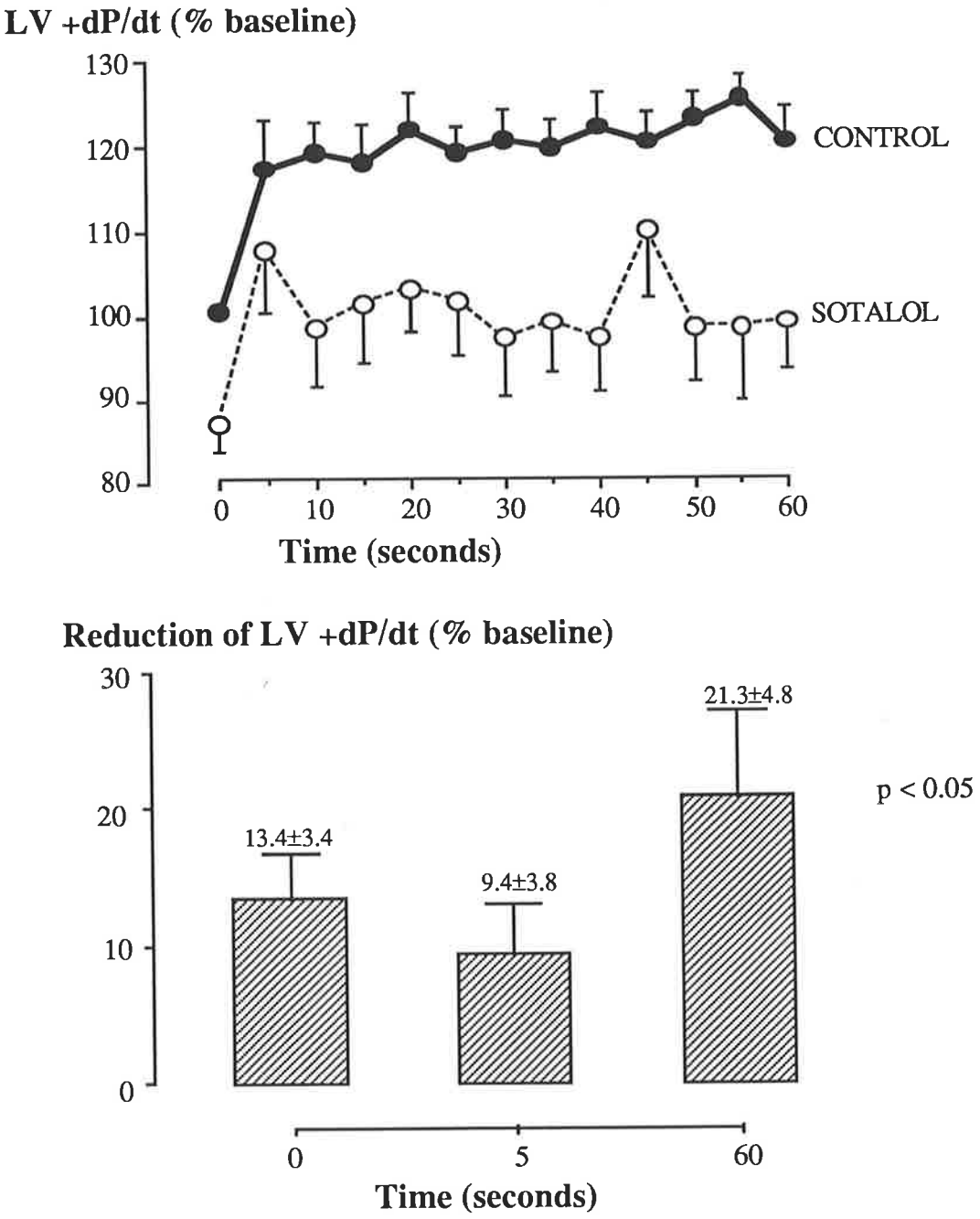


FIGURE 5.20 On the upper panel, the influence of 60s of rapid atrial pacing on LV+dP/dt, and on the lower panel, depression of LV+dP/dt before and after 5 and 60s of rapid atrial pacing

Considering specifically the sotalol-induced depression of LV+dP/dt, shown on the lower panel of Figure 5.20, sotalol induced a 13.4±3.4% negative inotropic effect at baseline cycle length, and 9.4±3.8% and 21.3±4.8% negative inotropic effects at 5 and 60s post institution of rapid pacing ($p<0.05$), indicating that the sotalol-induced depression of LV+dP/dt were progressively more marked during pacing-induced tachycardia.

The extent of sotalol-induced deterioration of dP/dt occurring with 60s of rapid atrial pacing, $\partial dP/dt_{PAC}$, was then calculated as the difference in sotalol-induced negative inotropic effects at 5 and 60s into the rapid pacing protocol :

$$\partial dP/dt_{PAC} = (control_{\partial 5} - sotalol_{\partial 5}) - (control_{\partial 60} - sotalol_{\partial 60})$$

where $control_{\partial 5}$ = increase in dP/dt at 5 seconds of pacing, prior to injection

$sotalol_{\partial 5}$ = increase in dP/dt at 5 seconds of pacing, post-sotalol

$control_{\partial 60}$ = increase in dP/dt at 60 seconds of pacing, prior to injection

$sotalol_{\partial 60}$ = increase in dP/dt at 60 seconds of pacing, post-sotalol.

Sotalol-induced deterioration in LV+dP/dt with rapid atrial pacing, $\partial dP/dt_{PAC}$, was -11.8±4.3% (range -42.0 to 6.6%), which was significantly less than zero ($p<0.02$), implying that deterioration in LV+dP/dt during rapid pacing with sotalol administration had indeed occurred in this group of patients. Thus, sotalol exerted negative inotropic effects which became somewhat more marked during sustained tachycardia, than at baseline.

5.3.14 Submaximal coronary vasodilator reserve

Submaximal coronary vasodilator reserve, a measure of the ability of the coronary vasculature to dilate with a certain submaximal increase in heart rate, was examined in 6 patients allocated to administration of intravenous sotalol. In this group of patients, the baseline cycle length was reduced from 798±60 to 519±27ms, representing an increased heart rate of 73±7%. Prior to injection, reserve was 1.33±0.20. Twenty minutes after injection, this had nonsignificantly fallen to 1.18±0.08 ($p=0.37$). Sotalol, at this dose at least, failed to influence submaximal coronary vasodilator reserve.

5.3.15 Summary of results

The major findings following an acute intravenous sotalol injection in the current study were :

(i) a reduction of LV+dP/dt at baseline heart rate, maximal at 11min;

(ii) a reduction of spontaneous heart rate, maximal at 10min;

(iii) a prolongation of PR intervals, maximal at 9min;

(iv) a prolongation of AH intervals, maximal at 10min;

(v) a prolongation of AV nodal effective refractory periods, maximal at 10min;

(vi) myocardial sotalol uptake of 2.1% of the dose peaking at 0.7min;

(vii) residual myocardial sotalol content at 17.5min 30% of maximal content;

(viii) accumulation of sotalol by the human myocardium was not enantioselective;

(ix) the extent of peak cardiac effects was not related to the extent of peak myocardial content;

(x) the time course of myocardial sotalol content and acute effects were not parallel;

(xi) the delay between content and effect was eliminated by predicting the amount of drug in a theoretical effect-site, using a pharmacokinetic/pharmacodynamic link model;

(xii) during the period of nett sotalol efflux from the myocardium, the drug redistributes to other vascular beds;

(xiii) redistribution of sotalol to other vascular beds was also not stereospecific;

(xiv) the negative inotropic effects of sotalol were preserved at isolated short extrasystolic intervals;

(xv) the negative inotropic effects of sotalol were preserved during trains of 8 beats of short cycle length;

(xvi) sotalol did not influence postextrasystolic potentiation studied in the absence of a compensatory pause;

(xvii) the negative inotropic effects of sotalol were more marked during pacing-induced tachycardia in patients with only mild angina

(xviii) no influence of sotalol was observed on submaximal coronary vasodilator reserve.

5.4 Discussion

The acute uptake of cardioactive drugs by the myocardium is a major predictor of the extent and time course of their pharmacodynamic effects (Horowitz and Powell 1986; Powell *et al* 1991b). The current study examined, for the first time in humans, myocardial uptake of sotalol, a common antiarrhythmic agent, and its relationship to the acute effects of the drug. Despite the limitation that technique used in the current study allowed only global estimation of myocardial sotalol content in the region drained by the coronary sinus, important information regarding the myocardial kinetics of this unique ß-adrenoceptor antagonist was acquired. The potential dependence of the negative inotropic effects of the ß-adrenoceptor-antagonist on rate in humans *in vivo* was also investigated for the first time.

Peak myocardial sotalol content, approximately 2.1% of the total injected dose of sotalol, was attained extremely rapidly, occurring 0.74min post administration. Subsequently, there was considerable nett efflux from the myocardium, with approximately 30% of peak content retained after 17.5 minutes.The femoral vascular bed was at least one site of redistribution during this time period. To date, sotalol myocardial uptake proceeds considerably more rapidly than for any other agent examined utilizing this technique, including verapamil, lignocaine, mexiletine, digoxin (Horowitz *et al* 1986; Powell *et al* 1990a, 1990b), and as demonstrated in Chapter 4 of this thesis, metoprolol. These findings with sotalol, which is a relatively water soluble ß-adrenoceptor antagonist (Arendt et al 1984), are in contrast to :

(i) the results of *in vitro* studies (Horowitz and Powell 1986), in myocardial accumulation was correlated with lipid solubility;

and (ii) the conventional pharmacokinetics of this drug (a longer elimination $t_{1/2}$ than metoprolol for example), which would imply a slower rate of absorption and elimination than observed by the human myocardium in the current study. From *in vitro* studies, in which lipophilicity determines myocardial drug uptake (Horowitz and Powell 1986), the uptake of metoprolol by the human myocardium would be expected to proceed more rapidly than that of the more hydrophilic sotalol. However, the current investigation demonstrated that the opposite was true. Possible explanation for this observation may include that sotalol, because of its lack

of lipophilicity, has fewer compartments within the heart to distribute into, and therefore equilibrates more rapidly.

Only limited information regarding sotalol distribution into the tissues in animals is currently available. However, uptake of sotalol by the feline cerebral cortex has been investigated, and was considerably lower than that of the more lipophilic propranolol (Arendt et al 1984). As lipophilicity should be an equally important determinant of rate and extent of myocardial drug accumulation (Horowitz and Powell 1986) as it is in the brain, a slow myocardial sotalol uptake, at least in feline models, could possibly be expected. The present investigation also demonstrated the acute myocardial kinetics of sotalol were not enantioselective : R- and S-sotalol displayed equivalent extent and trime course of absorption into and removal from the human myocardium, confirming previous findings in plasma (Carr *et al* 1992; Sallustio *et al* 1993).

In the current study, neither maximal myocardial sotalol uptake nor time to peak content were significantly influenced by the extent of fixed coronary artery disease or underlying LV systolic dysfunction in the present investigation. The finding that peak sotalol uptake is not affected by extent of fixed coronary artery disease of both normal and diseased hearts is in contrast with results obtained with verapamil, where peak myocardial content was inversely proportional to the extent of disease (Powell *et al* 1990b).

The predominant acute haemodynamic, ECG, and EP effects of a 20mg intravenous bolus of sotalol in the present investigation were :

(i) a progressive reduction of LV +dP/dt, peaking 11 minutes after injection;

(ii) significant diminution of spontaneous heart rate, maximal at 10 minutes;

(iii) prolongation of PR intervals at both fixed and spontaneous heart rates, maximal at 9 minutes;

and (iv) prolongation of AH intervals and AV nodal effective refractory periods, maximal at 10 minutes. All effects persisted for the duration of the study.

These findings were essentially consistent with those reported by previous investigators : after a single intravenous bolus dose (range 20-100mg), sotalol induced substantial reductions in spontaneous heart rate (range 11-23% reduction), and significantly prolonged PR (by 5-15%), QT (by 3-24%), and AH intervals (by 11-24%) at constant heart rate, and atrioventricular node effective refractory periods (by up to 25%), with minimal changes in mean arterial pressure, or HV intervals in a wide range of subjects, from healthy volunteers to patients with chronic atrial and ventricular dysrhythmias (Anttila *et al* 1976; Echt *et al* 1982; Ward *et al* 1979; Nathan *et al* 1982; Touboul *et al* 1984, 1987; McComb *et al* 1987; Huikuri *et al* 1992; Edvardsson *et al* 1980; Senges *et al* 1984; Nademanee *et al* 1985). In another study utilizing a similar dose, a significant negative inotropic effects, as demonstrated by reduced LV+dP/dt, was reported (Hutton *et al* 1972).

While the current study failed to demonstrate a significant prolongation of QT intervals at fixed heart rate, a definite but nonsignificant trend was observed with this 20mg dose, which is at the lower end of the range studied in the above-mentioned investigations. The influence of sotalol on action potential duration could not be examined in this study. Class III antiarrhythmic effects of the drug have been directly confirmed by prolongation of atrial and ventricular monophasic action potential duration at various stages of repolarization in patients following intravenous sotalol administration (Echt *et al* 1982; Edvardsson *et al* 1980).

The impairment of LV+dP/dt at the baseline pacing rate appeared to be constant at all extrasystolic intervals tested in construction of the short-cycle-length component of the MRC following sotalol administration (Figure 5.15). The parameter c , and hence the RDI, were not significantly influenced by sotalol administration (Figure 5.19). These findings statistically confirmed that the negative inotropic effects of the nonselective ß-adrenoceptor antagonist were not rate-related. Similar results were obtained utilizing the eight beat short-cycle-length MRC.

Reinforcing previous findings in the absence of drug administration, the contractile performance of the postextrasystolic beat (Figure 5.18) was augmented despite the lack of a compensatory pause (Chapter 3 of this dissertation; Hoffman *et al* 1956, 1965; Kuijer *et al* 1990; van der Werf *et al* 1976; Seed *et al* 1984), and was progressively potentiated with decreasing extrasystolic intervals (Seed *et al* 1984; Sung *et al* 1980). There was no trend for

any effect of sotalol on postextrasystolic potentiation, further confirming the lack of dependence of its negative inotropic effects on cycle length. Conversely, the negative inotropic effects of sotalol examined over one minute of rapid atrial pacing tended to be reduced initially, and then become progressively augmented (Figure 5.20), possibly implying a progressive deterioration in LV+dP/dt during pacing-induced tachycardia.

The influence of systolic interval on the negative inotropic effects of sotalol in the present investigation, which were more marked during tachycardia, are probably attributable to antagonism of ß-adrenoceptors. This is in contrast to the effect on the class III electrophysiologic effects reported by other investigators (Schmitt *et al* 1991, 1992; Lathrop *et al* 1989; Huikuri and Yli-Mayry 1992). Schmitt and coworkers observed reverse use-dependent effects of sotalol on the monophasic action potential duration in human right ventricle *in vivo* (Schmitt *et al* 1991). Similar results with sotalol, and the additional class III antiarrhythmic agent OPC-8212 in dog cardiac Purkinje fibres (Lathrop *et al* 1989; Schmitt *et al* 1992), and of d-sotalol in human right ventricle *in vivo* (Huikuri and Yli-Mayry 1992) have also been observed. Thus, the ß-adrenoceptor-antagonistic and the class III antiarrhythmic actions of sotalol are clearly functionally distinct.

One of the major hypotheses to be tested in the current study was that myocardial sotalol content is a direct determinant of the acute haemodynamic, ECG and EP effects of the drug. In contrast with the results of previous studies (Powell *et al* 1990b), peak myocardial sotalol content was not significantly correlated with peak changes in PR intervals, spontaneous heart rate, or LV+dP/dt, implying responsiveness to acute ß-adrenoceptor blockade in man is modulated extensively by factors which vary markedly between individuals. These may include changes in ß-adrenoceptor density and/or affinity as well as fluctuations in autonomic tone (Waagstein *et al* 1989; Colucci *et al* 1981; Fowler *et al* 1986). The influence of such modulating factors appears to have persisted despite prior withdrawal of ß-adrenoceptor antagonist therapy and attempted minimization of stress during the procedure.

Furthermore, peak haemodynamic, ECG and EP effects occurred considerably later than the time of peak myocardial sotalol content. The finding of hysteresis of sotalol efficacy on spontaneous heart rate (Figures 5.11 and 5.12) might theoretically reflect differential kinetics of

sotalol uptake into the sinus node and/or differential rates of onset of the effects of sotalol on central sympathetic mechanisms, although its relative lack of lipophilicity (Arendt *et al* 1984) would limit penetration of the blood : brain barrier, as demonstrated in in anaesthetized cats (Arendt *et al* 1984). These are not likely : from all data shown in Figures 5.11 and 5.12, the effects of sotalol on both atrioventricular nodal conduction and inotropic state also exhibit marked temporal fluctuations.

Definite anticlockwise hysteresis loops were constructed demonstrating the variable relationship between PR interval, spontaneous heart rate, and LV+dP/dt and myocardial sotalol content, suggesting that a distinct time interval must lapse prior to myocardial sotalol content eliciting peak effects. This time interval could not be explained in terms of a mediation of effect by a progressively generated metabolite, because sotalol has no active metabolites, and is predominantly excreted in the urine unchanged (Anttila *et al* 1976; Poirier *et al* 1981). The time course of interaction between a range of inotropic agents and cellular effector mechanisms has previously been shown to persist in the absence of diffusion barriers within myocardial cells and thus represents a true "biochemical lag phase" (Horowitz *et al* 1982; Barry *et al* 1985; Horowitz and Powell 1986). The time lag in the present study was also not attributable to stereoselective myocardial kinetics of sotalol, as no significant difference in the time course of MSC was observed between the R- and S-metoprolol enantiomers. The relationship between MSC and acute effects was analysed utilizing pharmacokinetic / pharmacodynamic link models. The amount of sotalol in the hypothetical effect compartment (Ce), postulated to directly determine acute myocardial effects (Holford and Sheiner 1981; Unadkat *et al* 1986), was linearly related to the acute effects of the drug.

Although the influence of hetereogenous sotalol effects on flow in ischaemic versus nonischaemic regions on myocardial sotalol uptake could not be examined in this model, no other study in humans to date has tackled this issue either. In a canine model of coronary partial occlusion-induced myocardial ischaemia, significant sotalol-induced increases in subendocardial blood flow, and the ratio of endocardial to epicardial flow, were observed in the ischaemic region following intravenous injection (Buck *et al* 1981b). Conversely, in the

nonischaemic region, sotalol elicited a significant reduction in tissue blood flow, without altering the ratio of endocardial to epicardial flow (Buck *et al* 1981b).

No information regarding uptake in patients during tachycardia could be derived from the present study, because content was only examined at fixed heart rates (70±3beats/min). Studies with both mexiletine and lignocaine (Horowitz *et al* 1986) indicated greater uptake of these agents by the human myocardium during periods of increased heart rate This finding suggests that myocardial uptake of metoprolol may also be enhanced in patients with tachycardia.

In conclusion, sotalol was rapidly and non-enantioselectively accumulated by the human myocardium, and was in no way impaired in patients with more severe ischaemic heart disease. Significant haemodynamic, ECG and EP effects were induced by this comparatively low dose, but were not maximal until some time later than peak myocardial content. Examination of the influence of short cycle length on the effects of sotalol on inotropic state demonstrated the reduction of LV+dP/dt elicited by sotalol was not modified by single premature beats. Incremental negative inotropic effects of sotalol during tachycardia tended to occur however, which may imply some limitations to the haemodynamic safety of sotalol in patients prone to tachyarrhythmias. Specifically, while sotalol greatly reduces the incidence of arrhythmias, if it fails to prevent an arrhythmia, the drug may not be as well tolerated as it is during sinus rhythm.

CHAPTER 6 :

ACUTE MYOCARDIAL UPTAKE OF MILRINONE : CORRELATION WITH ACUTE EFFECTS AND A BIOCHEMICAL MARKER IN HUMANS.

6.1 Backgrounds and aims

The phosphodiesterase III inhibitor milrinone exerts dual positive inotropic and vasodilatatory actions, and is administered intravenously as an alternative to catecholamines in the acute management of heart failure. However, little information regarding its myocardial kinetics following intravenous bolus injection are available. Conventional studies in normal individuals describe an elimination phase half-life of approximately 1h, and an apparent volume of distribution 0.3-0.4L/kg, whether orally or intravenously administered (Larsson *et al* 1986; Stroshane *et al* 1984a, 1984b). Longer elimination half-lives from 1.5 to 2.3h have been reported in patients with moderate to severe congestive heart failure (Cody *et al* 1984b; Stroshane *et al* 1984a, 1984b; Benotti *et al* 1984, 1985; Edelson et al 1986; Baim *et al* 1983; Likoff *et al* 1985). This drug has the shortest half-life and lowest volume of distribution of the three cardioactive agents examined in this thesis, suggestive of rapid elimination kinetics and reduced lipophilicity.

The aims of this chapter were :

(i) to specifically examine the process of acute myocardial milrinone uptake following intravenous bolus administration;

(ii) to determine the acute haemodynamic, ECG and EP effects of the drug in man;

(iii) to examine the time course of milrinone's phosphodiesterase III inhibition within the myocardium by measuring cyclic AMP concentrations in coronary sinus plasma;

(iv) to correlate myocardial content with these effects, in order to test the hypothesis: **"Myocardial milrinone content is a direct determinant of acute effect";** .

(v) to examine the potential dependence of the positive inotropic effects of milrinone on beat interval, utilizing the short-cycle-length component of the mechanical restitution curve (MRC) in humans *in vivo*, and the mathematical model described in Chapter 3 of this thesis, Determination of rate-related inotropic effects in humans

and (vi) to examine the influence of this novel ß-adrenoceptor antagonist on post-extrasystolic potentiation (PESP), for both post-extrasystolic intervals equal to, and greater than, the baseline cycle length.

6.2 Methods

Eleven patients undergoing diagnostic cardiac catheterization and coronary arteriography for the investigation of chest pain were selected for this study. The research procedure commenced at the end of the routine cardiac catheterization. The protocol for the research procedure was essentially as described in section 2.1.1 of this thesis : Protocol for cardiac catheterization for determination of myocardial drug uptake and measurement of haemodynamic effects in humans. However, an additional sample was drawn after 15s from the coronary sinus, to ensure myocardial drug uptake had indeed occurred. Also, the protocol was terminated at 15min in the majority of patients in view of the results of initial studies.

Myocardial milrinone uptake in this group of patients was calculated by determining the concentration of the drug in whole blood from femoral artery and coronary sinus, as described in section 2.2.3.2 of this thesis : HPLC quantitation of milrinone in human whole blood. Methodology developed for analysis of milrinone in human blood samples in the current investigation. The influence of milrinone on coronary venous plasma cAMP concentrations was determined utilising the methodology described in section 2.2.5 : RIA determination of cAMP concentrations in human plasma. The influence of hypoxia on the myocardial uptake profile of milrinone was not examined.

6.3 Results

6.3.1 Patient characteristics

In all, 11 male patients were studied, and their characteristics are summarized in Table 6.1. No patient had left ventricular systolic dysfunction, the ventriculography-derived ejection fraction consistently greater than fifty percent. Cardiac indices (CI) and baseline pulmonary capillary wedge pressure (PCWP) were all in the normal range, although 4 patients had CI below 2.5L/min/m^2. Haemodynamically significant coronary artery disease, defined by at least a fifty percent stenosis of one or more coronary arteries, was present in 7 of these patients. Baseline pacing rate throughout the study was at a mean cycle length of 753±24msec. Only 2 patients had not received antianginal therapy at any time prior to the investigation: 6 patients had been previously exposed to a ß-adrenoceptor antagonist, and 6 patients to a calcium channel antagonist, all of which were stopped at least five half-lives prior to the study. Nitrates had only ever been taken by 4 patients. The procedure was well tolerated and no patient developed adverse effects due to milrinone. Myocardial milrinone uptake was determined in 10 patients, while the potential for rate-related positive inotropic effects was explored in all patients in some form.

6.3.2 Acute haemodynamic effects of milrinone

The effects of milrinone on the various haemodynamic indices which were monitored after injection are summarized in Table 6.2, with the time course of these effects illustrated in Figures 6.1-6.4. The influence of milrinone on mean arterial (MAP), left ventricular systolic (LV SBP) and end-diastolic pressures (LV EDP) are illustrated in Figure 6.1. Milrinone did not alter LV EDP, but both LV SBP and MAP were significantly reduced by milrinone, with maximal reduction at 7.5±1.9 ($p<0.05$) and 8.2±1.1mins ($p<0.0001$) post administration respectively. Figure 6.2 portrays CI and coronary sinus flow (CSF) following milrinone intravenous bolus injection : neither were modified by milrinone administration.

TABLE 6.1 Patient characteristics prior to milrinone injection

Pt	Age (yr)	Sex	CI (L/min/m^2)	HR (beats/min)	LV EF %	PCWP (mmHg)	MAP (mmHg)	CAD	Prior Therapy
1	56	M	3.42	86	73	3	110	-	C
2	67	M	2.25	72	58	7	106	-	B, C, N
3	61	M	3.48	69	57	8	107	LAD,CIRC, RCA	-
4	65	M	2.73	78	72	6	86	-	B
5	44	M	2.75	78	64	5	108	-	C, N
6	43	M	4.27	80	65	10	128	LAD	B
7	72	M	2.09	53	56	13	91	LAD, CIRC	B
8	56	M	1.35	71	72	7	100	CIRC	C, N
9	61	M	2.57	62	72	10	125	LAD,CIRC, RCA	-
10	68	M	2.58	74	63	1	93	LAD	B, C, N
11	56	M	2.19	57	65	10	119	RCA	B, C
Mean	59		2.70	71	65	7	107		
SE	3		0.24	3	2	1	4		

B, ß-adrenoceptor antagonist; C, calcium channel antagonist; CAD, fixed coronary artery disease (>50% stenosis); CI, cardiac index; CIRC, left circumflex coronary artery; EF, ejection fraction; HR, heart rate; LAD, left anterior descending coronary artery; LV, left ventricle; M, male; MAP, mean arterial pressure; N, nitrate; PCWP, pulmonary capillary wedge pressure; Pt, patient; RCA, right coronary artery

TABLE 6.2 Acute haemodynamic effects of milrinone

Parameter (mean±SE)	Baseline value	Maximum change after milrinone	*p* value	Time (mins) of maximal effect
MAP (mmHg)	106±4	-7±1	0.0001	8.18±1.07
CI (L/min/m^2)	2.70±0.24	+0.56±0.17	NS	8.80±1.48
SVR (dynes.s.cm^{-5})	1650±150	-220±70	NS	8.64±1.41
LV+dP/dt ($mmHgs^{-1}$)	1500±100	+240±70	0.0005	7.80±1.16
LV SBP (mmHg)	142±7	-4±6	0.0363	7.45±1.94
LV EDP (mmHg)	15±2	-4±2	NS	4.77±1.21
CS flow (ml/min)	117±22	+1±16	NS	9.33±1.61
CVR (mmHg/ml/min)	1.15±0.18	-0.13±0.14	NS	8.30±1.51
Spontaneous HR (beats/min)	70.8±3.1	+7.0±2.1	0.0023	9.55±1.13

CI, cardiac index; CS, coronary sinus; CVR, coronary vascular resistance; HR, heart rate; LV+dP/dt, peak rate of rise of LV SBP; LV EDP, left ventricular end-diastolic pressure; LV SBP, left ventricular systolic blood pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance.

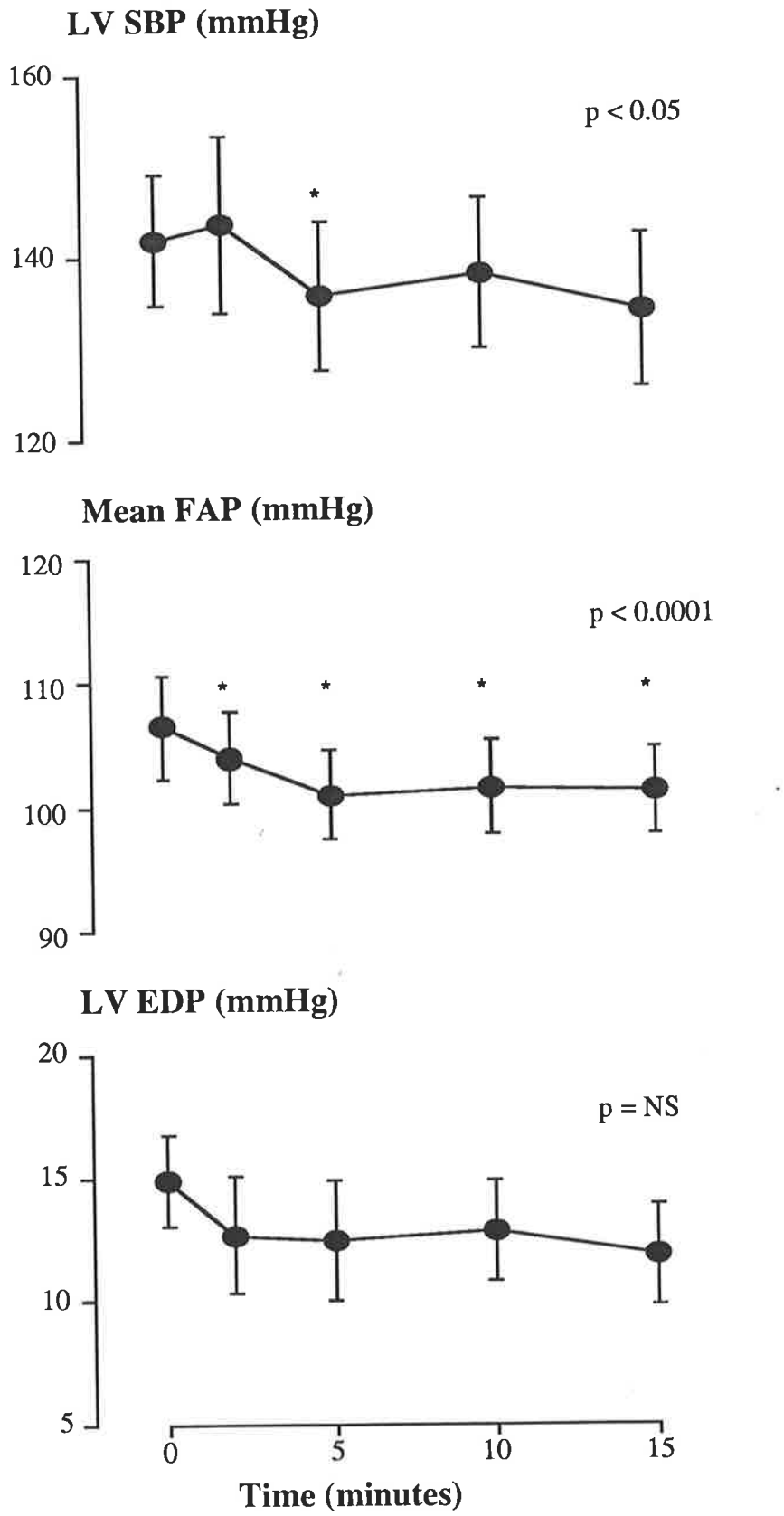


FIGURE 6.1 Time course of changes in left ventricular systolic (LV SBP), mean femoral arterial (mean FAP) and left ventricular end-diastolic (LV EDP) pressures for 15min after milrinone injection

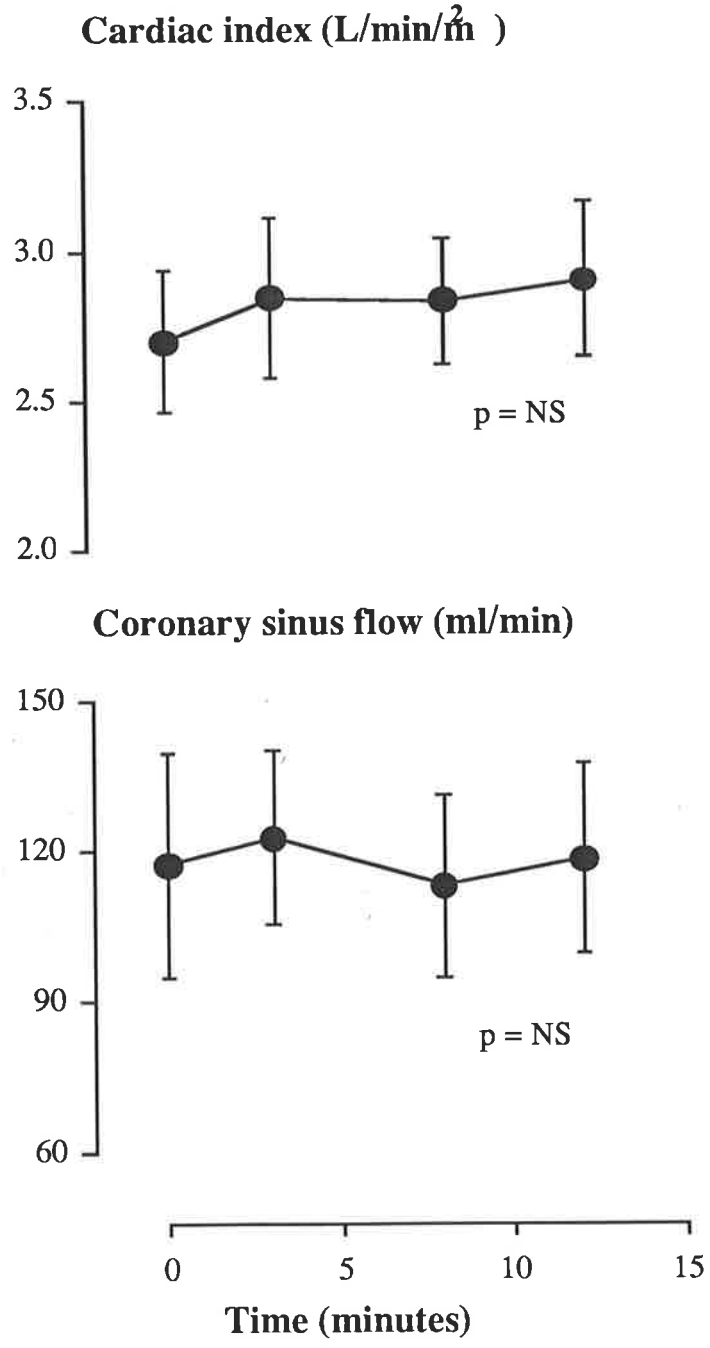


FIGURE 6.2 Time course of changes in cardiac index and coronary sinus flow for 15min after milrinone injection

The influence of the phosphodiesterase inhibitor on both systemic (SVR) and coronary vascular resistances (CVR) are illustrated in Figure 6.3. Although neither were significantly changed by milrinone, a nonsignificant trend for a reduction in SVR was seen (p=0.06), maximal 8.64±1.41mins post administration. Figure 6.4 shows the time course of spontaneous heart rate and LV+dP/dt (at fixed heart rate) following milrinone administration : both were significantly and markedly elevated, with maximal effects at 9.55±1.13 (p<0.005) and 7.80±1.16mins (p<0.0005) respectively.

6.3.3 Acute electrocardiographic effects of milrinone

The influence of milrinone on electrocardiograhic (ECG) intervals at either fixed or spontaneous heart rate are summarized in Table 6.3 and represented in Figures 6.5 and 6.6 respectively. Milrinone significantly reduced PR intervals at fixed heart rates, maximal at 6.68±1.50mins (p<0.05), without influence on PR intervals at spontaneous heart rates, or QT intervals.

6.3.4 Acute electrophysiologic effects of milrinone

The influence of milrinone on electrophysiologic (EP) intervals are also described in Table 6.3, and shown in Figures 6.7 and 6.8. Milrinone did not significantly modify AH or HV intervals, or the effective refractory periods of the atrioventricular node (AVN ERP) or right ventricle (RV ERP). Nonsignificant trends for reductions in both AH intervals (p=0.14) and AVN ERP (p=0.12) were observed, maximal at 7.84±1.61 and 4.75±1.19mins respectively.

6.3.5 Influence of milrinone on plasma cAMP concentrations

The influence of milrinone on plasma cAMP concentrations was investigated in 9 patients in coronary sinus (CS) samples for 15min, and is shown on the upper panel of Figure 6.9. Milrinone failed to significantly change plasma CAMP concentrations in the coronary sinus. A nonsignificant trend for lower concentrations of the nucleotide in CS (18.1±1.5nM) compared to the femoral artery (FA : 20.4±2.4nM) was observed at baseline (p=0.11).

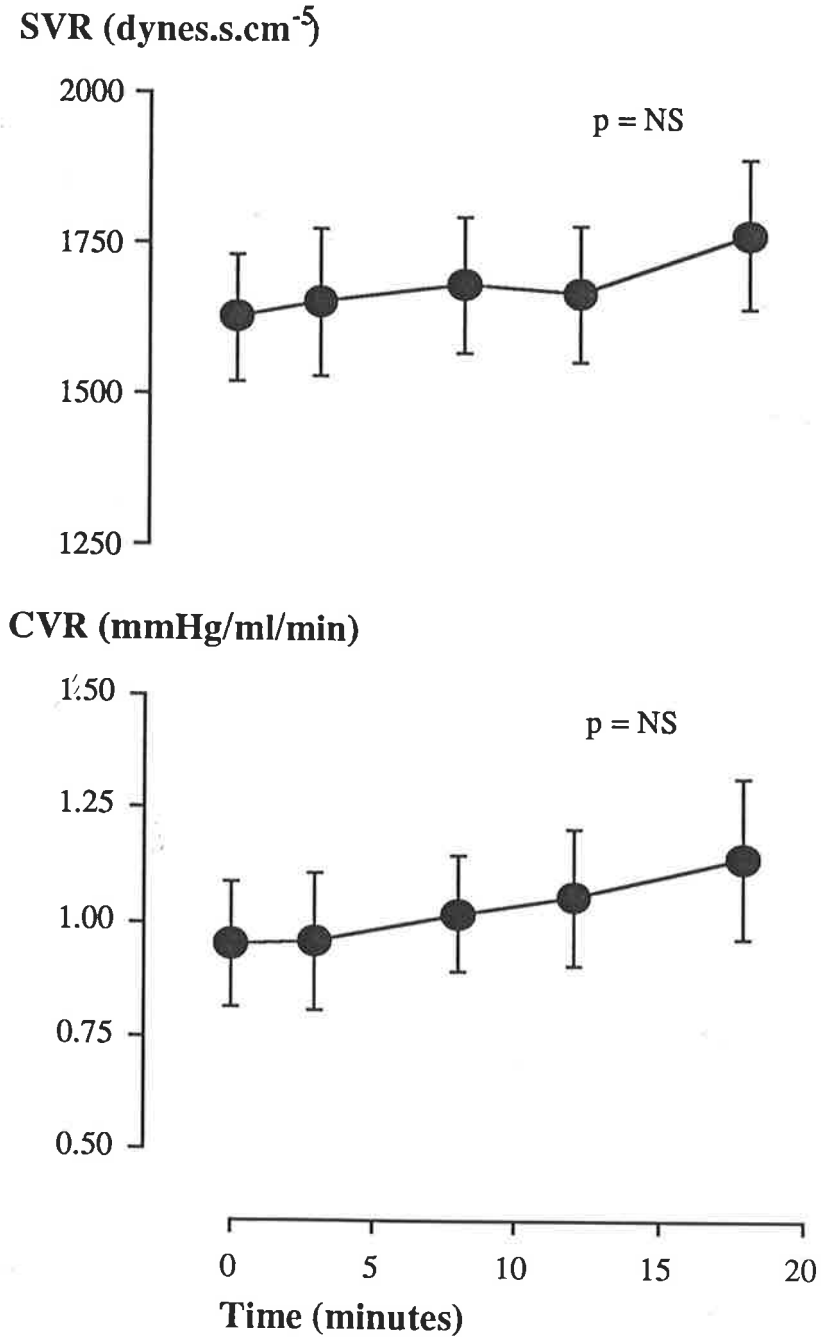


FIGURE 6.3 Time course of changes in systemic (SVR) and coronary (CVR) vascular resistances for 15min after milrinone injection

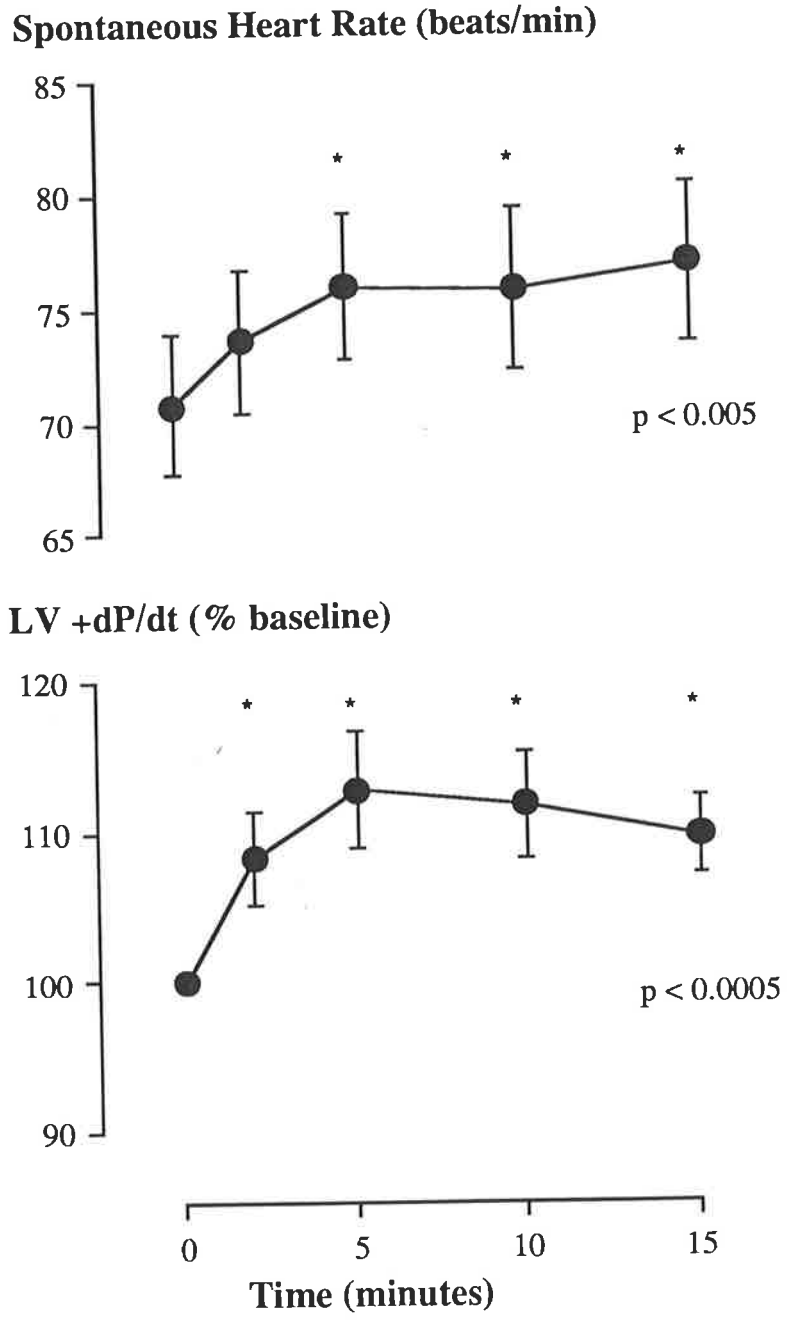


FIGURE 6.4 Time course of changes in spontaneous heart rate and LV+dP/dt for 15min after milrinone injection

TABLE 6.3 Electrocardiographic and electrophysiologic effects of milrinone

Parameter (mean±SE)	Baseline value	Maximum change after milrinone	*p* value	Time (mins) of maximal effect
Paced PR interval (msec)	194±8	-14±4	0.0102	6.68±1.50
Paced QT interval (msec)	367±7	-8±6	NS	8.83±1.76
Spontaneous PR interval (msec)	212±4	-7±5	NS	7.55±1.53
Spontaneous QT interval (msec)	369±5	+6±6	NS	9.89±1.53
AH interval (msec)	79.5±12.4	-4.4±3.0	NS	7.84±1.61
HV interval (msec)	49.3±3.2	-1.7±1.8	NS	8.82±1.84
AVN ERP (msec)	321±15	-18±8	NS	4.75±1.19
RV ERP (msec)	254±8	-9±7	NS	6.95±2.28

AVN ERP, atrioventricular nodal effective refractory period; RV ERP, right ventricular effective refractory period

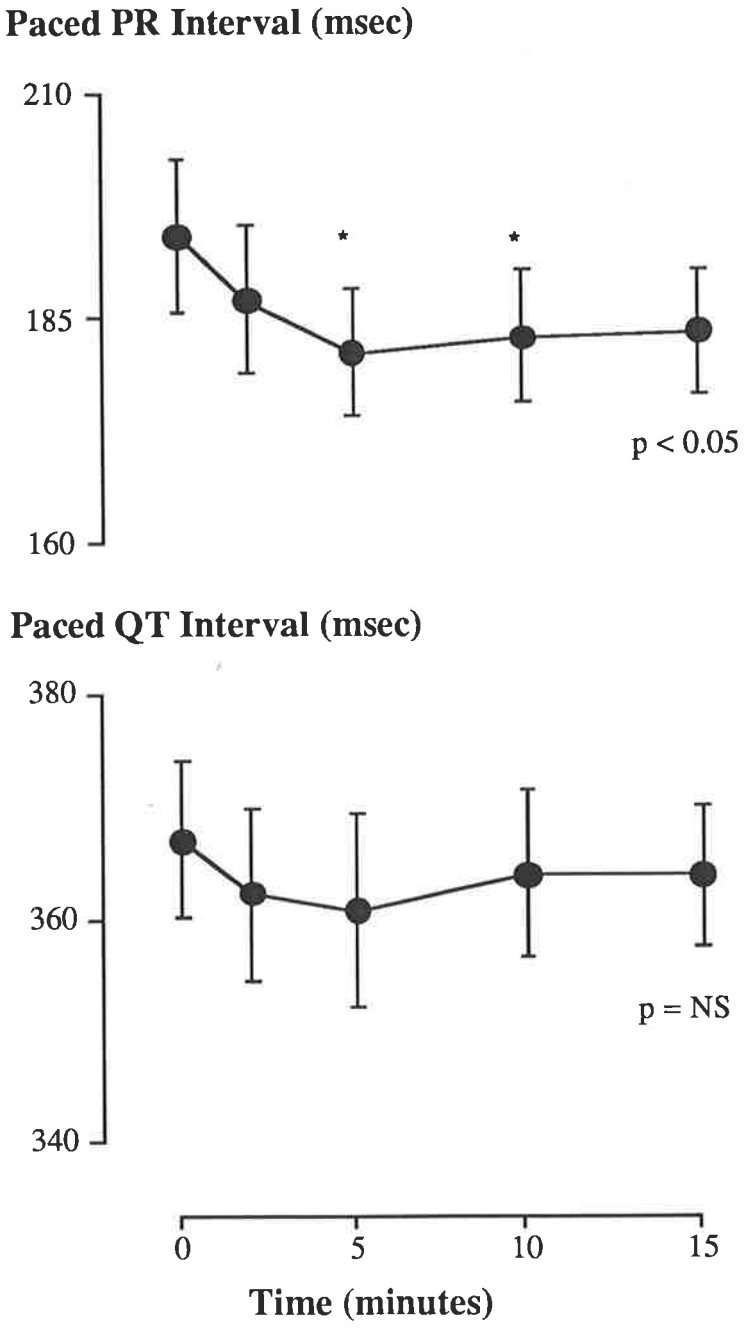


FIGURE 6.5 Time course of changes in electrocardiographic intervals at fixed heart rate for 15min after milrinone injection

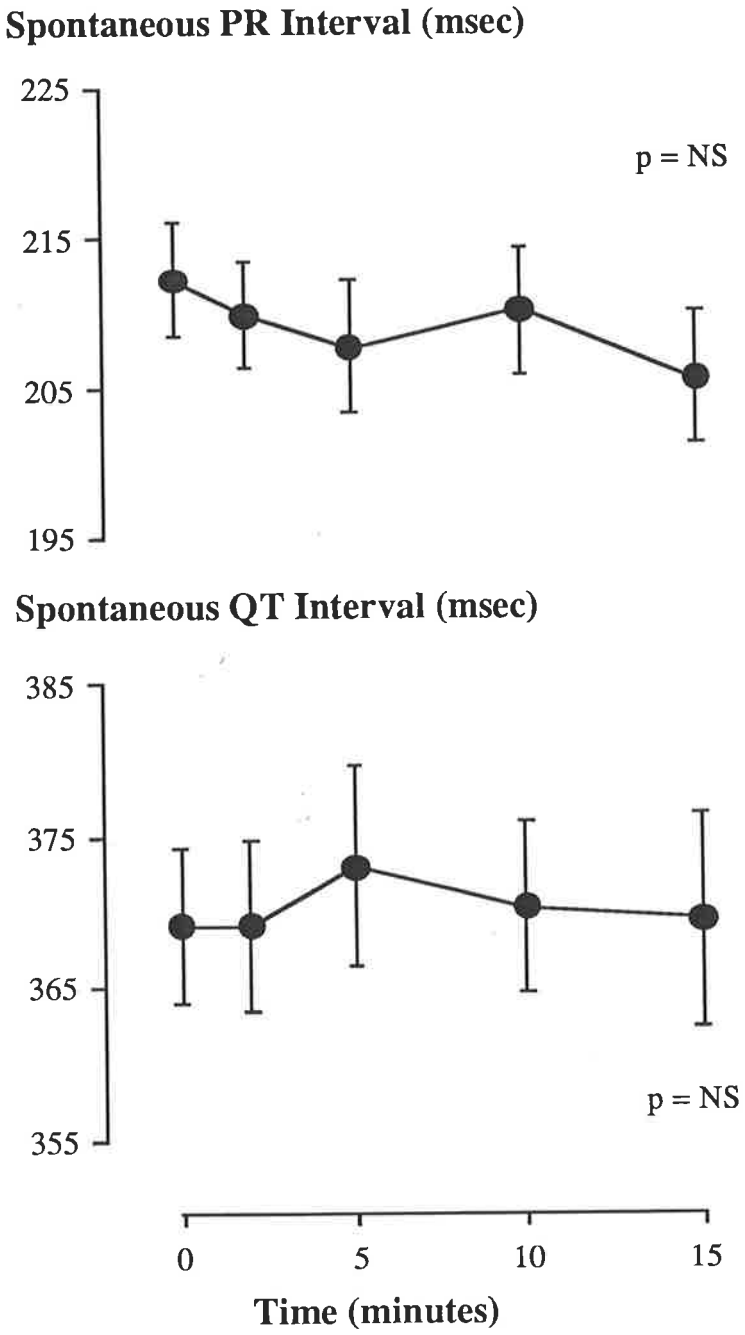


FIGURE 6.6 Time course of changes in electrocardiographic intervals at spontaneous heart rate for 15min after milrinone injection

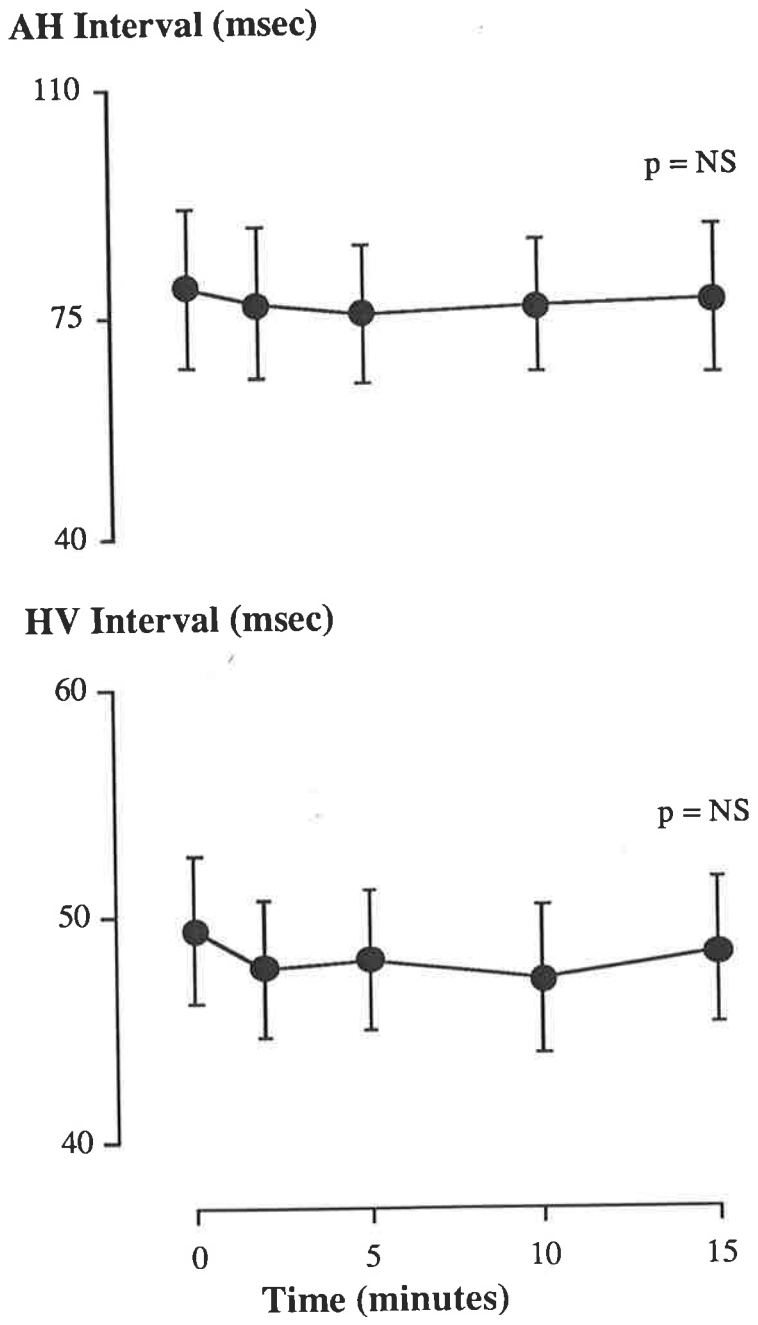


FIGURE 6.7 Time course of changes in electrophysiologic intervals for 15min after milrinone injection

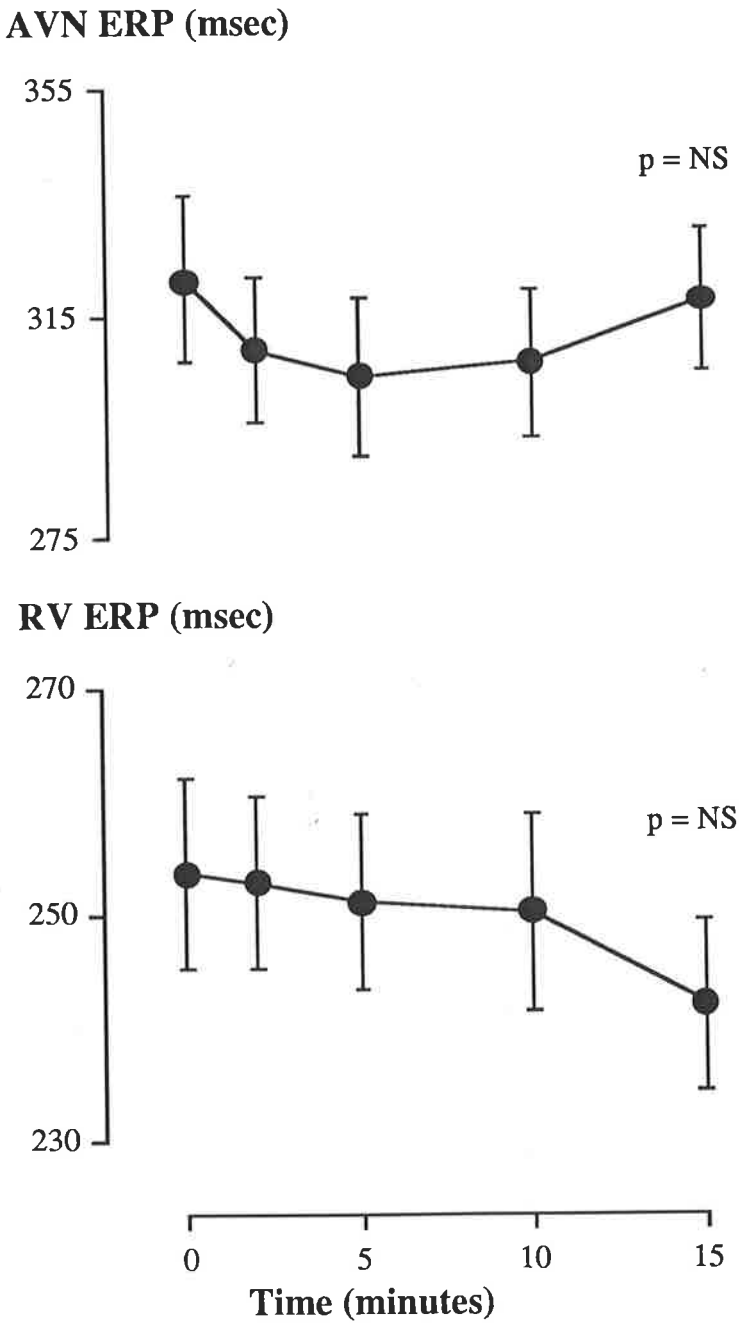


FIGURE 6.8 Time course of changes in atrioventricular (AVN ERP) and right ventricular (RV ERP) effective refractory periods for 15min after milrinone injection

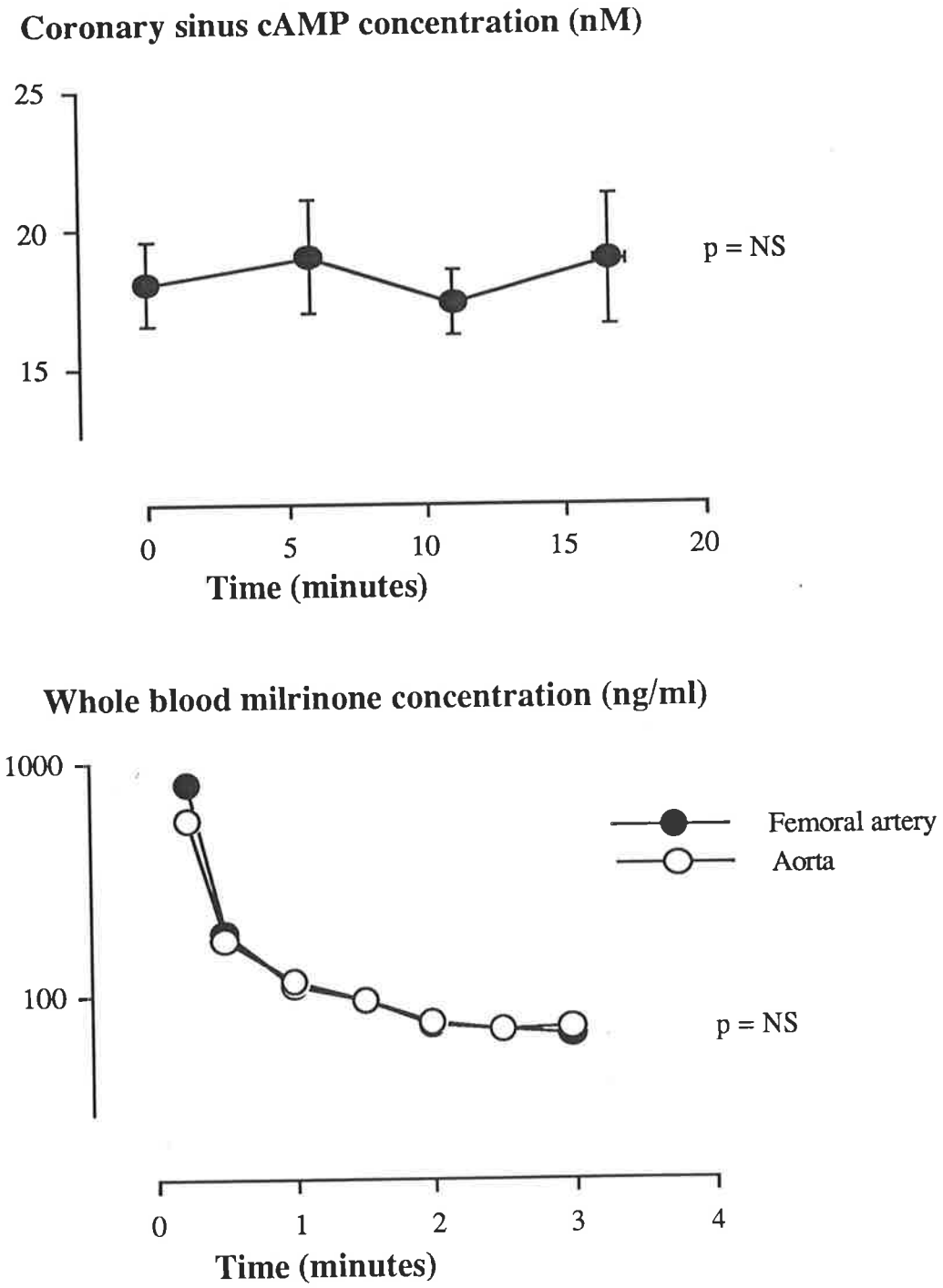


FIGURE 6.9 On the upper panel, the time course of changes in cAMP concentrations in coronary sinus plasma samples up to 15min in 9 patients, and on the lower panel, the time course of changes in whole blood milrinone concentrations in FA and aorta samples up to 3min in 1 patient, after milrinone injection

6.3.6 Validity of utilization of femoral arterial milrinone concentrations as a surrogate for those in the aorta

The validity of utilizing FA milrinone concentrations as a surrogate for those in the aorta (Ao) was assessed in one patient following intravenous bolus administration. As demonstrated in Figure 6.10, whole blood milrinone concentrations in the femoral artery and the aorta did not differ significantly (mean difference 2.48±4.15%, p=NS). The discrepancy between FA and Ao concentrations initially (the first Ao sample was approximately 30% different from the paired FA sample) may be related to the void volume of the sampling catheter positioned in the Ao in this patient.

6.3.7 Acute myocardial milrinone uptake

The concentration / time profile of milrinone in 10 patients after intravenous bolus injection in both the FA and CS whole blood is illustrated in the upper panel of Figure 6.10. FA milrinone whole blood concentrations were initially high, 900±154ng/ml at 0.26±0.01mins after injection. Arterial levels then rapidly declined to 25±2ng/ml at 15.2±0.1mins. Conversely, CS milrinone concentrations were considerably lower initially, 31.6±18.7ng/ml at 0.21±0.02mins, then extremely rapidly reaching a peak of 236±34ng/ml at 0.47±0.01mins, before also declining, to 24.7±2.7ng/ml at 15.3±0.3mins.

The time course of myocardial milrinone uptake (MMU) is shown on the lower panel of Figure 6.10. The initially large difference between FA and CS milrinone concentrations corresponded to markedly rapid nett uptake of the phosphodiesterase inhibitor by the human myocardium. Peak myocardial milrinone content (MMC), the timepoint at which whole blood concentrations in the FA and CS were equal, was also rapidly observed, at 0.56±0.06mins post administration, and was 182±32ng per ml/min when corrected for resting CS flow, representing 1.89±0.30% of the total injected dose.

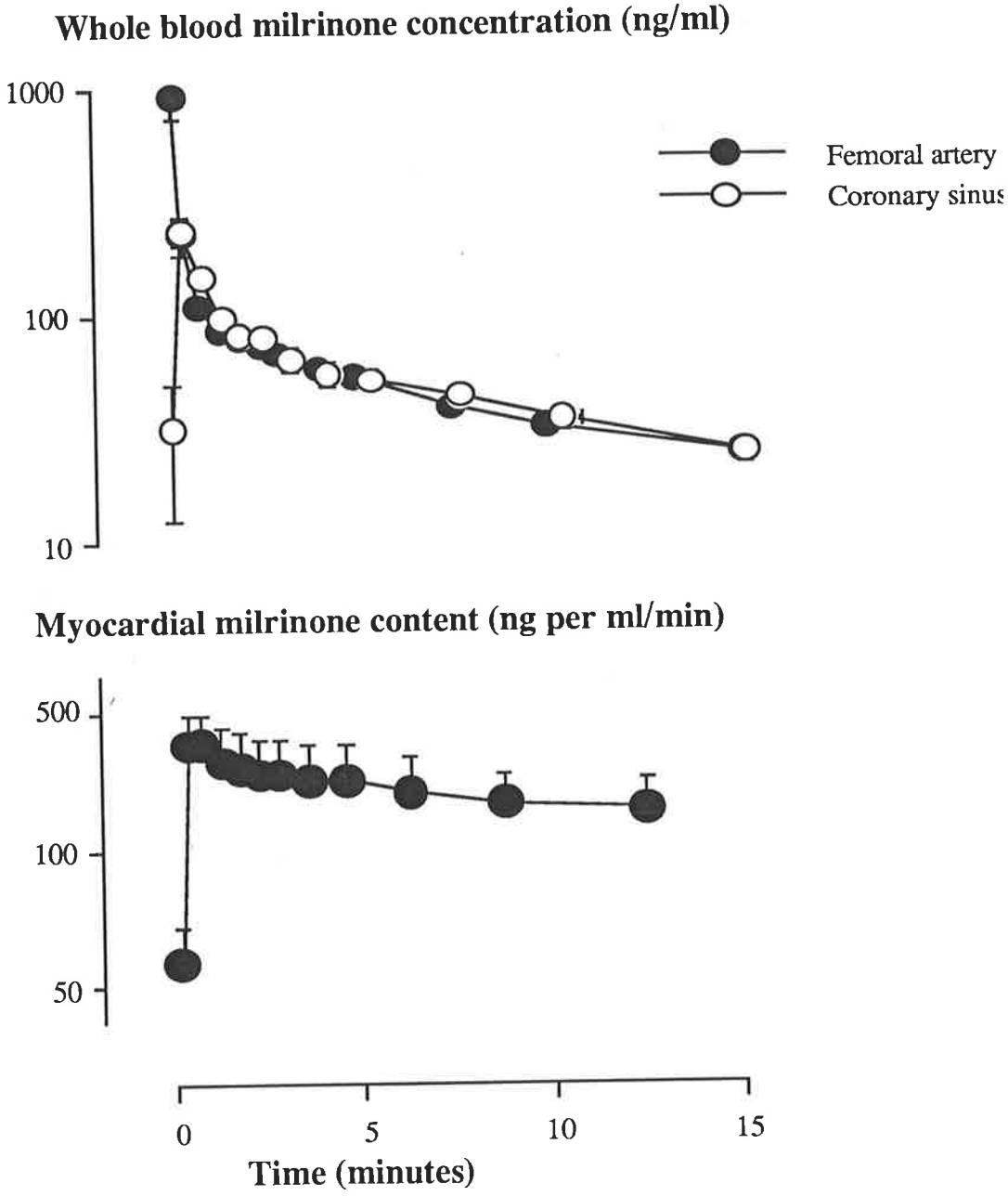


FIGURE 6.10 On the upper panel, the time course of milrinone concentrations in FA and CS, and on the lower panel, the time course of myocardial milrinone (relative to resting CS blood flow), for 15mins after milrinone injection

The characteristics of MMU in the individual patients, and their corresponding clinical characteristics at baseline, are listed in Table 6.4. Generally, the extent of peak MMC, or the time taken to achieve peak MMC (t_{max}), were not significantly dependent on individual patient characteristics at baseline, including resting cardiac index, ejection fraction, or the extent of fixed coronary artery disease in major branches of the left coronary artery. Subsequently, the transcoronary concentration gradient was slightly but consistently negative, indicative of a slow but progressive milrinone nett efflux phase from the myocardium. Thus, myocardial milrinone efflux was biphasic, with residual MMC 123±22ng per ml/min, or 69.1±5.7% of maximal content, retained at 12.5±0.0mins post administration.

6.3.8 Correlation between myocardial milrinone content and acute effects

As described in section 2.5 of this thesis, correlations were sought between :

(i) extent of maximum MMC and extent of maximum effects;

and (ii) the time course of MMC with that of the acute effects of the drug. Milrinone significantly reduced PR intervals and induced marked positive inotropic and chronotropic effects. However, the maximum changes in these parameters were not significantly correlated with peak MMC, shown in Figure 6.11. The extents of the positive chronotropic and inotropic effects were nonsignificantly related to maximal MMC ($p=0.07$ and $p=0.17$). Peak effects of the drug were attained much later than 0.6mins, time of maximum MMC.

Figure 6.12 describes the time course of these changes relative to simultaneous MMC. Significant fluctuations in the relationship between effect and myocardial content was observed for milrinone's acute effects, indicative of marked hysteresis between MMC and effect : the ratio of effect / content tended to increase over the first 6mins of the study, before reaching a plateau. The relationship between haemodynamic and ECG effects and the corresponding MMC for 15mins following milrinone administration is shown in Figure 6.13. This permits visual examination of the potential for hysteresis between peak content and effects, as suggested by the anticlockwise loops, perhaps representing a lag time for the onset and offset of effect due to drug-receptor interactions or bioconversion to second messenger compounds.

TABLE 6.4 Comparison of the individual patient characteristics of myocardial milrinone uptake with corresponding clinical characteristics at baseline

Patient	t_{max} mins	C_{max} ng/ml/min	C_{max}% %	CAD #	Age yrs	Sex	CI L/min/m^2	HR beats/min	LV EF %	PCWP mmHg	MAP mmHg
1	0.38	146	1.79%	0	56	M	3.42	86	73%	3	110
2	0.39	205	2.03%	0	67	M	2.25	72	58%	7	106
3	0.75	415	3.61%	2	61	M	3.48	69	57%	8	107
4	0.75	251	2.44%	0	65	M	2.73	78	72%	6	86
5	0.36	184	2.04%	0	44	M	2.75	78	64%	5	108
6	0.38	90	2.64%	1	43	M	4.27	80	65%	10	128
7	0.75	114	1.93%	2	72	M	2.09	53	56%	13	91
8	0.75	187	0.76%	1	56	M	1.35	71	72%	7	100
9	0.38	182	1.37%	2	61	M	2.57	62	72%	10	125
10	0.75	46	0.34%	1	68	M	2.58	74	63%	1	93
Mean	0.56	3.38	1.89%		59		2.75	72	65%	7	105
SE	0.06	0.41	0.30%		3		0.26	3	2%	1	4

CAD, fixed coronary artery disease in major branches of the left coronary artery (>50% stenosis); CI, cardiac index; C_{max}, peak myocardial milrinone content; C_{max}%, peak myocardial milrinone content as a percent of the total injected dose; EF, ejection fraction; HR, heart rate; LV, left ventricle; M, male; MAP, mean arterial pressure; PCWP, mean pulmonary capillary wedge pressure; t_{max}, time of peak myocardial milrinone content

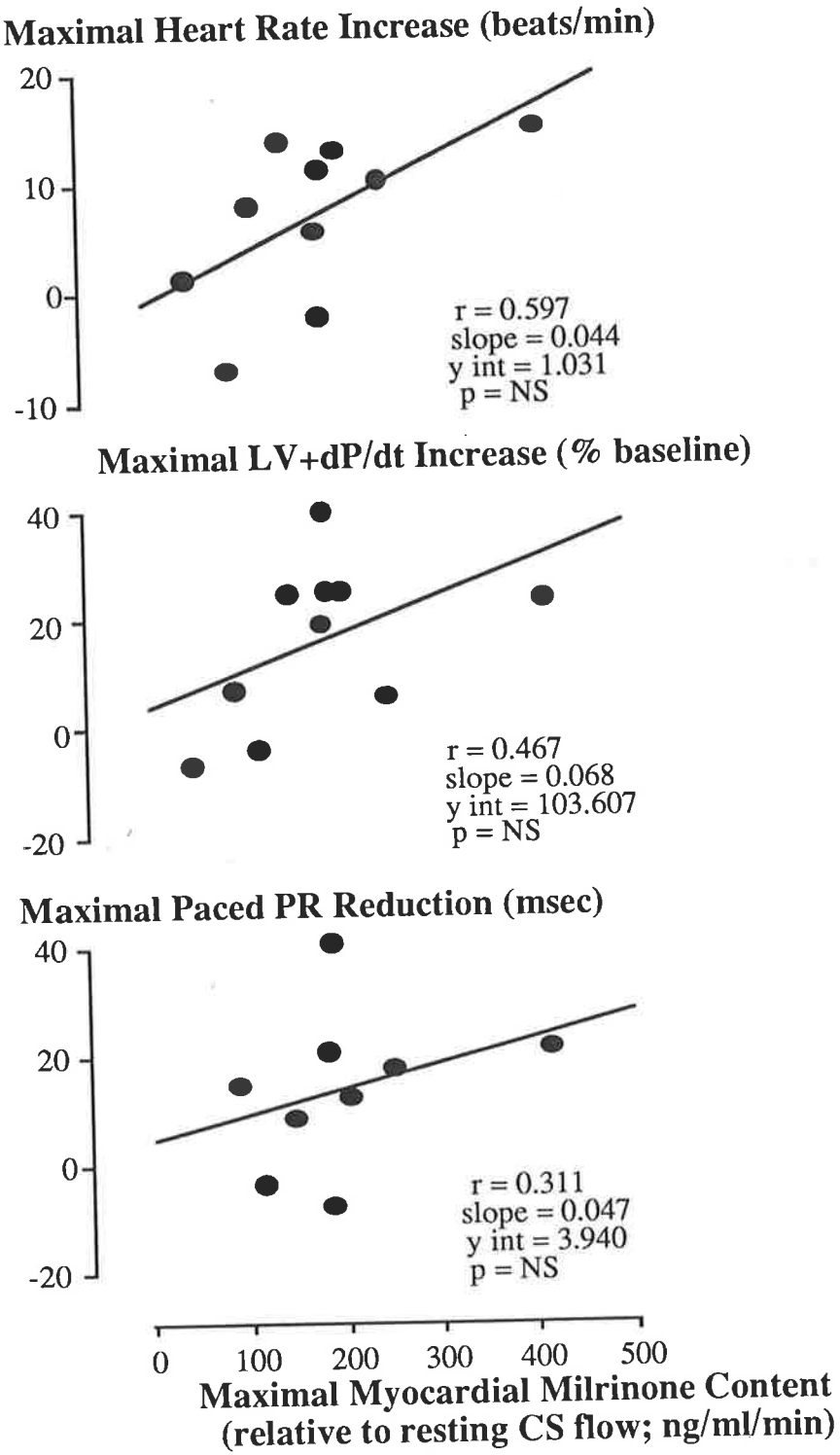


FIGURE 6.11 Individual maximal milrinone effects on heart rate, LV+dP/dt and paced PR intervals as a function of individual peak myocardial milrinone content (relative to resting CS flow)

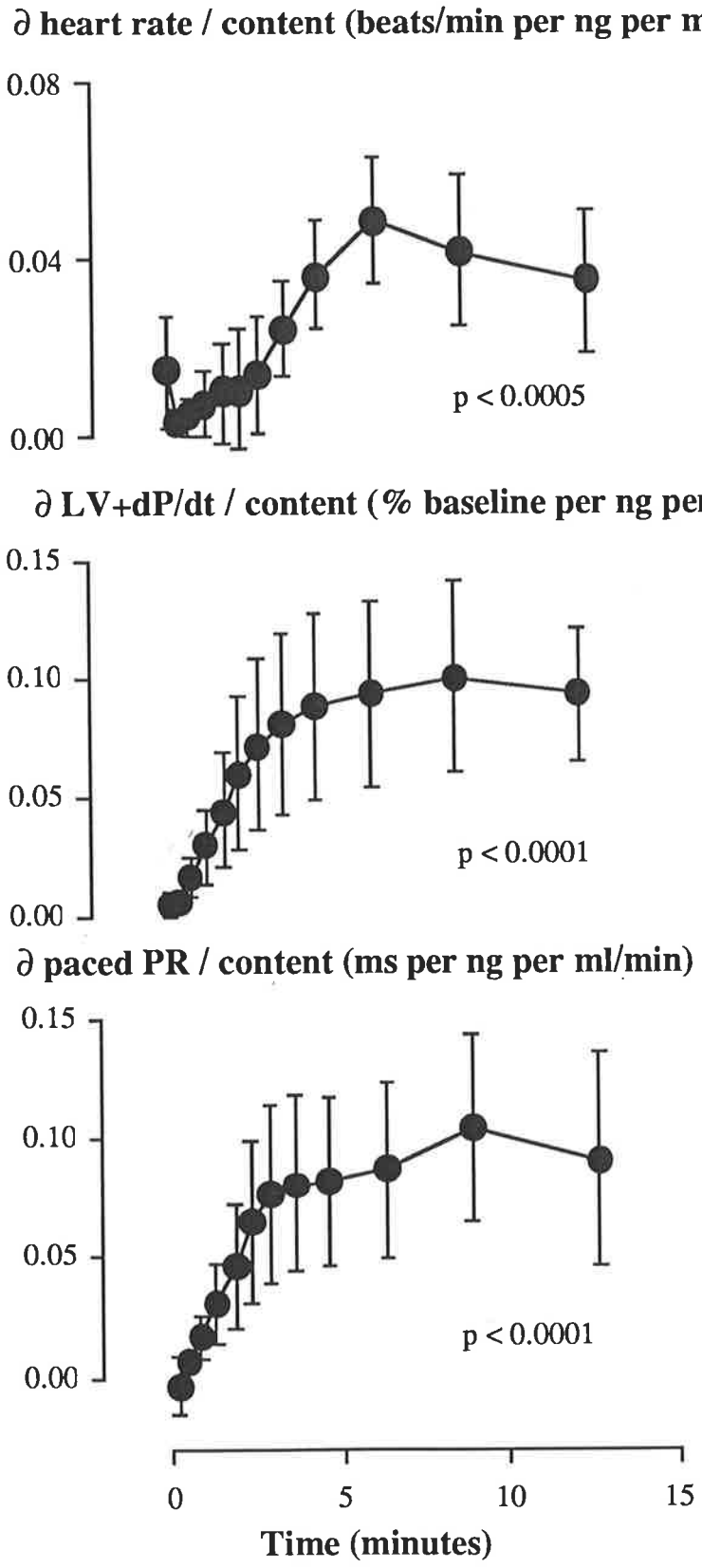


FIGURE 6.12 Time course of the ratio of effect and myocardial milrinone content (relative to resting CS flow)

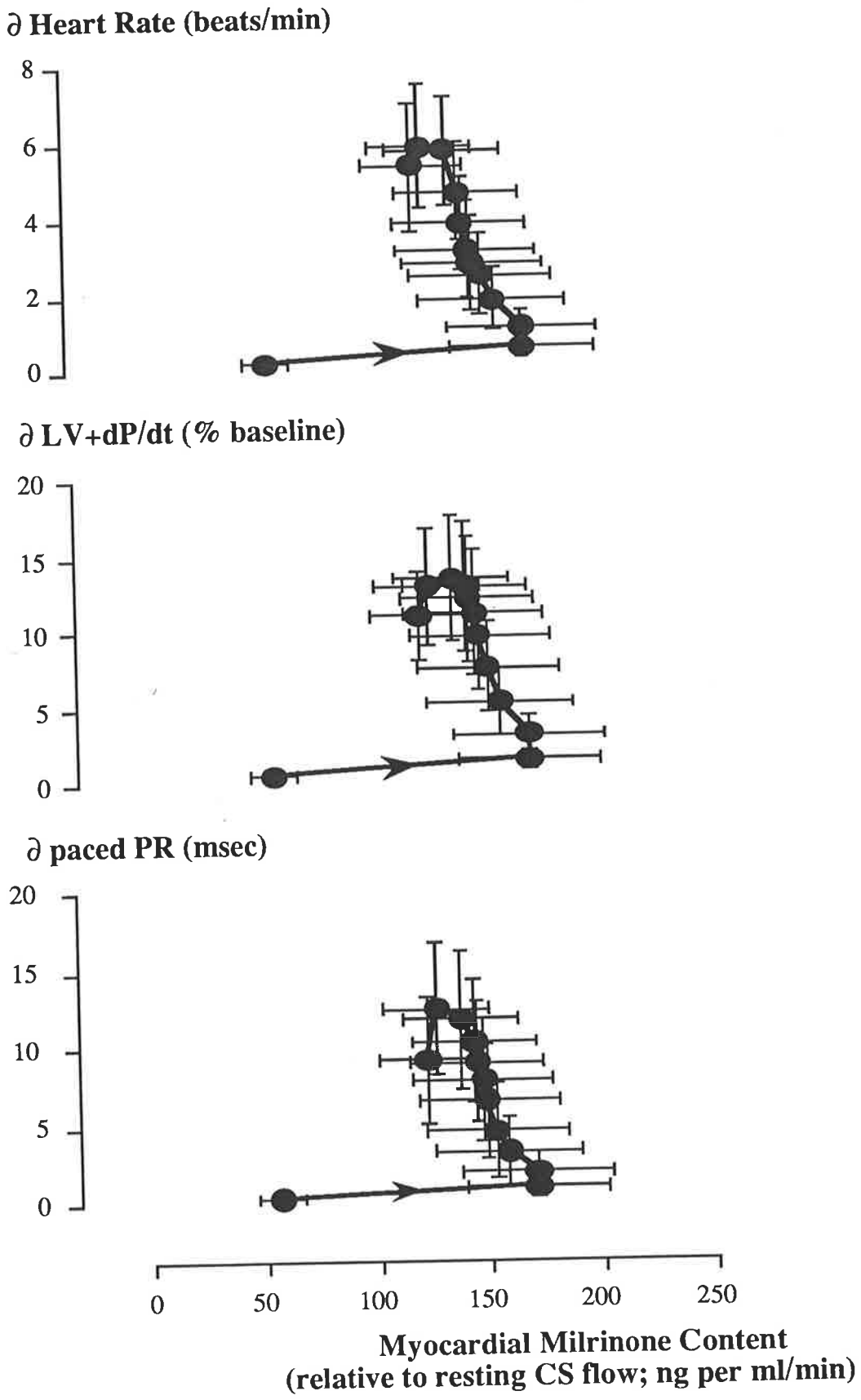


FIGURE 6.13 Relationship between changes in spontaneous HR, LV+dP/dt and paced PR intervals with myocardial milrinone content (relative to resting CS flow)

6.3.9 Milrinone redistribution into other vascular beds

The profile of milrinone concentrations in FA and femoral vein (FV) for 15mins following administration is illustrated in Figure 6.14. Milrinone concentrations in FV whole blood were 69.8±8.9ng/ml initially (2.07±0.01mins), before progressively declining to 27.7±2.1ng/ml 14.0±0.0mins post injection. As observed in the coronary circulation, the arteriovenous milrinone concentration gradient in the femoral vasculature was present but minimal, with FA concentrations slightly exceeding those in FV for approximately the first 6mins, representing nett uptake of the drug into the femoral bed. Uptake of milrinone into the lower limbs therefore continues for a considerably longer period than uptake into the myocardium.

6.3.10 Serial mechanical restitution curve construction

The potential for the positive inotropic effects of milrinone to be dependent on cycle length were examined utilizing serial construction of the *short-cycle length single-beat* MRC before, and at regular intervals after, milrinone injection in 10 patients, shown in Figure 6.15. Prior to injection, LV+dP/dt (percent of baseline pre-milrinone value) was progressively diminished with decreasing extrasystolic intervals (percent of baseline pre-milrinone value), from 100% at a cycle length of 100%, to 71.3±10.3% at a cycle length of 60.1±2.6%. The positive inotropic effects of milrinone at baseline cycle length have already been established : LV+dP/dt increased to 112±4% 10mins after injection (Figure 6.4). As the extrasystolic interval decreased, this positive inotropic effect was attenuated, and may even have become negatively inotropic at a cycle length of 60.0±2.7%, at which contractile force was only 56.2±12.4% of baseline. These results implied that the positive inotropic effects of milrinone were not constant at all RR intervals studied, but were possibly reverse-rate dependent. The effect of milrinone on this *short-cycle length single-beat* MRC appeared to be maximal at approximately 5mins post administration.

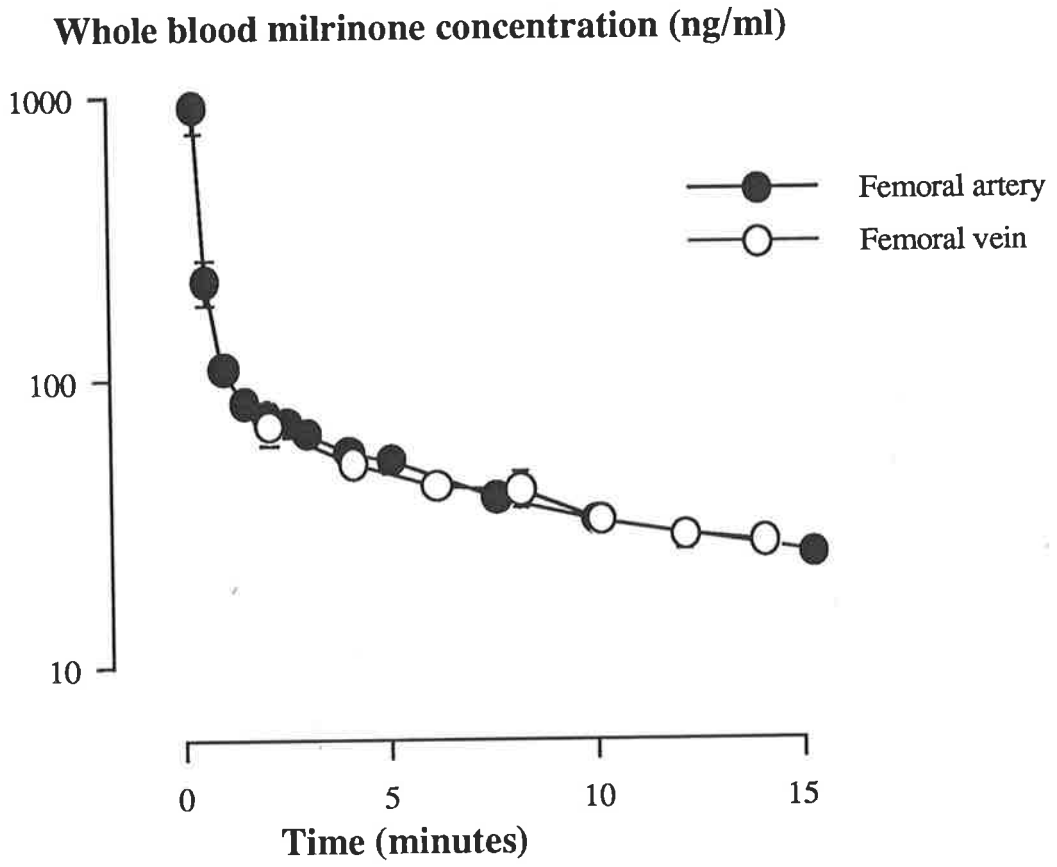


FIGURE 6.14 Time course of transvascular milrinone concentration gradient in the femoral bed

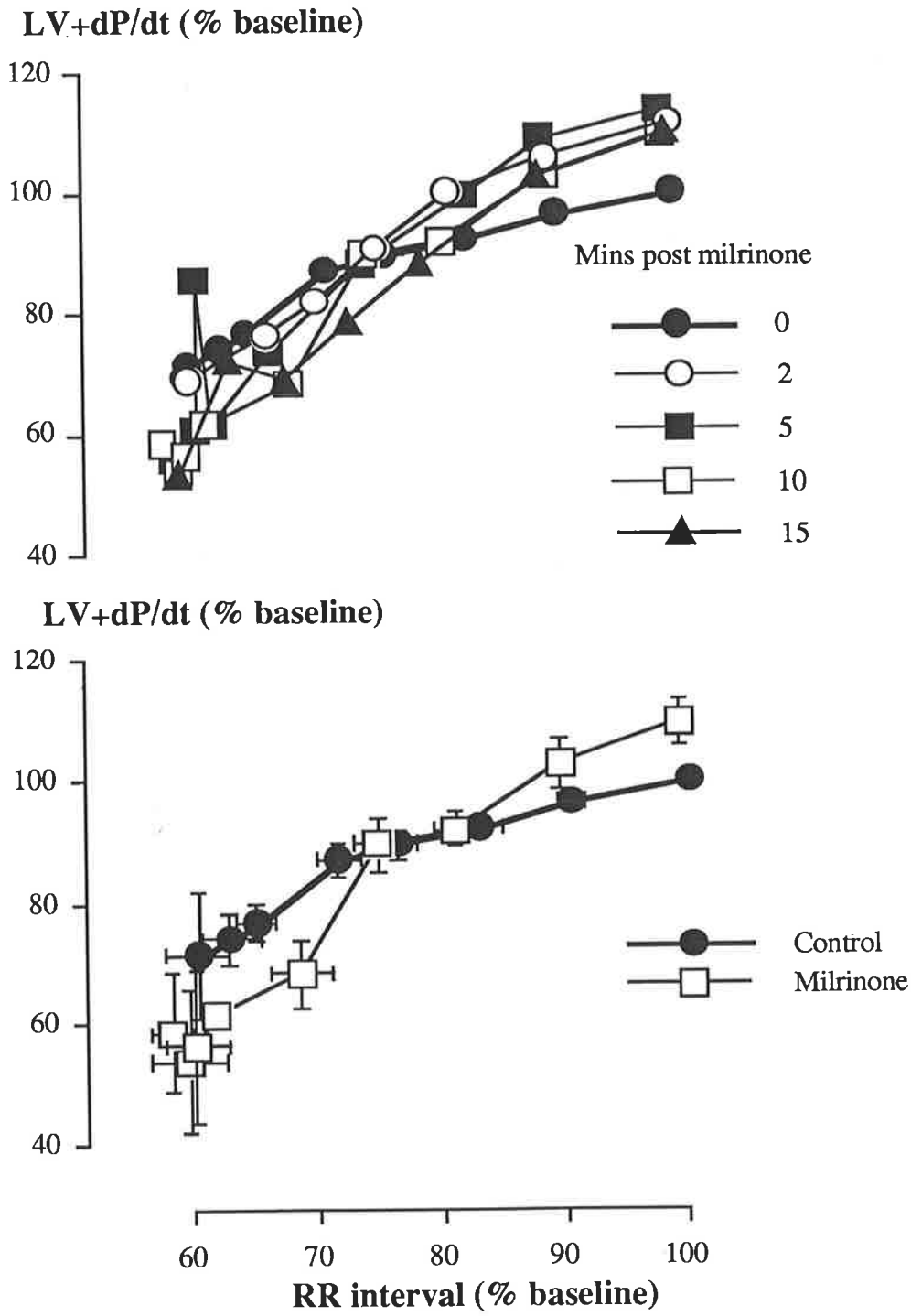


FIGURE 6.15 On the upper panel, the serial MRC obtained at all time points prior to and after injection, and on the lower panel, just the pre- and 10mins post-milrinone MRC: for ease of presentation, SE's not shown on upper panel

6.3.11 Post-extrasystolic potentiation without a compensatory pause

The potential for the positive inotropic effects of milrinone to be dependent on cycle length were also examined in 7 patients in terms of the degree of potentiation of the beat after each extrasystolic interval of the *short-cycle length single-beat* MRC, termed postextrasystolic potentiation (PESP). This beat was subjected to the baseline pacing rate, ie its RR interval was 100%. Despite the lack of a compensatory pause, the postextrasystolic beat was still potentiated. The influence of milrinone on PESP is shown in Figure 6.16. Prior to injection, LV+dP/dt (percent of baseline pre-milrinone value) of the postextrasystolic beat was progressively augmented from 6.6±0.8% to 18.4±3.8% greater than baseline, as the extrasystolic interval was decreased from 90.0±1.5% to 65.4±2.8% of the baseline pacing cycle length.

Ten minutes after milrinone injection, LV+dP/dt of the postextrasystolic beat was again progressively augmented from 6.6±2.0% to 25.7±4.0% greater than baseline, as the extrasystolic interval was decreased from 89.8±1.4% to 59.0±1.1% of the baseline pacing cycle length. As portrayed in Figure 6.16, milrinone did not appear to influence the relationship between potentiation of the postextrasystolic beat and the RR interval of the previous premature beat.

6.3.12 Post-extrasystolic potentiation with a compensatory pause

In 2 patients only, PESP was examined under circumstances permitting a compensatory pause following the premature stimulus. In the absence of drug, despite the resultant postextrasystole cycle length being approximately 150% of baseline, similar results were obtained for PESP as without the pause. Individual results for these 2 patients prior to and 10min after milrinone administration are displayed in Figure 6.17. In these 2 patients, PESP tended to be augmented by milrinone administration compared with control PESP curves ($p=0.09$ and $p<0.005$).

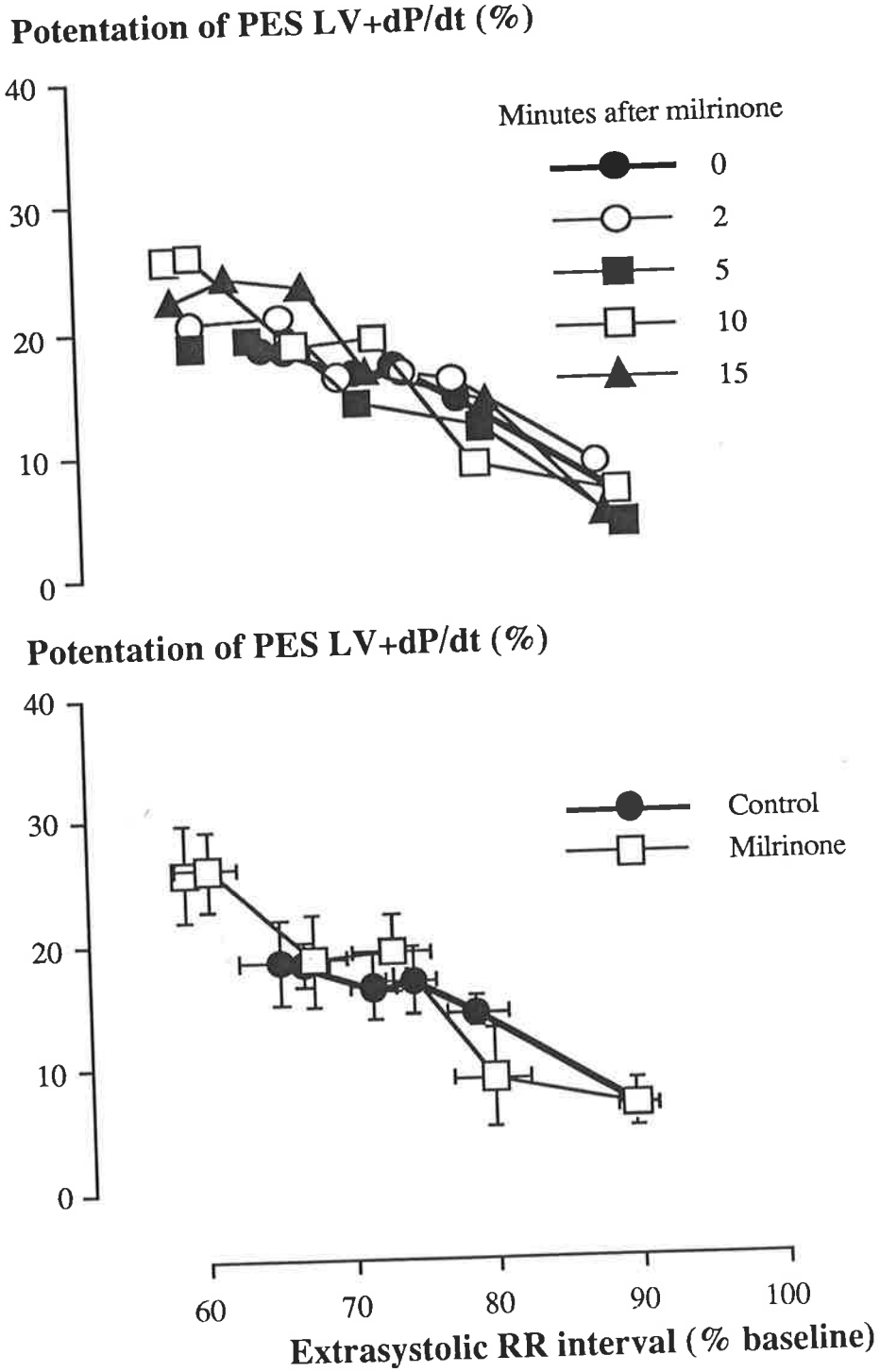


FIGURE 6.16 On the upper panel, the serial PESP curves obtained at all times before and after injection, and on the lower panel, the pre- and 10mins post-milrinone PESP curves : for ease of presentation, SE's not shown on upper panel

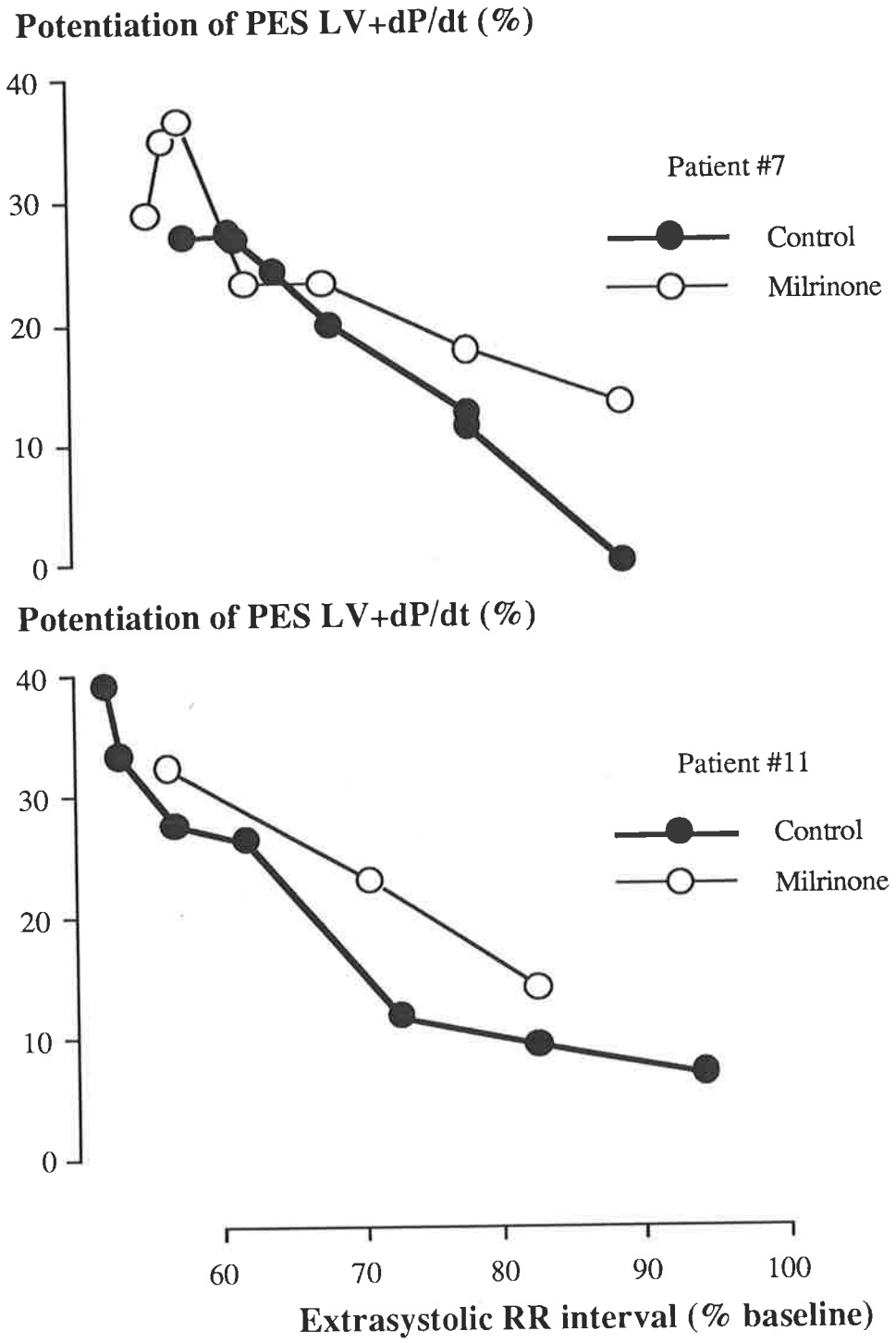


FIGURE 6.17 Individual PESP curves obtained in two patients prior to and 10mins post milrinone administration

6.3.13 Application of the curve-fitting model to mechanical restitution curves obtained post-milrinone injection

The curve-fitting model developed for the MRC to facilitate determination of the dependence of the inotropic effects of cardioactive agents on cycle length discussed in Chapter 3 was utilized. From fitting the data obtained from the MRC, values of c and the rate-dependence index (RDI) after milrinone administration were obtained, illustrated in Figure 6.18. In one patient allocated to milrinone, the model failed to provide positive values for c, because the shortest extrasystolic interval obtainable in this patient was consistently greater than 60% of the baseline cycle length. Results presented for the curve-fitting data are therefore from the remaining 9 patients. Milrinone significantly induced increases in both c and RDI, from 30.6±8.1% to 50.1±8.9% ($p<0.05$), and from 1 to 2.11±0.65 ($p<0.05$) respectively, at 10min. The phosphodiesterase inhibitor failed to induce significant effects on the horizontal and vertical asymptotes of the model however, represented in Table 6.5. Goodness-of-fit of the quantitative model, the residual SD's, was 6.92 (5.15, 8.70 95% confidence intervals, n=9).

6.3.14 Examination of hysteresis between myocardial milrinone content and the rate-dependence index

Having established a significant milrinone-induced elevation of the RDI, the potential for a delay between MMC and RDI reduction was examined in the 8 patients in whom both MMU and MRC curve-fitting was investigated. As represented in Figure 6.19, there was no correlation between peak MMC and peak RDI reduction, there was a significant increase in RDI reduction relative to simultaneous MMC up to 15min after administration ($p<0.0001$), and the relation between RDI reduction and MMC revealed an anticlockwise hysteresis loop. Thus, as for the other significant effects of metoprolol, the time course of the reverse use-dependence of the positive inotropic effects of milrinone was also not parallel with MMC.

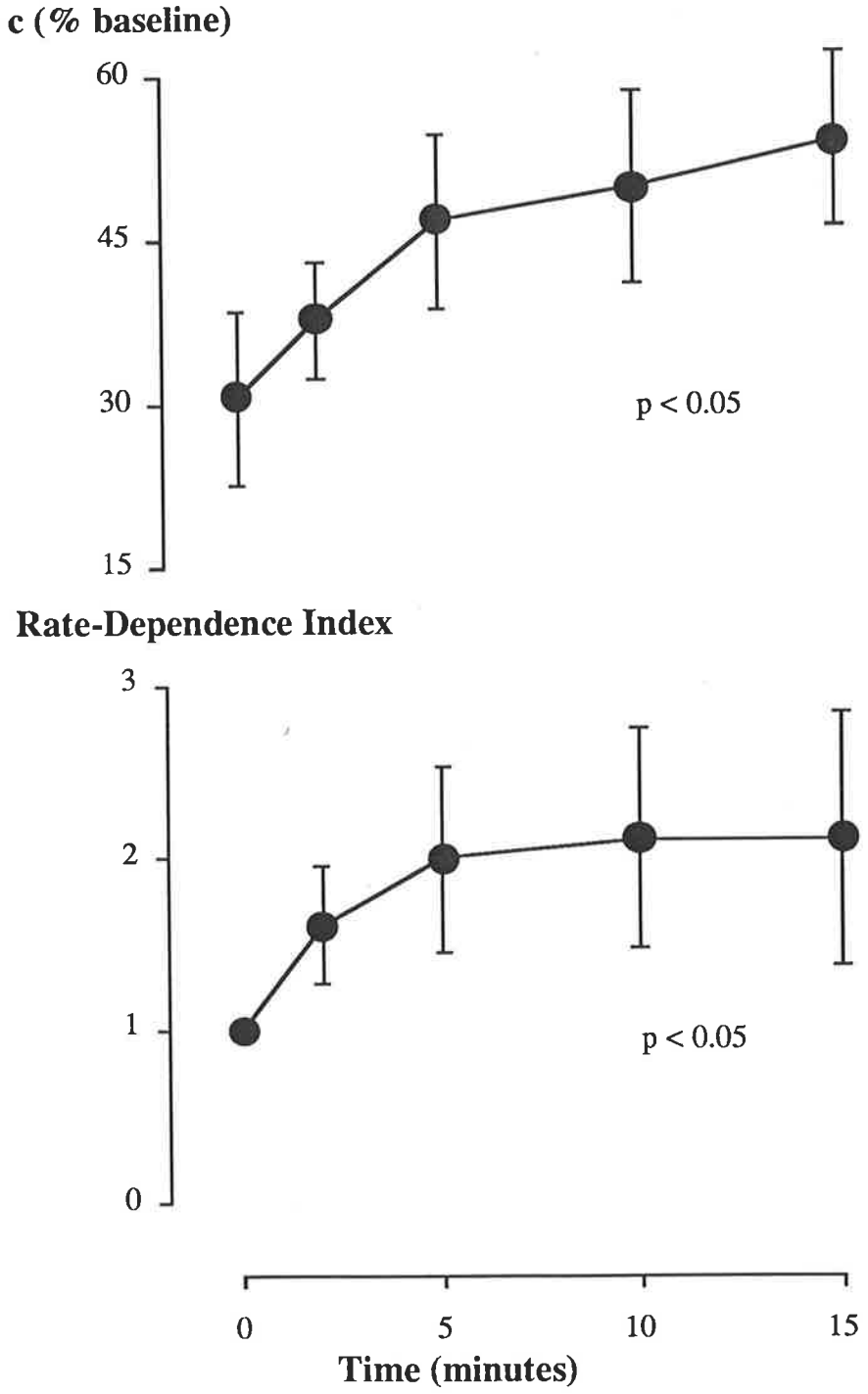


FIGURE 6.19 Time course of changes in *c* and the rate-dependence index after milrinone injection

TABLE 6.5 Time course of the asymptotes of the MRC curve-fitting model

Minutes post milrinone	0	2	5	10	15	p
a	115±5	144±10	153±12	166±16	215±37	NS
d	41±6	28±7	22±13	20±10	-7±25	NS

a , horizontal asymptote; *d* , vertical asymptote

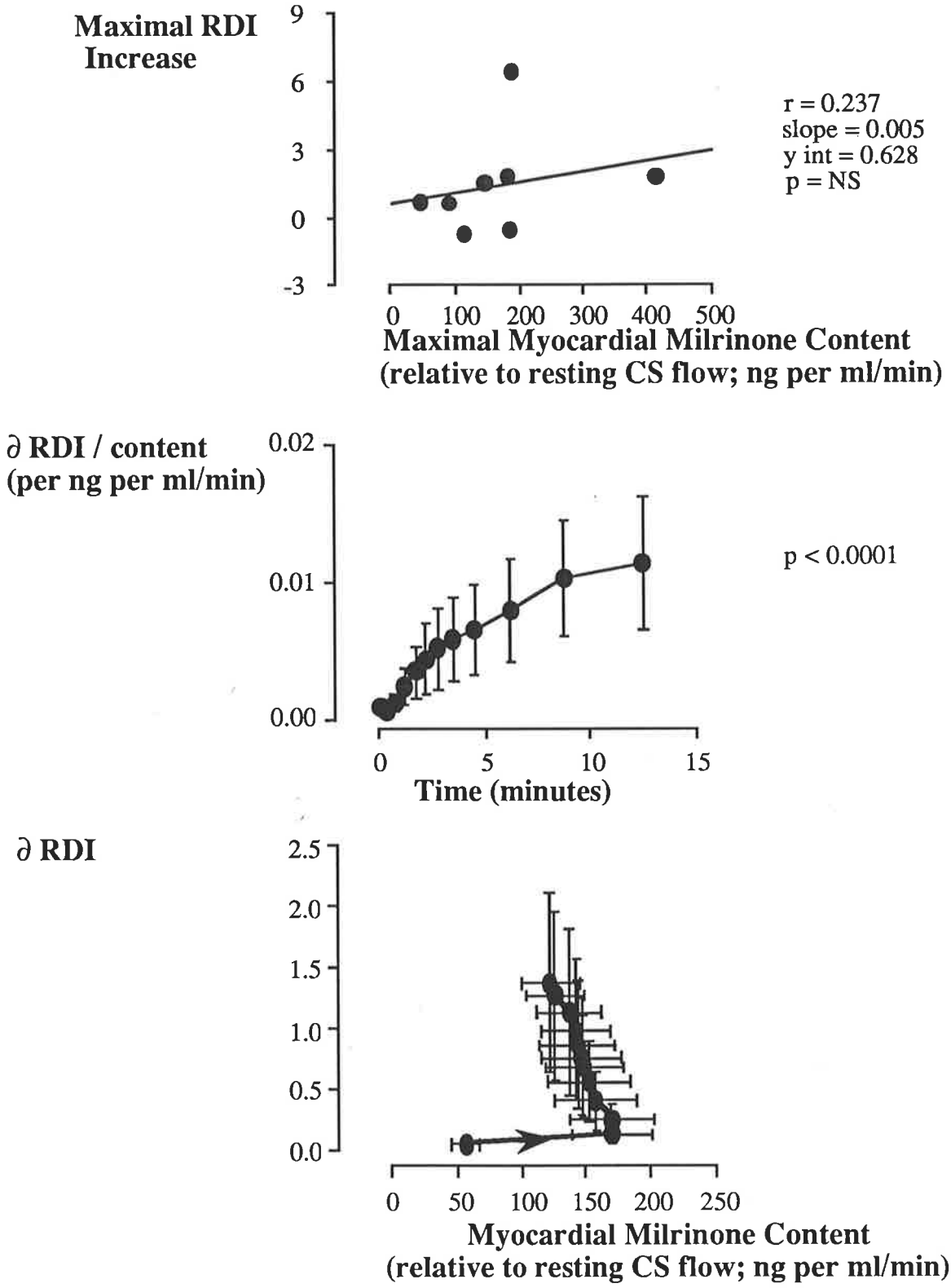


FIGURE 6.19 The relation between peak MMC and peak RDI reduction, the time course of ∂RDI : MMC, and anticlockwise hysteresis loop for MMC and ∂RDI

6.3.15 Influence of rapid atrial pacing on LV+dP/dt before and after milrinone injection

The effect of 60s of rapid atrial pacing on LV+dP/dt before and 10mins after milrinone was examined as an alternative method of assessing the potential for rate-related positive inotropic effects, in 8 selected patients with only mild angina. In this group of patients, the baseline cycle length was reduced from 747±26 to 490±6msec, representing an increased heart rate of 53±2beats/min : results are shown on the upper panel of Figure 6.20. Prior to drug administration, onset of pacing produced a 29.0±5.8% increase in LV+dP/dt, which then remained stable. At 60s, dP/dt was 23.0±6.4% of that prior to onset of pacing. After milrinone administration, pacing induced an 18.9±9.3% positive inotropic effect, which was not different to the increases in dP/dt obtained prior to injection. Following 60sec of rapid atrial pacing, LV+dP/dt was 28.9±12.1% above that obtained prior to the onset of pacing, not significantly different from the milrinone-induced increase in LV+dP/dt observed prior to and 5s after institution of pacing-induced tachycardia.

Considering specifically the milrinone-induced exaggeration of LV+dP/dt, illustrated in the lower panel of Figure 6.20, milrinone induced a 10.1±4.4% positive inotropic effect at baseline cycle length, and 0.1±14.0% and 16.0±15.5% positive inotropic effects at 5 and 60sec post institution of rapid pacing (p=NS), indicating that the milrinone-induced augmentation of LV+dP/dt did not significantly vary with time after institution of rapid pacing.

The extent of milrinone-induced elevation of LV+dP/dt observed with 60s of rapid atrial pacing, $\partial dP/dt_{PAC}$, was then calculated as the difference in milrinone-induced positive inotropic effects at 5 and 60s into the rapid pacing protocol :

$$\partial dP/dt_{PAC} = (control_{\partial 5} - milrinone_{\partial 5}) - (control_{\partial 60} - milrinone_{\partial 60})$$

where
- $control_{\partial 5}$ = increase in dP/dt at 5 seconds of pacing, prior to injection
- $milrinone_{\partial 5}$ = increase in dP/dt at 5 seconds of pacing, post-milrinone
- $control_{\partial 60}$ = increase in dP/dt at 60 seconds of pacing, prior to injection
- $milrinone_{\partial 60}$ = increase in dP/dt at 60 seconds of pacing, post-milrinone

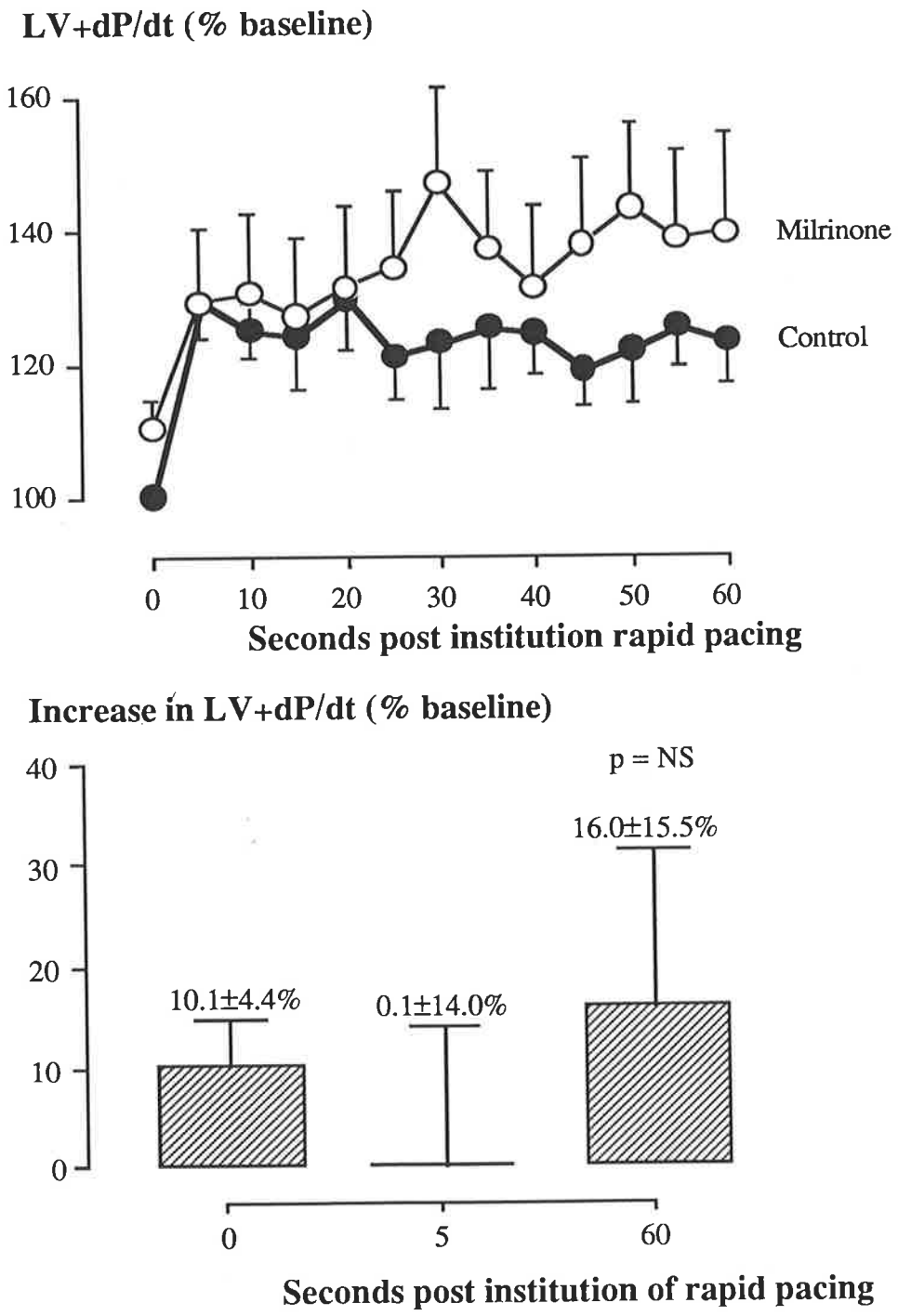


FIGURE 6.20 Influence of 60s of rapid atrial pacing on LV+dP/dt before and after milrinone : on the upper panel, the time course of LV+dP/dt, and on the lower panel, the augmentation of LV+dP/dt before and after 5 and 60sec of rapid atrial pacing

The milrinone-induced increase in LV+dP/dt attained with rapid atrial pacing, $\partial dP/dt_{PAC}$, was -15.9±11.5% (range -27.3 to 82.7%), which tended to be greater than zero, but failed to reach statistical significance (p=0.21), implying that LV+dP/dt during rapid pacing with milrinone had not changed significantly.

6.3.16 Submaximal coronary vasodilator reserve

Submaximal coronary vasodilator reserve is a measure of the ability of the coronary vasculature to dilate with a certain increase in heart rate, in order to meet the additional oxygen requirements of such an increase. Reserve was examined in 8 patients allocated to administration of intravenous milrinone. In this group of patients, the baseline cycle length was reduced from 747±26 to 490±6msec, representing an increased heart rate of 53±2beats/min. Prior to injection, reserve was 1.50±0.16. Ten minutes after injection, reserve tended to decrease to 1.18±0.05, however the decrease was not significant (p=0.37). Milrinone, at this dose at least, failed to influence submaximal coronary vasodilator reserve.

6.3.17 Summary of results

The major findings following an acute intravenous milrinone injection in the current study were:

(i) increased LV+dP/dt at baseline heart rate maximal at 8min;

(ii) increased spontaneous heart rate maximal at 10min;

(iii) prolongation of PR intervals at fixed heart rate maximal at 7min;

(iv) reduction of mean arterial pressure maximal at 8min;

(v) reduction of LV systolic pressure maximal at 7min;

(vi) no change in coronary sinus plasma cAMP concentrations;

(vii) myocardial milrinone uptake of 1.9% of the dose peaking at 0.6min;

(viii) residual myocardial milrinone content at 12.5min 70% of maximal content;

(ix) the extent of peak effects was not significantly related to the extent of peak myocardial content;

(x) the time course of myocardial milrinone content and acute effects were not parallel;

(xi) during the period of nett milrinone efflux from the myocardium, the drug does not extensively redistribute to other vascular beds;

(xii) the positive inotropic effects of milrinone were attenuated at isolated premature beats of hort cycle length;

(xiii) milrinone did not influence postextrasystolic potentiation studied in the absence of a compensatory pause;

(xiv) milrinone may influence postextrasystolic potentiation studied in the presence of a compensatory pause (only examined in 2 patients);

(xv) the positive inotropic effects of milrinone were preserved during pacing-induced tachycardia in patients with only mild angina

(xvi) no influence of milrinone on submaximal coronary vasodilator reserve was observed.

6.4 Discussion

On the basis that the acute uptake of cardioactive drugs by the myocardium is a major predictor of the extent and time course of pharmacodynamic effects (Horowitz and Powell 1986; Powell *et al* 1991b), the current study examined, for the first time in any experimental model, myocardial uptake of milrinone, a phosphodiesterase III inhibitor with combined positive inotropic and vasodilator actions, and its relationship to the acute effects of the drug. No previous studies have been described investigating milrinone distribution to any of the various body tissues in animals or humans *in vivo* . Important information regarding the myocardial kinetics of the agent was acquired, despite only global estimation of myocardial milrinone content in the region drained by the coronary sinus. In humans *in vivo* for the first time, the potential dependence of the inotropic effects of the drug on cycle length was also investigated.

Following intravenous milrinone bolus injection, the drug was very rapidly taken up by the human myocardium, as indicated by peak myocardial content 0.56min post administration. Contentr of milrinone were observed at this peak represented approximately 1.9% of the total injected dose. Appreciable myocardial milrinone content was still present 12.5min post administration, with minimal redistribution to other vascular beds during this time. Myocardial uptake of milrinone proceeded markedly more rapidly than any other agent examined utilising this technique (Horowitz *et al* 1986; Powell *et al* 1990a, 1990b). These findings with milrinone are not contradictory to the conventional pharmacokinetics of this drug, which are also rapid (Larsson *et al* 1986; Stroshane *et al* 1984a, 1984b).

In contrast with results obtained with verapamil (Powell *et al* 1990b), the current study failed to identify a direct relationship between extent of, or time to, maximal myocardial milrinone content and the degree of fixed coronary artery disease or underlying LV systolic dysfunction.

In the present investigation, the predominant acute haemodynamic, ECG, and EP effects of the 1mg intravenous milrinone bolus were maximal 7 - 10min post injection, and persisted for the duration of the study. These effects included:

(i) a progressive marked increase in LV+dP/dt;

(ii) significant considerable acceleration of spontaneous heart rate;

(iii) reduction of mean arterial pressure;

(iv) slight diminution of LV systolic pressure;

(v) a slight reduction in PR intervals at fixed heart rates

Despite primarily investigating the acute haemodynamic effects of an intravenous milrinone bolus in patients with moderate to severe congestive heart failure, the findings of previous investigators (Table 1.7) were essentially consistent with the present study : after a single intravenous bolus dose (range 12.5-125μg/kg), milrinone induced dose-related increases in spontaneous heart rate (range 0-16% increase), and significantly reduced mean arterial pressure (by -4-14%), and systemic vascular resistance (by 15-42%), accompanied by a 1-32% increase in LV+dP/dt (Benotti *et al* 1984, 1985; Benotti and Hood 1984; Sonnenblick *et al* 1986; Jaski *et al* 1985; Le Jemtel *et al* 1984; Likoff *et al* 1985; Grose *et al* 1986; Maskin *et al* 1983; Baim *et al* 1983; Wright and Sherry 1991; Feneck *et al* 1991; Dubois-Rande *et al* 1991; Klocke *et al* 1991; Mager *et al* 1991; Anderson *et al* 1991; Villeri *et al* 1992; Pflugfelder *et al* 1991; Colucci *et al* 1985; Ludmer *et al* 1986; Monrad *et al* 1984). These studies often also demonstrated significant dose-dependent reductions in both PCWP and LV end-diastolic pressure, and increased cardiac indices. The former was not examined in the current investigation, but the latter two findings contrast to results obtained here; the difference may be specific to the presence of systolic heart failure. Similarly, while milrinone appeared to induce a slight reduction in LV EDP and increase in cardiac index, these effects were not marked or consistent. However, many of these previous studies used doses up to almost ten-fold that utilized in the present study. The lack of effect of milrinone on LV EDP or cardiac index here is probably therefore attributable to this lower dose.

Despite the predominant mechanism of action of milrinone being attributable to phosphodiesterase III inhibition (Vandenplassche *et al* 1992; Komai *et al* 1991), the drug failed to influence plasma cAMP concentrations in coronary sinus plasma samples in the present investigation. However, significant haemodynamic and ECG effects were recorded following intravenous milrinone administration - this discrepancy might be attributed to one or more of the

following factors :

(i) plasma concentrations of cAMP may not necessarily reflect modulation of the nucleotide within the cell (no previous studies have documented significant milrinone effects on this parameter in man);

(ii) phosphodiesterase III inhibition may not be the predominant mechanism of action of milrinone in this patient group (a positive inotropic effect has previously been reported with the drug in canine ventricular muscle before the increase in tissue cAMP was observed : Endoh *et al* 1986);

(iii) the time required for the biochemical process of phosphodiesterase inhibition may be greater than the time period utilized in the present study;

(iv) an additional mechanism of action of milrinone may have been involved (a role of inhibition of the inhibitory G protein associated with the ß-adrenoceptor has been identified, which stimulates adenylate cyclase to also bring about increased myocardial contractility : Parsons and Stiles 1987).

However, it should be noted that the positive inotropic effect discussed in point (ii) above was effectively antagonized by carbachol, implicating some role of cAMP accumulation (Endoh *et al* 1986), and therefore the first possibility of a lack of association between plasma and intracellular concentrations of cAMP probably explains this discrepancy.

The milrinone-induced increase of LV+dP/dt observed at the baseline pacing rate appeared to become progressively attenuated as the systolic interval decreased in construction of the short-cycle-length component of the MRC (Figure 6.15). The parameter c , and hence the RDI, were significantly augmented by milrinone administration (Figure 6.18). These findings statistically confirmed that the positive inotropic effects of the phosphodiesterase inhibitor were reverse rate-dependent under these circumstances.

Reinforcing previous findings in the absence of drug administration, the contractile performance of the postextrasystolic beat was augmented both in the absence (Figure 6.16) and presence (Figure 6.17) of a compensatory pause (Chapter 3 of this dissertation; Hoffman *et al* 1956, 1965; Kuijer *et al* 1990; van der Werf *et al* 1976; Seed *et al* 1984; Dyke *et al* 1974; Sung *et al* 1980; Di Donato *et al* 1990), and was progressively potentiated with decreasing

extrasystolic intervals (Seed *et al* 1984; Sung *et al* 1980). While there was no trend for any effect of milrinone on the potentiation of the postextrasystolic beat in the absence of a compensatory pause, a tendency for augmented potentiation was observed in the two patients in whom such a pause was permitted. However, firm conclusions cannot be drawn from the investigation of two patients alone; this area merits further investigation utilizing paired comparison of both PESP methods. Conversely, the positive inotropic effects of the drug were preserved during one minute of rapid atrial pacing.

The reverse use-dependence of the positive inotropic effects of milrinone observed in studies utilizing the MRC in the present investigation appear, on the surface, as in direct contrast to the findings reported by Alousi and Johnson (1986). In the only previous study of the influence of rate on these effects of milrinone to date, these investigators observed increased positive inotropic effects of the drug in isolated guinea pig papillary muscles with increasing frequency of stimulation (up to 1Hz), and postulated the existence of a milrinone-induced frequency-dependent enhancement of calcium entry across the sarcolemma at these frequencies (Alousi and Johnson 1986). Increasing the stimulation frequency higher than 1Hz however, was linked to a slight decrease in the inotropic response of milrinone (Alousi and Johnson 1986). This decrease was attributed to the reduced time of calcium influx resulting from reduced total contractile time duration (Alousi and Johnson 1986). Similar conclusions could also be drawn from the present study in man *in vivo* , attributing the decreased positive inotropic effects of milrinone at short cycle lengths during MRC construction to reduced time for the milrinone-augmented calcium entry to demonstrate its effects. Reverse use-dependent effects have only been previously reported for the electrophysiological effects of class III antiarrhythmic agents, including sotalol and OPC-8212 (Lathrop *et al* 1989; Sager *et al* 1991; Knilans *et al* 1991; Schmitt *et al* 1991, 1992; Huikuri and Yli-Mayry 1992).

In previous investigations, cardioactive drugs reported to influence PESP include the calcium antagonists verapamil, diltiazem and diperdipine (Kerker *et al* 1985; Iinumato and Kato 1978; Di Donato *et al* 1990; Marchionni *et al* 1992), isoprenaline (Iinumato and Kato 1978) and caffeine (Iinumato and Kato 1978). Other cardioactive agents investigated include glyceryl trinitrate (presumably via relief of ischaemia : Banka *et al* 1976) and ryanodine (Bose *et al*

1988). All of the previous studies in which drugs influenced the extent of PESP permitted a compensatory pause after the extrasystolic interval (Kerker *et al* 1985; Iinumato and Kato 1978; Di Donato *et al* 1990; Marchionni *et al* 1992). This suggests that by disallowing the pause in most of the patients in the current study, significantly limited the potential for observing milrinone effects on PESP. Contractile force of the postextrasystolic beat was significantly reduced by diltiazem, verapamil, caffeine and isoprenaline (Kerker *et al* 1985; Iinumato and Kato 1978; Di Donato *et al* 1990), while diperdipine displayed additive effects on the improved ejection fraction and regional wall motion of PESP (Marchionni *et al* 1992).

One of the major hypotheses to be tested in this study was that myocardial milrinone content is a direct determinant of the acute hemodynamic effects of the drug. Peak myocardial milrinone content was not significantly correlated with peak effects, including changes in spontaneous heart rate, LV+dP/dt, PR intervals at fixed heart rate, or the rate-dependence index. This is in contrast with the results of previous studies with verapamil (Powell *et al* 1990b). This negative finding might imply considerable variation exists between one or more of : fundamental contractile reserve, the ability of blood vessels to dilate, intrinsic plasma and intracellular cAMP concentrations at the time of the procedure, and possibly also autonomic tone, previously demonstrated to influence milrinone's effects (Shaffer *et al* 1986; Hasking *et al* 1987). Nevertheless, the actual finding of $r=0.47$, $p=0.17$ as regards the relationship between increase in LV+dP/dt and myocardial milrinone content raises the possibility that a larger study might have revealed a significant correlation : a similar possibility exists for the heart rate / content relationship.

Furthermore, the hysteresis observed between the time course of MMC and haemodynamic and ECG effects (Figures 6.12, 6.13 and 6.19) might theoretically result from hetereogenous kinetics of milrinone uptake into the sinus node and/or differential rates of onset of the effects of the drug on the functionally distinct vasodilatatory and positive inotropic actions (Gosgnach *et al* 1991; Steffen and Wastila 1992; Lebedinsky *et al* 1992; Colucci *et al* 1985; Ludmer *et al* 1986). From all data shown in Figures 6.12, 6.13 and 6.19, it appears not improbable that all of the effects of milrinone exhibit marked temporal fluctuations as functions of simultaneous MMC. Definite hysteresis loops were constructed, inferring a defined time interval must lapse

prior to myocardial content eliciting peak effects, not attributable to a progressively generated metabolite, because milrinone is not extensively metabolized, with no pharmacologically active metabolites : the majority of the drug excreted unchanged in the urine at 24h (Stroshane *et al* 1984b). As previously mentioned in other chapters, a true "biochemical lag phase" has previously been shown to persist for the time course of interaction between cardioactive drugs and cellular effector mechanisms in the absence of diffusion barriers within myocardial cells (Horowitz *et al* 1982; Barry *et al* 1985; Horowitz and Powell 1986).

The variability in uptake theoretically imposed by hetereogenous milrinone effects on flow in ischaemic versus nonischaemic regions could not be examined in this model, nor were the effects of induction of ischaemia, or local coronary occlusion, on myocardial milrinone uptake examined. None of these have been attempted in any experimental model to date. The myocardial kinetics of milrinone in regions of ischaemia may be significantly slower, as is the case for the conventional pharmacokinetics of the drug in patients with moderate to severe congestive heart failure (Benotti *et al* 1984, 1985; Baim *et al* 1983; Likoff *et al* 1985; Cody *et al* 1984b; Edelson *et al* 1986; Stroshane *et al* 1984a). No information regarding uptake in patients during tachycardia could be derived from the present study, because content was only examined at fixed heart rates (71beats/min). On the basis of previous studies with other drugs, the myocardial uptake of milrinone may also be enhanced in patients with tachycardia (Horowitz *et al* 1986), although in the current study, spontaneous heart rate at baseline was not correlated with extent or time of peak MMC.

Milrinone has been associated with increased mortality with long-term oral administration (Packer *et al* 1991a, 1991b). This observation, the primary reason for premature termination of the PROMISE (Prospective Randomized Milrinone Survival Evaluation), may reflect a fundamental arrhythmogenic property of the drug, or another mechanism which has not yet been elucidated. The nonsignificant tendency for milrinone to reduce AVN ERP may well indicate pro-arrhythmia as the culprit. However, another possibility is that onset of tachycardia in patients already identified as having severely impaired LV function triggers haemodynamic deterioration in the presence of milrinone. While the current study could not confirm this as a possible mechanism of action of the deleterious effects of the drug, results presented in Figure

6.15 suggested that the positive inotropic effects of milrinone during sinus rhythm, may become negatively inotropic with particularly short cycle lengths, of the order easily achieved during sustained tachycardia. Pacing-induced tachycardia in this group of patients after milrinone injection did not result in deterioration of LV+dP/dt in the present investigation, but systolic dysfunction was not present in any of the patients examined, and only the patients reporting particularly mild symptoms of angina pectoris were selected for the rapid pacing protocol. These limitations could therefore easily mask such a deleterious effect.

In conclusion, the human myocardium accumulated milrinone extraordinarily rapidly, which was not influenced by the degree of pre-existing ischaemic heart disease. Significant haemodynamic and ECG effects were induced by this low dose, but were not maximal until some time later than peak myocardial content. Examination of the influence of short cycle length on the effect of milrinone on inotropic state implied milrinone-induced augmentation of LV+dP/dt was progressively impaired in single premature beats, but not influenced significantly during tachycardia, which implies limitations to the haemodynamic safety of the drug in patients prone to tachyarrhythmias, already a sensitive issue.

CHAPTER 7 : GENERAL DISCUSSION

7.1 Overview

This thesis has examined the acute haemodynamic, electrocardiographic (ECG) and (to some extent) the electrophysiologic (EP) effects of three cardioactive agents (metoprolol, sotalol, and milrinone), and determined the influence of both simultaneous myocardial drug content and the systolic interval on these effects. The experiments described here were performed predominantly in humans *in vivo*, and have yielded new information regarding the determinants of acute pharmacodynamic effects, short-term myocardial kinetics, and the dependence of inotropic effects on rate both in general, and for the 3 agents investigated specifically. Basic to these experiments was the refinement of currently available, or development of, in the case of milrinone, HPLC assays for determination of these cardioactive drugs in human whole blood.

Significant effects on spontaneous heart rate, LV+dP/dt at constant heart rate, and ECG intervals were induced by all three agents investigated, with minor fluctuations in blood pressure. Each compound was rapidly taken up into the human heart, and the rate of uptake was not related to resting cardiac function. Partially because of consistent hysteresis between myocardial drug content (MDC) and acute effects, MDC was not a statistically significant predictor of effect for any of the agents investigated. On the assumption that MDC should determine effect, pharmacokinetic / pharmacodynamic link models were utilized to calculate a theoretical effect-site concentration, Ce. Additionally, the influence of all three drugs on contractile state was demonstrated as dependent on cycle length.

7.2 Acute effects of cardioactive drugs

The selective $ß_1$-adrenoceptor antagonist metoprolol predominantly reduced spontaneous heart rate and LV+dP/dt at fixed heart rate, and prolonged the PR interval. The drug also tended to slightly improve LV EDP. No influence on mean arterial or LV systolic pressures, cardiac index, systemic or coronary vascular resistances, coronary flow, or QT intervals was observed.

Similarly, the nonselective ß-adrenoceptor antagonist sotalol also exerted negative chronotropic and inotropic effects, and prolonged the PR interval. Effects of prolonged AH intervals and effective refractory periods of the atrioventricular node were demonstrated with sotalol.

Conversely, the phosphodiesterase III inhibitor milrinone significantly increased spontaneous heart rate and LV+dP/dt. This was accompanied by reductions in LV systolic and mean arterial pressures, systemic vascular resistance and PR intervals at fixed heart rate. Nonsignificant trends for minor reductions in AH intervals and effective refractory periods of the atrioventricular node were also observed. No influence on cardiac index, coronary vascular resistances, coronary flow, or QT intervals was observed following intravenous bolus administration of any of these three cardioactive agents.

7.3 Determinants of drug effects : influence of myocardial drug uptake in the present investigation

The major predictors of cardioactive drug effects are purported to include :

(i) the acute uptake process,

(ii) the myocardium as a substrate for drug effects,

(iii) instantaneous cardiac state, and finally,

(iv) the determinants of receptor sensitivity to the drug.

In the present study, the concentration of cardioactive drug within the myocardium was not a major determinant of acute effect. This in direct contrast to results described by other investigators in both animal and human experimental models (Anderson *et al* 1980a, 1980b; Gillis and Kates 1986; Keefe and Kates 1982; Horowitz *et al* 1986; Upton *et al* 1988; Powell *et al* 1990b; Gillis and Keashly 1991). A definite hysteresis between MDC and effect was demonstrated for each of the three drugs studied in the current investigation.

The protracted time course of interaction between inotropic agents and cellular effector mechanisms has previously been shown to persist in a monolayer culture of isolated myocardial cells, in the absence of significant diffusion barriers within myocardial cells and the perfusate and thus represents a true "biochemical lag phase" (Horowitz *et al* 1982; Barry *et al* 1985; Horowitz and Powell 1986). In these *in vitro* studies, the time course of accumulation of calcium antagonists was not parallel to the time course of negative inotropic effects, despite minimal obstacles between uptake and effect (Horowitz *et al* 1982; Barry *et al* 1985; Horowitz and Powell 1986). This time-lag represents another factor contributing to marked end-organ variability in sensitivity to ß-adrenoceptor blockade / phosphodiesterase inhibition (another factor may include sympathetic tone).

The relationship between MDC and acute effects was further analyzed with the use of pharmacokinetic / pharmacodynamic link models : the amount of drug in a hypothetical effect compartment (Ce) was derived from the time course of both MDC and effect, which was then successfully assessed for a linear relation with effect. This Ce is purely hypothetical, and may

represent an as yet unidentified process which must proceed between global myocardial drug accumulation and induction of effect. Examples may include distribution of the drug to the sinoatrial node, internalization to permit binding with intracellular receptors, or the time interval required for a biochemical process, such as second messenger formation.

Because of ethical implications, the influence of prolonged ischaemia on accumulation of drugs by the myocardium *per se* could not be examined. However, in an isolated perfused rat heart preparation, the effect of a period of hypoxia on myocardial metoprolol accumulation was determined. While characteristics of drug uptake appeared unchanged by the hypoxic conditions, efflux was significantly impaired. Thus, the concept of the phenomenon of reduced myocardial cardioactive drug concentrations in regions of ischaemia / hypoxia (Ablad *et al* 1987; Avitall *et al* 1990; Wenger *et al* 1978, 1980; Ku 1983; Patterson *et al* 1982; Ryden *et al* 1990, 1991; Wenger *et al* 1980; Gillis and Keashly 1991) must be extended to also include impaired efflux.

In the present investigation, the myocardium as a substrate for direct drug effects could not be investigated in isolation : any effects of the drugs administered were the combined results of cardiac and extracardiac actions. The influence of instantaneous cardiac state on the acute effects of these pharmacologic agents was also not investigated, but on the basis of previous investigations, tachycardia would be expected to augment myocardial drug accumulation (Horowitz *et al* 1986; Lullmann *et al* 1979; Lloyd and Taylor 1978). Also not addressed in this study were the determinants of receptor sensitivity to a drug. These may influence acute effects, and include up- or down-regulation, and ischaemia-related receptor interactions. However, rate-related receptor interactions were extensively studied in humans *in vivo* for all three cardioactive agents investigated, and are discussed below.

7.4 Myocardial drug uptake of cardioactive agents : comparison with previous studies

A limited number of studies in man have previously been reported utilizing the technique of coronary sinus catheterization for determination of myocardial drug uptake, including lignocaine, mexiletine, verapamil and digoxin (Horowitz *et al* 1986; Powell *et al* 1990a, 1990b). This technique, while applicable to patients undergoing routine cardiac catheterization, has a number of limitations : it allows only global estimation of MDC in the region drained by the coronary sinus; it does not measure total MDC, nor indicate drug concentrations at specific sites of action; nor is it easily possible to incorporate into the study design paired placebo control patients, despite the theoretical desirability of such observations. Additionally, hetereogenous drug effects on flow in ischaemic versus nonischaemic regions (which could also contribute to variability in uptake) can also not be readily incorporated into the protocol.

Despite these limitations discussed above, the technique utilized in the present investigation for determination of MDC in man has many advantages, primarily the facility for accurate serial determination of MDC at any time up to 30mins after intravenous injection, without the necessity of considerable expense or prolonged radiation exposure. None of these advantages apply to the other techniques previously reported for determining myocardial content in man, including myocardial tissue biopsies collected from patients at autopsy, during cardiac surgery or diagnostic cardiac catheterization, or with imaging techniques (Latini *et al* 1987; Jogestrand 1980; Plachetka *et al* 1981; Padrini *et al* 1985; Hartel *et al* 1976; Charbonneau *et al* 1986).

Both milrinone and sotalol were accumulated by the human myocardium *in vivo* much more rapidly than any other agent previously investigated with this method (Horowitz *et al* 1986; Powell *et al* 1990a, 1990b). Interestingly, the MDC profile with respect to time of both of these compounds appeared biphasic in nature, comprising a rapid followed by a slow phase of uptake, unlike metoprolol in the present study, or lignocaine, mexiletine, or verapamil in previous investigarions (Horowitz *et al* 1986; Powell *et al* 1990b). The myocardial uptake of digoxin was described as biphasic also, but such that the peak digoxin content was not

observed even up to 30min post injection in some patients (Powell *et al* 1990a). Peak myocardial metoprolol content was achieved at a similar time to lignocaine, but more rapidly than digoxin, mexiletine or verapamil (Horowitz *et al* 1986; Powell *et al* 1990a, 1990b).

Comparing the conventional and myocardial kinetics of all cardioactive agents now studied utilizing this method, including those from the current investigation, it appears that the time of peak MDC of a cardioactive drug may be related to its elimination half-life, a short $t_{1/2}$ corresponding to an early time of peak MDC. The lipophilicity of cardioactive agents is also positively correlated with tissue accumulation in *in vitro* studies (Lullmann *et al* 1979). However, determination of the MDC : time profile still needs to be determined for all widely employed cardioactive agents injected intravenously for rapid response, such that the precise extent and time of maximal content, and subsequent extent and time of maximal effects, is available in the emergency situation. The results in the present investigation with sotalol further emphasize the necessity for determination of MDC of cardioactive drugs : the myocardial uptake of sotalol in humans was more rapid than expected for a compound with and a relative lack of lipophilicity (Arendt *et al* 1984), and an elimination $t_{1/2}$ in man of 5-17h (Anttila *et al* 1976; Tjandramaga *et al* 1976; Kahela *et al* 1979; Ishizaki *et al* 1980; Blair *et al* 1981; Sundquist *et al* 1980; Carr *et al* 1992). This is longer than the elimination $t_{1/2}$ of either metoprolol (Jordo *et al* 1980; Regardh *et al* 1980, 1981) or verapamil (Powell *et al* 1990b).

In summary, a broad understanding of the acute myocardial drug uptake profile of a cardioactive agent permits a better understanding of the basis of a cardioactive agent's acute effects, in terms of both the time course of these effects, and accurate differentiation between cardiac and extracardiac effects. This may assist the evaluation of the optimal clinical role of the drug, so it may be administered more wisely. Antiarrhythmic, antiischaemic and positive inotropic agents are often administered to patients intravenously in the in-hospital phase. Precise knowledge of the rate and extent of myocardial drug disposition can potentially facilitate selection of the most appropriate agent.

7.5 Influence of cardioactive drugs on contractile state : modulation by changes in cycle length

Some cardioactive agents are postulated to undergo rate-dependent interactions with their receptor mechanisms (Davis *et al* 1986; Chappell *et al* 1985), which might contribute to the acute haemodynamic deterioration described following initiation of intravenous therapy with several class 1A and 1C antiarrhythmic agents and calcium antagonists in patients prone to tachyarrhythmias (Sharma *et al* 1990; Hammermeister 1990; Akiyama *et al* 1991; Stewart *et al* 1987; Rankin *et al* 1987; Buxton *et al* 1987; Switzer *et al* 1986). With regard to the cardioactive agents selected for study in the current investigation, such problems are rarely reported for the ß-adrenoceptor antagonists, but long-term oral administration of the phosphodiesterase inhibitor milrinone has recently been associated with increased cardiovascular mortality, particularly in patients with the markedly severe congestive heart failure (Packer et al 1990a, 1990b). The mechanism for this is essentially unclear, and may be pro-arrhythmic (demonstrated in coronary occlusion-induced myocardial infarction in dogs : Trolese-Mongheal *et al* 1992) and/or rate-related, as explored in the present study, in origin.

In the current investigation, the negative inotropic effects of metoprolol and the positive inotropic effects of milrinone examined with analysis of serial MRC construction were progressively attenuated at short cycle lengths, while not significantly altered by pacing-induced tachycardia. Conversely, the negative inotropic effects of sotalol only appeared rate-dependent during the rapid pacing protocol, and not by MRC, with deterioration of LV+dP/dt after 60sec of tachycardia. Some suggestion of conversion of the inotropic effects of milrinone from positive at baseline cycle length to negative at particularly short cycle lengths was apparent in analysis of MRC, but was not further examined.

The apparent disparity between results obtained from MRC analysis and the rapid pacing protocol with all three agents studied can probably be explained in terms of two major points :

(i) analysis of serial MRC permits examination of drug-induced effects on contractile performance at short cycle lengths without changing the loading conditions,

ventricular dimensions, or ischaemia induction, and can therefore be more readily studied in patients with some degree of LV dysfunction : any modification of drug effects on contractile performance observed with MRC can only reflect altered calcium mobilization, without the conflicting effects of increased sympathetic tone and catecholamine release.

(ii) while one minute of pacing-induced tachycardia might on the surface appear closer to the "real life" situation, the results discussed from the present investigation will have been influenced by catecholamine release etc. More importantly, the rapid pacing protocol was only studied in patients describing mild anginal pain with good LV function. Quite different results may well have been obtained in patients with more severe ischaemic heart disease and/or heart failure, but could not be sought for ethical reasons. Of particular relevance may be the example cited above, where the adverse effects of milrinone were noted most commonly in patients with New York Heart Association heart failure class IV (Packer et al 1990a, 1990b).

In the present investigation it can therefore be concluded that :

(i) the negative inotropic effects of metoprolol are less marked at short cycle lengths, and are therefore reverse use-dependent, suggesting that if the worst occurs and an arrhythmia occurs during metoprolol administration, then haemodynamic deterioration is less likely to result;

(ii) sotalol may exhibit some deterioration of LV performance during prolonged tachycardia, and may on the surface therefore appear less favourable than metoprolol. However, recent findings from the ESVEM (Electrophysiologic Study Versus Electrocardiographic Monitoring) study indicate this ß-adrenoceptor antagonist / class III antiarrhythmic agent is more efficacious than six other common antiarrhythmic agents (comprising imipramine, mexiletine, pirmenol, procainamide, propafenone, and quinidine) in preventing death and recurrent arrhythmia (Mason *et al* 1993a, 1993b);

(iii) the positive inotropic effects of milrinone are less marked at short cycle lengths, and may even become negative at particularly short cycle lengths, also indicative of reverse use-dependence. Furthermore, the phosphodiesterase inhibitor was regarded as haemodynamically safe prior to the publication of the findings of the PROMISE study of long-term oral administration in patients with severe heart failure (Packer et al 1990a, 1990b), under which circumstances a negative inotropic effect of the drug may be unmasked.

The phenomenon of a use-dependent receptor interaction is simply explained as the substrate interacting preferentially with its receptor when the membrane is depolarized, demonstrated by augmented calcium-channel blocking effects of verapamil at more rapid rates of stimulation *in vitro* (Ehara and Kaufmann 1978). From this, and the findings of the present investigation, it is suggested that metoprolol has a lower affinity and/or potency as regards its interaction with the $ß_1$-adrenoceptor at short cycle lengths, and milrinone for inhibition of the phosphodiesterase III enzyme. The nexus between sotalol and cardiac ß-adrenoceptors may not be uninfluenced by shorter cycle lengths, but this modification may be masked by the drug's additional properties of $ß_2$-adrenoceptor antagonism and/or class III electrophysiologic properties.

7.6 Conclusions

From the experiments described in this thesis, it is apparent that the pharmacodynamics of cardioactive drugs are quite probably determined by their uptake and efflux from the myocardium subsequent to administration. However, an as yet unidentified biochemical lag phase often separates the timing of maxima of the two. These acute effects can also be potentially modified by both changes in systolic interval and hypoxia (as it influences MDC). Additionally, the force-interval relationship in humans can be easily described by a simple equation, facilitating analysis of the relationship in a range of clinical settings. Future studies arising from these findings may include :

(i) acute myocardial disposition studies of other cardioactive agents injected intravenously in emergency situations, including amiodarone, angiotensin converting enzyme inhibitors and antagonists, organic nitrates and perhexiline;

(ii) utilization of the model of the MRC to facilitate analysis of the potential for rate-related effects of other negative and positive inotropic agents, including verapamil, xamoterol, digoxin, adrenaline, and magnesium;

(iii) examination of the influences of cardioactive agents on contractile performance *in vitro* , in both whole heart and isolated cellular preparations, perhaps elucidating the mechanism of actions of these ß-adrenoceptor antagonists on reducing, and the phosphodiesterase inhibitor on augmenting, cardiovascular mortality : the contribution of outward rectifier potassium channel antagonism on the effects of sotalol administration could also be examined in such *in vitro* preparations, to elucidate the ß-adrenoceptor and potassium channel effects of the drug;

(iv) finally, yet perhaps most interesting, would be the study of the potential for the effects of d-sotalol to be influenced by systolic interval. The d-enantiomer of the drug is devoid of significant ß-adrenoceptor antagonists activity at pharmacological concentrations, but still possesses class III antiarrhythmic effects : this isomer has the potential to be the "new amiodarone", efficacious in arrhythmia prevention yet almost completely eliminated from the body in 24h, if cessation of therapy is desired.

In summary, original studies investigating the acute myocardial uptake of three pharmacologic agents, metoprolol, sotalol, and milrinone, the subsequent acute haemodynamic, ECG and EP effects of the drugs, and the influence of systolic interval on these effects in humans are presented in this thesis, and related to the current available literature on both myocardial disposition and use-dependent effects of these and other cardioactive drugs.

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