ACUTE DRUG EFFECTS ON THE HEART-
HAEMODYNAMIC, PHARMACOLOGIC AND
METABOLIC CORRELATIONS

Thesis submitted for the degree of
DOCTOR OF PHILOSOPHY

by

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DECLARATION

I declare that this thesis is of my own composition, is of less than 50,000 words in length exclusive of tables, figures and bibliography, and is a record of original work conducted between February 1996 and January 1999 in the Cardiology Unit, Department of Medicine, University of Adelaide, The Queen Elizabeth Hospital, Adelaide.

The work described has not been submitted for any other degree or award.

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CHRIS
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This work was carried out under the supervision of Prof John Horowitz whose passion for research is highly contagious and a constant source of inspiration. He has been a friend, colleague and mentor and I am deeply privileged to have studied with him.

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Finally, I acknowledge the tremendous sacrifices of my wife, Kathryn, over the past 3 years, without whose support, I would not have achieved so much. To my son James and daughter, Emma, who have only ever known PhD, daddy’s back!
PUBLICATIONS AND COMMUNICATIONS TO LEARNED SOCIETIES

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

Publications


Abstracts


ABSTRACT

Chapter 1
This introductory chapter provides a brief review of the relevant literature as regards the acute myocardial uptake of drugs. It explores the available methodologies for assessing acute drug effects with particular reference to the analysis of the left ventricular force-interval relationship. It outlines the current series of experiments conducted.

Chapter 2
This chapter examines the process of the acute myocardial uptake of the angiotensin converting enzyme inhibitors, perindoprilat and enalaprilat, in humans, utilizing cardiac catheterisation, including the insertion of coronary sinus catheters. The uptake of these agents, described here for the first time is examined together with the haemodynamic, metabolic and biochemical effects. In particular, the impact of these agents on angiotensin and bradykinin peptides both within the heart and peripherally is described. The selective intracoronary bolus administration of ACE inhibitors is also examined using the same methodology.

Chapter 3
The left ventricular force-interval relationship is a major determinant of contractile function. The acute effects of a range of cardioactive drugs upon this relationship, in humans, in vivo, are examined using three techniques: mechanical restitution curve construction, post extra-systolic potentiation, and frequency potentiation. Finally, the determinants of the response to frequency potentiation are sought.

Chapter 4
Provides conclusions, some potential limitations and consideration of the implications and potential future research possibilities on the basis of the current study.
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CHAPTER 1: REVIEW: EVALUATING ACUTE MYOCARDIAL DRUG UPTAKE

INTRODUCTION

A number of commonly and chronically utilized cardioactive drugs exert both direct and indirect effects on cardiac function, for example, by a combination of inotropic, coronary vasomotor, peripheral vasomotor and central effects. In order to elucidate the precise mechanisms of therapeutic drug effect, or of adverse reactions, it is necessary to control for indirect effects. One approach to this problem involves invasive investigation via cardiac catheterisation, including the selective examination of drug effects on the myocardium and coronary circulation. This requires the insertion of catheters into the coronary sinus to permit assessment of transcoronary gradients of drugs and/or metabolites together with fluctuations in coronary blood flow; this may permit correlation of the process of drug uptake into the myocardium with determination of the acute effects of such drugs (Horowitz and Powell 1986).

Drug effects on the heart have previously been examined in various ways. Studies have utilized cellular preparations, isolated working hearts, in vivo animal experimentation with serial myocardial biopsy and/or sacrifice of the animal, through to in vivo human experimentation (Bellemann and Scholzl 1975; Carr, Carroll et al. 1979; Horowitz, Barry et al. 1982; Powell, Horowitz et al. 1990; Khaw, Torchilin et al. 1995). In vivo experimentation has significant advantages over other techniques in allowing determination of factors that may modulate the rate and extent of acutely administered drug uptake. Such factors include the mechanical activity of the heart itself, heart rate, rate of coronary blood flow, extent of coronary artery disease and/or regional ischaemia and the presence of acute inflammation (Horowitz and Powell 1986; Reske, Schon et al. 1986; Powell, Horowitz et al. 1990; Morgan 1996).

Recognition of these modulating factors has enabled significant clinical advancement in some areas. For example, the potential for coronary artery disease to cause
ischaemia and, therefore, differential uptake of radio-labeled drugs into the heart is used to both diagnose and quantitate the extent of inducible ischaemia non-invasively in humans (Primeau, Taillefer et al. 1991). This technique can also be used to establish myocardial viability following acute ischaemic syndromes and, in the future, may permit the rapid, non-invasive determination of the presence and extent of early cellular injury (Ogiu, Nakai et al. 1994; Nakata and Shimamoto 1998). However, perhaps the most important aspect to the acute myocardial uptake of drugs is the ability to observe this phenomenon in humans in vivo.

**ACUTE DRUG UPTAKE INTO THE HUMAN HEART**

The acute uptake of drugs into the human heart in vivo can be studied in various ways but ultimately involves either invasive or non-invasive techniques. There are clear advantages in using non-invasive techniques in that they expose patients to negligible risk and enable the possibility for serial investigations in individuals. However, there are significant practical limitations imposed. Both positron emission tomographic (PET) scanning and magnetic resonance imaging (MRI) have been used to examine aspects of acute myocardial uptake of compounds (Ragosta and Beller 1993; Brudin, Valind et al. 1994; Fowler, Ding et al. 1994; Knuuti, Maki et al. 1995; Monti, Lucignani et al. 1995; Inoue, Kim et al. 1997). Monitoring of acute flux of myocardial drug content involves dynamic scanning which is limited in speed and is costly. Furthermore, the requirements of scanning are such that adequate additional data on drug effects can not readily be gathered concurrently, significantly limiting the utility of the data gathered. While the information available from these non-invasive methods may be complementary to that gathered invasively, invasive investigations remain the ‘gold standard’.

Quantification of the uptake of acutely administered drugs into the heart in humans in vivo is most reliably performed using cardiac catheterisation. It is necessary to take blood samples from either side of the myocardial vascular bed, being the central aorta and the coronary sinus. Whilst there is a single source of blood entering the
coronary circulation i.e. the central aorta, there is not a single site from which venous blood leaves the coronary vascular bed. The majority of the venous drainage of the heart ends in the coronary sinus as a continuation of the great cardiac vein. However, the anterior cardiac vein, which collects blood from the anterior surface of the right ventricle, drains directly into the right atrium and some additional small veins open directly into the right atrium. The right coronary vein whilst draining into the coronary sinus, does so at the right extremity of this vessel (Gray 1978). As a result of the above, a blood sample drawn through a catheter sited in the coronary sinus will not represent the entire venous drainage of the heart. However, it will represent the venous drainage of the vast majority of the left ventricle and, in particular, the left coronary circulation (comprising the left anterior descending and circumflex arteries). Because the right coronary vein drains into the terminal portion of the coronary sinus, sampling of this territory can not be guaranteed. Calculation of acute myocardial drug uptake therefore, of necessity, is limited to that portion of the myocardium drained by the proximal coronary sinus and, furthermore, assumes that drug uptake is equivalent amongst the various regions of the heart being drained by the coronary sinus.

Once the heart is effectively ‘isolated’ in terms of its blood supply, as described above, it is possible to determine the uptake of drugs into the heart when they are administered as a rapid intravenous bolus injection. Simultaneous paired blood samples are drawn from the aorta and coronary sinus at frequent and serial time points, enabling the calculation of the flux in aorto-coronary sinus drug concentration gradients over time. This enables accurate assessment of the time course of net uptake of drug into the heart and the time course of subsequent efflux of drug. The catheter positioned in the coronary sinus is not only used for blood sampling but can also be used to calculate coronary sinus blood flow by the thermodilution method (Ganz, Tamura et al. 1971). The combination of the time course of aorto-coronary sinus drug concentration gradient and coronary sinus blood flow enables the calculation of myocardial drug content at any point in time, for correlation with effects.
It must be noted that there are a number of potential limitations to this technique. First, the coronary sinus catheter cannot be positioned in such a way that sampling takes place at the distal end of the coronary sinus without risking contamination via reflux of right atrial blood (and also invalidation of flow measurements). Thus sampling from the circumflex bed is variably incomplete. Second, the position of the catheter cannot be correlated precisely with a known proportion of total coronary blood flow sampling in any one case: hence myocardial drug uptake and resultant content can be expressed most validly relative to resting coronary sinus blood flow (Horowitz and Powell 1986). This limitation imposes further priority on avoidance of any shift in catheter position during the procedure. Finally, as distinct from direct estimation of coronary arterial flow, coronary sinus flow determination can not generally be correlated precisely to any single coronary artery. However, selective catheterisation of the great cardiac vein has been utilized to obtain blood from the distribution of the left anterior descending artery (Feldman, Nichols et al. 1981).

The above description proposes sampling of arterial blood from the central aorta. Whilst this is possible, it is made technically difficult by limitations on the number of catheters which can safely be positioned within the body at any one time. However, it is possible to readily sample arterial blood from a femoral arterial sheath, without the need for placement of additional catheters. The femoral arterial blood sample, subject to validation studies, therefore may become a surrogate for central arterial blood (Horowitz, Dynon et al. 1986). Given that the aorta is effectively a large conduit that rapidly delivers blood from the central aorta to the femoral artery, there is minimal theoretical opportunity for variation of drug concentration and no basis for assuming a drug concentration gradient between these two sites.

Further problems inherent in the assessment of acute drug uptake by the myocardium in this way (involving the so-called “mass balance principles”) are:
(1) Ethical considerations preclude prolonged evaluation. Hence there may be incomplete information for agents for which uptake is prolonged and/or efflux is very slow.

(2) Accuracy of the calculation of cardiac drug content depends on the precision of the assay methods utilized as regards the determination, not of a drug concentration, but on a gradient between two simultaneous drug concentrations.

(3) The results of assessed drug content at any instant are not indicative of drug distribution within the heart and thus do not necessarily bear a defined relationship to global or regional myocardial drug concentration, nor to drug concentration at site(s) of action (e.g. receptors).

EVALUATING ACUTE DRUG EFFECTS
Although the current studies are principally concerned with drug effects on the heart, it is apparent that an acute intravenously administered drug in a human subject will have measurable effects on the whole organism. Extra-cardiac drug effects may influence changes within the heart, affecting interpretation of intra-cardiac drug effects. The evaluation of drug effects therefore involves several aspects.

Intra-cardiac haemodynamic effects
The evaluation of intra-cardiac haemodynamic effects involves those aspects of cardiac function that can, by altering, induce changes in measured haemodynamic variables. Some changes, such as variation in coronary blood flow and coronary vascular resistance are limited to the heart, whilst other changes, such as induction of ischaemia, may induce significant changes in filling pressures and cardiac output affecting peripheral haemodynamic parameters (1992). In addition to conventional haemodynamic measures, cardiac contractile function can also be determined. Contractile or inotropic function is determined by a number of factors, including preload, afterload, contractility and the force-interval relationship (Mahler F 1975; Anderson, Manring et al. 1979; Manring and Anderson 1980). Whilst a change in any
one of these variables may be associated with a change in contractile function, a change in function that is not associated with a change in preload, afterload or heart rate is therefore a sensitive measure of change in contractility.

**Measurement of contractility in vivo**

Indices of contractility can broadly be considered as either isovolumic or ejection phase, each having both strengths and weaknesses. The ejection phase indices of left ventricular contractile function include fractional shortening, ejection fraction, mean velocity of circumferential fiber shortening and left ventricular systolic work index (Grossman W 1991). By the very nature of being ejection phase indices, they are highly sensitive to alterations in loading conditions. Therefore, any alteration in an ejection phase index can only be interpreted as a change in contractility if the loading conditions of the ventricle have remained constant (Karliner JS 1971; Ross 1976; Grossman W 1991). Nevertheless, these parameters are in general easily measured, often non-invasively, and therefore clinically useful.

Contractility can alternatively be assessed utilizing measures of the isovolumic phase of contraction. As implied, the closed mitral and aortic valves during this phase should make this index relatively insensitive to loading conditions. Whilst not a “pure” isovolumic index, the maximal rate of rise of left ventricular systolic pressure, $\frac{dP}{dt_{max}}$, is widely used as a reliable index (Gleason WL 1962). Extensive investigations of the effects of various changes in loading conditions have revealed that $\frac{dP}{dt_{max}}$ is relatively insensitive to changes in loading conditions that are within the physiological range (Mahler F 1975; Barnes, Horwitz et al. 1979; Broughton and Korner 1980). Alternative isovolumic indices have been proposed, including $(dP/dt)/P$, where $P$ is left ventricular pressure, $(\frac{dP}{dt})/IIT$, where $IIT$ is integrated isovolumic tension and $(dP/dt)/CPIP$, where $CPIP$ is common developed isovolumic pressure (Mason 1969; Sonnenblick, Parmley et al. 1969; Falsetti, Mates et al. 1971; Peterson, Skloven et al. 1974; Stein, McBride et al. 1975). None of these indices has been shown to be consistently superior to $dP/dt$ (Peterson, Skloven et al. 1974; Mahler F 1975; Broughton and Korner 1980).
A further alternative methodology involves recording of left ventricular pressure-volume loops (Sasayama, Nonogi et al. 1984). This is done by recording both left ventricular pressure and volume throughout a cardiac cycle and recording this graphically with left ventricular pressure on the y-axis and left ventricular volume on the x-axis. By varying loading conditions or contractile force, a series of different loops can be formed (Kass 1992). The resulting relationship between end systolic pressure and end systolic volume is virtually linear (Aroney, Herrmann et al. 1989). A change in the slope of the end-systolic pressure-volume relationship is therefore a very sensitive and load independent measure of left ventricular performance. However, there are some practical limitations to the serial use of this technique in a single subject, although recent advances in catheter technology are addressing this issue (Baan, van der Velde et al. 1984; Kass, Midei et al. 1988; Kass 1992). Furthermore, there is a need to vary loading conditions in order to produce a range of pressure-volume loops, enabling the calculation of end-systolic pressure-volume relationship. This is not only time consuming but interferes with monitoring of haemodynamic affects of acutely administered drugs.

**Effects on the left ventricular force-interval relationship:**

One of the major determinants of left ventricular contractile function is heart rate. The impact of heart rate on contractile function was first described in detail over 100 years ago (Bowditch 1871). Since this time, a large body of literature has been published regarding this interaction (Gwathmey, Slawsky et al. 1990; Schmidt, Hajjar et al. 1995). Attempts to unravel the complex cellular processes determining the force-frequency relationship continue to be made. These processes are likely to be of critical importance. For example, in heart failure, the force-frequency relationship is reversed, such that tachycardia results in no incremental positive inotropic effect, but rather, in a negative inotropic effect (Ezzaher, el Houda Bouanani et al. 1992; Mulieri, Hasenfuss et al. 1992; Eising, Hammond et al. 1994; Hasenfuss, Holubarsch et al. 1994; Hasenfuss, Reinecke et al. 1996). An understanding of the cellular
processes in both normal and failing myocardium might enable the development of more effective therapeutic strategies for heart failure.

It has long been recognized that some cardioactive drugs may have variable inotropic and haemodynamic effects depending on heart rate (McCans, Lindenmayer et al. 1974; Artman, Graham et al. 1985; Bohm, Diet et al. 1988; Kambayashi, Miura et al. 1992; Holubarsch, Schneider et al. 1995; Ross, Miura et al. 1995). The presence of heart failure, or impaired left ventricular systolic function, may have a further impact on these frequency-related drug effects. This has important implications for both the short and long term use of cardioactive drugs where there is the potential for tachycardia to occur, for example, during exercise.

The force-frequency relationship can be studied in various ways. In vitro studies provide a means for examination of the relationship in the absence or presence of spontaneous ventricular contraction (e.g. with isolated papillary muscle preparations or whole beating hearts respectively) (Manring and Anderson 1980; Becker and Gerlach 1984). The use of papillary muscle preparations provides an ideal method for studying the full range of the force-interval relationship, but that component occurring at very low stimulation frequencies is of limited physiological interest. In vitro experiments commonly use frequency potentiation, or the rate staircase. This involves examining inotropic function during a pacing-induced tachycardia at various rates of pacing. It is difficult to apply this methodology to the in vivo setting. Sustained pacing-induced tachycardia may result in neurohumoral activation (Twidale, Rayner et al. 1993) and, in patients with coronary artery disease, is likely to induce ischaemia. Furthermore, in patients with impaired left ventricular systolic function, pacing-induced tachycardia has the potential to induce haemodynamic compromise (Schmidt, Hajjar et al. 1995).

The force-frequency relationship in man can alternatively be investigated by examining mechanical restitution, a potential single-beat surrogate for tachycardia (Anderson, Manring et al. 1979). Mechanical restitution refers to the recovery of
contractile function following a non-steady state beat. In man, due to spontaneous sinus node activity, mechanical restitution can only be examined for premature non-steady state beats. It has recently been shown that mechanical restitution curves, based upon the component of the mechanical restitution curve occurring at cycle lengths shorter than those observed spontaneously, are highly reproducible in patients with ischaemic heart disease and may be utilized to examine rate-dependent inotropic phenomena (Ritchie, Wuttke et al. 1995). This methodology requires a high fidelity pressure manometer to be positioned in the left ventricle for determination of pressure and its first derivative, LV dP/dt, and a temporary bipolar pacing lead to be positioned in the right atrium to enable programmed atrial stimulation. Both aspects can be readily incorporated into studies of acute myocardial drug uptake, enabling examination of both sustained tachycardia and mechanical restitution. Post extra-systolic potentiation, another aspect of the force-interval relationship can also be examined in this way.

**Extra-cardiac haemodynamic effects:**

Given that a significant component of the injected dose of a drug is distributed to areas other than the heart it is not surprising that extra-cardiac haemodynamic effects can result. This can occur, for example, due to direct effects of the administered drug on peripheral arteriolar tone such as occurs with calcium antagonists (Tsoucaris, Benetos et al. 1995). Alternatively, a drug such as prazosin can induce reflex effects on autonomic function (Ajayi and Raji 1994). There may be modulation of autacoid release as a result of either cardiac or extra-cardiac effects such as occurs with inhibitors of the angiotensin converting enzyme (Littler 1990; Bottcher, Frost et al. 1992). Finally, uptake of drugs into the central nervous system can occur with direct actions on central cardiovascular controlling centers (Cook, Doherty et al. 1984). Such effects as those described above may begin rapidly, being distorting to apparent correlation between myocardial drug uptake and haemodynamic and other effects (Powell, Horowitz et al. 1990).
In order to fully evaluate the haemodynamic effects of acutely administered drugs, it is therefore necessary to monitor both intra and extra-cardiac parameters in order to detect the relative contribution of various changes to the overall effects of the drug. This is most reliably performed with invasive cardiac catheterisation but it may be necessary to perform additional investigations (e.g. plasma concentrations of catecholamines, autacoids).

Despite the extensive measures detailed above it is not always possible to differentiate between central and peripheral effects of a drug. One method by which this can be overcome is to administer a small dose of the drug being tested directly into the coronary circulation. Ideally, such administration of a drug should have no measurable direct peripheral effects. Any changes observed under these circumstances can therefore reliably be ascribed to direct effects of the drug on the heart but the possibility of reflexly mediated changes (e.g. due to hypotension) cannot be excluded without prior autonomic blockade. Conversely, direct effects of agents on peripheral vasomotor tone and to some extent venous capacitance can be studied more accurately via intra-arterial infusion of small concentrations of the agent into regional vascular beds, such as the forearm.

Determinants of disposition
The natural variability between patients undergoing investigation of the effects of acutely administered drugs provides the opportunity for examining the interaction between these variables and the kinetics of drug uptake and subsequent effects. Factors that may influence the uptake of drugs into the myocardium include basal heart rate and extent of coronary artery disease. Other factors such as left ventricular ejection fraction and loading conditions may directly modulate drug effects on haemodynamics.
Metabolic effects:
Cardioactive agents may affect myocardial oxygen supply/demand relationships, and hence inducibility of myocardial ischaemia. There is also, however, increasing evidence that many such agents will have direct effects on efficiency of myocardial oxygen utilization, producing modification of myocardial metabolism independent of any other haemodynamic effects. Nitric oxide donors, catecholamines, modulators of mitochondrial potassium channels and carnitine palmitoyl transferase-I inhibitors provide some examples of such activity (Mueller 1978; Bunger, Permanetter et al. 1983; Isono, Sato et al. 1993; Kennedy, Unger et al. 1996; Grover 1997; Szekeres, Dezsi et al. 1997).

Detection of changes in myocardial metabolism induced by acute drug administration may be performed at rest or during tachycardia-induced stress: the latter circumstance is relevant in particular to the potential induction of ischaemia. In this regard, both MRI techniques (de Roos and van der Wall 1994; Ishikawa, Mori et al. 1995; Pluim, Lamb et al. 1998) and myocardial metabolic imaging with positron emission tomography (PET) or single photon emission computed tomography (SPECT) (Valkema, van Eck-Smit et al. 1994; Bergmann, Weinheimer et al. 1996) have advantages over cardiac catheterisation as regards localization of changes, and also specific quantitation of high energy phosphate content (MRI) or specific patterns of metabolism (PET/SPECT) at rest. However, cardiac catheterisation is more adaptable for comparison of metabolism at rest and during tachycardia, and permits specific measurement of myocardial handling of labeled or unlabelled metabolites. For example, myocardial oxygen consumption and net lactate consumption/production are easily determined (Chiong and Parker 1975). For assessment of trans myocardial glucose or fatty acid metabolism, labeled metabolite must be utilized (Wisneski, Gertz et al. 1985).

Other biochemical effects:
This aspect of acute drug effects on the heart extends observation beyond the primary observed effects and aims to elicit the mechanism by which such changes
occur. The measurements made in this regard are therefore specific to the drug being investigated and depend on the existing knowledge regarding the potential mechanisms of action.

An example of this level of investigation is the examination of the acute effects of angiotensin converting enzyme (peptidyl dipeptidase) inhibitors. These agents are able to affect, from a primary point of view, two separate biochemical pathways, one involving the formation of angiotensin II from angiotensin I and the second involving the catabolism of the nonapeptide bradykinin(1-9) to the heptapeptide bradykinin(1-7). Inhibiting the formation of end products of both reactions could theoretically result in similar acute haemodynamic effects. It is therefore critical in evaluating the acute effects of such drugs to not only perform superficial observations of haemodynamic and metabolic effects but to also examine the direct effects on the various biochemical pathways. Such examination can itself be performed in various ways. For example, specific inhibitors of one or other pathway could be co-administered with the angiotensin converting enzyme inhibitor or their effects compared with it. Alternatively, inhibitors of the end products of either pathway could be administered and, again, the results compared with the effects of angiotensin converting enzyme inhibitors. However, the most reliable and appropriate investigation involves the assaying of the various compounds involved in the biochemical cascades, enabling accurate correlation between the demonstrable biochemical effects with the direct effects of the drugs.

CORRELATIONS BETWEEN PHARMACOKINETICS AND PHARMACODYNAMICS

Pharmacokinetics

Traditionally, the myocardium has been considered part of a “well-perfused compartment” in classical pharmacokinetic models of acute drug disposition. Thus it is anticipated that drug uptake into the myocardium should be generally very rapid and closely correlated with the α-half life of single-dose pharmacokinetic studies.
This correlation has previously been examined for some agents (Horowitz, Dynon et al. 1986). While some correlation exists, it is likely that potentially ischaemic zones of myocardium do not fit such a model. In addition, myocardial uptake of some agents is best considered as a 2-compartment model (Morgan, Horowitz et al. 1989).

**Effect compartments**

A more recent study attempted to compare pharmacokinetic parameters of uptake of metoprolol and dl-sotalol into the myocardium with their theoretical concentrations in an “effect” compartment based on negative inotropic effects at constant heart rate (Ritchie, Morgan et al. 1998). The results were consistent with the suggestion that the “effect” compartment was peripheral for both agents. Whether this surprising finding results from a delay in onset of drug effect due to gradual induction of changes in intracellular metabolism remains to be determined.

**EXPERIMENTAL DESIGN: PRACTICAL CONSIDERATIONS FOR INDIVIDUAL AGENTS**

A number of general points need to be considered when planning the type of experiments broadly described above. Any drug being considered for such experimentation needs to be available for intravenous administration. Furthermore, the vehicle in which the drug is dissolved must be free of any effects on the parameters described above which are to be analyzed (Gough, Zeiler et al. 1982). The drug must be administered in a dose that is sufficient to result in measurable effects while, at the same time, representing a dose that is relevant to routine clinical practice. Lastly, given the limited time course available for experimentation, it is important to administer drugs with biological activity, avoiding pro-drugs that must first be metabolized to their active form.

**CURRENT INADEQUACIES OF KNOWLEDGE**

The acute myocardial drug uptake and disposition of a number of cardioactive drugs have previously been described. Among the many, there remain three broad
aspects to this work that remain largely neglected to date, which are particularly relevant to the myocardial disposition and effects of cardioactive drugs.

A. Myocardial disposition and effects of angiotensin converting enzyme inhibitors
Angiotensin converting enzyme (ACE) inhibitors have been shown to produce beneficial effects on systemic haemodynamic status in patients with symptomatic heart failure, and after acute myocardial infarction, particularly in the presence of impairment of left ventricular systolic function (1987; Pfeffer, Lamas et al. 1988; 1991; 1992; Cohn 1992; Pfeffer, Braunwald et al. 1992; Yusuf, Pepine et al. 1992). However, despite these beneficial effects, a number of important questions remain unanswered concerning both the mechanism of action and therapeutic potential of ACE inhibitors. These include:

(i) Mediators of effect.
ACE has a variety of substrates: it may induce, notably both the bioconversion of angiotensin I to angiotensin II, as well as the inactivation of bradykinin (BK), by cleavage to form its inactive heptapeptide metabolite. There is increasing evidence to suggest that increased BK is important as regards some of the acute cardioprotective effects of ACE inhibitors in acute ischaemic models, and also their effects on neointimal proliferation (Linz and Scholkens 1992; Farhy, Carretero et al. 1993). However, such inferences have generally been drawn via studies utilizing the type-2 BK receptor antagonist Hoe140, rather than via correlation between BK concentrations and ACE inhibitor effect. This issue is likely to be of pivotal importance in predicting the relative roles of ACE inhibitors and the recently developed angiotensin II receptor antagonists, which do not affect BK turnover.

Furthermore, with respect to the effects of ACE inhibitors on cardiac levels of angiotensin II, Urata and colleagues have proposed that the major pathway of conversion of angiotensin I to angiotensin II in the heart is the heart chymase, an enzyme which is not inhibited by ACE inhibitors (Urata, Kinoshita et al. 1990).
(ii) Relative significance of effects in tissues vs plasma.

ACE is present in high concentrations in many tissues, including the myocardium and heart valves. However, it remains uncertain to what extent the effect of ACE inhibitors, both haemodynamic and therapeutic, reflect interaction with tissue, rather than plasma, ACE. It is also not known to what extent various ACE inhibitors are taken up into the human myocardium acutely or during chronic therapy, although it is clear cut that chronic ACE inhibitor treatment in animal models results in inhibition of tissue ACE activity.

(iii) Effects of ACE inhibitors on myocardial ischaemia.

Studies of the effect of ACE inhibitors on inducibility of angina pectoris have reached variable conclusions, irrespective of the left ventricular function of the patient group tested and the ACE inhibitor studied (Abrams 1990; Linder and Heusch 1990). The relationship between ACE inhibitor therapy, its biochemical effects, and changes in myocardial metabolism in patients with exercise-induced angina pectoris has also been minimally studied.

B. Acute drug effects on the left ventricular force-interval relationship

Prediction of potentially beneficial or indeed deleterious symptomatic effects of cardioactive agents on the basis of their acute haemodynamic effects at rest has proved unreliable (Dawson, Canepa-Anson et al. 1983; Cowley and Skene 1994). Differences between observations at rest and those relevant to non-basal conditions (for example during exercise tachycardia, acute atrial fibrillation or ventricular tachycardia) are readily explicable in terms of changes in neurohumoral milieu (Horowitz, Dyon et al. 1984) and/or rate related variability in pharmacological properties of the agents concerned (Applegate, Walsh et al. 1987; Sharma, Purves et al. 1990; Remme 1993). However, previous acute haemodynamic investigations have not generally utilized methodology to take such possible eventualities into account.

There is a solid basis for considering potential effects of cardioactive drugs on the left ventricular force-interval relationship. A number of investigations, both in vitro and in vivo, have shown that the positive inotropic effects of β-adrenoceptor agonists
and antagonists are frequency-dependent in animal models and in human subjects with these effects varying with left ventricular systolic function (Kambayashi, Miura et al. 1992; Ross, Miura et al. 1995). Furthermore, *in vitro* data has revealed the potential for the L-calcium channel antagonist, verapamil, to demonstrate use-dependent negative inotropic effects (Applegate, Walsh et al. 1987; Remme 1993). Clinical data have reported acute haemodynamic deterioration following initiation of intravenous therapy during tachycardia with class I and IV anti-arrhythmic agents in patients prone to tachyarrhythmias (Buxton, Marchlinski et al. 1987; Rankin, Rae et al. 1987; Sharma, Purves et al. 1990).

Whilst the acute myocardial drug uptake of both verapamil and digoxin have previously been described in detail, the effects on the left ventricular force-interval relationship have not (Powell, Horowitz et al. 1990; Powell, Horowitz et al. 1990). This may have implications for the relative safety and clinical efficacy of these agents.

To date, there has been minimal work performed investigating the effects of drugs on the force-interval relationship. Cardioactive drugs are commonly prescribed to patients who are either at increased risk of serious tachyarrhythmias or who have significant impairment of contractile function. It is therefore, potentially, of critical importance to understand the relative effects of these drugs in non-basal conditions. Furthermore, previous experience with translating promising haemodynamic effects of drugs at rest into significant benefits from chronic therapy has yielded disastrous results on a number of occasions (1989; Packer, Carver et al. 1991; Waldo, Camm et al. 1995). The incremental knowledge gained from examining drug effects on the force-interval relationship may offer the potential to improve predictive accuracy when determining long term therapeutic strategies.

C. Assessment of the left ventricular force-interval relationship

Coupled with the paucity of *in vivo* data regarding analysis of the left ventricular force-interval relationship is the issue of how best to assess this parameter *in vivo*. 
Various methodologies have been utilized previously, predominantly involving a pacing induced tachycardia at several stimulation frequencies in order to produce a 'staircase' response (Hasenfuss, Holubarsch et al. 1994). Recently, the construction if incomplete mechanical restitution curves in vivo in humans has been reported, yielding highly reproducible results in patients with ischaemic heart disease (Ritchie, Wuttke et al. 1995).

The study of the force-interval relationship of the left ventricle in vivo is limited by several factors. Given that the study population is largely ischaemic the use of pacing induced tachycardia has the potential to invoke variable and unpredictable degrees of ischaemia. Coupled but not necessarily secondary to this is the potential for neurohumoral activation, such as catecholamine release (Twidale, Rayner et al. 1993). Furthermore, in patients with significant underlying impairment of systolic function there is potential for haemodynamic deterioration during tachycardia, limiting the scope for study in these patients (Ezzahe, el Houda Bouanani et al. 1992; Mulieri, Hasenfuss et al. 1992; Eising, Hammond et al. 1994; Hasenfuss, Holubarsch et al. 1994; Hasenfuss, Reinecke et al. 1996).

Mechanical restitution and frequency potentiation involve somewhat differing physiological processes. However, they are both measures of the force-interval relationship and do share some common aspects. There has been no previous attempt in vivo to correlate these two measures. Given that the analysis of mechanical restitution is highly unlikely to induce ischaemia or neurohumoral activation and can be safely performed in patients with significant impairment of systolic function, its adequacy as a measure of the left ventricular force-interval relationship needs to be established.

**SCOPE OF THE PRESENT STUDY**

This thesis examines the uptake process of acutely administered cardioactive drugs into the human heart in vivo and uses a range of methodologies to examine the
subsequent effects of these drugs both on the heart itself and on the peripheral circulation. This approach facilitates differentiation between mechanisms of drug action, specifically isolating primary sites of effect (cardiac versus extracardiac). Such experiments provide an important contribution to knowledge of drug effects. This is particularly the case when considering various disease states that can have diverse effects on the heart versus the rest of the body. Increased knowledge regarding the differential effects of these drugs is therefore likely to translate into modified clinical use.

A series of experiments were designed for the present study in order to gather detailed information relating to the myocardial uptake of acutely administered cardioactive drugs. These data were then correlated with effects of the drugs on the parameters discussed above.

The first series of experiments involved the acute intravenous bolus administration of the angiotensin converting enzyme inhibitors, perindoprilat and enalaprilat to patients undergoing elective cardiac catheterisation and coronary angiography for the investigation of chest pain. In order to allow determination of acute myocardial drug uptake, a radioinhibitor binding displacement assay for determination of whole blood concentrations of perindoprilat and enalaprilat was developed. The effects of these drugs on haemodynamic parameters, inotropic function, left ventricular force-interval relationship and trans-cardiac gradients of angiotensin and bradykinin peptides were examined both at resting heart rate and during pacing induced tachycardia, as a potential mechanism for inducing myocardial ischaemia.

A further series of experiments involved the acute intracoronary injection of the same angiotensin converting enzyme inhibitors in an attempt to isolate the cardiac effects from those on the rest of the body.

The second series of experiments involved a detailed evaluation of effects of several agents on the left ventricular force-interval relationship. These experiments utilized
existing knowledge regarding the acute myocardial drug uptake of the negatively inotropic agent, verapamil, and the positively inotropic agent, digoxin, as the framework for a 'second generation' series of experiments evaluating drug effects on haemodynamics and inotropic function at both resting heart rates and during pacing induced tachycardia. Drug effects on the force-interval relationship were further evaluated by examining incomplete mechanical restitution curves and post extrasystolic potentiation. The results from these experiments were then compared with available data for other negatively and positively inotropic agents.

A further investigation, performed in all patients with data available on both pacing induced tachycardia and mechanical restitution, sought the extent of potential correlation between these two measures of the force-interval relationship, and also other possible determinants of extent of frequency potentiation.
CHAPTER 2: MYOCARDIAL UPTAKE OF PERINDOPRILAT AND ENALAPRILAT AND THEIR EFFECTS

INTRODUCTION

BACKGROUND

The renin-angiotensin system has long been recognized as playing an important role in the maintenance of normal homeostasis, particularly as regards blood pressure. Renin, released from the kidneys in response to a fall in perfusion pressure in the glomerular apparatus, is converted to angiotensin I, predominantly in the lungs, and finally converted into angiotensin II by angiotensin converting enzyme which is widely present in serum and tissues. Angiotensin II is both a powerful peripheral vasoconstrictor and stimulates aldosterone secretion to retain sodium and thus elevate blood pressure.

Over the past three decades, the role of this system in response to acute myocardial infarction and/or the development of congestive cardiac failure has been well documented (Weber, Sun et al. 1995; Megarry, Sapsford et al. 1997; Parmley 1998; Sun, Zhang et al. 1993). Furthermore, the development of specific inhibitors of the angiotensin converting enzyme has resulted in a class of drugs which are widely prescribed for the therapy of hypertension and cardiac failure (1987; Pfeffer, Lamas et al. 1988; 1991; 1992; Pfeffer, Braunwald et al. 1992). Recently, evidence has been produced to show a substantial reduction in morbidity and mortality when ACE inhibitors are prescribed early following acute myocardial infarction (Opie 1994; 1995).

Complementing the advancements in clinical information has been the development of a greater understanding of the basic processes involved at a cellular level. Angiotensin II has been demonstrated to exert its effects via at least two different receptors, the AT₁ and AT₂ receptors (Capponi 1996; Chung, Stoll et al. 1996; Regitz-Zagrosek, Neuss et al. 1996; Thomas, Thekkumkara et al. 1996; Matsubara 1998). The
AT₁ receptor, of which there are two subtypes, is responsible for virtually all of the physiological actions of angiotensin II. The receptor is coupled to a G protein activating phospholipase C, which mobilizes intracellular calcium stores and activates protein kinase C. The AT₂ receptor is expressed in high numbers during fetal life but at low levels in the adult heart (Matsubara 1998). It can, however, be re-expressed in the setting of heart failure or neointima formation after vascular injury. Binding of angiotensin II to this receptor enhances cell death. However, it also exerts an inhibitory influence on AT₁ mediated mitogenic signals and synthesis of extracellular matrix protein. Furthermore, it has been shown to activate the kinin/nitric oxide system in the heart (Porsti, Bara et al. 1994; Auch-Schwelk, Duske et al. 1995; Gorelik, Carbini et al. 1998).

The beneficial effects of ACE inhibitors were traditionally considered due to inhibition of the formation of angiotensin II from angiotensin I. However, a number of factors have caused the reappraisal of this view. Firstly, ACE, a peptidyl dipeptidase, is not the only enzymatic pathway for the formation of angiotensin II. A cardiac distribution of chymase within the human heart is able to cleave angiotensin I to form angiotensin II and, indeed, it has been proposed that this is the major route for angiotensin II formation in humans (Urata, Hoffmann et al. 1994; Urata, Nishimura et al. 1995; Urata, Nishimura et al. 1996). In conjunction with this concept is the finding that angiotensin II levels, while being suppressed following acute therapy with ACE inhibitors can return to pre-treatment levels during chronic therapy (Liao and Husain 1995).

ACE is not an angiotensin I specific enzyme. It is also one of the major pathways for the degradation of bradykinin 1-9 to the inactive heptapeptide, bradykinin 1-7. Bradykinin 1-9, acting predominantly via the type-2 bradykinin receptor(B2) mediates both nitric oxide and prostacyclin production resulting in vasodilatation (Mombouli 1997). There is now good evidence to show that the cardioprotective effects of ACE inhibitors in the setting of acute myocardial infarction, with particular reference to limitation of infarct size and recovery of contractile function, are
mediated through bradykinin (Duncan, Burrell et al. 1996; Linz, Wiemer et al. 1997; Remme 1997). The relative roles of inhibition of angiotensin II formation and prevention of bradykinin 1-9 breakdown in the beneficial effects of ACE inhibitors, both acutely and chronically have therefore been the source of some debate. This issue has taken on potentially greater importance with the introduction of specific inhibitors of the angiotensin II receptor, although the majority of these agents are specific for the AT₁ subtype, leaving the AT₂ receptor free for activation by angiotensin II, potentially activating the kinin/nitric oxide/cyclicGMP system in the heart. This does however assume that the AT₂ receptor has been re-expressed in response to the underlying process (Matsubara 1998).

COMPARISON OF TWO ACE INHIBITORS

The current project involves the comparison of two different ACE inhibitors, perindoprilat and enalaprilat. This approach has been taken in order to compare the relative impact of two inhibitors with different affinities for tissue, as opposed to plasma, ACE. Perindoprilat is a more potent inhibitor of tissue ACE than is enalaprilat (Johnston, Fabris et al. 1989). This approach has been taken in order to help determine the relative importance of tissue versus plasma ACE inhibition as regards the acute effects of these agents. The actual choice of ACE inhibitors ultimately came down to an issue of funding and drug availability. The current study was funded by Servier laboratories and the perindoprilat provided as a gift. The enalaprilat was a gift from Merck, Sharp & Dohme.

In both cases, the active metabolite of the clinically prescribed drug is administered. Both perindopril and enalapril must be metabolized to the active form, the pro-drug having no significant biological activity. The need for metabolism results in peak haemodynamic effects being delayed for at least one hour following acute administration (Sakaguchi, Jackson et al. 1988; Mendelsohn, Pupic et al. 1991). Due to the highly invasive nature of the current investigation, data collection for this
length of time was not considered practical. The active metabolite was therefore administered in both cases.

RATIONALE FOR PERINDOPRILAT AND ENALAPRILAT DOSES
The current study was designed to examine the effects of ACE inhibitors on a range of haemodynamic and biochemical pathways. In order to critically examine this area, it was therefore desired to administer a sufficient dose of ACE inhibitor to largely, if not totally, inhibit serum ACE and, potentially, tissue ACE activity. Furthermore, it was necessary to review the safety data for both perindoprilat and enalaprilat, given that neither agent was marketed in Australia for parenteral administration.

Perindoprilat
There has been a modest amount of previous experience with the intravenous and intracoronary administration of perindoprilat in humans (Reid, Lees et al. 1987; MacFadyen, Lees et al. 1991; MacFadyen, Lees et al. 1992; MacFadyen, Lees et al. 1993; Reid, MacFadyen et al. 1993; Reid, MacFadyen et al. 1993; Haber, Powers et al. 1994; MacFadyen, Lees et al. 1994; Antony, Lerebours et al. 1995; Antony, Lerebours et al. 1996). These studies have largely been performed in patients with either class II-IV heart failure or with hypertension, initial studies having been performed in normals. They have used intravenous infusions of perindoprilat which have been given over various intervals from 20 minutes to 6 hours resulting in effects ranging from no change in systolic blood pressure, through to a 10% decrease. There is, however, no evidence to suggest incremental haemodynamic effects at doses sufficient to largely inhibit serum ACE activity. Acute intravenous doses of 1-4mg of perindoprilat have resulted in similar extent of blockade of tissue ACE activity (Lees and Reid 1987). Given the relatively short time course for the experimental protocol (less than 30 minutes), the dose selected was therefore at the lower end of this range, being 1.25mg.
The subsequent selection of an intracoronary dose for perindoprilat was made on the basis of wanting to limit the extent of peripheral effects, whilst attaining intracoronary drug concentrations sufficient to inhibit ACE activity. In the absence of previous publications regarding bolus administration, a dose of 0.25mg was chosen.

**Enalaprilat**

There has been a greater experience with the acute intravenous and intracoronary administration of enalaprilat to humans (Kubo, Cody et al. 1985; Hornung and Hillis 1987; Rutledge, Ayers et al. 1988; Neutel, Luther et al. 1991; Boldt, Knothe et al. 1994; Haber, Powers et al. 1994; Hirschl, Binder et al. 1995; Hirschl, Binder et al. 1997). Furthermore, parenteral formulations of enalaprilat are available for clinical use in some countries. The recommended initial dose of enalaprilat in the USA is 0.625mg. This dose is recognized to be the minimum dose necessary to substantially inhibit serum ACE. The literature records the administration of single intravenous doses of enalaprilat up to 5mg in the absence of any incremental haemodynamic effects (Hirschl, Binder et al. 1995). Superficially, once a sufficient dose is given to inhibit ACE, incremental dosing is unlikely to result in incremental effect. Whilst the doses administered have resulted in up to a 35 mmHg reduction in mean arterial pressure, this has occurred in the setting of significant falls in pulmonary capillary wedge pressure and systemic vascular resistance, and a significant increase in cardiac index. Peak haemodynamic effects were generally observed within 30 minutes of drug administration.

The dosing levels chosen for the current investigation were on both the basis of the above information and the desire to administer two different ACE inhibitors with resultant similar ACE inhibition i.e., to allow for the different potency of the drugs. The intravenous dose of enalaprilat was therefore set at 2.5 mg and the intracoronary dose at 0.5 mg.
MYOCARDIAL DRUG UPTAKE OF ACE INHIBITORS

The acute myocardial drug uptake of ACE inhibitors has not previously been studied in human subjects. While it is recognized that chronic therapy with ACE inhibitors results in effective inhibition of cardiac tissue ACE, in animal models, the extent to which these agents are taken up acutely by the human heart is not known.

SCOPE OF THE PRESENT STUDY

The current inadequacies of knowledge have been highlighted previously, the current study being designed to address several of these issues. This study involves the acute administration of the active metabolites of two commonly used ACE inhibitors with different degrees of tissue ACE activity. Utilizing invasive haemodynamic, metabolic and pharmacological measures, the acute effects of these agents both at rest and during pacing induced tachycardia will be compared with each other and with baseline observations. Intracoronary dosing will be used in a cohort of patients in an attempt to separate central and peripheral effects.

METHODOLOGY

DATA COLLECTION

Patient Selection
Patients were selected from those presenting for elective cardiac catheterisation and coronary angiography for the investigation of chest pain. Patients were required to be clinically stable with all cardioactive medications withheld for at least five half lives prior to the research procedure. Exclusion criteria included:

- Unstable angina pectoris
- Q-wave myocardial infarction in the preceding three months
- Haemodynamically significant valvular heart disease or left main coronary artery stenosis (>50% reduction in luminal diameter)
- Concomitant therapy with angiotensin converting enzyme inhibitors or long-acting nitrates
- Previous adverse reactions to angiotensin converting enzyme inhibitors
• Clinically significant impairment of hepatic or renal function
• Atrial fibrillation

The protocol was approved by the Queen Elizabeth Hospital Ethics of Human Research Committee and written, informed consent was obtained prior to the procedure in all cases.

**Instrumentation**

Femoral arterial and femoral venous sheaths were inserted via the right groin (Barry, Levin et al. 1979). Routine coronary angiography was performed using non-ionic contrast media with a minimum of 20 minutes elapsing between the last injection of contrast and the commencement of the research protocol (Judkins 1968). A 7F Swan-Ganz catheter was positioned via the femoral venous sheath for serial determination of pulmonary capillary wedge pressure and cardiac output, utilizing the thermodilution method, the reading at each time point being the average of at least three recordings (Sorensen, Bille-Brahe et al. 1976). A 4F micromanometer-tipped catheter (Millar Instruments, Texas) was positioned in the left ventricle, via the femoral arterial sheath, for determination of instantaneous left ventricular pressure and its first derivative, dP/dt. A coronary sinus catheter (Cordis Webster, California) was inserted via the left cubital fossa using a cut-down approach to isolate a suitable vein. The final position of the distal end of the catheter within the coronary sinus was confirmed by the injection of a small amount of non-ionic radiographic contrast (3-5 mls), principally to demonstrate that the external thermistor was clear of the coronary sinus ostium, minimizing the potential for distortion of flow measurements by the reflux of right atrial blood into the coronary sinus. The coronary sinus catheter was externally fixed in position and periodically checked to exclude subsequent movement. This catheter was utilized for serial blood sampling, thermodilution flow measurements and for atrial pacing (Ganz, Tamura et al. 1971). Femoral arterial pressure was constantly monitored via the femoral arterial sheath. A 2-channel surface electrocardiograph was constantly recorded. In the case of
patients receiving intracoronary drug injections, a second femoral arterial sheath was inserted.

**Experimental Protocol**

Each patient underwent a series of three experimental protocols, receiving one of four possible angiotensin converting enzyme (ACE) inhibitor administrations (either enalaprilat or perindoprilat as either an intravenous or intracoronary injection).

**Baseline measures:**

Once all instrumentation was completed, a series of baseline measurements were made prior to the series of experimental protocols. These measurements included electrocardiographic intervals both at spontaneous heart rate and during atrial pacing at a rate just above spontaneous rate, with all other measurements being made during atrial pacing. These included phasic and mean femoral arterial pressure, the maximal positive deflection of the first derivative of left ventricular pressure, LV+dP/dt$_{\text{max}}$, pulmonary capillary wedge pressure, cardiac output as determined by the thermodilution method and coronary sinus blood flow, also using the thermodilution method, the latter two measures always being performed at separate time points. Spontaneous heart rate was serially determined during transient interruption of atrial pacing. Paired femoral arterial and coronary sinus blood samples were taken for subsequent determination of oxygen saturation, plasma ACE concentration, lactate concentration and angiotensin and kinin peptide levels. Data were gathered for construction of mechanical restitution curves, the detailed methodology of which is described in chapter 3.

**Experiment 1:**

Effects of atrial pacing on myocardial metabolism and handling of angiotensin and bradykinin peptides.
Atrial pacing at a cycle length of 400-500 msec (approximately 60% of baseline paced cycle length) was performed for 2 min. During this period, mean arterial pressure, pulmonary capillary wedge pressure and dP/dt were continuously recorded and coronary sinus blood flow was measured after approximately one minute of rapid pacing. All haemodynamic and inotropic parameters were measured again approximately one minute following the restoration of the baseline atrial pacing cycle length. Paired blood samples (arterial and coronary sinus) were drawn at baseline, during rapid pacing, and 2 minutes following restoration of the baseline atrial pacing cycle length for determination of oxygen saturation, plasma ACE activity and lactate concentration. Further assays to be performed on these samples included angiotensin I, angiotensin II and angiotensin(1-7) concentrations and bradykinin(1-7), bradykinin(1-8) and bradykinin(1-9) levels.

**Experiment 2A:**

Acute uptake of intravenously injected ACE inhibitors into the heart: correlation of effects.

As various ACE inhibitors may have differing effects due possibly to different rates of tissue uptake, both enalaprilat and perindoprilat were studied. ACE inhibitors were injected intravenously as a bolus, as previously described for analogous myocardial drug uptake studies (Horowitz and Powell 1986). Enalaprilat dosage was 2.5mg; perindoprilat dosage was 1.25mg.

Paired arterial and coronary sinus blood samples were drawn for estimation of ACE inhibitor concentration at baseline, 10, 20, 30, 45, 60, 75 and 90 seconds and 2, 3, 4, 5, 7.5, 10, 15, and 20 minutes after drug injection. Coronary sinus flow was measured at frequent intervals post injection to permit calculation of ACE inhibitor uptake into the myocardium. Serial determination of pulmonary capillary wedge pressure and cardiac output was made at frequent intervals following drug injection. Mean
arterial pressure and dP/dt were constantly measured. Data for construction of mechanical restitution curves was gathered at 5 minute intervals.

Paired arterial and coronary sinus blood samples were drawn for determination of plasma ACE activity, and blood concentrations of angiotensin I, angiotensin II, angiotensin(1-7), bradykinin(1-7), bradykinin(1-8) and bradykinin(1-9) at baseline, 2, 5, 10, and 20 minutes post drug injection.

**Experiment 2B:**
Direct effects of ACE inhibitors on the heart and coronary circulation.

Both enalaprilat and perindoprilat were studied with direct injection into the left coronary artery in order to attempt to limit the extracoronary effects. Enalaprilat dosage was 0.5mg; perindoprilat dosage was 0.25mg.

Serial measurements of the following parameters were performed:
1. Cardiac effects: coronary sinus flow, LV dP/dt, and spontaneous heart rate.
2. Peripheral arterial concentrations of ACE inhibitor (in order to assess the extent of penetration of the drug into the systemic circulation).
3. Changes in plasma ACE activity, and blood concentrations of angiotensin I, angiotensin II, angiotensin(1-7), bradykinin(1-7), bradykinin(1-8) and bradykinin(1-9) peptides at baseline, 2, 5, 10, and 20 minutes post drug injection, as described for Experiment 2A.

**Experiment 3:**
Effects of ACE inhibitor on induction of ischaemia: relevance of cardiac and extracardiac effects.

All patients underwent repeat atrial pacing 10-15 minutes after intravenous or intracoronary injection of ACE inhibitor. The protocol was identical to that utilized
in Experiment 1. During this period, mean arterial pressure, pulmonary capillary wedge pressure and dP/dt were continuously recorded and coronary sinus blood flow was measured after approximately one minute of rapid pacing. All haemodynamic and inotropic parameters were measured again approximately one minute following the restoration of the baseline atrial pacing cycle length. Blood samples were taken as in Experiment 1 in order to study the effects of the ACE inhibitor on pacing-induced myocardial ischaemia, myocardial metabolism (oxygen saturation and lactate) and cardiac metabolism of angiotensin and bradykinin peptides.

ASSAY FOR WHOLE BLOOD LEVELS OF PERINDOPRILAT AND ENALAPRILAT

In order to correlate the acute effects of the injected angiotensin converting enzyme inhibitors, perindoprilat and enalaprilat, with their effects, acute myocardial drug uptake was determined by examining the gradient in drug concentration across the coronary circulation over time (Horowitz and Powell 1986). This involved the determination of whole blood concentrations of the relevant drugs at frequent time points. Although previous methodology had been published outlining the determination of serum concentrations of various angiotensin converting enzyme inhibitors both in animal models and in humans (Fabris, Chen et al. 1990), no previous methodology existed for the determination of whole blood concentrations in either model.

Available methods for determination of serum ACE inhibitor concentrations.

The peptidyl carboxypeptidase, angiotensin converting enzyme, bioconverts the decapeptide angiotensin I to the pressor peptide, angiotensin II. The activity of this enzyme is competitively inhibited by a variety of therapeutic agents that have a common mechanism of action but may differ in their tissue distribution (Fabris, Chen et al. 1990; Herman 1992). Activity of the enzyme cannot reliably be determined based on the bioconversion of angiotensin I to angiotensin II owing to the lack of specificity for this pathway. However, alternative methodology has been
developed which quantitates the product of a tripeptide, specifically cleaved by angiotensin converting enzyme e.g. production of hippuric acid from hippuryl-L-histidyl-L-leucine (Cushman D W 1971).

From the above, it is apparent that a number of options exist for measuring the concentration of angiotensin converting enzyme inhibitors present in vivo in humans. These include both direct and functional assays. Previous studies examining the pharmacokinetics and pharmacodynamics of a range of angiotensin converting enzyme inhibitors have used various methodologies to assay drug concentrations in serum, plasma and tissue. The disposition and metabolism of enalapril maleate has been examined using an enzyme inhibition assay. The substrate carbobenzoxy carbonyl-L-phenylalanine-L-histidyl-L-leucine was used to determine the activity of angiotensin converting enzyme. ACE inhibitor concentrations were then determined by the extent of ACE inhibition, compared to standards (Tocco, deLuna et al. 1982). Radioimmunoassay, coupled with anion-exchange chromatography, has been used to directly assay concentrations of perindopril and its metabolites in human plasma and urine (Doucet, De Veyrac et al. 1990; Louis, Conway et al. 1992). A radioinhibitor binding displacement assay has been described which has the advantage of using a single method to accurately record levels of a range of angiotensin converting enzyme inhibitors and, unlike radioimmunoassay, does not require a new antibody for each new inhibitor studied (Jackson, Cubela et al. 1987; Jackson, Cubela et al. 1988; Johnston, Cubela et al. 1988).

To date, however, no data have been published regarding the determination of whole blood drug levels, a methodology essential for the determination of acute myocardial drug uptake. In the current project, it was decided to adapt the radioinhibitor binding displacement assay method to the determination of whole blood concentrations of enalaprilat and perindoprilat, this being the most accurate methodology suitable for examining a range of different inhibitors.
Overview of Radioinhibitor Binding Displacement Assay

This methodology involves the use of $^{125}$I-labelled MK351A (N-(1-carboxy-3-phenylpropyl)-L-lysyl-[eN-4hydroxy benzimido]-L-proline), (*MK351A) a potent and competitive inhibitor of angiotensin converting enzyme, and an analog of the angiotensin converting enzyme inhibitor enalapril. Aliquots of *MK351A are combined with aliquots of angiotensin converting enzyme. To each sample a known concentration of the ACE inhibitor being studied is added, resulting in a variable extent of displacement of *MK351A from ACE by competitive binding. Following a period of incubation, the ACE-*MK351A complexes are precipitated, the supernatant removed and the pellet radioactivity counted. A standard curve can therefore be constructed against which unknown samples of the same ACE inhibitor can be compared and the concentrations determined.

Angiotensin Converting Enzyme Inhibitor Assay

Samples:

Whole blood samples were collected from patients undergoing cardiac metabolic studies. One to two milliliter paired aliquots were taken from the femoral artery and the coronary sinus at 10, 20, 30, 45 and 60 seconds following rapid intravenous bolus injection of either perindoprilat 1.25mg (Servier) or enalaprilat 2.5mg (Merck, Sharp and Dohme), and at 2, 3, 4, 5, 7.5, 10, 15 and 20 minutes. Additional whole blood was collected as a pool for diluting samples as required for the assay procedure. Samples were stored at -20°C until the assay was performed.

Solutions:

$I^{125}$-labelled MK351A was provided by Austin Biomedical Services, University of Melbourne, Department of Medicine. This was diluted in 0.1M ammonium acetate buffer (pH 3.5) such that 50 microliter aliquots provided about 35000 counts per minute with fresh label. Rat serum was used as a source of high ACE activity. This was diluted in 0.05M Tris buffer, pH 7.0, containing 0.3% bovine serum albumen,
75mM NaCl and 50μM ZnSO₄·7H₂O in order to give approximately 50% binding of *MK351A.

Assay Procedure:
The assay procedure was based on that reported by Jackson et al (Frej Fyhrquist 1984; Jackson, Cubela et al. 1987). Duplicate serial dilutions of the angiotensin converting enzyme inhibitor being studied were used to gather data for construction of a standard curve. 20 μl aliquots of perindopril (dose range, 3.1 x 10⁻⁷ to 4.2 x 10⁻¹⁰M) or enalapril (dose range, 1.43 x 10⁻⁷ to 2.81 x 10⁻¹⁰M) were added to 250 μl of high ACE activity serum diluted in Tris buffer and 50μl of *MK351A in ammonium acetate buffer. 20μl of whole blood (drawn at baseline from the same patient as the samples for assay) was added to each tube for the standard curve. Samples from each time point were assayed in triplicate. If dilution of the sample was required, this was performed in whole blood, drawn at baseline, from the same patient. Non-specific binding was determined by adding 250μl of high ACE activity serum, diluted in Tris buffer, to 20μl of either perindopril 3.82 x 10⁻⁴M or enalapril 4.29 x 10⁻³M and 20μl of whole blood from the same patient, drawn at baseline; the mixture then being combined with 50μl *MK351A in ammonium acetate buffer. The ‘zero’ binding reference, B₀, was determined by adding 270μl of high ACE activity serum, diluted in Tris buffer, to 20μl whole blood and 50μl *MK351A in ammonium acetate buffer.

All tubes were then vortexed well before being allowed to stand overnight at room temperature. The next day each sample was precipitated by adding 1ml absolute ethanol. All samples were then placed in a centrifuge at 3,000rpm for 10-15 minutes at 4°C. The resulting supernatant was aspirated and the pellets counted for 3 minutes to give an average 1 minute count for each tube. The standard curve was then constructed by subtracting the non-specific binding count from each sample count and expressing the result as a ratio of B₀. Samples with an unknown concentration of angiotensin converting enzyme inhibitor were diluted in order to
give counts which fell on the steep portion of the resulting sigmoid-shaped curve, the concentration being computer calculated and subsequently adjusted for the extent of dilution. The reported concentration at each time point was the average of all three readings. A typical standard curve is illustrated for both perindoprilat (Figure 2.1a) and enalaprilat (Figure 2.1b).

**Analytical Variables**

**Source of ACE:**
Initial experiments were performed using pooled sera from patients having high ACE activity, usually on the basis of secreting tumors. Unfortunately these samples were in limited supply and, furthermore, the dilutions required to give approximately 50% binding of tracer resulted in unacceptable levels of non-specific binding (>10%). A commercial source of ACE was tested (Sigma) but had a low specificity for the angiotensin converting enzyme inhibitors being assayed. Ultimately, it was decided to use pooled sera from rats with high ACE activity, allowing dilutions that resulted in acceptable levels of non-specific binding.

**Effects of time:**
All assays of patient samples were performed with an incubation period of 18-24 hours. During assay development, different durations of incubation were examined with no difference in results being found following either 48 or 72 hour incubation times. Although a one week incubation period did increase labeled ACE inhibitor binding to serum (from 27% to 43%), there was no associated increase in sensitivity of the assay.

**Other variables:**
Previous data have demonstrated that labeled ACE inhibitor binding to serum is increased in the presence of sulfate and chloride, is reduced with reduction in
incubation temperature and is optimized at pH 7.0 (Frej Fyhrquist 1984). Hence the rationale for the contents of the Tris buffer solution used. The impact of temperature on extent of binding was examined in the current study. A similar phenomenon was observed, also having an impact on the accuracy of the assay (Table 2.1). All experiments were therefore performed with all components at room temperature, incubation also occurring at room temperature.

**Table 2.1: Effect of Temperature on Standard Curve Construction**

<table>
<thead>
<tr>
<th></th>
<th>Room Temperature</th>
<th>4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding (*MK351A to ACE)</td>
<td>39%</td>
<td>16%</td>
</tr>
<tr>
<td>Non-specific binding</td>
<td>4.2%</td>
<td>3.7%</td>
</tr>
<tr>
<td>ED-50</td>
<td>6.492 pmol/ml</td>
<td>15.35 pmol/ml</td>
</tr>
<tr>
<td>QC 1</td>
<td>2.368</td>
<td>5.232</td>
</tr>
<tr>
<td>QC 2</td>
<td>14.369</td>
<td>25.501</td>
</tr>
</tbody>
</table>

**Figure 2.1a: A typical standard curve for perindoprilat**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-specific binding</td>
<td>5%</td>
</tr>
<tr>
<td>Binding ratio</td>
<td>42%</td>
</tr>
<tr>
<td>ED-20</td>
<td>18.06 pmol/ml</td>
</tr>
<tr>
<td>ED-50</td>
<td>6.187 pmol/ml</td>
</tr>
<tr>
<td>ED-80</td>
<td>1.93 pmol/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.340</td>
</tr>
</tbody>
</table>

**Standard Curve (Perindoprilat)**

- (B-NSB)/(Bo-NSB)%
- Log[Perindoprilat]
Figure 2.1b: A typical standard curve for enalaprilat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-specific binding</td>
<td>6%</td>
</tr>
<tr>
<td>Binding ratio</td>
<td>71%</td>
</tr>
<tr>
<td>ED-20</td>
<td>15.575 pmol/ml</td>
</tr>
<tr>
<td>ED-50</td>
<td>5.129 pmol/ml</td>
</tr>
<tr>
<td>ED-80</td>
<td>2.026 pmol/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.353</td>
</tr>
</tbody>
</table>

**Standard Curve (Enalaprilet)**

**B** = Binding of *MK351A to sample
**NSB** = Non-specific binding
**Bo** = Binding of *MK351A to sample with no ACE present
For each assay of patient samples, the standard curve was constructed utilizing whole blood collected at baseline from the same patient as the samples being assayed. This was necessary in view of the fact that each patient sample, by virtue of its composition, contained additional angiotensin converting enzyme, in excess to that contained in the standard serum being used as a source of angiotensin converting enzyme. Furthermore, as demonstrated by the measurement of serum ACE activity in the patient population being studied, there is significant inter-individual variation. The only way to control for this variability is to use each patient as his own control, thus ensuring that the amount of angiotensin converting enzyme present in each sample and each standard is identical. However, this creates difficulty in assessing inter-assay variability using quality control samples. The same quality control source, by definition, must be used for each assay. However, the angiotensin converting enzyme activity of the whole blood containing the quality control will be different to the angiotensin converting enzyme activity of the patient samples being assayed. This is likely to explain the extent of variability observed for the quality control results determined in this investigation, rather than simply reflecting a high degree of inter-assay variability.

**STATISTICAL ANALYSES**

All data are recorded as mean ± standard error of the mean. The time course of haemodynamic drug effects were analyzed using repeated measures analysis of variance with Dunnett's correction. The time course of drug effects on peptides were analyzed using time series repeated measures (Mixed model). Haemodynamic and metabolic effects of induction of tachycardia were analyzed by performing t tests on the change in variable between baseline pacing and tachycardia in the absence and presence of drug. The recovery data displayed are not included in this analysis. The changes in peptide levels following the induction of tachycardia and subsequent return to baseline pacing were analyzed using doubly repeated measures (Mixed model). The comparison of the two populations receiving perindoprilat and enalaprilat intravenously was performed using non-paired t tests.
All peptide data analysis was performed on log transformed data in order to normalize the distribution of the data. The graphical displays however are of the actual raw data.

Statistical analyses were performed using Statistica version 5.0 with the exception of Mixed model analyses that used SAS. The Mixed model is based on the maximum likelihood estimation and does not exclude cases with single missing data points, which does occur with ANOVA.

Significant results were accepted as being indicated by a p value of <0.05. Analyses with p values > 0.1 are recorded as being not significant (NS).
RESULTS

A total of 33 patients consented to the research protocol with 31 subsequently being studied. One patient was considered too unstable following routine coronary angiography and one patient could not be studied due to tortuosity of the coronary sinus catheter preventing sampling and flow measurements. Of the 31 patients studied, 16 received perindoprilat and 15 received enalaprilat as either an intravenous or intracoronary bolus injection. The results for each of these four possible treatments are detailed below.

INTRAVENOUS PERINDOPRILAT

A total of 12 patients received a 1.25mg intravenous bolus injection of perindoprilat with subsequent determination of myocardial uptake being made. Their baseline characteristics are recorded in Table 2. The planned protocol was completed for all patients with no adverse events being experienced. The effects of perindoprilat on haemodynamic and inotropic status and angiotensin and bradykinin peptides are presented with data both at resting heart rates and during rapid atrial pacing.

Effects of intravenous perindoprilat at resting heart rate

Haemodynamic and electrocardiographic effects:

All patients were paced from the right atrium at a constant rate that was 6.2 ± 1.5% above that occurring spontaneously. The baseline haemodynamics and electrocardiographic intervals are recorded in Table 2.3. Following the acute intravenous bolus administration of 1.25mg perindoprilat, these variables were monitored for up to 20 minutes. The time to maximum effect of perindoprilat on these measured variables is recorded in Table 2.4.

Perindoprilat effects on haemodynamic parameters are presented in Figures 2.2–2.7. Perindoprilat resulted in a 7.0 ± 3.5% (p=0.03) decrease in systolic blood pressure 2 minutes following administration with no significant reduction being evident at 10
minutes (-5.4 ± 2.9%, p=ns). Mean arterial blood pressure was reduced by 2.5 ± 1.4% (p=0.049) 4 minutes following drug administration, with no significant effect observed at 10 minutes (-2.2 ± 1.4%, p=ns). There was no significant effect on diastolic blood pressure (-0.4 ± 4.3%, p=ns) during and at 10 minutes following perindoprilat. Mean pulmonary capillary wedge pressure was marginally reduced (-9.7 ± 9.2%, p=ns) and LV+dP/dt_max (-2.5 ± 3.2%, p=ns) and spontaneous heart rate (-1.6 ± 1.6%, p=ns) were unchanged 10 minutes after drug administration. There was a significant and sustained reduction in cardiac index that was evident at 2 minutes (-8.6 ± 2.3%, p=0.014), maximal at 6 minutes (-9.5 ± 2.1%, p=0.009) and persisting at 10 minutes (-6.7 ± 2.8%, p=0.02). This translated into a marked increase in systemic vascular resistance index that was maximal at 2 minutes (22.4 ± 7.1% increase, p=0.005), remaining significant at 10 minutes (19.9 ± 6.8% increase, p=0.01). However, coronary sinus blood flow, although moderately reduced at 4 minutes (-9.0 ± 4.7%, p=ns) was not significantly changed at 10 minutes (-2.7 ± 6.3%, p=ns), with coronary vascular resistance index remaining unchanged throughout (5.8 ± 7.3%, p=ns 10 minutes following perindoprilat). Left ventricular stroke work was not significantly affected on ANOVA, although it was reduced 6 minutes following perindoprilat administration (by 13.2 ± 4.3%, p=0.03) with this reduction no longer being evident after 10 minutes i.e. stroke work was reduced at the time of peak haemodynamic effect of perindoprilat but this effect did not persist significantly. There was no correlation between changes in left ventricular stroke work and (the lack of change in) coronary vascular resistance. Neither PR (+1.4 ± 1.9% at 10 minutes, p=ns) nor QT interval (-0.0 ± 1.0% at 10 minutes, p=ns) were modified following perindoprilat.

The maximal haemodynamic effects of perindoprilat were evident within 10 minutes of drug administration. Further monitoring of these variables for up to 20 minutes revealed no incremental effects during this period (data not shown).
### Table 2.2: Patient Characteristics- Intravenous perindoprilat group

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age</th>
<th>BSA (m²)</th>
<th>CI (L/min/m²)</th>
<th>LVEF (%)</th>
<th>Coronary Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>60</td>
<td>2.02</td>
<td>1.95</td>
<td>52</td>
<td>LAD</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>69</td>
<td>1.88</td>
<td>2.84</td>
<td>63</td>
<td>LAD, Cx, RCA</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>70</td>
<td>1.60</td>
<td>2.26</td>
<td>70</td>
<td>LAD, Cx, RCA</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>71</td>
<td>1.97</td>
<td>2.46</td>
<td>75</td>
<td>LAD, Cx, RCA</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>57</td>
<td>2.04</td>
<td>2.38</td>
<td>74</td>
<td>LAD</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>48</td>
<td>1.91</td>
<td>3.29</td>
<td>72</td>
<td>LAD</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>64</td>
<td>2.02</td>
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</tr>
<tr>
<td>11</td>
<td>M</td>
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<td>LAD</td>
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<tr>
<td>12</td>
<td>M</td>
<td>57</td>
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<td>2.03</td>
<td>73</td>
<td>LAD, Cx</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>74</td>
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<td>2.37</td>
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</tr>
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<td>30</td>
<td>M</td>
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<td>LAD</td>
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<tr>
<td>31</td>
<td>F</td>
<td>41</td>
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<td>3.49</td>
<td>74</td>
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<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>60</td>
<td>1.95</td>
<td>67</td>
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</tr>
<tr>
<td>SEM</td>
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<td>0.05</td>
<td>0.15</td>
<td></td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

BSA = Body surface area  
CI = Cardiac Index  
LVEF = Left ventricular ejection fraction as measured on contrast ventriculography  
Coronary Disease = >50% luminal stenosis  
Cx = Circumflex, LAD = Left anterior descending, RCA = Right coronary artery
Table 2.3: Baseline haemodynamic and electrocardiographic parameters immediately prior to perindoprilat administration- intravenous perindoprilat group

<table>
<thead>
<tr>
<th>Number</th>
<th>MAP</th>
<th>PCWP</th>
<th>LV+dP/dt\textsubscript{max}</th>
<th>SVRI</th>
<th>CSBF</th>
<th>PR Interval</th>
<th>QT Interval</th>
<th>Paced heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>16</td>
<td>1430</td>
<td>4430</td>
<td>84</td>
<td>180</td>
<td>350</td>
<td>80</td>
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<tr>
<td>2</td>
<td>130</td>
<td>12</td>
<td>1640</td>
<td>3670</td>
<td>171</td>
<td>220</td>
<td>440</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>11</td>
<td>1090</td>
<td>3010</td>
<td>92</td>
<td>230</td>
<td>420</td>
<td>63</td>
</tr>
<tr>
<td>7</td>
<td>86</td>
<td>9</td>
<td>1100</td>
<td>2800</td>
<td>NR</td>
<td>210</td>
<td>430</td>
<td>60</td>
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<td>1470</td>
<td>4110</td>
<td>116</td>
<td>140</td>
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<td>75</td>
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<td>125</td>
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<td>1840</td>
<td>3040</td>
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<td>1290</td>
<td>4020</td>
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<td>2130</td>
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<td>360</td>
<td>100</td>
</tr>
<tr>
<td>Mean</td>
<td>111</td>
<td>11</td>
<td>1460</td>
<td>3480</td>
<td>105</td>
<td>183</td>
<td>392</td>
<td>73</td>
</tr>
<tr>
<td>SEM</td>
<td>5</td>
<td>1</td>
<td>90</td>
<td>200</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

MAP = Mean Arterial Pressure (mmHg)  
PCWP = Mean Pulmonary Capillary Wedge Pressure (mmHg)  
SVRI = Systemic Vascular Resistance Index (dynes.sec.cm\textsuperscript{-5}m\textsuperscript{2})  
NR = Not recorded  
CSBF = Coronary Sinus Blood Flow (mls/min)
Table 2.4: Haemodynamic and electrocardiographic effects of Perindoprilat

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>Maximal Change From Baseline</th>
<th>Time to Maximal Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>111 ± 5</td>
<td>108 ± 5</td>
<td>4 mins</td>
<td>0.049</td>
</tr>
<tr>
<td>Cardiac Index (L/min/m²)</td>
<td>2.62 ± 0.15</td>
<td>2.33 ± 0.13</td>
<td>6 mins</td>
<td>0.009</td>
</tr>
<tr>
<td>Mean PCWP (mmHg)</td>
<td>10.9 ± 0.8</td>
<td>9.4 ± 1.2</td>
<td>10 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic Vasc Resistance Index (dynes.sec.cm⁻¹.m²)</td>
<td>3480 ± 200</td>
<td>4190 ± 250</td>
<td>2 mins</td>
<td>0.014</td>
</tr>
<tr>
<td>LV+dP/dtmax (mmHg/sec)</td>
<td>1460 ± 90</td>
<td>1410 ± 100</td>
<td>4 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary Sinus Blood Flow (mls/min)</td>
<td>105 ± 10</td>
<td>93 ± 8</td>
<td>6 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary Vasc Resistance Index (dynes.sec.cm⁻¹.m²)</td>
<td>94000 ± 11000</td>
<td>112000 ± 14000</td>
<td>4 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Spontaneous Heart Rate</td>
<td>73 ± 4</td>
<td>72 ± 5</td>
<td>8 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Left Ventricular Stroke Work (gm.m)</td>
<td>142 ± 10</td>
<td>118 ± 8</td>
<td>6 mins</td>
<td>0.03</td>
</tr>
<tr>
<td>PR Interval (msec)</td>
<td>183 ± 9</td>
<td>185 ± 9</td>
<td>10 mins</td>
<td>NS</td>
</tr>
<tr>
<td>QT Interval (msec)</td>
<td>395 ± 11</td>
<td>393 ± 9</td>
<td>5 mins</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 2.2: Perindoprilat effects on Systolic and Diastolic Blood Pressure

* p < 0.05, ANOVA p=0.046, F = 2.434

p = ns
Figure 2.3: Perindoprilat effects on Mean Arterial and Pulmonary Capillary Wedge Pressure

*\(p<0.05\), ANOVA \(p=0.049\) \(F=2.400\)

\[\text{Mean Arterial Pressure (mmHg)}\]

\[\text{Mean PCWP (mmHg)}\]

\(p = \text{ns}\)
Figure 2.4: Perindoprilat effects on $LV^+dP/dt_{max}$ during constant pacing at baseline rate

![Graph showing the effect of Perindoprilat on $LV^+dP/dt_{max}$](image)

$p = \text{ns}$

Minutes

Figure 2.5: Perindoprilat effects on Cardiac Index

![Graph showing the effect of Perindoprilat on Cardiac Index](image)

$* p < 0.05, \quad ** p < 0.01$

ANOVA $p=0.0009, \quad F = 4.941$
Figure 2.6: Perindoprilat effects on Systemic Vascular Resistance Index and Left Ventricular Stroke Work

** Systemic Vascular Resistance Index (dynes.sec.cm⁻²/m²)

** p < 0.01, ANOVA  p<0.0001, F = 7.165

** Left Ventricular Stroke Work (gm.m)

* p<0.05, ANOVA, F=2.082  p=ns
Figure 2.7:  Perindopril effects on Coronary Sinus Blood Flow and Coronary Vascular Resistance Index

- Coronary Sinus Blood Flow (mls/min)
  - Flow decreases over time with a significant increase at 46 minutes.
  - Statistical significance: p = ns

- Coronary Vascular Resistance Index (dynes.sec.cm⁻⁵.m⁻²)/000
  - Resistance index decreases over time with a significant increase at 46 minutes.
  - Statistical significance: p = ns
Effects on the renin-angiotensin system and kinin peptides:

The rapid intravenous bolus administration of 1.25mg perindoprilat resulted in an abrupt, complete and sustained suppression of plasma ACE activity as demonstrated in Figure 2.8 (from 90.4 ± 11.1 to 2.8 ± 0.3 U/L, p = 0.0002 for femoral artery and from 88.3 ± 10.2 to 3.6 ± 0.8 U/L, p = 0.0002 for coronary sinus). The absolute femoral arterial whole blood concentration of angiotensin I (AI) abruptly increased (from 2.0 ± 0.6 to 5.7 ± 1.4 fmol/ml, p = 0.0032) within 2 minutes, the extent of the increase being less marked but still significant at 10 minutes (4.5 ± 1.2 fmol/ml, p = 0.041). The level of angiotensin II (AII) abruptly decreased (from 1.8 ± 0.7 to 0.4 ± 0.1 fmol/ml, p = 0.0002) within 2 minutes, again the change being less marked but still significant at 10 minutes (0.9 ± 0.7 fmol/ml, p = 0.029). Correspondingly, there was an abrupt and large reduction in the AII/AI ratio (from 1.1, ± 0.3 to 0.1 ± 0.1, p = 0.0001) within 2 minutes, the reduction remaining significant 10 minutes following administration of perindoprilat (0.5 ± 0.4 fmol/ml, p = 0.016) (Figures 2.9-2.11). However, while the peptide levels in coronary sinus blood shifted in the same directions (from 2.6 ± 0.5 to 3.0 ± 1.3 fmol/ml for AI, from 1.1 ± 0.5 to 0.9 ± 0.5 fmol/ml for AII, and from 0.5 ± 0.2 to 0.4 ± 0.2 for the AII/AI ratio), 2 minutes following perindoprilat and to 5.2 ± 1.2 fmol/ml for AI, 0.6 ± 0.4 fmol/ml for AII, and 0.2 ± 0.1 for the AII/AI ratio, 10 minutes following perindoprilat administration, none of these changes were significant (Figures 2.9-2.11). Furthermore, in comparison to the observed changes in femoral arterial peptide levels, there were no early abrupt changes noted in the coronary sinus.

In contrast to the angiotensin peptide data, perindoprilat induced no significant changes in the femoral arterial whole blood concentrations of bradykinin[1-9] (BK1-9) (from 2.5 ± 1.1 to 4.4 ± 0.9 fmol/ml, p = ns), its major metabolite bradykinin[1-7] (BK1-7) (from 3.9 ± 0.8 to 4.8 ± 1.1 fmol/ml, p = ns), or the ratio BK1-7/BK1-9 (from 3.4 ± 1.8 to 1.3 ± 0.3 fmol/ml, p = ns), as depicted in Figures 2.12-2.14, 10 minutes following administration. Furthermore, there was no change in the level of bradykinin[1-8] (BK1-8) (from 2.2 ± 0.6 to 3.7 ± 1.0 fmol/ml, p = ns) which represents
an alternative degradation pathway for BK1-9 (Figure 2.15). However, examination of coronary sinus whole blood concentrations of these same peptides revealed a significant reduction in BK1-7 (from $13.2 \pm 4.2$ to $6.1 \pm 1.0$ fmol/ml, $p = 0.002$), an increase in BK1-8 (from $1.7 \pm 0.3$ to $4.9 \pm 1.0$ fmol/ml, $p = 0.02$), no change in BK1-9 (from $16.5 \pm 5.4$ to $13.3 \pm 3.2$ fmol/ml, $p = ns$) but a reduction in the BK1-7/BK1-9 ratio (from $1.0 \pm 0.2$ to $0.5 \pm 0.1$, $p = 0.013$) 10 minutes following perindoprilat (Figures 2.12-2.15).

**Figure 2.8:** Perindoprilat effects on plasma ACE activity

![Graph showing ACE activity over time for Femoral Artery and Coronary Sinus](image)

**Figure 2.9:** Perindoprilat effects on whole blood angiotensin I levels

![Graph showing Angiotensin I levels over time for Femoral Artery and Coronary Sinus](image)
Figure 2.10: Perindoprilat effects on angiotensin II levels

![Graph showing the effects of Perindoprilat on angiotensin II levels in Femoral Artery and Coronary Sinus.](image)

- Femoral Artery
- Coronary Sinus

FA p=0.002
CS p=ns

Figure 2.11: Perindoprilat effects on angiotensin II/angiotensin I ratio

![Graph showing the effects of Perindoprilat on the ratio of angiotensin II to angiotensin I in Femoral Artery and Coronary Sinus.](image)

- Femoral Artery
- Coronary Sinus

FA p=0.0006
CS p=ns
Figure 2.12: Perindoprilat effects on Bradykinin-(1-7) levels

Figure 2.13: Perindoprilat effects on Bradykinin-(1-8) levels
Figure 2.14: Perindoprilat effects on Bradykinin-(1-9)

![Graph showing Perindoprilat effects on Bradykinin-(1-9)](image)

- **Femoral Artery**
- **Coronary Sinus**

- FA $p=ns$
- CS $p=ns$

Figure 2.15: Perindoprilat effects on Bradykinin-(1-7)/Bradykinin-(1-9) ratio

![Graph showing Perindoprilat effects on Bradykinin-(1-7)/Bradykinin-(1-9) ratio](image)

- **Femoral Artery**
- **Coronary Sinus**

- FA $p=ns$
- CS $p=0.013$
Myocardial Perindoprilat Uptake:

A total of 11 patients received an acute intravenous bolus injection of 1.25mg/10ml perindoprilat, enabling determination of acute myocardial drug content. The data from the standard curves constructed for each patient are detailed in Table 2.5. The data gathered for acute myocardial drug uptake are recorded in Table 2.6 and displayed in Figure 2.16.

**Table 2.5: Standard Curve Descriptors for Patients Receiving Intravenous Perindoprilat**

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Binding</th>
<th>Non-specific Binding</th>
<th>ED-20</th>
<th>ED-50</th>
<th>ED-80</th>
<th>QC1</th>
<th>QC2</th>
<th>cv for Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48%</td>
<td>6%</td>
<td>15.2</td>
<td>4.58</td>
<td>1.05</td>
<td>13.0</td>
<td>1.22</td>
<td>1.60±0.40</td>
</tr>
<tr>
<td>2</td>
<td>34%</td>
<td>7%</td>
<td>10.5</td>
<td>1.57</td>
<td>0.19</td>
<td>12.4</td>
<td>1.62</td>
<td>2.31±0.66</td>
</tr>
<tr>
<td>3</td>
<td>46%</td>
<td>9%</td>
<td>9.20</td>
<td>4.14</td>
<td>1.46</td>
<td>37.6</td>
<td>4.88</td>
<td>1.40±0.21</td>
</tr>
<tr>
<td>7</td>
<td>42%</td>
<td>7%</td>
<td>8.74</td>
<td>2.74</td>
<td>0.69</td>
<td>31.4</td>
<td>3.60</td>
<td>1.84±0.71</td>
</tr>
<tr>
<td>8</td>
<td>46%</td>
<td>9%</td>
<td>8.49</td>
<td>3.26</td>
<td>1.09</td>
<td>37.5</td>
<td>10.7</td>
<td>1.38±0.29</td>
</tr>
<tr>
<td>9</td>
<td>30%</td>
<td>8%</td>
<td>15.6</td>
<td>5.29</td>
<td>1.35</td>
<td>37.4</td>
<td>11.9</td>
<td>1.88±0.44</td>
</tr>
<tr>
<td>10</td>
<td>64%</td>
<td>7%</td>
<td>13.4</td>
<td>5.89</td>
<td>2.39</td>
<td>29.5</td>
<td>8.36</td>
<td>1.15±0.27</td>
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<tr>
<td>11</td>
<td>70%</td>
<td>7%</td>
<td>18.9</td>
<td>7.23</td>
<td>2.78</td>
<td>33.4</td>
<td>5.10</td>
<td>1.54±0.47</td>
</tr>
<tr>
<td>12</td>
<td>71%</td>
<td>5%</td>
<td>15.5</td>
<td>6.68</td>
<td>2.55</td>
<td>37.6</td>
<td>6.11</td>
<td>0.70±0.16</td>
</tr>
<tr>
<td>28</td>
<td>67%</td>
<td>9%</td>
<td>20.7</td>
<td>8.25</td>
<td>3.86</td>
<td>23.6</td>
<td>2.96</td>
<td>1.05±0.15</td>
</tr>
<tr>
<td>30</td>
<td>58%</td>
<td>9%</td>
<td>23.5</td>
<td>9.02</td>
<td>4.28</td>
<td>37.9</td>
<td>5.09</td>
<td>1.03±0.23</td>
</tr>
<tr>
<td>Mean</td>
<td>52.4</td>
<td>7.6</td>
<td>14.5</td>
<td>5.3</td>
<td>2.0</td>
<td>30.1</td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>4.4</td>
<td>0.4</td>
<td>1.5</td>
<td>0.7</td>
<td>0.4</td>
<td>2.9</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

cv = coefficient of variation (%) based on counts per minute for paired standards

Assays for the first two patients were performed using a human source of angiotensin converting enzyme, with all subsequent assays using rat sera. New QC’s were also used following the first two patient assays.
Peak myocardial drug uptake gradient of perindoprilat occurred 20.7 ± 1.5 seconds following rapid intravenous bolus administration. At this time point, the femoral artery/coronary sinus gradient of perindoprilat was 17,700 ± 4,400 pmol/ml/minute, equal to 5.8 micrograms/ml/minute. The peak myocardial content of perindoprilat accounted for 0.46 ± 0.13% of the injected dose and occurred 25.7 ± 2.7 seconds following drug administration. Five minutes following drug administration, myocardial drug content accounted for 0.24 ± 0.095% of the injected dose and after 10 minutes accounted for 0.16 ± 0.13%.

Table 2.6: Acute Myocardial Drug Content of Perindoprilat

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Peak gradient (pmol/ml/min)</th>
<th>Time to peak gradient (sec)</th>
<th>Peak content (% of dose)</th>
<th>Time to peak content (sec)</th>
<th>Cv for samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44,700</td>
<td>22.5</td>
<td>0.117</td>
<td>45</td>
<td>9.61±1.40</td>
</tr>
<tr>
<td>2</td>
<td>1,880</td>
<td>30</td>
<td>0.093</td>
<td>30</td>
<td>12.98±1.80</td>
</tr>
<tr>
<td>3</td>
<td>24,100</td>
<td>22.5</td>
<td>1.01</td>
<td>22.5</td>
<td>3.70±0.66</td>
</tr>
<tr>
<td>7</td>
<td>4,010</td>
<td>22.5</td>
<td>0.205</td>
<td>22.5</td>
<td>6.73±1.28</td>
</tr>
<tr>
<td>8</td>
<td>21,200</td>
<td>22.5</td>
<td>0.811</td>
<td>22.5</td>
<td>3.74±0.86</td>
</tr>
<tr>
<td>9</td>
<td>34,100</td>
<td>22.5</td>
<td>0.089</td>
<td>22.5</td>
<td>4.61±0.49</td>
</tr>
<tr>
<td>10</td>
<td>27,800</td>
<td>15</td>
<td>1.14</td>
<td>15</td>
<td>3.17±0.62</td>
</tr>
<tr>
<td>11</td>
<td>2,430</td>
<td>15</td>
<td>0.112</td>
<td>15</td>
<td>4.00±0.69</td>
</tr>
<tr>
<td>12</td>
<td>4,180</td>
<td>15</td>
<td>0.144</td>
<td>25</td>
<td>2.26±0.29</td>
</tr>
<tr>
<td>28</td>
<td>8,250</td>
<td>25</td>
<td>0.417</td>
<td>37.5</td>
<td>7.24±0.85</td>
</tr>
<tr>
<td>30</td>
<td>22,100</td>
<td>15</td>
<td>0.876</td>
<td>25</td>
<td>4.20±0.51</td>
</tr>
<tr>
<td>Mean</td>
<td>17,700</td>
<td>20.7</td>
<td>0.46</td>
<td>25.7</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>4,400</td>
<td>1.5</td>
<td>0.13</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

cv = average coefficient of variation (%) based on counts per minute for triplicate samples
Figure 2.16: (a) Average Femoral arterial and coronary sinus perindoprilat concentration for 20 minutes post injection

(b) Average Perindoprilat concentrations for 3 minutes post injection
Correlation between myocardial perindoprilat content and haemodynamic effects

Of the 4 haemodynamic variables that were significantly altered by perindoprilat, correlations were sought between changes in myocardial perindoprilat content and acute drug effect on mean arterial and systolic blood pressure, cardiac index and systemic vascular resistance index. None of these variables was significantly correlated with myocardial drug content. The hysteresis diagrams depicted in Figure 2.17 reveal that all drug effects occurred well beyond the time of peak content (Powell, Horowitz et al. 1990). Nevertheless, the time course of effects of perindoprilat exhibited significant hysteresis in all cases, with peak haemodynamic effects occurring at about 5 minutes post drug administration, compared with peak myocardial drug content occurring within 30 seconds.

There was no statistically significant correlation between the maximal myocardial content of perindoprilat and any baseline patient characteristics, including left
ventricular ejection fraction, cardiac index, basal paced heart rate, extent of fixed coronary artery disease and coronary sinus blood flow. Time to maximal myocardial content of perindoprilat was also independent of these factors.

Figure 2.17  Hysteresis between myocardial perindopril content and effects on
(a) Systolic BP  (b) Mean Arterial Pressure
(c) Cardiac Index  (d) Systemic Vascular Resistance Index

(a)

Systolic Blood Pressure (mmHg)

170  
160  
150  
140  

0 20 40 60 80 100

Myocardial Perindoprilat Content (%maximum)

(b)

Mean Arterial Pressure (mmHg)

120  
115  
110  
105  
100  

0 20 40 60 80 100

Myocardial Perindoprilat Content (%maximum)

(c)

Cardiac Index (L/min/m²)

2.8  
2.6  
2.4  
2.2  
2.0  
1.8  

0 20 40 60 80 100

Myocardial Perindoprilat Content (%maximum)

(d)

Systemic Vascular Resistance Index (dynes.sec.cm⁻⁵.m²)

4400  
3600  

0 20 40 60 80 100

Myocardial Perindoprilat Content (%maximum)
Effects of Perindoprilat during pacing induced tachycardia

In addition to the observations made during atrial pacing at just above spontaneous heart rate, tachycardia was induced both in the absence and presence of perindoprilat in order to examine its effects on induction of ischaemia. Detailed data examining perindoprilat effects on the left ventricular force-interval relationship were also gathered. However, this component is presented in Chapter 3.

Of the 12 patients who received an intravenous bolus injection of 1.25mg perindoprilat, 11 underwent atrial pacing at a cycle length that was 62.2 ± 1.9% of the baseline paced cycle length, equal to a heart rate of 126 ± 5 beats per minute. This procedure was performed at baseline and repeated 14.6 ± 1.4 minutes following perindoprilat administration. Of the 11 patients studied, 9 had significant coronary artery disease involving the left anterior descending artery. The results are presented both for the total cohort of 11 patients and for the 9 patients for whom induction of ischaemia during tachycardia is likely.

Effects on haemodynamic parameters

The haemodynamic and metabolic responses to the induction of a pacing induced tachycardia in both the absence and presence of perindoprilat are presented in Figures 2.18-2.21 below. In the absence of perindoprilat, the induction of pacing induced tachycardia produced a significant increase in coronary sinus blood flow, from 105 ± 10 to 142 ± 21 mls/min, p=0.04. This was not associated with any significant change in coronary vascular resistance (-14.5 ± 10.5%, p=0.12) or mean arterial pressure (+2.2 ± 1.8%, p=ns). There was, however, a moderate increase in the mean pulmonary capillary wedge pressure (from 9.8 ± 0.9 to 13.2 ± 1.2 mmHg, p=0.03) and LV+dP/dt_{max} (from 1510 ± 100 to 1750 ± 100 mmHg/sec, p=0.0006). Only 3 patients demonstrated net lactate production during tachycardia whilst oxygen extraction for the group was significantly increased (by 29 ± 11%, p=0.03).
In the presence of perindoprilat, the haemodynamic and metabolic responses to tachycardia were somewhat modified. Coronary sinus blood flow increased from 105 ± 10 to 147 ± 17 mls/min, p=0.001. This was associated with a significant reduction in coronary vascular resistance (by 22.4 ± 7.5%, p=0.03) but no change in mean arterial pressure (+3.0 ± 1.8%, p=ns). There was, however, no increase in pulmonary capillary wedge pressure (+8.2 ± 8.6%, p=ns). LV+dP/dt\text{max} increased from 1480 ± 90 to 1700 ± 80 mmHg/sec (p=0.0008). Net lactate production was not observed for any patient following perindoprilat, the extraction of lactate increasing by 18.6 ± 9.8%, p=0.09. Oxygen extraction was increased by 37 ± 10%, p=0.001.

*Figure 2.18*  Response of coronary sinus blood flow to pacing induced tachycardia in the absence and presence of perindoprilat

![Graph showing response of coronary sinus blood flow to pacing induced tachycardia in the absence and presence of perindoprilat. Baseline and perindoprilat conditions are compared at rest, tachycardia, and recovery phases. Significant differences are indicated with p=ns for effect of perindoprilat.]
Figure 2.19  Response to pacing induced tachycardia in the absence and presence of Perindoprilat for (a) Coronary Vascular Resistance Index and (b) Mean Arterial Pressure.
Figure 2.20  
Response of (a) mean pulmonary capillary wedge pressure and (b) $LV+dP/dt_{max}$ to the induction of tachycardia in the absence and presence of perindoprilat.

(a) 
![Graph showing mean pulmonary capillary wedge pressure (mmHg) at rest, tachycardia, and recovery with baseline and perindoprilat conditions.]

(b) 
![Graph showing $LV+dP/dt_{max}$ (mmHg/sec) at rest, tachycardia, and recovery with baseline and perindoprilat conditions.]

- Baseline
- Perindoprilat

$p=0.07$ for effect of perindoprilat

$p=ns$ for effect of perindoprilat
Figure 2.21  Effects of perindoprilat on (a) myocardial oxygen extraction and (b) myocardial lactate production/extraction, during a pacing induced tachycardia
As mentioned previously, 9 patients had haemodynamically significant stenoses in one or more major epicardial coronary arteries with the disease involving the left anterior descending artery in all cases, and thus with coronary anatomy suitable for sampling coronary sinus blood from potentially ischaemic myocardium. The experiments examining the response to the induction of tachycardia were performed in order to determine if perindoprilat had an impact on the induction of myocardial ischaemia. It is therefore appropriate to consider the subgroup of patients with significant coronary disease separately for the purposes of such analysis.

The haemodynamic and metabolic responses to the induction of a pacing induced tachycardia in both the absence and presence of perindoprilat are presented in Figures 2.22-2.25 below. In the absence of perindoprilat, the induction of pacing induced tachycardia produced only a slight increase in coronary sinus blood flow (from 103 ± 12 to 123 ± 18 mls/min, p=0.1) consistent with the impaired coronary vasodilator reserve frequently observed in the setting of significant coronary artery disease. In keeping with this, there was no significant change in coronary vascular resistance (-7.8 ± 11.7%, p=ns). There was, however, a moderate increase in the mean pulmonary capillary wedge pressure (from 10.5 ± 0.9 to 14.9 ± 0.5 mmHg, p=0.006) and LV+dP/dtmax (from 1480 ± 100 to 1710 ± 110 mmHg/sec, p=0.004), mean arterial pressure remaining unchanged (+2.6 ± 1.9%, p=ns). Only 2 patients demonstrated net lactate production during tachycardia whilst oxygen extraction was not significantly increased (by 20 ± 11%, p=ns).

In the presence of perindoprilat, the haemodynamic and metabolic responses to tachycardia were somewhat modified. Coronary sinus blood flow now increased markedly from 103 ± 12 to 146 ± 20 mls/min, p=0.005. This was associated with a modest reduction in coronary vascular resistance (by 22.3 ± 9.2%, p=0.06) but no change in mean arterial pressure (+3.4 ± 2.1%, p=ns). The baseline increase in pulmonary capillary wedge pressure was no longer evident (+0.0 ± 4.2%, p=ns). LV+dP/dtmax increased from 1470 ± 110 to 1690 ± 100 mmHg/sec (p=0.006). Net lactate production was not observed for any patient following perindoprilat, the
extraction of lactate increasing by 22.4 ± 11.5%, p=0.09. Oxygen extraction was increased by 40 ± 12%, p=0.009.

In summary, following the induction of tachycardia, perindoprilat induced a significant increase in coronary sinus blood flow (p=0.006 vs pre-perindoprilat), a corresponding reduction in coronary vascular resistance (p=0.04 vs pre-perindoprilat), prevented the rise in pulmonary capillary wedge pressure (p=0.04 vs pre-perindoprilat) and substantially increased oxygen extraction (p=0.03 vs pre-perindoprilat) in this group of patients with significant coronary artery disease. There was a modest trend towards increased lactate extraction (p=0.1 vs pre-perindoprilat).

Figure 2.22  Effect of perindoprilat on response of Coronary Sinus Blood Flow to the induction of tachycardia in patients with coronary artery disease
Figure 2.23  Effects of perindoprilat on (a) Coronary Vascular Resistance Index and (b) Mean Arterial Pressure during induction of tachycardia in patients with coronary artery disease.
Figure 2.24  Effects of Perindoprilat on (a) Mean Pulmonary Capillary Wedge Pressure and (b) LV+dP/dt\text{max} during the induction of tachycardia in patients with coronary artery disease.
Figure 2.25  Effects of perindoprilat on (a) Myocardial Oxygen Extraction and (b) Myocardial Lactate Production during the induction of tachycardia in patients with coronary artery disease.
Effects on renin-angiotensin system and Bradykinin peptides

As previously described, the activity of serum ACE was markedly attenuated following the administration of perindoprilat (Figure 2.8). In addition, significant differences in the levels of various angiotensin and bradykinin peptides during the induction of tachycardia were also noted (Figures 2.26-2.32).

Prior to the administration of perindoprilat, the induction of tachycardia exhibited no significant effects on peripheral angiotensin or bradykinin peptides. There was however an increase in coronary sinus angiotensin II levels and the AII/AI ratio, with evidence of a 'washout' effect during the recovery period immediately after restoration of basal paced heart rate. The coronary sinus bradykinin peptide levels all fell during tachycardia, the BK(1-7)/BK(1-9) ratio showing a modest increase.

In the presence of perindoprilat, there was significant modulation of angiotensin and bradykinin peptide levels during the induction of tachycardia. Femoral arterial AII/AI and BK(1-7)/BK(1-9) ratios were both significantly suppressed, in keeping with the extent of inhibition of serum ACE. Similarly, the coronary sinus AII/AI and BK(1-7)/BK(1-9) ratios were also significantly suppressed, whilst BK(1-8) levels were raised. In the case of angiotensin, this effect was consequent upon both a suppression of AII and an increase in AI levels. However, in the case of bradykinin, the fall in BK(1-7)/BK(1-9) was almost entirely due to suppression of BK(1-7) generation, the BK(1-9) levels remaining unchanged. The changes in peptide levels observed were independent of the presence of coronary artery disease, the results presented being those for all patients receiving intravenous perindoprilat.
Figure 2.26 Effects of perindoprilat on angiotensin I during pacing induced tachycardia

Femoral Artery

- Baseline
- • Perindoprilat

Rest Tachycardia Recovery

Ang I (fmol/ml)

0 2 4 6 8 10 12

p=0.074

Coronary Sinus

- Baseline
- • Perindoprilat

Rest Tachycardia Recovery

Ang I (fmol/ml)

0 2 4 6 8 10 12

p=0.0001
Figure 2.27  Effects of perindoprilat on angiotensin II during pacing induced tachycardia

Femoral Artery

- Baseline
- • Perindoprilat

Ang II (fmol/ml)

Rest  Tachycardia  Recovery

p=0.0005

Coronary Sinus

- Baseline
- • Perindoprilat

Ang II (fmol/ml)

Rest  Tachycardia  Recovery

p=0.0001
Figure 2.28  Effects of perindopril on AII/AI during pacing induced tachycardia

Femoral Artery

![Graph showing the effects of perindopril on Ang II/Ang I ratio during rest, tachycardia, and recovery in the femoral artery.]

Coronary Sinus

![Graph showing the effects of perindopril on Ang II/Ang I ratio during rest, tachycardia, and recovery in the coronary sinus.]

p = 0.0011

p = 0.0001
Figure 2.29  Effects of perindoprilat on Bradykinin 1-7 during pacing induced tachycardia

**Femoral Artery**

- Baseline
- • Perindoprilat

p=ns

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<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Perindoprilat</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Tachycardia</td>
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<tr>
<td>Recovery</td>
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</tbody>
</table>

**Coronary Sinus**

- Baseline
- • Perindoprilat

p=0.0001

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Perindoprilat</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Tachycardia</td>
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</tr>
<tr>
<td>Recovery</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.30  Effects of perindoprilat on Bradykinin 1-8 during pacing induced tachycardia
Figure 2.31  Effects of perindoprilat on Bradykinin 1-9 during pacing induced tachycardia

**Femoral Artery**

![Graph showing effects of perindoprilat on Bradykinin 1-9 in femoral artery.](image)

- **Baseline**
- **Perindoprilat**

p=0.0001

**Coronary Sinus**

![Graph showing effects of perindoprilat on Bradykinin 1-9 in coronary sinus.](image)

- **Baseline**
- **Perindoprilat**

p=ns
Figure 2.32  Effects of perindoprilat on BK 1-7/BK 1-9 during pacing induced tachycardia

**Femoral Artery**

- Baseline
- Perindoprilat

p=0.0001

**Coronary Sinus**

- Baseline
- Perindoprilat

p=0.0045
Summary of Intravenous Perindoprilat Effects

The intravenous bolus administration of 1.25mg perindoprilat to the 12 patients studied produced significant changes both at resting heart rate and during the induction of tachycardia using atrial pacing.

During atrial pacing at just above spontaneous heart rate, perindoprilat was rapidly taken up by myocardium, peak drug content being achieved in less than 30 seconds, accounting for about 0.5% of the injected dose. Perindoprilat caused modest reductions in both systolic and mean arterial blood pressure and a more marked reduction in cardiac index. This translated into a significant increase in systemic vascular resistance index despite the absence of a significant negative inotropic effect as determined using LV+dP/dt\textsubscript{max} (although left ventricular stroke work was reduced). Coronary haemodynamics were not affected. The peak haemodynamic effects of perindoprilat occurred at about 4 minutes following drug injection, with significant effects persisting for at least 10 minutes.

The dose of perindoprilat administered was sufficient to cause an abrupt and almost total suppression of serum ACE activity. This was accompanied by a significant suppression of the peripheral AII/AI ratio, but not that of the coronary sinus, and a marked reduction in the coronary sinus BK1-7/BK1-9 ratio, but not that of the peripheral blood.

During the induction of tachycardia, perindoprilat had its most marked effect in those patients with significant fixed coronary artery disease. It caused a significant increase in coronary sinus blood flow and prevented the rise in pulmonary capillary wedge pressure that had been observed for this group. This was accompanied by suppression of the AII/AI and BK1-7/BK1-9 ratios in both the peripheral and coronary sinus blood.
INTRACORONARY PERINDOPRILAT

A total of 4 patients received a selective left main intracoronary injection of 0.25mg perindoprilat. One patient developed sustained atrial fibrillation with a rapid ventricular response during the drug-free examination of atrial pacing induced tachycardia. Spontaneous reversion to sinus rhythm occurred 3 hours later. The procedure was well tolerated in all other patients. The baseline characteristics of these 4 patients are recorded in Table 2.7.

Due to the requirement of multiple femoral arterial sheaths, only one of the patients studied had a Millar catheter inserted, enabling serial determination of LV+dP/dt_max. For patient 6, variant anatomy prevented the passage of a Swan-Ganz catheter from either the femoral or brachial approach. The baseline haemodynamic parameters of the patients are recorded in Table 2.8.

Effects of intracoronary perindoprilat at resting heart rate

Following intracoronary administration of perindoprilat, during baseline atrial pacing at a rate 9.7 ± 2.9% above spontaneous heart rate, haemodynamic variables were monitored for up to 20 minutes. The maximal changes in these variables are recorded in Table 2.9. Diastolic blood pressure was the only parameter to be significantly altered in the presence of perindoprilat. However, in view of the very small sample, the trends observed for the other variables may not have reached statistical significance due to Type 2 error.

| Table 2.7 Patient characteristics – intracoronary perindoprilat |
|--------------------|-----------------|-----------------|----------------|----------------|-----------------|-----------------|
| Number  | Sex  | Age  | BSA (m²)  | CI (L/min/m²) | LVEF (%)  | Coronary Disease |
| 4       | F    | 59   | 1.77      | 2.38          | 76        | Nil             |
| 5       | M    | 66   | 2.26      | 2.10          | 71        | LAD, Cx, RCA    |
| 6       | M    | 58   | 2.04      | NR            | 70        | Nil             |
| 13      | M    | 46   | 1.98      | 3.23          | 76        | LAD, Cx         |
Table 2.8  
**Baseline haemodynamic parameters – intracoronary perindoprilat**

<table>
<thead>
<tr>
<th>Number</th>
<th>MAP</th>
<th>PCWP</th>
<th>SVRI</th>
<th>CSBF</th>
<th>Paced heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>108</td>
<td>10</td>
<td>4840</td>
<td>146</td>
<td>86</td>
</tr>
<tr>
<td>5</td>
<td>130</td>
<td>8</td>
<td>3780</td>
<td>49</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>NR</td>
<td>NR</td>
<td>52</td>
<td>67</td>
</tr>
<tr>
<td>13</td>
<td>86</td>
<td>8</td>
<td>2800</td>
<td>130</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 2.9  
**Peak haemodynamic effects of intracoronary perindoprilat**

<table>
<thead>
<tr>
<th></th>
<th>Baseline Measure</th>
<th>Maximal Change From Baseline</th>
<th>Time to Maximal Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>113 ± 11</td>
<td>116 ± 11</td>
<td>2 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>168 ± 18</td>
<td>170 ± 18</td>
<td>4 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>87 ± 8</td>
<td>81 ± 11</td>
<td>10 mins</td>
<td>0.01</td>
</tr>
<tr>
<td>Cardiac Index (L/min/m²)</td>
<td>2.57 ± 0.34</td>
<td>2.70 ± 0.50</td>
<td>12 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic Vasc Resistance Index (dynes.sec.cm⁻⁵.m⁻²)</td>
<td>3810 ± 590</td>
<td>3940 ± 680</td>
<td>8 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary Sinus Blood Flow (mls/min)</td>
<td>108 ± 26</td>
<td>142 ± 29</td>
<td>10 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary Vasc Resistance Index (dynes.sec.cm⁻⁵.m⁻²)</td>
<td>104000 ± 24000</td>
<td>76000 ± 23000</td>
<td>10 mins</td>
<td>NS</td>
</tr>
</tbody>
</table>

NR = not recorded  
MAP = Mean Arterial Pressure (mmHg)  
PCWP = Mean Pulmonary Capillary Wedge Pressure (mmHg)  
SVRI = Systemic Vascular Resistance Index (dynes.sec.cm⁻⁵.m⁻²)  
CSBF = Coronary Sinus Blood Flow (mls/min)
The effects of intracoronary perindoprilat on the renin-angiotensin system and bradykinin peptides are recorded in Figures 2.33-2.36. Although selective intracoronary dosing of a small amount of perindoprilat was performed, there was widespread and marked suppression of plasma ACE activity. Furthermore, despite the small sample size, a significant decrease in the peripheral AII/AI ratio was detected, the change in the coronary sinus ratio being of only borderline significance. The only impact on bradykinin peptides noted was a transient increase in femoral arterial bradykinin-1-9 levels, there being no effect noted in the coronary sinus samples.

*Figure 2.33  Perindopril effects on (a) ACE activity and (b) Angiotensin I*
Figure 2.34  Perindoprilat effects on (a) Angiotensin II and (b) AII/AI ratio

(a)

![Graph showing Angiotensin II levels over time.](image)

- Femoral Artery
- Coronary Sinus

FA  p=0.036
CS  p=ns

(b)

![Graph showing AII/AI ratio over time.](image)

- Femoral Artery
- Coronary Sinus

FA  p=0.0052
CS  p=0.052
Figure 2.35 Perindoprilat effects on (a) Bradykinin 1-7 and (b) Bradykinin 1-8
Figure 2.36  Perindoprilat effects on (a) Bradykinin 1-9 and (b) BK 1-7/BK 1-9 ratio

(a)

- ● Femoral Artery
- ○ Coronary Sinus

FA  p=0.008
CS  p=ns

Minutes

(b)

- ● Femoral Artery
- ○ Coronary Sinus

FA  p=0.09
CS  p=ns

Minutes
Effects of Intracoronary Perindoprilat during tachycardia

Of the 4 patients receiving an intracoronary dose of 0.25mg perindoprilat, 3 were examined during a pacing induced tachycardia in both the absence and presence of drug. The fourth patient developed sustained atrial fibrillation during initial atrial pacing and was therefore excluded from this component of the protocol. Atrial pacing was performed at a cycle length that was \(59 \pm 1\%\) of the baseline paced cycle length for 2 minutes. The effects on coronary haemodynamic and metabolic parameters are recorded in Figures 2.37 and 2.38. The effects on the renin-angiotensin system and bradykinin peptides are presented in Figures 2.39-2.44.

Coronary sinus blood flow was increased during induction of tachycardia, the magnitude of this increase being maintained in the presence of perindoprilat. Myocardial lactate extraction was significantly increased following perindoprilat and there was an increase in net myocardial extraction of oxygen. No other haemodynamic parameters were significantly affected.

The impact of a small intracoronary dose of perindoprilat on serum ACE activity was previously described (Figure 2.33a). There was a significant reduction in both the peripheral and intracoronary AII/AI ratio (Figure 2.41), this being almost totally due to a reduction in the generation of angiotensin II (Figure 2.40), angiotensin I levels remaining relatively constant (Figure 2.39). Perindoprilat induced no substantial changes in bradykinin peptide levels, either peripherally or within the coronary sinus (Figures 2.42-2.44).
Figure 2.37  Effects of Perindoprilat on response to tachycardia for
(a) Coronary Sinus Blood Flow, and
(b) Coronary Vascular Resistance Index
Figure 2.38  Effects of Perindoprilat on response to tachycardia for
(a) Myocardial Oxygen Extraction and
(b) Myocardial Lactate Production

(a) Myocardial Oxygen Extraction

(b) Myocardial Lactate Production
Figure 2.39  Effects of perindoprilat on angiotensin I levels

Femoral Artery

![Graph showing effects of perindoprilat on angiotensin I levels in the femoral artery.](image)

Coronary Sinus

![Graph showing effects of perindoprilat on angiotensin I levels in the coronary sinus.](image)
Figure 2.40  Effects of perindopril on angiotensin II levels

**Femoral Artery**

- Baseline
- Perindoprilat

p=0.0009 for effect of perindoprilat

<table>
<thead>
<tr>
<th>.Rest</th>
<th>Tachycardia</th>
<th>Recovery</th>
</tr>
</thead>
</table>

**Coronary Sinus**

- Baseline
- Perindoprilat

p=0.003 for effect of perindoprilat

<table>
<thead>
<tr>
<th>.Rest</th>
<th>Tachycardia</th>
<th>Recovery</th>
</tr>
</thead>
</table>
Figure 2.41  Effects of perindoprilat on AII/AI ratio

Femoral Artery

- Baseline
- O Perindoprilat

p=0.014 for effect of perindoprilat

Coronary Sinus

- Baseline
- O Perindoprilat

p=0.0008 for effect of perindoprilat
Figure 2.42  Effects of perindoprilat on Bradykinin 1-7 levels

**Femoral Artery**

- Baseline
- Perindoprilat

- p=ns for effect of perindoprilat

**Coronary Sinus**

- Baseline
- Perindoprilat

- p=0.05 for effect of perindoprilat
Figure 2.43  Effects of perindoprilat on Bradykinin 1-9 levels

Femoral Artery

- Baseline
- O- Perindoprilat

p=ns for effect of perindoprilat

<table>
<thead>
<tr>
<th>Rest</th>
<th>Tachycardia</th>
<th>Recovery</th>
</tr>
</thead>
</table>

Coronary Sinus

- Baseline
- O- Perindoprilat

p=0.05 for effect of perindoprilat

<table>
<thead>
<tr>
<th>Rest</th>
<th>Tachycardia</th>
<th>Recovery</th>
</tr>
</thead>
</table>
Figure 2.44  Effects of perindoprilat on BK1-7/BK1-9 ratio

**Femoral Artery**

![Graph showing effects of perindoprilat on BK1-7/BK1-9 ratio in Femoral Artery]

- Baseline
- -• Perindoprilat

p=ns for effect of perindoprilat

**Coronary Sinus**

![Graph showing effects of perindoprilat on BK1-7/BK1-9 ratio in Coronary Sinus]

- Baseline
- -• Perindoprilat

p=ns for effect of perindoprilat
Summary of Intracoronary Perindoprilat effects

The intracoronary administration of 0.25mg perindoprilat was examined in only 4 patients. Data collection was limited by the induction of atrial fibrillation in one patient and by the inability to pass a Swan-Ganz catheter in another. The same experimental protocol was followed as for those patients receiving intravenous perindoprilat.

For almost all haemodynamic parameters examined during baseline atrial pacing at just above spontaneous heart rate, no substantial change was found. However, serum ACE activity in both the coronary sinus and the femoral artery was significantly suppressed, this being accompanied by a reduction in the AII/AI ratio that was more marked in the femoral artery than in the coronary sinus. The level of bradykinin peptides was not significantly altered with a tendency to more marked shifts being observed in the periphery.

During the induction of tachycardia, intracoronary perindoprilat caused a significant increase in the myocardial extraction of oxygen and lactate. There were no changes in haemodynamic parameters observed. There was a significant suppression of the AII/AI ratio in both the coronary sinus and the periphery with no effect on bradykinin peptide levels being found.
INTRAVENOUS ENALAPRILAT

A total of 10 patients received a 2.5mg intravenous bolus injection of enalaprilat with subsequent determination of myocardial uptake being made. Their baseline characteristics are recorded in Table 2.10. The planned protocol was completed for all patients with no adverse events being experienced. The effects of enalaprilat on haemodynamic and inotropic status and angiotensin and bradykinin peptides are presented with data both at resting heart rates and during rapid atrial pacing.

Effects of intravenous enalaprilat at resting heart rate

Haemodynamic and electrocardiographic effects:

All patients were paced from the right atrium at a constant rate that was $7.7 \pm 3.2\%$ above that occurring spontaneously. The baseline haemodynamics and electrocardiographic intervals are recorded in Table 2.11. Following the acute intravenous bolus administration of 2.5mg enalaprilat, these variables were monitored for up to 20 minutes. The time to maximum effect of enalaprilat on these measured variables is recorded in Table 2.12.

Enalaprilat effects on haemodynamic parameters are presented in Figures 2.45-2.50. Enalaprilat resulted in a significant decrease in systolic, diastolic and mean arterial blood pressure. Mean arterial pressure fell abruptly within 2 minutes of drug administration (by $4.7 \pm 2.1\%, p=0.02$), the fall being maximal at 15 minutes ($-6.4 \pm 1.6\%, p=0.004$). Both systolic and diastolic blood pressure were maximally reduced 15 minutes after enalaprilat (by $6.0 \pm 2.5\%, p=0.008$, and by $7.5 \pm 3.4\%, p=0.005$ respectively). Mean pulmonary capillary wedge pressure was not affected ($-2.6 \pm 17.5\%, p=ns$ 12 minutes following enalaprilat) and spontaneous heart rate was unchanged during the first 15 minutes after drug administration. There was a significant reduction in cardiac index that was first evident 12 minutes following enalaprilat, the change being maximal at 15 minutes ($-12.4 \pm 1.9\%, p=0.003$). The reductions in mean arterial pressure and cardiac index were proportional such that systemic vascular resistance index was unchanged during the first 15 minutes.
Coronary sinus blood flow, although relatively stable immediately following drug administration was significantly reduced by 10 minutes (-9.6 ± 6.7%, p=0.02), with the reduction being maximal by 15 minutes (-17.3 ± 6.3%, p=0.007). Coronary vascular resistance index remaining unchanged throughout, indicating proportional reductions in mean arterial pressure and coronary sinus blood flow. Left ventricular contractility, as measured by LV+dp/dt_max, was marginally reduced 15 minutes following enalaprilat (-5.2 ± 3.5%, p=0.03; ANOVA, p=ns). Left ventricular stroke work gradually reduced with time, the change being maximal at 15 minutes (-17.0 ± 3.2%, p=0.0006). This reduction in stroke work was significantly correlated with the reduction in coronary sinus blood flow (r=0.7, p=0.036). Neither PR (+1.4 ± 1.9% at 10 minutes, p=ns) nor QT intervals (-0.0 ± 1.0% at 10 minutes, p=ns) were modified following enalaprilat.

Table 2.10: Patient Characteristics- intravenous enalaprilat group

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age</th>
<th>BSA (m²)</th>
<th>CI (L/min/m²)</th>
<th>LVEF (%)</th>
<th>Coronary Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>M</td>
<td>66</td>
<td>1.88</td>
<td>2.51</td>
<td>80</td>
<td>LAD, Cx</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>67</td>
<td>2.02</td>
<td>3.06</td>
<td>75</td>
<td>Nil</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>67</td>
<td>1.97</td>
<td>2.08</td>
<td>62</td>
<td>LAD, Cx</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>49</td>
<td>1.88</td>
<td>2.48</td>
<td>78</td>
<td>Cx, RCA</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>49</td>
<td>1.86</td>
<td>2.31</td>
<td>75</td>
<td>RCA</td>
</tr>
<tr>
<td>22</td>
<td>M</td>
<td>69</td>
<td>2.16</td>
<td>2.50</td>
<td>81</td>
<td>LAD, Cx, RCA</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>55</td>
<td>2.01</td>
<td>2.40</td>
<td>65</td>
<td>LAD, Cx</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>52</td>
<td>1.96</td>
<td>2.47</td>
<td>86</td>
<td>LAD</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>57</td>
<td>2.11</td>
<td>1.79</td>
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<td>LAD, Cx, RCA</td>
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<td>29</td>
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<td>3.34</td>
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<td></td>
<td>1.96</td>
<td>2.49</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

BSA = Body surface area  
CI = Cardiac Index  
LVEF = Left ventricular ejection fraction as measured on contrast ventriculography  
Coronary Disease = >50% luminal stenosis  
Cx = Circumflex, LAD = Left anterior descending, RCA = Right coronary artery
Table 2.11: Baseline haemodynamic and electrocardiographic parameters immediately prior to intravenous enalaprilat administration

<table>
<thead>
<tr>
<th>Number</th>
<th>MAP</th>
<th>PCWP</th>
<th>LV+(dP/dt_{max})</th>
<th>SVRI</th>
<th>CSBF</th>
<th>PR Interval</th>
<th>QT Interval</th>
<th>Paced heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>111</td>
<td>10</td>
<td>1410</td>
<td>3540</td>
<td>118</td>
<td>180</td>
<td>430</td>
<td>60</td>
</tr>
<tr>
<td>15</td>
<td>92</td>
<td>4</td>
<td>1270</td>
<td>2410</td>
<td>91</td>
<td>190</td>
<td>390</td>
<td>71</td>
</tr>
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<td>142</td>
<td>10</td>
<td>1530</td>
<td>5470</td>
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<tr>
<td>Mean</td>
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<td>9</td>
<td>1450</td>
<td>3710</td>
<td>101</td>
<td>199</td>
<td>409</td>
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</tr>
<tr>
<td>SEM</td>
<td>4</td>
<td>1</td>
<td>60</td>
<td>300</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

MAP = Mean Arterial Pressure (mmHg)  
PCWP = Mean Pulmonary Capillary Wedge Pressure (mmHg)  
SVRI = Systemic Vascular Resistance Index (dynes.sec.cm²m⁻²)  
CSBF = Coronary Sinus Blood Flow (mls/min)
Table 2.12:  **Haemodynamic and electrocardiographic effects of Enalaprilat**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>Maximal Change From Baseline</th>
<th>Time to Maximal Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>112 ± 4</td>
<td>105 ± 4</td>
<td>4 &amp; 15 mins</td>
<td>0.004</td>
</tr>
<tr>
<td>Cardiac Index (L/min/m²)</td>
<td>2.49 ± 0.14</td>
<td>2.18 ± 0.13</td>
<td>15 Mins</td>
<td>0.003</td>
</tr>
<tr>
<td>Mean PCWP (mmHg)</td>
<td>8.9 ± 1.3</td>
<td>7.3 ± 0.9</td>
<td>12 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic Vasc Resistance Index (dynes.sec.cm⁻⁵.m⁻²)</td>
<td>3710 ± 300</td>
<td>4000 ± 360</td>
<td>15 Mins</td>
<td>NS</td>
</tr>
<tr>
<td>LV+dP/dt_max (mmHg/sec)</td>
<td>1450 ± 60</td>
<td>1380 ± 80</td>
<td>15 mins</td>
<td>0.03</td>
</tr>
<tr>
<td>Coronary Sinus Blood Flow (mls/min)</td>
<td>101 ± 7</td>
<td>85 ± 6</td>
<td>15 mins</td>
<td>0.007</td>
</tr>
<tr>
<td>Coronary Vasc Resistance Index (dynes.sec.cm⁻⁵.m⁻²)</td>
<td>94600 ± 10400</td>
<td>102200 ± 8200</td>
<td>15 Mins</td>
<td>NS</td>
</tr>
<tr>
<td>Spontaneous Heart Rate</td>
<td>69 ± 4</td>
<td>67 ± 5</td>
<td>15 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Left Ventricular Stroke Work (gm.m)</td>
<td>130 ± 9</td>
<td>107 ± 7</td>
<td>15 mins</td>
<td>0.0006</td>
</tr>
<tr>
<td>PR Interval (msec)</td>
<td>199 ± 7</td>
<td>195 ± 8</td>
<td>10 mins</td>
<td>NS</td>
</tr>
<tr>
<td>QT Interval (msec)</td>
<td>409 ± 12</td>
<td>401 ± 12</td>
<td>10 mins</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure 2.45: Enalaprilat effects on Systolic and Diastolic Blood Pressure

**Systolic Blood Pressure (mmHg)**

![Systolic Blood Pressure Graph]

* p<0.01, ANOVA p=0.023, F=2.565

**Diastolic Blood Pressure (mmHg)**

![Diastolic Blood Pressure Graph]

* p<0.05, ** p<0.01
ANOVA p=0.026, F=2.496
Figure 2.46: Enalaprilat effects on Mean Arterial and Pulmonary Capillary Wedge Pressure

**Figure Legend**

- **Top Graph:** Mean Arterial Pressure (mmHg)
- **Bottom Graph:** Mean PCWP (mmHg)

- *p<0.05, **p<0.01
- ANOVA p=0.0008, F=4.143
- p=ns
Figure 2.47: Enalaprilat effects on LV+dP/dt\(\text{max}\) during constant pacing at baseline rate

\[ \text{LV+dP/dt}_{\text{max}} \text{ (mmHg/sec)} \]

\[ \begin{array}{c|c|c|c}
0 & 5 & 10 & 15 \\
\hline
1200 & 1300 & 1400 & 1500 & 1600 \\
\end{array} \]

\[ *p<0.05, \text{ANOVA } F=1.489, \text{ p}=\text{ns} \]

Figure 2.48: Enalaprilat effects on Cardiac Index

\[ \text{Cardiac Index} \text{ (L/min/m}^2\text{)} \]

\[ \begin{array}{c|c|c|c}
0 & 5 & 10 & 15 \\
\hline
1.75 & 2.00 & 2.25 & 2.50 & 2.75 & 3.00 \\
\end{array} \]

\[ *p<0.01, \text{ANOVA } p=0.0014, F=3.891 \]
Figure 2.49: Enalaprilat effects on Systemic Vascular Resistance Index and Left Ventricular Stroke Work

Systemic Vascular Resistance Index (dyne.sec.cm⁻².m⁻¹)

Left Ventricular Stroke Work (gm.m)

*p<0.01, ANOVA p<0.0001, F=6.466
**Figure 2.50: Enalaprilat effects on Coronary Sinus Blood Flow and Coronary Vascular Resistance Index**

*Coronary Sinus Blood Flow (mls/min)*

*Coronary Vascular Resistance Index (dynes.sec.cm⁻¹.m⁻².l⁻¹)*

*p<0.05, **p<0.01  
ANOVA p=0.0055, F=3.229  

p=ns
Effects on the renin-angiotensin system and kinin peptides:

The rapid intravenous bolus administration of 2.5mg enalaprilat resulted in an abrupt, complete and sustained suppression of plasma ACE activity as demonstrated in Figure 2.51 (from 81.2 ± 4.2 to 3.1 ± 0.4 U/L, p<0.0001 for femoral artery and from 82.5 ± 4.4 to 3.9 ± 1.0 U/L, p<0.0001 for coronary sinus, after 10 minutes). The absolute femoral arterial whole blood concentration of angiotensin I (AI) increased (from 1.4 ± 0.6 to 3.1 ± 0.9 fmol/ml, p=0.015) within 2 minutes, the extent of the increase being more marked at 10 minutes (4.7 ± 1.2 fmol/ml, p=0.0001). The level of angiotensin II (AII) abruptly decreased (from 2.1 ± 0.6 to 0.7 ± 0.2 fmol/ml, p=0.02) within 2 minutes, and remained significantly depressed at 10 minutes (0.7 ± 0.2 fmol/ml, p=0.02). Correspondingly, there was an abrupt and large reduction in the AII/AI ratio (from 2.7 ± 1.0 to 0.4 ± 0.2, p=0.0005) within 2 minutes, the reduction remaining significant 10 minutes following administration of enalaprilat (0.3 ± 0.1 fmol/ml, p=0.0001) (Figures 2.52-2.54). The corresponding peptide levels in coronary sinus blood shifted in the same directions and with similar magnitudes (from 1.5 ± 0.5 to 3.6 ± 1.2 fmol/ml (p=0.002) for AI, from 1.4 ± 0.5 to 0.7 ± 0.2 fmol/ml (p=ns) for AII, and from 1.4 ± 0.7 to 0.4 ± 0.1 (p=0.004) for the AII/AI ratio), 2 minutes following enalaprilat and to 3.9 ± 1.0 fmol/ml (p=0.002) for AI, 0.7 ± 0.1 fmol/ml (p=ns) for AII, and 0.3 ± 0.1 (p=0.002) for the AII/AI ratio, 10 minutes following enalaprilat administration (Figures 2.52-2.54).

Enalaprilat induced a significant increase in the femoral arterial whole blood concentration of bradykinin[1-9] (BK1-9) (from 1.0 ± 0.3 to 3.0 ± 0.3 fmol/ml, p=0.0001) after 10 minutes, without significantly impacting on the level of its major metabolite bradykinin[1-7] (BK1-7) (from 2.8 ± 0.4 to 3.5 ± 0.6 fmol/ml, p=ns). Overall, however, there was a significant suppression of the ratio BK1-7/BK1-9 (from 4.2 ± 1.0 to 1.2 ± 0.2 fmol/ml, p=0.0004), as depicted in Figures 2.55,2.57 and 2.58, 10 minutes following administration. Furthermore, there was a modest increase in the level of bradykinin[1-8] (BK1-8) (from 0.5 ± 0.1 to 0.8 ± 0.2 fmol/ml, p=0.0001) which represents an alternative degradation pathway for BK1-9 (Figure 2.56).
However, examination of coronary sinus whole blood concentrations of these same peptides revealed no significant reduction in BK1-7 (from 5.0 ± 1.4 to 3.0 ± 0.4 fmol/ml, p=ns), a non-significant increase in BK1-8 (from 0.6 ± 0.2 to 1.3 ± 0.4 fmol/ml, p=ns), no change in BK1-9 (from 9.9 ± 4.0 to 7.3 ± 3.8 fmol/ml, p=ns) and no effect on the BK1-7/BK1-9 ratio (from 1.2 ± 0.3 to 0.9 ± 0.2, p=ns) 10 minutes following enalaprilat (Figures 2.55-2.58).

**Figure 2.51: Enalaprilat effects on plasma ACE activity**

![Graph showing decrease in ACE activity](image)

**Figure 2.52: Enalaprilat effects on whole blood angiotensin I levels**

![Graph showing increase in angiotensin I levels](image)
Figure 2.53: Enalaprilat effects on angiotensin II levels

![Graph showing changes in angiotensin II levels over time.]

- Femoral Artery
- Coronary Sinus

FA $p=0.027$
CS $p=0.083$

Figure 2.54: Enalaprilat effects on angiotensin II/angiotensin I ratio

![Graph showing changes in angiotensin II/angiotensin I ratio over time.]

- Femoral Artery
- Coronary Sinus

FA $p=0.0001$
CS $p=0.0085$
Figure 2.55: Enalaprilat effects on Bradykinin-(1-7) levels

![Graph showing Enalaprilat effects on Bradykinin-(1-7) levels](image)

- Femoral Artery
- Coronary Sinus

FA $p=\text{ns}$
CS $p=0.1$

Figure 2.56: Enalaprilat effects on Bradykinin-(1-8) levels

![Graph showing Enalaprilat effects on Bradykinin-(1-8) levels](image)

- Femoral Artery
- Coronary Sinus

FA $p=0.032$
CS $p=0.097$
**Figure 2.57: Enalaprilat effects on Bradykinin-(1-9)**

![Graph showing Enalaprilat effects on Bradykinin-(1-9)]

- Femoral Artery
- Coronary Sinus

FA $p=0.0001$
CS $p=ns$

**Figure 2.58: Enalaprilat effects on Bradykinin-(1-7)/Bradykinin-(1-9) ratio**

![Graph showing Enalaprilat effects on Bradykinin-(1-7)/Bradykinin-(1-9) ratio]

- Femoral Artery
- Coronary Sinus

FA $p=0.0007$
CS $p=ns$
Myocardial Enalaprilat Uptake:
A total of 10 patients received an acute intravenous bolus injection of 1.25mg/10ml perindoprilat, enabling determination of acute myocardial drug uptake gradient. The data from the standard curves constructed for each patient are detailed in Table 2.13. The data gathered for acute myocardial drug uptake is recorded in Table 2.14 and displayed in Figure 2.59.

Table 2.13: Standard Curve Descriptors for Patients Receiving Intravenous Enalaprilat

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Binding</th>
<th>Non-specific Binding</th>
<th>ED-20</th>
<th>ED-50</th>
<th>ED-80</th>
<th>QC1</th>
<th>QC2</th>
<th>cv for Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>44%</td>
<td>5%</td>
<td>6.81</td>
<td>2.17</td>
<td>0.60</td>
<td>1.40</td>
<td>5.63</td>
<td>2.78±1.18</td>
</tr>
<tr>
<td>15</td>
<td>51%</td>
<td>6%</td>
<td>8.66</td>
<td>2.93</td>
<td>0.82</td>
<td>2.35</td>
<td>7.82</td>
<td>1.76±0.30</td>
</tr>
<tr>
<td>16</td>
<td>43%</td>
<td>7%</td>
<td>10.9</td>
<td>3.37</td>
<td>0.94</td>
<td>1.89</td>
<td>7.81</td>
<td>1.15±0.28</td>
</tr>
<tr>
<td>19</td>
<td>54%</td>
<td>6%</td>
<td>7.83</td>
<td>2.51</td>
<td>0.84</td>
<td>2.03</td>
<td>6.64</td>
<td>2.17±0.62</td>
</tr>
<tr>
<td>20</td>
<td>54%</td>
<td>7%</td>
<td>7.54</td>
<td>2.37</td>
<td>0.62</td>
<td>2.29</td>
<td>6.89</td>
<td>0.88±0.25</td>
</tr>
<tr>
<td>22</td>
<td>45%</td>
<td>12%</td>
<td>15.2</td>
<td>6.24</td>
<td>2.02</td>
<td>1.83</td>
<td>7.79</td>
<td>1.21±0.36</td>
</tr>
<tr>
<td>23</td>
<td>77%</td>
<td>10%</td>
<td>19.3</td>
<td>8.25</td>
<td>4.08</td>
<td>1.89</td>
<td>7.12</td>
<td>1.02±0.29</td>
</tr>
<tr>
<td>26</td>
<td>58%</td>
<td>10%</td>
<td>15.1</td>
<td>6.71</td>
<td>3.09</td>
<td>1.39</td>
<td>6.62</td>
<td>1.32±0.39</td>
</tr>
<tr>
<td>27</td>
<td>56%</td>
<td>10%</td>
<td>19.7</td>
<td>8.03</td>
<td>3.57</td>
<td>2.03</td>
<td>6.89</td>
<td>1.54±0.45</td>
</tr>
<tr>
<td>29</td>
<td>68%</td>
<td>9%</td>
<td>22.0</td>
<td>8.88</td>
<td>4.18</td>
<td>2.22</td>
<td>7.95</td>
<td>0.87±0.23</td>
</tr>
<tr>
<td>Mean</td>
<td>55</td>
<td>8.2</td>
<td>13.3</td>
<td>5.1</td>
<td>2.1</td>
<td>1.9</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>3.4</td>
<td>0.7</td>
<td>1.8</td>
<td>0.9</td>
<td>0.5</td>
<td>0.1</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

cv = average coefficient of variation (%) based on counts per minute for paired standards
ED-20 = Enalaprilat concentration (pmol/ml) at 20% of maximal response
ED-50 = Enalaprilat concentration (pmol/ml) at 50% of maximal response
ED-80 = Enalaprilat concentration (pmol/ml) at 80% of maximal response
QC’s = Quality controls
Peak myocardial drug uptake gradient of enalaprilat occurred $18.3 \pm 2.4$ seconds following rapid intravenous bolus administration. At this time point, the femoral artery/coronary sinus gradient of enalaprilat was $19,500 \pm 4,900$ pmol/ml/minute, equal to $5.68$ micrograms/ml/minute. The peak myocardial content of enalaprilat accounted for $0.23 \pm 0.06\%$ of the injected dose and occurred $28.4 \pm 3.6$ seconds following drug administration. Five minutes following drug administration, myocardial drug content accounted for $0.083 \pm 0.028\%$ of the injected dose and after 10 minutes accounted for $0.075 \pm 0.028\%$. 

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Peak gradient (pmol/ml/Min)</th>
<th>Time to peak gradient (sec)</th>
<th>Peak content (% of dose)</th>
<th>Time to peak content (sec)</th>
<th>cv for samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>43,400</td>
<td>15</td>
<td>0.506</td>
<td>25.2</td>
<td>3.72±0.68</td>
</tr>
<tr>
<td>15</td>
<td>2,390</td>
<td>15</td>
<td>0.028</td>
<td>25.2</td>
<td>3.20±0.66</td>
</tr>
<tr>
<td>16</td>
<td>20,700</td>
<td>37.8</td>
<td>0.241</td>
<td>52.8</td>
<td>3.67±0.77</td>
</tr>
<tr>
<td>19</td>
<td>13,500</td>
<td>15</td>
<td>0.158</td>
<td>25.2</td>
<td>3.57±0.93</td>
</tr>
<tr>
<td>20</td>
<td>3,100</td>
<td>15</td>
<td>0.036</td>
<td>15</td>
<td>3.37±1.40</td>
</tr>
<tr>
<td>22</td>
<td>25,000</td>
<td>25.2</td>
<td>0.291</td>
<td>37.8</td>
<td>3.79±0.63</td>
</tr>
<tr>
<td>23</td>
<td>24,200</td>
<td>15</td>
<td>0.282</td>
<td>25.2</td>
<td>5.72±1.41</td>
</tr>
<tr>
<td>26</td>
<td>44,7</td>
<td>15</td>
<td>0.520</td>
<td>25.2</td>
<td>6.64±1.48</td>
</tr>
<tr>
<td>27</td>
<td>16,000</td>
<td>15</td>
<td>0.186</td>
<td>37.8</td>
<td>5.26±1.62</td>
</tr>
<tr>
<td>29</td>
<td>2,400</td>
<td>15</td>
<td>0.028</td>
<td>15</td>
<td>3.92±0.78</td>
</tr>
<tr>
<td>Mean</td>
<td>19,500</td>
<td>18.3</td>
<td>0.23</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>4,900</td>
<td>2.4</td>
<td>0.06</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

cv = average coefficient of variation(%) based on counts/min for triplicate samples
Figure 2.59: (a) Average Femoral Arterial and Coronary Sinus Enalaprilat Concentrations for 20 minutes post injection
(b) Average Femoral Arterial and Coronary Sinus Enalaprilat Concentrations for 3 minutes post injection
Correlation between myocardial enalaprilat content and haemodynamic effects

It is evident that the time course of effect of enalaprilat exhibited significant hysteresis with regard to myocardial content. Although peak content occurred within 30 seconds of enalaprilat administration, peak haemodynamic effects were not observed until up to 15 minutes had elapsed, this hysteresis being apparent for all measured variables.

Of the haemodynamic variables that were significantly altered by enalaprilat, correlation between peak myocardial drug content and peak haemodynamic effect was sought. Mean arterial pressure, LV+dP/dt\text{max} and coronary sinus blood flow were not significantly correlated with myocardial drug content. Changes in cardiac index, however, were correlated with evidence of hysteresis; the myocardial drug content at 2 minutes being correlated with cardiac index at 4 minutes (r=0.68,
p=0.03). The hysteresis diagrams depicted in Figure 2.60 reveal that all drug effects occurred well beyond the time of peak content.

There was no statistically significant correlation between the maximal myocardial content of enalaprilat and any baseline patient characteristics, including left ventricular ejection fraction, cardiac index, basal paced heart rate, extent of fixed coronary artery disease and coronary sinus blood flow. Time to maximal myocardial content of enalaprilat was also independent of these factors.

**Figure 2.60** *Hysteresis between myocardial enalaprilat content and effects on*

(a) **Cardiac Index**
(b) **Mean Arterial Pressure**
(c) **LV+ dP/dt\(_{max}\)**
(d) **Coronary Sinus Blood Flow**

![Graphs showing hysteresis between myocardial enalaprilat content and effects on cardiac index, mean arterial pressure, LV+ dP/dt\(_{max}\), and coronary sinus blood flow.](attachment:image)
Effects of Enalaprilat during pacing induced tachycardia

In addition to the observations made during atrial pacing at just above spontaneous heart rate, tachycardia was induced both in the absence and presence of enalaprilat in order to examine its effects on induction of ischaemia. Detailed data examining enalaprilat effects on the left ventricular force-interval relationship were also gathered. However, this component is presented in Chapter 3.

Of the 10 patients who received an intravenous bolus injection of 2.5mg enalaprilat, 9 underwent atrial pacing at a cycle length that was 58.5 ± 2.2% of the baseline paced cycle length, equal to a heart rate of 127 ± 5 beats per minute. One patient did not have this procedure performed due to the spontaneous onset of angina during atrial pacing, at baseline cycle length, 12 minutes following enalaprilat administration. Rapid atrial pacing was repeated 13.0 ± 0.5 minutes following enalaprilat administration. Of the 9 patients studied, 7 had significant coronary artery disease involving the left anterior descending artery, the circumflex artery or both. The results were analyzed both for the total cohort of 9 patients and for the 7 patients for whom induction of ischaemia, in the vascular territory drained by the coronary sinus, during tachycardia was likely.

Effects on haemodynamic parameters

The haemodynamic and metabolic responses to the induction of a pacing induced tachycardia in both the absence and presence of enalaprilat are presented in Figures 2.61-2.64 below. In the absence of enalaprilat, the induction of pacing induced tachycardia produced a significant increase in coronary sinus blood flow, from 104 ± 8 to 155 ± 15 mls/min, p=0.01. This was associated with a modest reduction in coronary vascular resistance index (-29.4 ± 8.5%, p=0.02) but no change in mean arterial pressure (+0.6 ± 3.1%, p=ns). There was, however, a moderate increase in the mean pulmonary capillary wedge pressure (from 9.0 ± 1.3 to 12.8 ± 1.7 mmHg, p=0.0006) and LV+dP/dtmax (from 1440 ± 60 to 1780 ± 90 mmHg/sec, p=0.002). No
patient demonstrated net lactate production during tachycardia, extraction increasing by $28.3 \pm 13.3$ mg/ml/min ($p=0.07$), whilst oxygen extraction for the group was significantly increased (by $51 \pm 16\%$, $p=0.01$).

In the presence of enalaprilat, the haemodynamic and metabolic responses to tachycardia were not substantially modified. Coronary sinus blood flow increased from $86 \pm 5$ to $113 \pm 13$ mls/min, $p=0.02$. This was associated with a small reduction in coronary vascular resistance (by $21.4 \pm 7.8\%$, $p=0.04$) but no change in mean arterial pressure ($-1.7 \pm 2.1\%$, $p=ns$). The increase in pulmonary capillary wedge pressure was still observed ($+42.7 \pm 13.5\%$, $p=0.02$). LV+dP/dt$_{max}$ increased from $1370 \pm 90$ to $1620 \pm 120$ mmHg/sec ($p=0.009$). Net lactate production was observed for 2 patients following enalaprilat, the extraction of lactate increasing by just $5.4 \pm 6.3$ mg/ml/min ($p=ns$). Oxygen extraction was moderately increased by $21 \pm 8\%$, $p=0.03$.

**Figure 2.61**  *Response of coronary sinus blood flow to pacing induced tachycardia in the absence and presence of enalaprilat*

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Figure 2.62  Response to pacing induced tachycardia in the absence and presence of enalaprilat for (a) Coronary Vascular Resistance Index and (b) Mean Arterial Pressure
Figure 2.63  Response of (a) mean pulmonary capillary wedge pressure and (b) $LV+dP/dt_{max}$ to the induction of tachycardia in the absence and presence of enalaprilat.
Figure 2.64  Effects of enalaprilat on (a) myocardial oxygen extraction and (b) myocardial lactate production/extraction, during a pacing induced tachycardia.
When the impact of enalaprilat on the haemodynamic and metabolic responses to the induction of tachycardia was examined for the 7 patients with significant coronary artery disease, almost identical results were obtained, the effect on coronary sinus blood flow now being significant (Figure 2.65).

Figure 2.65 Enalaprilat effects on response to tachycardia for (a) Coronary sinus blood flow and (b) Coronary Vascular Resistance Index, in patients with significant fixed coronary artery disease.
Effects on renin-angiotensin system and Bradykinin peptides

As previously described, the activity of serum ACE was markedly attenuated following the administration of enalaprilat (Figure 2.51). In addition, significant differences in the levels of various angiotensin and bradykinin peptides during the induction of tachycardia were also noted (Figures 2.66-2.72).

Prior to the administration of enalaprilat, the induction of tachycardia exhibited no significant effects on peripheral angiotensin or bradykinin peptides. Examination of coronary sinus peptide levels also revealed no impact from the induction of tachycardia. In the presence of enalaprilat, there was significant modulation of angiotensin and bradykinin peptide levels during tachycardia. Femoral arterial AII/AI and BK(1-7)/BK(1-9) ratios were both significantly suppressed, in keeping with the extent of inhibition of serum ACE. Similarly, the coronary sinus AII/AI ratio was also significantly suppressed, whilst none of the bradykinin peptides or ratios were significantly altered. The observed effects on the AII/AI ratio was consequent upon both a suppression of AII and an increase in AI levels. However, in the case of bradykinin, the fall in femoral arterial BK(1-7)/BK(1-9) ratio was almost entirely due to increased BK(1-9) levels, the level of BK(1-7) remaining unchanged. The changes in peptide levels observed were independent of the presence of coronary artery disease, the results presented being those for all patients receiving intravenous enalaprilat.
Figure 2.66  Effects of enalaprilat on angiotensin I during pacing induced tachycardia

**Femoral Artery**

Baseline
- Enalaprilat

Rest  Tachycardia  Recovery

p=0.0006 for effect of enalaprilat

**Coronary Sinus**

Baseline
- Enalaprilat

Rest  Tachycardia  Recovery

p=0.0002 for effect of enalaprilat
Figure 2.67  Effects of enalaprilat on angiotensin II during pacing induced tachycardia

Femoral Artery

- Baseline
- • Enalaprilat

p=0.0001 for effect of enalaprilat

Coronary Sinus

- Baseline
- • Enalaprilat

p=0.0004 for effect of enalaprilat
Figure 2.68  Effects of enalaprilat on AII/AI during pacing induced tachycardia

Femoral Artery

Coronary Sinus

p=0.0001 for effect of enalaprilat
Figure 2.69  Effects of enalaprilat on Bradykinin 1-7 during pacing induced tachycardia

Femoral Artery

Coronary Sinus
Figure 2.70  Effects of enalaprilat on Bradykinin 1-8 during pacing induced tachycardia

Femoral Artery

Coronary Sinus

p=ns for effect of enalaprilat
Figure 2.71  Effects of enalaprilat on Bradykinin 1-9 during pacing induced tachycardia

**Femoral Artery**

![Femoral Artery Graph]

- Baseline
- Enalaprilat

\[ p=0.0001 \text{ for effect of enalaprilat} \]

**Coronary Sinus**

![Coronary Sinus Graph]

- Baseline
- Enalaprilat

\[ p=ns \text{ for effect of enalaprilat} \]
Figure 2.72  Effects of enalaprilat on BK 1-7/BK 1-9 during pacing induced tachycardia

**Femoral Artery**

![Graph showing effects of enalaprilat on BK 1-7/BK 1-9 in the femoral artery.](image)

- Baseline
- Enalaprilat

p=0.0001 for effect of enalaprilat

**Coronary Sinus**

![Graph showing effects of enalaprilat on BK 1-7/BK 1-9 in the coronary sinus.](image)

- Baseline
- Enalaprilat

p=ns for effect of enalaprilat
Summary of Intravenous Enalaprilat Effects

The intravenous bolus administration of 2.5mg enalaprilat to the 10 patients studied produced significant changes both at resting heart rate and during the induction of tachycardia using atrial pacing.

During atrial pacing at just above spontaneous heart rate, enalaprilat was rapidly taken up by myocardium, peak drug content being achieved in less than 30 seconds, accounting for 0.23% of the injected dose. Enalaprilat caused a moderate reduction in mean arterial pressure and a less marked reduction in cardiac index. Although there was also a trend towards a negative inotropic effect (as determined using LV+dP/dt max) systemic vascular resistance was not altered. There was a significant reduction in both coronary sinus blood flow and left ventricular stroke work. The peak haemodynamic effects of enalaprilat occurred at about 15 minutes following drug injection with significant, but not incremental effects persisting for at least 20 minutes.

The dose of enalaprilat administered was sufficient to cause an abrupt and almost total suppression of serum ACE activity. This was accompanied by a significant suppression of both the peripheral AII/AI ratio and that of the coronary sinus. There was a marked reduction in the femoral artery BK1-7/BK1-9 ratio, with that of the coronary sinus showing no change.

Enalaprilat effects during tachycardia did not vary markedly according to the presence/absence of coronary artery disease. It caused a modest decrease in coronary sinus blood flow with trends towards both a negative inotropic effect and a fall in oxygen extraction. This was accompanied by suppression of the AII/AI ratio in both the peripheral and coronary sinus blood. The BK1-7/BK1-9 ratio while being significantly reduced in the peripheral circulation was not altered in the coronary sinus. BK1-8 levels were not increased.
INTRACORONARY ENALAPRILAT

A total of 5 patients received a selective left main intracoronary injection of 0.5mg enalaprilat. Despite denial by one patient of angiotensin converting enzyme inhibitor therapy, it became apparent during analysis of results that chronic exposure to such a drug was occurring and the results for this patient were therefore excluded from subsequent analysis. One patient was noted to develop significant ST elevation on the 2-channel ECG one minute following intracoronary enalaprilat that gradually resolved within 2 minutes. This was not associated with any symptoms. Confirmation of the presence of coronary artery spasm was not sought at the time. The procedure was well tolerated in all other patients. The baseline characteristics of these 4 patients are recorded in Table 2.14 and their baseline haemodynamic parameters in Table 2.15.

Effects of intracoronary enalaprilat at resting heart rate

Effects on haemodynamic parameters

Following intracoronary administration of enalaprilat, during baseline atrial pacing at a rate 56.8 ± 3.8% above spontaneous heart rate, haemodynamic variables were monitored for up to 20 minutes. The maximal changes in these variables are recorded in Table 2.16. The results obtained were similar to those for intravenous enalaprilat, there being a decrease in blood pressure and cardiac index and a borderline decrease in LV+dP/dt\text{max} and stroke work. There was, however, no change in coronary haemodynamics. These results are graphically displayed in Figures 2.73-2.76.
### Table 2.14  Patient characteristics – intracoronary enalaprilat

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age (a)</th>
<th>BSA (m²)</th>
<th>CI (L/min/m²)</th>
<th>LVEF (%)</th>
<th>Coronary Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>F</td>
<td>56</td>
<td>1.79</td>
<td>2.32</td>
<td>66</td>
<td>Nil</td>
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<tr>
<td>21</td>
<td>F</td>
<td>75</td>
<td>1.78</td>
<td>2.84</td>
<td>77</td>
<td>Nil</td>
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<tr>
<td>24</td>
<td>M</td>
<td>55</td>
<td>2.11</td>
<td>3.18</td>
<td>81</td>
<td>LAD, Cx</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>64</td>
<td>1.98</td>
<td>2.05</td>
<td>52</td>
<td>LAD, Cx, RCA</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>63</td>
<td>1.92</td>
<td>2.60</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>5</td>
<td>0.08</td>
<td>0.25</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2.15  Baseline haemodynamic parameters – intracoronary enalaprilat

<table>
<thead>
<tr>
<th>Number</th>
<th>MAP</th>
<th>PCWP</th>
<th>SVRI</th>
<th>CSBF</th>
<th>Paced heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>99</td>
<td>11</td>
<td>3410</td>
<td>41</td>
<td>71</td>
</tr>
<tr>
<td>21</td>
<td>128</td>
<td>12</td>
<td>3610</td>
<td>204</td>
<td>92</td>
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<tr>
<td>24</td>
<td>108</td>
<td>6</td>
<td>2710</td>
<td>107</td>
<td>92</td>
</tr>
<tr>
<td>25</td>
<td>112</td>
<td>19</td>
<td>4370</td>
<td>89</td>
<td>55</td>
</tr>
<tr>
<td>Mean</td>
<td>112</td>
<td>12</td>
<td>3520</td>
<td>111</td>
<td>70</td>
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<tr>
<td>SEM</td>
<td>6</td>
<td>3</td>
<td>340</td>
<td>34</td>
<td>8</td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure (mmHg)
PCWP = mean pulmonary capillary wedge pressure (mmHg)
SVRI = systemic vascular resistance index (dynes.sec.cm⁻⁵.m²⁻¹)
CSBF = coronary sinus blood flow (mls/min)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline Measure</th>
<th>Maximal Change From Baseline</th>
<th>Time to Maximal Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>112 ± 6</td>
<td>105 ± 6</td>
<td>6 &amp; 15 mins</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>155 ± 11</td>
<td>141 ± 7</td>
<td>4 &amp; 15 mins</td>
<td>0.007</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>84 ± 4</td>
<td>78 ± 3</td>
<td>15 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac Index (L/min/m²)</td>
<td>2.60 ± 0.25</td>
<td>2.35 ± 0.19</td>
<td>4 mins</td>
<td>0.0077</td>
</tr>
<tr>
<td>Mean PCWP (mmHg)</td>
<td>12 ± 3</td>
<td>13 ± 2</td>
<td>2 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic Vasc Resistance Index (dynes.sec.cm⁻⁵.m²)</td>
<td>3520 ± 340</td>
<td>3670 ± 380</td>
<td>4 mins</td>
<td>NS</td>
</tr>
<tr>
<td>LV+dP/dtₘₙₙx (mmHg/sec)</td>
<td>1620 ± 150</td>
<td>1490 ± 100</td>
<td>15 mins</td>
<td>0.014</td>
</tr>
<tr>
<td>Coronary Sinus Blood Flow (mls/min)</td>
<td>111 ± 34</td>
<td>96 ± 28</td>
<td>2 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary Vasc Resistance Index (dynes.sec.cm⁻⁵.m²)</td>
<td>106000 ± 31000</td>
<td>119000 ± 35000</td>
<td>2 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Spontaneous Heart Rate</td>
<td>70 ± 8</td>
<td>69 ± 9</td>
<td>15 mins</td>
<td>NS</td>
</tr>
<tr>
<td>Left Ventricular Stroke Work (gm.m)</td>
<td>93 ± 8</td>
<td>80 ± 9</td>
<td>4 mins</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Figure 2.73  Effects of intracoronary enalaprilat on (a) Systolic and (b) Diastolic blood pressure

(a)

Systolic Blood Pressure (mmHg)

* p<0.05,  ** p<0.01
ANOVA p=0.01,  F=3.592

(b)

Diastolic Blood Pressure (mmHg)

p=ns
Figure 2.74  Effects of intracoronary enalaprilat on (a) Mean arterial pressure and (b) Mean pulmonary capillary wedge pressure

(a)

Mean Arterial Pressure (mmHg)

Minutes

* p<0.05  ANOVA p=0.09

(b)

Mean PCWP (mmHg)

Minutes

p=ns
Figure 2.75  Effects of intracoronary enalaprilat on (a) Cardiac Index and (b) LV+dP/dt_max

(a) Cardiac Index (L/min/m²)

(b) LV+dP/dt_max (mmHg/sec)

Minutes

*p<0.05, **p<0.01
ANOVA p=0.048, F=2.522

*p<0.05  ANOVA p=0.052
Figure 2.76  Effects of intracoronary enalaprilat on (a) Coronary sinus blood flow and (b) Stroke Work

(a) Coronary Sinus Blood Flow (mls/min)

(b) Left Ventricular Stroke Work (gm.m)

Minutes

Coronary Sinus Blood Flow

Minutes

Left Ventricular Stroke Work (gm.m)

* p<0.05  ANOVA p=0.09
Effects on the renin-angiotensin system and bradykinin peptides

The effects of intracoronary enalaprilat on the renin-angiotensin system and bradykinin peptides are recorded in Figures 2.77-2.80. Although selective intracoronary dosing of a small amount of enalaprilat was performed, there was widespread and marked suppression of plasma ACE activity. A suppression of the AII/AI ratio was detected, the change in the coronary sinus ratio being more marked than that occurring peripherally. The only significant impact on bradykinin peptides noted was a decrease in the femoral arterial BK1-7/BK1-9 ratio, consequent on increased Bradykinin 1-9 levels. However, the decrease in the BK1-7/BK1-9 ratio was of borderline significance in the coronary sinus.

Figure 2.77  Enalaprilat effects on (a) ACE activity and (b) Angiotensin I

(a)  

![ACE activity graph](image)

FA p<0.0001
CS p<0.0001

(b)  

![Angiotensin I graph](image)

FA p=ns
CS p=0.051
Figure 2.78  Enalaprilat effects on (a) Angiotensin II and (b) AII/AI ratio

(a)

(b)

- Femoral Artery
- Coronary Sinus

FA  p=0.065
CS  p=ns

FA  p=ns
CS  p=0.0003
Figure 2.79  Enalaprilat effects on (a) Bradykinin 1-7 and (b) Bradykinin 1-8

(a)

Bradykinin 1-7 (fmol/ml)

- Femoral Artery
- Coronary Sinus

FA p=ns
CS p=0.07

(b)

Bradykinin 1-8 (fmol/ml)

- Femoral Artery
- Coronary Sinus

FA p=ns
CS p=ns
Figure 2.80  Enalaprilat effects on (a) Bradykinin 1-9 and (b) BK 1-7/BK 1-9 ratio

(a)

- Femoral Artery
- Coronary Sinus

FA  p=0.0004  
CS  p=ns

(b)

- Femoral Artery
- Coronary Sinus

FA  p=0.046  
CS  p=0.058
Effects of Intracoronary Enalaprilat during tachycardia

Of the 4 patients receiving an intracoronary dose of 0.5mg enalaprilat, all were examined during a pacing induced tachycardia in both the absence and presence of drug. Atrial pacing was performed at a cycle length that was 57 ± 4% of the baseline paced cycle length for 2 minutes. The effects on coronary haemodynamic and metabolic parameters are recorded in Figures 2.81 and 2.82. The effects on the renin-angiotensin system and bradykinin peptides are presented in Figures 2.83-2.89.

Coronary vasodilator reserve was abnormal in this group of patients, with coronary sinus blood flow decreasing during induction of tachycardia (from 106 ± 36 to 92 ± 7 mls/min, p=ns). Furthermore, this abnormal decrease was exacerbated following the administration of enalaprilat. Myocardial lactate production was observed in one patient prior to enalaprilat administration and in 2 patients subsequently, the net increase in lactate production being significantly increased following enalaprilat (from -0.4 ± 9.7 to 5.7 ± 9.6 mg/ml/min). At baseline, there was an increase in net myocardial extraction of oxygen during tachycardia. Following enalaprilat, the increase was somewhat attenuated (by 19 ± 8%, p=0.097). The hypotensive effects of enalaprilat observed during baseline pacing were further exacerbated during the induction of tachycardia, there being a further 6.1 ± 1.9% (p=0.049) fall in mean arterial pressure during tachycardia in the presence of enalaprilat. No other haemodynamic parameters were significantly affected.

The impact of a small intracoronary dose of enalaprilat on serum ACE activity was previously described (Figure 2.77a). There was a significant reduction in both the peripheral and intracoronary AII/AI ratio (Figure 2.85), this being almost totally due to a reduction in the generation of angiotensin II (Figure 2.84), angiotensin I levels remaining relatively constant (Figure 2.83), except in the coronary sinus where an increase was observed. Enalaprilat induced no substantial changes in bradykinin peptide levels within the coronary sinus but did produce a marked suppression of the peripheral BK1-7/BK1-9 ratio (Figures 2.86-2.89).
Figure 2.81  Effects of Enalaprilat on response to tachycardia for
(a) Coronary Sinus Blood Flow, and (b) Mean Arterial Pressure

(a) Coronary Sinus Blood Flow (mls/min)

- Baseline
- Enalaprilat

p=ns for effect of enalaprilat

Rest  Tachycardia  Recovery

(b) Mean Arterial Pressure (mmHg)

- Baseline
- Enalaprilat

p=0.049 for effect of enalaprilat

Rest  Tachycardia  Recovery
Figure 2.82  Effects of Enalaprilat on response to tachycardia for
(a) Myocardial Oxygen Extraction and (b) Myocardial Lactate Production

(a)

Myocardial Oxygen Extraction (% baseline)

--- Baseline
- • Enalaprilat

p=0.097 for effect of enalaprilat

Rest Tachycardia Recovery

(b)

Net Myocardial Lactate Production (mg/ml/min)

--- Baseline
- • Enalaprilat

p=0.033 for effect of enalaprilat

Rest Tachycardia Recovery
Figure 2.83 Effects of enalaprilat on angiotensin I levels

**Femoral Artery**

![Graph showing effects of enalaprilat on angiotensin I levels in femoral artery.](image)

- Baseline
- Enalaprilat

$p = \text{ns}$

**Coronary Sinus**

![Graph showing effects of enalaprilat on angiotensin I levels in coronary sinus.](image)

- Baseline
- Enalaprilat

$p = 0.012$
Figure 2.84  Effects of enalaprilat on angiotensin II levels

Femoral Artery

![Graph showing effects of enalaprilat on angiotensin II levels in the femoral artery.]

- Baseline
- Enalaprilat

p=0.0003

Coronary Sinus

![Graph showing effects of enalaprilat on angiotensin II levels in the coronary sinus.]

- Baseline
- Enalaprilat

p=0.025
Figure 2.85  Effects of enalaprilat on AII/AI ratio

Femoral Artery

- Baseline
- Enalaprilat

Baseline

Enalaprilat

p=0.0003

Rest  Tachycardia  Recovery

Coronary Sinus

- Baseline
- Enalaprilat

Baseline

Enalaprilat

p=0.0018

Rest  Tachycardia  Recovery
Figure 2.86  Effects of enalaprilat on Bradykinin 1-7 levels

**Femoral Artery**

- Baseline
- Enalaprilat

Rest  | Tachycardia  | Recovery
---|---|---

**Coronary Sinus**

- Baseline
- Enalaprilat

Rest  | Tachycardia  | Recovery
---|---|---

p=ns
Figure 2.87  Effects of enalaprilat on Bradykinin 1-8 levels.

Femoral Artery

![Graph showing the effects of enalaprilat on Bradykinin 1-8 levels in the femoral artery.]

- Baseline
- Enalaprilat

\[ p=0.028 \]

Coronary Sinus

![Graph showing the effects of enalaprilat on Bradykinin 1-8 levels in the coronary sinus.]

- Baseline
- Enalaprilat

\[ p=\text{ns} \]
Figure 2.88  Effects of enalaprilat on Bradykinin 1-9 levels

Femoral Artery

![Graph showing effects of enalaprilat on Bradykinin 1-9 levels in femoral artery. Baseline and Enalaprilat conditions are compared with p=0.0006.]

Coronary Sinus

![Graph showing effects of enalaprilat on Bradykinin 1-9 levels in coronary sinus. Baseline and Enalaprilat conditions are compared with p=ns.]
Figure 2.89  Effects of enalaprilat on BK1-7/BK1-9 ratio

Femoral Artery

Coronary Sinus

p=0.0009

p=ns
Summary of Intracoronary Enalaprilat effects

The intracoronary administration of 0.5mg enalaprilat was examined in 4 patients. The same experimental protocol was followed as for those patients receiving intravenous enalaprilat. One patient experienced transient, asymptomatic ST elevation following drug administration.

Despite the small number of patients examined, changes in several haemodynamic parameters, examined during baseline atrial pacing at just above spontaneous heart rate, were found. These changes were similar to those seen after intravenous drug administration, including falls in systolic and mean arterial blood pressure, cardiac index, LV+dP/dt\textsubscript{max} and left ventricular stroke work. Serum ACE activity in both the coronary sinus and the femoral artery was significantly suppressed, this being accompanied by a reduction in the AII/AI ratio which was more marked in the coronary sinus than in the femoral artery. The level of bradykinin peptides was not significantly altered in the coronary sinus but a significant increase in peripheral BK1-9 was noted, causing a moderate decrease in the BK1-7/BK1-9 ratio, a similar trend being noted in the coronary sinus.

During the induction of tachycardia, intracoronary enalaprilat caused a significant decrease in mean arterial pressure, a decrease in the myocardial extraction of oxygen and a significant increase in net myocardial lactate production. There was a significant suppression of the AII/AI ratio in both the coronary sinus and the periphery with a reduction in the BK1-7/BK1-9 ratio being observed in the periphery only. These observations regarding angiotensin and bradykinin peptides were virtually identical to those made following intravenous enalaprilat administration.
COMPARISON OF INTRAVENOUS PERINDOPRILAT AND ENALAPRILAT

It is apparent from reviewing the above data that whilst both perindoprilat and enalaprilat produce an abrupt and sustained suppression of serum ACE activity, the effects on haemodynamic and metabolic parameters are somewhat different. The study protocol was designed for the comparison of two ACE inhibitors with different affinity for tissue versus serum ACE. This approach was taken in order to help address the issue as to the relative contribution of tissue versus serum ACE inhibition in the haemodynamic, metabolic and biochemical effects of these agents. Perindoprilat has a much higher affinity for tissue ACE than enalaprilat with this affinity being very high for cardiac ACE. Therefore, the comparison of the effects of these two agents should afford insight as to the relative importance of affinity for cardiac ACE.

The two groups were well matched at baseline (Table 2.17) with a slightly higher left ventricular ejection fraction for the enalaprilat group, but overall the majority of patients having normal systolic function. There was some disparity between the groups with respect to bradykinin peptides. The perindoprilat group had a significantly higher level of both bradykinin 1-7 and bradykinin 1-8 at baseline, in comparison to the enalaprilat group (Table 18). The reason for this was not sought at the time. However, it is noted that the perindoprilat group was potentially more ischaemic, as indicated by the impairment of coronary vasodilator reserve at baseline. It is possible that this situation involves proportionately greater production of bradykinin 1-9, this being metabolized quite rapidly to the octa- and heptapeptide. Alternatively, the increased levels could represent artefactual generation of bradykinin by local trauma caused by the insertion of a catheter into the coronary sinus. However, the same procedure was followed in all cases with mechanisms in place to minimize the potential for artefactual generation of bradykinin peptides.

The effects of enalaprilat and perindoprilat on haemodynamic parameters during baseline atrial pacing shared both some similarities and some differences (Table 19).
Whilst both agents were rapidly taken up into the myocardium, the time to peak haemodynamic effect for enalaprilat was longer than that for perindoprilat, taking 13.3 ± 0.7 minutes versus 5.9 ± 0.8 minutes respectively (p<0.0001). However, these effects tended to persist and, for both drugs, significant effects on these parameters were still evident when rapid atrial pacing was performed. Therefore, it is possible to directly compare the effects of these drugs during rapid atrial pacing.

The myocardial handling of ACE inhibitor was slightly different for the two drugs studied. Perindoprilat exhibited a slightly higher peak uptake and a higher peak content in the heart, although neither of these differences was significant. However, the hysteresis of effect was significantly different as indicated above.

The haemodynamic and metabolic responses to tachycardia are recorded in Table 2.19. This records the impact of tachycardia, on the measured parameters, both in the absence and presence of drug. Because the effects of perindoprilat were more apparent in patients with significant fixed coronary artery disease, Table 2.21 excludes those patients with no significant coronary artery stenoses. The corresponding angiotensin and bradykinin peptide data for Table 2.19 are presented in Table 2.20 and for Table 2.21, presented in Table 2.22.

Perindoprilat increased coronary sinus blood flow during tachycardia, relative to enalaprilat, having a similar effect on oxygen extraction (Table 2.19). These effects were even more marked in patients with significant coronary artery disease. Both perindoprilat and enalaprilat decreased AII release during tachycardia, there being a marked suppression of the AII/AI ratio in both cases. However, only perindoprilat resulted in a relative increase in bradykinin 1-9 release during tachycardia (Table 2.20).
Table 2.17  
Baseline haemodynamic and patient characteristics for groups receiving intravenous perindoprilat and enalaprilat

<table>
<thead>
<tr>
<th></th>
<th>Perindoprilat</th>
<th>Enalaprilat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59 ± 3</td>
<td>61 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>10M : 1F</td>
<td>9M : 1F</td>
<td>NS</td>
</tr>
<tr>
<td>Coronary Disease</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF</td>
<td>66 ± 4</td>
<td>76 ± 3</td>
<td>0.07</td>
</tr>
<tr>
<td>MAP</td>
<td>113 ± 4</td>
<td>109 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac Index</td>
<td>2.64 ± 0.16</td>
<td>2.50 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>PCWP</td>
<td>11.0 ± 0.8</td>
<td>9.0 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>LV+dp/dt\text{max}</td>
<td>1510 ± 100</td>
<td>1440 ± 60</td>
<td>NS</td>
</tr>
<tr>
<td>CSBF</td>
<td>105 ± 10</td>
<td>104 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Paced heart rate</td>
<td>76.2 ± 3.6</td>
<td>71.9 ± 3.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Coronary disease = number of major epicardial coronary arteries with >50% stenosis

Table 2.18  
Baseline angiotensin and bradykinin coronary sinus peptide data for groups receiving intravenous perindoprilat and enalaprilat

<table>
<thead>
<tr>
<th></th>
<th>Perindoprilat (fmol/ml)</th>
<th>Enalaprilat (fmol/ml)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>91 ± 10</td>
<td>84 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>2.6 ± 0.8</td>
<td>2.3 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>1.2 ± 0.3</td>
<td>2.7 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>AII/AI</td>
<td>0.56 ± 0.22</td>
<td>2.34 ± 1.09</td>
<td>NS</td>
</tr>
<tr>
<td>BK 1-7</td>
<td>22.7 ± 4.4</td>
<td>6.7 ± 2.5</td>
<td>0.007</td>
</tr>
<tr>
<td>BK 1-8</td>
<td>3.6 ± 0.7</td>
<td>1.6 ± 0.5</td>
<td>0.04</td>
</tr>
<tr>
<td>BK 1-9</td>
<td>29.6 ± 9.4</td>
<td>18.5 ± 7.7</td>
<td>NS</td>
</tr>
<tr>
<td>BK 1-7/BK 1-9</td>
<td>1.32 ± 0.45</td>
<td>0.66 ± 0.16</td>
<td>NS</td>
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</tbody>
</table>
Table 2.19  Haemodynamic and metabolic changes at rest and during tachycardia- perindoprilat vs enalaprilat

<table>
<thead>
<tr>
<th></th>
<th>PERINDOPRILAT</th>
<th></th>
<th>ENALAPRILAT</th>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug effect at</td>
<td>Tachycardia effect</td>
<td>Drug effect</td>
<td>Tachycardia effect</td>
<td>Drug effect at</td>
<td>Tachycardia effect</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mins (rest)</td>
<td>(rest)</td>
<td>Drug free</td>
<td>Drug</td>
<td>15 mins (rest)</td>
<td>Drug free</td>
<td>Drug free</td>
<td>Drug free</td>
</tr>
<tr>
<td></td>
<td>(%change)</td>
<td>(%change)</td>
<td>% change</td>
<td>% Change</td>
<td>(%change)</td>
<td>% Change</td>
<td>% Change</td>
<td>% Change</td>
</tr>
<tr>
<td>MAP</td>
<td>-2.2 ± 1.4</td>
<td>2.9 ± 1.7</td>
<td>3.0 ± 1.8</td>
<td>-6.4 ± 1.6</td>
<td>0.6 ± 3.1</td>
<td>-1.7 ± 2.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PCWP</td>
<td>-9.7 ± 9.2</td>
<td>38.2 ± 13.6</td>
<td>8.2 ± 8.6</td>
<td>-2.6 ± 17.5</td>
<td>58.2 ± 14.9</td>
<td>42.7 ± 13.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LV+dP/dt_{max}</td>
<td>-2.5 ± 3.2</td>
<td>16.9 ± 3.7</td>
<td>16.2 ± 4.1</td>
<td>-5.2 ± 3.5</td>
<td>25.7 ± 6.1</td>
<td>18.1 ± 5.4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CSBF</td>
<td>-2.7 ± 6.3</td>
<td>34.9 ± 13.6</td>
<td>43.5 ± 11.1</td>
<td>-17.3 ± 6.3</td>
<td>58.3 ± 17.0</td>
<td>25.9 ± 8.9</td>
<td>NS</td>
<td>0.046</td>
</tr>
<tr>
<td>CVRI</td>
<td>5.8 ± 7.3</td>
<td>-14.5 ± 10.5</td>
<td>-22.4 ± 7.5</td>
<td>13.9 ± 10.2</td>
<td>-29.4 ± 8.5</td>
<td>-21.4 ± 7.8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate</td>
<td>-16.5 ± 9.7</td>
<td>-7.1 ± 7.0</td>
<td>-18.6 ± 9.8</td>
<td>-13.8 ± 7.3</td>
<td>-28.3 ± 13.3</td>
<td>-5.4 ± 6.3</td>
<td>NS</td>
<td>0.09</td>
</tr>
<tr>
<td>Oxygen</td>
<td>1.1 ± 4.7</td>
<td>28.5 ± 10.7</td>
<td>37.0 ± 10.0</td>
<td>-12.0 ± 8.6</td>
<td>51.4 ± 16.5</td>
<td>21.4 ± 8.1</td>
<td>NS</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Column data represents the percent change in variables with onset of tachycardia
p value 1 = comparison of the 2 patient groups, for changes induced by tachycardia, in the absence of drug (i.e., column 2 vs column 5)
p value 2 = comparison of the differences between columns 2&3 (the effect of drug on response to tachycardia) for each drug
MAP = mean arterial pressure
PCWP = mean pulmonary capillary wedge pressure
CSBF = coronary sinus blood flow
CVRI = coronary vascular resistance index
Lactate = myocardial lactate production- figures quoted are absolute changes (mg/ml/min)
Oxygen = myocardial oxygen extraction
### Table 2.20  Changes in coronary sinus angiotensin and bradykinin peptides during tachycardia

<table>
<thead>
<tr>
<th></th>
<th>PERINDOPRILAT</th>
<th>ENALAPRILAT</th>
<th>p value 1</th>
<th>p value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Change at baseline</td>
<td>Change after drug</td>
<td>Change at baseline</td>
<td>Change after drug</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>-0.50 ± 0.85</td>
<td>1.73 ± 0.73</td>
<td>-0.58 ± 0.82</td>
<td>0.24 ± 0.51</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>0.88 ± 0.11</td>
<td>0.0 ± 0.28</td>
<td>-1.14 ± 0.58</td>
<td>0.36 ± 0.32</td>
</tr>
<tr>
<td>AII/AI</td>
<td>1.40 ± 0.69</td>
<td>-0.21±0.13</td>
<td>-0.19 ± 1.65</td>
<td>0.07 ± 0.04</td>
</tr>
<tr>
<td>Bradykinin 1-7</td>
<td>-9.55 ± 3.8</td>
<td>0.07 ± 0.99</td>
<td>0.69 ± 2.32</td>
<td>2.4 ± 1.77</td>
</tr>
<tr>
<td>Bradykinin 1-8</td>
<td>-1.78 ± 0.53</td>
<td>4.3 ± 1.76</td>
<td>0.28 ± 0.79</td>
<td>-0.03±0.54</td>
</tr>
<tr>
<td>Bradykinin 1-9</td>
<td>-13.5 ± 9.9</td>
<td>0.8 ± 6.44</td>
<td>0.51 ± 4.22</td>
<td>0.3 ± 2.26</td>
</tr>
<tr>
<td>BK1-7/BK1-9</td>
<td>0.71 ± 0.43</td>
<td>0.45 ± 0.27</td>
<td>0.16 ± 0.35</td>
<td>0.4 ± 0.53</td>
</tr>
</tbody>
</table>

Column data represents the percent change in variables with onset of tachycardia

p value 1 = comparison of the 2 patient groups in the absence of drug
(i.e.. column 1 vs column 3)

p value 2 = comparison of the differences between columns 1&2 for each drug
Table 2.21  Haemodynamic and metabolic changes during tachycardia for patients with significant fixed coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>PERINDOPRILAT (n=9)</th>
<th>ENALAPRILAT (n=7)</th>
<th>p value 1</th>
<th>p value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change at baseline</td>
<td>Change after drug</td>
<td>Change at baseline</td>
<td>Change</td>
<td>p value</td>
</tr>
<tr>
<td>MAP (%change)</td>
<td>2.6 ± 1.9</td>
<td>3.4 ± 2.1</td>
<td>2.5 ± 3.6</td>
<td>-1.5 ± 2.7</td>
</tr>
<tr>
<td>PCWP (%change)</td>
<td>44.2 ± 9.3</td>
<td>0.0 ± 4.2</td>
<td>62.6 ± 19.1</td>
<td>39.4 ± 17.2</td>
</tr>
<tr>
<td>LV+dP/dt_{max} (%change)</td>
<td>16.4 ± 4.3</td>
<td>16.4 ± 5.2</td>
<td>27.6 ± 7.8</td>
<td>20.9 ± 6.1</td>
</tr>
<tr>
<td>CSBF (%change)</td>
<td>22.0 ± 11.5</td>
<td>46.0 ± 13.3</td>
<td>73.2 ± 18.0</td>
<td>30.1 ± 11.1</td>
</tr>
<tr>
<td>CVRI (%change)</td>
<td>-7.8 ± 11.7</td>
<td>-22.3 ± 9.2</td>
<td>-34.8 ± 9.8</td>
<td>-22.6 ± 10.1</td>
</tr>
<tr>
<td>Lactate (%change)</td>
<td>-2.8 ± 5.7</td>
<td>-22.4 ± 11.5</td>
<td>-33.4 ± 16.9</td>
<td>-5.4 ± 7.9</td>
</tr>
<tr>
<td>Oxygen (%change)</td>
<td>19.9 ± 10.8</td>
<td>40.2 ± 11.8</td>
<td>64.6 ± 18.3</td>
<td>25.4 ± 10.0</td>
</tr>
</tbody>
</table>

Column data represents the percent change in variables with onset of tachycardia
p value 1 = comparison of the 2 patient groups in the absence of drug
(i.e., column 1 vs column 3)
p value 2 = comparison of the differences in columns for each drug
MAP = mean arterial pressure
PCWP = mean pulmonary capillary wedge pressure
CSBF = coronary sinus blood flow
CVRI = coronary vascular resistance index
Lactate = myocardial lactate production
Oxygen = myocardial oxygen extraction
Table 2.22 Changes in coronary sinus angiotensin and bradykinin peptides during tachycardia for patients with significant fixed coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>PERINDOPRILAT (fmol/ml)</th>
<th>ENALAPRILAT (fmol/ml)</th>
<th>p value 1</th>
<th>p value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Change at baseline</td>
<td>Change after drug</td>
<td>Change at baseline</td>
<td>Change after drug</td>
</tr>
<tr>
<td>Angiotensin I</td>
<td>-1.1 ± 1.0</td>
<td>0.53 ± 0.94</td>
<td>-0.42 ± 1.07</td>
<td>0.43 ± 0.67</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>0.83 ± 0.15</td>
<td>0.0 ± 0.37</td>
<td>-1.37 ± 0.76</td>
<td>0.45 ± 0.43</td>
</tr>
<tr>
<td>AII/AI</td>
<td>1.56 ± 0.96</td>
<td>-0.21±0.16</td>
<td>-0.26 ± 2.25</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td>Bradykinin 1-7</td>
<td>-14.8 ± 4.3</td>
<td>1.03 ± 1.1</td>
<td>-1.33 ± 2.6</td>
<td>2.6 ± 2.03</td>
</tr>
<tr>
<td>Bradykinin 1-8</td>
<td>-2.0 ± 0.62</td>
<td>3.25 ± 1.82</td>
<td>0.33 ± 0.91</td>
<td>-0.19±0.51</td>
</tr>
<tr>
<td>Bradykinin 1-9</td>
<td>-24.5 ± 11.3</td>
<td>2.78 ± 5.88</td>
<td>-0.53 ± 4.73</td>
<td>0.69 ± 2.57</td>
</tr>
<tr>
<td>BK1-7/BK1-9</td>
<td>0.20 ± 0.50</td>
<td>0.47 ± 0.26</td>
<td>0.21 ± 0.4</td>
<td>-0.02±0.37</td>
</tr>
</tbody>
</table>

Column data represents the percent change in variables with onset of tachycardia
p value 1 = comparison of the 2 patient groups in the absence of drug
(i.e., column 1 vs column 3)
p value 2 = comparison of the differences between columns 1&2 for each drug

Summary of comparison data

The 2 patient groups were well matched at baseline, the only borderline difference being a higher left ventricular ejection fraction in the intravenous enalaprilat group, which may at least partially be due to the slightly lower heart rate in this group.
The haemodynamic and metabolic responses to the induction of tachycardia were similar at baseline but were significantly different in the presence of the 2 drugs. Intravenous perindoprilat resulted in a significantly greater increase in coronary sinus blood flow when compared to enalaprilat, this being associated with increased myocardial oxygen extraction and a trend towards less net myocardial lactate production. When only those patients with significant fixed coronary artery disease were considered separately, the 2 groups were no longer matched in terms of the baseline haemodynamic and metabolic response to tachycardia. The perindoprilat group had relative impairment of coronary vasodilator reserve with less myocardial oxygen extraction and a tendency to greater net myocardial lactate production. Despite correcting for these baseline differences by comparing the differences in responses, the two drugs showed even more apparent differences. Perindoprilat significantly improved coronary vasodilator reserve in comparison to enalaprilat, this being associated with a relative fall in coronary vascular resistance. Net myocardial oxygen extraction during tachycardia was significantly reduced by enalaprilat and lactate production significantly increased, as compared to the effects of perindoprilat.

The perindoprilat group produced more angiotensin II during the induction of tachycardia at baseline with reductions in bradykinins 1-7, 1-9 and to a lesser extent 1-8; the AII/AI and BK1-7/BK1-9 ratios being comparable between groups. Perindoprilat was significantly more effective at suppressing the generation of angiotensin II, without impacting significantly on the AII/AI ratio. Furthermore, perindoprilat significantly increased bradykinin 1-9 levels relative to enalaprilat effects with an associated marked increase in bradykinin 1-8 levels, a change not observed in the presence of enalaprilat. When only those patients with significant fixed coronary disease were considered separately, a similar pattern of responses was observed.
DISCUSSION

SUMMARY OF MAJOR FINDINGS

The acute intravenous or intracoronary administration of either perindoprilat or enalaprilat resulted in the following effects:

(1) Following bolus intravenous administration, both perindoprilat and enalaprilat were rapidly taken up into the heart, with a slower net efflux rate. Maximal proportional cardiac content of perindoprilat was, if anything, greater than that of enalaprilat (0.46 ± 0.13% versus 0.23 ± 0.06%; p=ns).

(2) Both perindoprilat and enalaprilat rapidly and extensively inhibited ACE activity in circulating blood. Associated with this, there was a rapid reduction in AII/AI ratios in peripheral blood.

(3) Only enalaprilat selectively reduced AII/AI ratios in coronary sinus blood. However, perindoprilat significantly increased bradykinin-1-9/1-7 ratios in coronary sinus blood, suggesting selective inhibition of intracardiac bradykinin catabolism by perindoprilat.

(4) The only significant systemic haemodynamic effect of either perindoprilat or enalaprilat was negative inotropy. While peak effects appeared more rapidly for perindoprilat than for enalaprilat, maximal changes were similar for both agents.

(5) After intracoronary administration of perindoprilat or enalaprilat, there were similar haemodynamic changes. However, there was extensive inhibition of peripheral ACE, suggesting absence of "selective" intracardiac effect.

(6) Perindoprilat tended to decrease coronary vasomotor tone relative to changes observed with enalaprilat, both at rest and during pacing-induced tachycardia.

(7) Both perindoprilat and enalaprilat suppressed cardiac angiotensin II release during tachycardia, but only perindoprilat increased the BK1-9/BK1-7 ratio.
ACUTE MYOCARDIAL UPTAKE OF ACE INHIBITOR

The acute myocardial drug uptake of either perindoprilat or enalaprilat was studied in 22 patients. The performance of the assay utilized was very good with the cv’s for both duplicate and triplicate samples being very low. At first glance, a significant variability in the QC’s is noted. This does not reflect a high degree of inter-assay inaccuracy but rather is a reflection on the methodology of the assay itself.

The radioinhibitor binding displacement assay compared the displacement of an unknown quantity of ACE inhibitor from ACE, by *MK351A. This assay had not previously been described for whole blood samples but had been used for serum samples. Due to the nature of the assay, the sample being analyzed contained ACE. Thus the ACE available for binding of either *MK351A or the unknown quantity of ACE inhibitor consisted of the ACE contained in the rat serum added to each sample and the ACE contained in each sample per se. This therefore introduced a source of variability for inter-assay comparisons, each patient having a different level of ACE activity. However, because each assay used the patient’s own blood for constructing the standard curves and for diluting any samples that required it, the intra-assay variability will have been very low and, probably, reflected by the observed cv’s (particularly for triplicate samples). Therefore, rather than using a methodology that provided excellent inter-assay but poor intra-assay accuracy, it was decided to accept a poor marker of inter-assay comparison as a sacrifice to enhanced intra-assay accuracy. The variability in QC results therefore reflects this fact.

This study is the first to assess the acute myocardial uptake of perindoprilat and enalaprilat in vivo. It has previously been established that chronic therapy with various ACE inhibitors results in significant inhibition of cardiac tissue ACE (Fabris, Chen et al. 1990; Fabris, Yamada et al. 1990; Kinoshita, Urata et al. 1993). However, the time interval required for tissue versus plasma ACE inhibition has not been extensively investigated, largely due to the limitations imposed by the methodology used. The finding that both perindoprilat and enalaprilat are taken up by the heart extremely rapidly suggests that tissue and plasma ACE inhibition may proceed at
similar rates, although this assumes that myocardial uptake occurs directly into an effect compartment. Nevertheless, the haemodynamic responses observed in the current study, following both intravenous and intracoronary dosing are consistent with an early inhibitory effect on cardiac tissue ACE.

The results do not reveal significant differences in kinetics of acute myocardial drug handling between perindoprilat and enalaprilat. Peak myocardial drug content tended to be higher for perindoprilat but this difference was not statistically significant. Theoretically, the major determinant of extent of myocardial drug uptake at matched heart rates should be lipid solubility. In the case of perindoprilat and enalaprilat, this would imply that the expected relative uptake should reflect the octanol:water partition coefficients for these agents. Indeed there is partial supportive evidence for this. The partition coefficient for enalaprilat is <0.001 at pH 7 and 0.3 for the zwitterionic species (Ranadive, Chen et al. 1992), enalaprilat being 3 times less lipophilic than enalapril. Although the partition coefficients for perindoprilat have not been published, the comparative coefficients for enalapril and perindopril are 0.2 and 0.32 respectively (Drummer, Nicolaci et al. 1990), the primary metabolite thus probably maintaining superior lipophilicity to enalaprilat.

Given the lack of previous data evaluating uptake of these agents directly, only loosely inferential conclusions can be drawn from examination of relative extent of inhibition of intracardiac ACE activity, as this reflects affinity of binding to ACE rather than total tissue distribution.

INTRACORONARY VERSUS INTRAVENOUS DOSING

The study of a cohort of patients following selective intracoronary administration of either perindoprilat or enalaprilat was performed with the intention of studying the intracardiac effects of these drugs, in the absence of significant peripheral effects. In the case of both agents, peripheral effects on angiotensin II formation and bradykinin 1-9 catabolism were evident, often to a greater extent than was evident
within the coronary sinus. However, the haemodynamic effects of intravenously administered ACE inhibitors appeared to be confined almost totally to the heart. Therefore, the aim of studying direct cardiac effects, in the absence of significant peripheral effects may have already been achieved with intravenous dosing, there being no apparent significant incremental information to be gathered utilizing the current protocol for intracoronary administration. Previous investigations have examined the direct effects of the intracoronary administration of enalaprilat to patients with significant aortic stenosis or dilated cardiomyopathy (Foult, Tavolaro et al. 1989; Friedrich, Lorell et al. 1994). However, rather than utilizing bolus drug administration, a slow intracoronary infusion (0.05mg/minute) has been used, with no change in peripheral serum ACE activity being noted. In the current study, the source of systemic appearance of ACE inhibitor activity after bolus intracoronary drug administration may have been the coronary venous circulation rather than direct arterial overflow from the site of injection. In the absence of detectable systemic haemodynamic effects, the main issue of importance is whether direct cardiac effects of ACE inhibitors were accentuated following intracoronary bolus administration. In fact, the available data provide no suggestion that the negative inotropic effects of either perindoprilat or enalaprilat were accentuated following intracoronary injection. There are two possible explanations for this observation:

1. Both intravenous and intracoronary administration of both perindoprilat and enalaprilat induced 'complete' inhibition of intracardiac ACE
2. Bolus intracoronary drug administration did not result in any increment in myocardial drug uptake relative to intravenous administration

These two possibilities cannot be resolved completely with the methodology utilized. Despite the fact that myocardial uptake of both ACE inhibitors was less than 1% with peripheral injection, it is likely that the intracoronary injection would have increased proportional uptake: this could not be ascertained utilizing mass-balance uptake methodology.
Furthermore, an important issue is whether the intracoronary injection might potentially induce selective inhibition of cardiac ACE activity. This could be addressed, in retrospect, only via the utilization of a considerably smaller bolus dose of ACE inhibitor, or perhaps via prolonged intracoronary ACE inhibitor infusion.

EFFECTS OF ACE INHIBITION

At rest
There is good evidence to show that the acute haemodynamic effects of ACE inhibition, in the setting of hypertension or normal controls, are maximal when complete inhibition of serum ACE activity has occurred, with incremental dosing resulting in no incremental haemodynamic effects (Reid, Lees et al. 1987; MacFadyen, Lees et al. 1991; MacFadyen, Lees et al. 1992; MacFadyen, Meredith et al. 1993; Reid, MacFadyen et al. 1993). However, this does not guarantee equivalence of tissue ACE effects. Indeed, the intention of this study was to have potentially differential effects on tissue ACE and, given the equivalent effects on serum ACE, it is possible that the differential effects of the two ACE inhibitors studied reflect the impact of tissue ACE inhibition rather than non-equivalent doses.

The intracoronary dosing of perindoprilat and enalaprilat produced marked suppression of serum ACE activity both in the periphery and in the coronary sinus. Furthermore, the haemodynamic effects observed were similar to those observed with the larger intravenous doses. While the impact of intracoronary dosing is likely to be greater as regards regional tissue ACE inhibition, it is apparent that the haemodynamic responses to the two ACE inhibitors remain similar over a broad dose range that virtually totally inhibits serum ACE activity. This is further evidence in terms of dose equivalence.

During baseline atrial pacing at just above spontaneous heart rate, the acute administration of 1.25mg perindoprilat intravenously caused a wide range of effects. Although the fall in mean arterial pressure was similar to that previously reported
(MacFadyen, Lees et al. 1991; MacFadyen, Lees et al. 1992; MacFadyen, Lees et al. 1993; Reid, MacFadyen et al. 1993; MacFadyen, Lees et al. 1994), the unexpected finding was the significant fall in cardiac index, associated with a substantial increase in systemic vascular resistance index and fall in left ventricular stroke work. These changes represent a significant negative inotropic effect of acutely administered perindoprilat, despite the absence of a significant effect on L.V+dP/dt_{max}. Enalaprilat, administered as a 2.5mg intravenous bolus injection, exhibited a similar range of effects to perindoprilat, the differences being a more marked decrease in LV+dP/dt_{max} and a less marked increase in systemic vascular resistance index. Thus overall enalaprilat had a slightly more marked negative inotropic effect than perindoprilat.

Review of previous acute haemodynamically orientated investigations with perindoprilat and enalaprilat reveals rather different findings to those in the current study. A number of previous studies have examined the acute haemodynamic effects of both intravenous and intracoronary administration of ACE inhibitors, in a broad range of pathological conditions. This experience is greatest for enalaprilat which has been available for intravenous administration in various clinical settings. Whilst a large component of the literature deals with the acute effects of enalaprilat on haemodynamic profiles for patients with chronic stable heart failure, it has also been evaluated in patients with acute heart failure, essential hypertension, aortic stenosis, hypertrophic cardiomyopathy and hypertensive crises, it being rare for patients with significant coronary artery disease to be examined (De Marco, Daly et al. 1987; Dickstein, Aarsland et al. 1987; Murphy, Vaughan et al. 1989; MacFadyen, Lees et al. 1993; Varriale, David et al. 1993; Boldt, Muller et al. 1995; Hirschl, Binder et al. 1995; Annane, Bellissant et al. 1996). The intravenous administration of enalaprilat to patients with both acute and chronic heart failure has almost universally resulted in reduction of pulmonary capillary wedge pressure, mean arterial pressure and systemic vascular resistance. This has been accompanied by improvements or no change in indices of systolic function. The intravenous bolus administration of enalaprilat to patients with congestive heart failure has resulted in
 reductions in mean arterial pressure of up to 30 mmHg, significant effects being evident by 15 minutes and maximal by 75 minutes (Dickstein, Aarsland et al. 1987). It is further noteworthy that bolus administration of enalaprilat to normal patients prior to the induction of anaesthesia has demonstrated that significant hypotension occurs with sparing of autonomic reflexes (Murphy, Vaughan et al. 1989).

A number of investigations have examined the impact of intracoronary enalaprilat administration, often as an infusion, and occasionally using a bilateral administration technique (Foult, Tavolaro et al. 1988; Foult, Tavolaro et al. 1989; Friedrich, Lorell et al. 1994; Haber, Powers et al. 1994; Kuga, Mohri et al. 1997; Rundqvist, Eisenhofer et al. 1997; Kyriakidis, Triposkiadis et al. 1998). The administration of intracoronary enalaprilat to hypertensive patients improved active (calcium-dependent) relaxation, the improvement being proportional to the extent of left ventricular hypertrophy (Haber, Powers et al. 1994). Whilst there was a moderate reduction in left ventricular systolic pressure, there was no change in systemic vascular resistance and significant reductions in stroke volume index and stroke work index, there being a 15% reduction in cardiac index with the highest dose (5mg) used (Haber, Powers et al. 1994). Foult et al have utilized a bilateral intracoronary infusion technique to examine the effects of enalaprilat in patients with dilated cardiomyopathy (Foult, Tavolaro et al. 1988). Whilst there was a 19% increase in coronary sinus blood flow, accompanied by an 18% reduction in coronary vascular resistance, during the 15-20 minute infusion, there was a deterioration in all indices of left ventricular function, there being a 9% fall in cardiac index, a 12% fall in ejection fraction and an 8% fall in the end-systolic stress/volume ratio (Foult, Tavolaro et al. 1988).

The published experience with the parenteral administration of perindoprilat is less extensive, largely due to the fact that it is not in routine clinical use (Omvik and Lund-Johansen 1989; Benetos, Santoni et al. 1990; MacFadyen, Lees et al. 1992; MacFadyen, Lees et al. 1993; Reid, MacFadyen et al. 1993; MacFadyen, Lees et al. 1994; Antony, Lerebours et al. 1995; Antony, Lerebours et al. 1996; Antony and
Nitenberg 1997). Investigations have largely centered on patients with hypertension and cardiac failure with further studies into effects on coronary vasomotion. Intravenous administration has frequently been performed using a continuous infusion rather than bolus drug administration with consistent demonstration of a reduction in mean arterial pressure, largely due to a reduction in systemic vascular resistance. Intracoronary drug administration has concentrated almost exclusively on effects on coronary vasomotion with no substantial data available to either confirm or refute the possibility of a negative inotropic effect. Coronary blood flow and vascular resistance were unchanged in hypertensive patients following perindoprilat but endothelial dependent vasodilatation to cold pressor testing was restored (Antony, Lerebours et al. 1996).

Thus the administration of both ACE inhibitors had a significant negative inotropic effect that was not necessarily anticipated on the basis of previous experience with the exception of the data of Foulτ et al (Foulτ, Tavolaro et al. 1988). The mechanism of this negative inotropic effect is not known but may involve several possible mechanisms. Although angiotensin II has not generally been regarded as having a significant positive inotropic effect on the ventricles in vitro (Lenz, Schmid et al. 1995), it may be having a small effect in vivo, possibly via catecholamines (Foucart, de Champlain et al. 1991; Valles Prats, Matas Serra et al. 1996; Bernier and Perry 1997). Indeed, there is a sound theoretical basis for an effect on intracellular calcium availability to the contractile apparatus (Dosemeci, Dhallan et al. 1988). Furthermore, angiotensin II is known to activate phospholipase C, increasing the inositol phosphates, promoting the release of intracellular calcium and delaying its reuptake. It has also been shown to increase the L-type calcium current, independently of adenylate cyclase (Allen, Cohen et al. 1988) and to increase myofilament calcium sensitivity (Dosemeci, Dhallan et al. 1988). A positive intotropic effect of angiotensin II has been demonstrated in a number of models (Freer, Pappano et al. 1976; Kobayashi, Furukawa et al. 1978; Baker, Campanile et al. 1984; Hirakata, Fouad-Tarazi et al. 1990) and angiotensin II receptors have been clearly demonstrated in both the myocardium and cardiac nerve terminals in humans (Blumberg, Ackerly et
A reduction in angiotensin II levels may therefore be accompanied by a reduction in free intracellular calcium and, therefore, a negative inotropic effect.

An alternative explanation for the acute negative inotropic effects of perindoprilat and enalaprilat lies with the effects on bradykinin metabolism. By significantly suppressing the BK1-7/BK1-9 ratio, this may result in increased activation of type-2 bradykinin receptors via BK1-9, stimulating nitric oxide release that, in small doses, may exert a positive inotropic effect, but in larger doses exerts a significant negative inotropic effect (Mohan, Sys et al. 1995; Zhu, Zaugg et al. 1995; Mohan, Brutsaert et al. 1996). However, the relative impact of this aspect is debatable given the greater negative inotropic effect of enalaprilat and the lack of impact of enalaprilat on coronary sinus bradykinin catabolism. The importance of differential effects of enalaprilat and perindoprilat on bradykinin metabolism may, more importantly, relate to the other haemodynamic responses observed. Perindoprilat administration not only resulted in a negative inotropic effect but peripheral vasoconstriction whereas this was not observed following enalaprilat. Perindoprilat exerted no substantial effect on peripheral bradykinin metabolism whereas enalaprilat did (the reverse being the case for intracardiac effects), bradykinin effects on peripheral venodilatation possibly impacting on this observed difference in systemic vascular resistance. Paradoxically, Gomez Llambi et al have demonstrated that angiotensin II may inhibit tonic positive inotropic effects of catecholamines via release of nitric oxide (Gomez Llambi H 1996). This provides a possible mechanism whereby ACE inhibition might have had positive inotropic effects provided a background of significant β-adrenoceptor agonist activity.

Although previous experience with parenteral administration of ACE inhibitors has not exhibited such marked negative inotropic effects, the majority of these studies have been performed utilizing intravenous infusions for up to 6 hours (MacFadyen, Lees et al. 1991; MacFadyen, Lees et al. 1991; MacFadyen, Lees et al. 1992; MacFadyen, Lees et al. 1993; MacFadyen, Meredith et al. 1993; Reid, MacFadyen et
It is possible that the current observations are due to acute and abrupt ACE inhibition causing the acute haemodynamic changes, most likely due to abrupt inhibition of AII formation. Indeed, reflex stimulation in response to this is likely to be the basis of the observed vasoconstrictor effect, particularly for perindoprilat. Alternatively, the negative inotropic effect may be centrally mediated. It is known that angiotensin II can stimulate catecholamine release from the area postrema (Brosnihan, Ferrario et al. 1981). Inhibition of this effect may potentially therefore result in a negative inotropic effect. However, previous investigation of the intracoronary administration of enalaprilat has demonstrated no change in noradrenaline spillover either at rest or during exercise (Rundqvist, Eisenhofer et al. 1997).

The most surprising aspect of the systemic haemodynamic effects of perindoprilat and enalaprilat is the complete lack of evidence for peripheral vasodilation: in fact, as already stated, there was a trend towards constriction. As there was very extensive peripheral ACE inhibition, it is most likely that this finding reflects predominance of an indirect vasoconstrictor response (perhaps mediated via baroreceptor sensitization in response to negative inotropy-induced hypotension. Demonstration of underlying direct changes in vasomotor tone consequent upon reduced circulating angiotensin II effect would theoretically be demonstrable via autonomic blockade.

Both perindoprilat and enalaprilat exhibited significant hysteresis for time course of effects, although this was more marked for enalaprilat. It is tempting to speculate that this difference is due to differential access to the physiological "effect compartment" represented by tissue ACE. The greater access of perindoprilat to this compartment would cause the earlier onset of effects, in comparison to enalaprilat. However, there are two issues that weigh somewhat against this argument. Firstly, the time course of effect of perindoprilat on coronary sinus AII levels was more gradual than that observed for enalaprilat or in the periphery. Secondly, the haemodynamic effects of enalaprilat were, if anything, more marked than those of
perindoprilat. Given that the predominant haemodynamic effect of both ACE inhibitors was negative inotropy, it suggests that local impact on cardiac ACE is the major pathway of effect.

Perindoprilat induced peptide changes that were diverse among vascular beds. The femoral arterial AII/AI ratio was abruptly and dramatically suppressed within two minutes of drug administration, rebounding somewhat but remaining suppressed compared to baseline. On the other hand, although there was a gradual decline in coronary sinus AII/AI ratio throughout the first ten minutes following drug administration, this did not achieve significance. This may suggest that the peripheral and cardiac effect compartments for perindoprilat, as regards suppression of AII formation, provide differential access to perindoprilat. The fact that perindoprilat was capable of exerting a significant negative inotropic effect therefore suggests that the negative inotropic effects of perindoprilat may reflect, at least in part, reduced concentrations of angiotensin II reaching the heart, rather than purely intracardiac angiotensin II formation. Peak haemodynamic effects of perindoprilat were observed approximately 4 minutes following drug administration, at a time when peripheral suppression of the AII/AI ratio was more marked than that seen centrally. An alternative explanation for these somewhat paradoxical observations is that the observed fluxes in coronary sinus angiotensin peptides are not fully reflective of the extent of tissue ACE inhibition, the observed changes rather reflecting similar changes in peripheral and central AII/AI ratios. Indeed, if all patients receiving perindoprilat (intravenous and intracoronary) are examined, the suppression of femoral arterial and coronary sinus AII/AI ratios are similar. Furthermore, the considerable wash-out of angiotensin II from the coronary sinus, observed following the drug-free examination of response to tachycardia induction, may have been a further confounding factor in the subsequent observation of intracardiac perindoprilat effects. On the other hand, it is possible that cardiac ACE activity is a less important determinant of intracardiac angiotensin I bioconversion than is ACE activity in peripheral tissues and plasma (Urata, Nishimura et al. 1996).
The acute intravenous bolus administration of enalaprilat resulted in abrupt and significant reduction in both femoral arterial and coronary sinus AII/AI ratios. Despite this, peak haemodynamic effects tended to be observed at 15 minutes, although some effects were evident within a few minutes. Although delayed access to a physiological "effect compartment" for enalaprilat versus perindoprilat is possible, this explanation would not fully resolve the paradoxical observations with regard to coronary sinus AII/AI ratios.

The above discussion is based on the assumption that the dosing of the two ACE inhibitors used was equivalent with any observed differences being due to different properties of the drugs. The use of a dose of perindoprilat half of that for enalaprilat was based on the previous estimates of relative potency of the two drugs with regard to ACE inhibition (Brunner, Nussberger et al. 1985). At least one other comparative study has demonstrated that the selected doses are approximately equivalent (MacFadyen, Lees et al. 1993).

In contrast to the effects of perindoprilat on angiotensin peptide levels, the impact on bradykinin catabolism was apparently confined to the coronary circulation. Femoral arterial levels of bradykinin peptides were not altered following drug administration, whereas there was a gradual and sustained suppression of the bradykinin 1-7/1-9 ratio, associated with a significant surge in bradykinin 1-8 formation and unchanged levels of bradykinin 1-9, suggesting that this was being metabolised to BK1-8 rather than BK1-7. Enalaprilat exerted no significant intracardiac effects on bradykinin catabolism. It is well recognized that ACE contributes to intracardiac bradykinin catabolism and that bradykinin is a high affinity substrate for ACE (Mombouli, Illiano et al. 1992; Blais, Drapeau et al. 1997; Gibbons 1997; Remme 1997). Despite the significant impact of perindoprilat on bradykinin catabolism and the absence of an effect of enalaprilat, the responses of coronary haemodynamics to the administration of both agents were similar, with no suggestion of coronary vasodilatation under basal conditions. It is noted that there was a significant negative inotropic effect of both ACE inhibitors that might lead to
decreased myocardial perfusion requirements, perhaps explaining the lack of coronary vasodilator effect observed by others (Kuga, Mohri et al. 1997). It appears probable that the finding that only perindoprilat significantly suppresses intracardiac bradykinin catabolism reflects a truly "cardio-selective" component of the effects of the drug. On the other hand, the fact that only perindoprilat exerted a significant effect on intracardiac bradykinin metabolism tends to suggest that angiotensin II, rather than bradykinin/nitric oxide, mediated the approximately equivalent negative inotropic effects of perindoprilat and enalaprilat.

The interaction between ACE inhibition, intracardiac bradykinin metabolism and coronary haemodynamics has previously been examined. Whilst investigations in isolated rat myocardium have demonstrated that only about 50% of bradykinin breakdown occurs via ACE, interstitial metabolism of bradykinin occurs almost exclusively to bradykinin-1-7, this pathway being inhibited by ACE inhibition (Dendorfer, Wolfrum et al. 1997). Although initial experiments in dogs suggested that the cardiac effects of bradykinin were mediated largely via prostaglandins, with no evidence for a significant contribution via nitric oxide, subsequent studies in human subjects have suggested that both pathways are important for mediation of bradykinin effects (Ehring, Baumgart et al. 1994; Kuga, Mohri et al. 1997). Intracoronary administration of bradykinin increases coronary blood flow and diameter, this effect being significantly suppressed by blockade of nitric oxide and restored by the administration of enalaprilat 50mcg (Kuga, Mohri et al. 1997). Importantly, the interaction between ACE inhibition and bradykinin appears to be dependent on bradykinin secretion, with ACE inhibition prolonging the effects of intracoronary bradykinin administration but not enhancing the effect (Houel, Su et al. 1997).

In the current study, coronary blood flow was reduced following ACE inhibitor administration. However, there was also a significant negative inotropic effect for both agents; hence an important question is whether there was any change in coronary vasomotor tone, or simply a reduction in myocardial oxygen demand.
There is no single parameter that adequately describes left ventricular systolic function. As evident in this series, negative inotropism can impact to varying degrees on a number of different parameters. However, one of the better indicators of overall function is left ventricular stroke work which takes into account loading conditions of the ventricle. The impact of ACE inhibitor therapy on left ventricular stroke work was compared with effects on coronary sinus blood flow. Enalaprilat produced a $17 \pm 3\%$ reduction in stroke work and perindoprilat a $13 \pm 4\%$ reduction. For patients receiving an intravenous bolus dose of enalaprilat, the reduction in left ventricular stroke work was significantly correlated with reduction in coronary sinus blood flow ($r = 0.7, p = 0.036$), suggesting that, relative to metabolic demands, there was no change in coronary sinus blood flow following enalaprilat. However, no such correlation was noted for patients receiving perindoprilat, at least 3 patients demonstrating an increase in coronary sinus blood flow despite a more than 20% reduction in left ventricular stroke work. Enalaprilat resulted in a $10 \pm 7\%$ reduction in coronary sinus blood flow 10 minutes following administration and a $17 \pm 6\%$ reduction after 15 minutes, compared to the $3 \pm 6\%$ reduction for perindoprilat. These data would suggest that perindoprilat in fact exhibited a modest coronary vasodilatory effect at basal heart rate. Given the differing effects of the two ACE inhibitors on intracardiac bradykinin metabolism, this difference is most likely to be due to accumulation of bradykinin-1-9 following perindoprilat administration.

The observations made with respect to the acute bolus administration of ACE inhibitors are somewhat different to those made during slow intravenous infusions (MacFadyen, Lees et al. 1992; MacFadyen, Lees et al. 1993; Reid, MacFadyen et al. 1993; MacFadyen, Lees et al. 1994). This may have implications regarding the clinical use of these agents. It is now clear that administration of ACE inhibitors soon after acute myocardial infarction provides benefits in terms of morbidity and mortality both short and longer term (1994; 1995). There is also evidence from the management of heart failure that the largest possible dose of an ACE inhibitor should be given (Hobbs 1998). The results of the current investigation may potentially temper enthusiasm for rapid introduction of high dose ACE inhibitor therapy. This is
particularly relevant when one considers the one "negative" ACE inhibitor trial, involving the intravenous administration of enalaprilat to patients with acute myocardial infarction (Swedberg, Held et al. 1992). There was a significant incidence of early hypotension, necessitating interruption of treatment (12% versus 3%) in the active treatment group, and a strong trend (p=0.06) towards increased incidence of death due to progressive heart failure following treatment with enalaprilat, with potential relevance to the acute negative inotropic effects demonstrated in the current study.

A further issue that potentially impacts on the interpretation of the data is the interaction between inhibition of cardiac and circulatory ACE. Kyriakidis et al investigated the effects of intracoronary enalaprilat in patients with hypertrophic cardiomyopathy (Kyriakidis, Triposkiadis et al. 1998). This resulted in an increase in coronary blood flow and coronary flow reserve as well as improved diastolic function. However, a subsequent sublingual dose of captopril reversed all of these effects such that they were now unchanged from baseline. The variable contributions of cardiac versus circulatory ACE inhibition are likely to be complex and dependent on the underlying disease process (eg hypertrophic versus dilated cardiomyopathy), as suggested by the difficulties experienced in interpreting the results of the current study in comparison to previously published experience. Nevertheless, it would seem prudent to recommend that, where parenteral administration of ACE inhibitor is considered, gradual infusion rather than bolus administration is likely to minimise the potential for substantial impact of this factor.

The current study has enrolled only a single patient with impaired systolic function at rest (left ventricular ejection fraction < 50%). It is therefore not possible to comment on the effect of this variable on the results. However, it is reasonable to speculate, on the basis of the data presented, that incremental haemodynamic effects may be observed in a group of patients with impaired systolic function, given the potential for increased importance of AII (Hall and Karlberg 1986; Ji, Ren et al. 1996). The one patient studied with a lower ejection fraction (42%) had no associated
coronary artery disease and experienced an 18% increase in systemic vascular resistance following perindoprilat without any other substantial haemodynamic changes apart from a fall in cardiac index (maximally 6%).

There was a single patient studied with an intracoronary dose of enalaprilat, for whom subsequent analyses revealed that ACE inhibitor therapy must have been chronically taken, despite denial of this. Whilst this patient was excluded from further analysis, it is worth examining the haemodynamic effects observed. In the 15 minutes following enalaprilat administration, there was a 31% reduction in mean arterial pressure, an 18% reduction in cardiac index, a 38% reduction in LV+dP/dt\textsubscript{max}, and a 48% reduction in left ventricular stroke work. These marked effects were observed despite complete suppression of circulating ACE being present at baseline. This is at odds with previous suggestions that incremental dosing with ACE inhibitors, once complete suppression of serum ACE has been achieved, results in no incremental haemodynamic effects and raises the possibility that the procedure itself may somehow have resulted in haemodynamic compromise, perhaps due to fluid depletion.

**During tachycardia**

The effects of perindoprilat were more marked during the induction of tachycardia. In patients with haemodynamically significant coronary disease, all of the haemodynamic changes identified were consistent with the relief of ischaemia. Perindoprilat increased coronary sinus blood flow and decreased coronary vascular resistance during the induction of tachycardia. Furthermore, the increase in pulmonary capillary wedge pressure was abolished and myocardial oxygen extraction was increased, there being a trend towards reduced lactate production, consistent with a relative increase in aerobic metabolism.

The changes in peptide levels induced by perindoprilat during tachycardia were similar to those occurring at rest but more marked. Both the AII/AI and BK1-7/BK1-9 ratios were significantly suppressed in both the femoral artery and
coronary sinus. Once more, there was a significant increase in the coronary sinus level of BK1-8. Of these three major changes in peptide balance, it is not clear which represents the basis for the haemodynamic and metabolic improvements seen or, indeed, if the changes represent causes and effect. It is clear however that this component of the experiment was performed at a time when maximal haemodynamic and peptide metabolic effects were occurring.

The finding of increased coronary sinus levels of BK1-8 is relevant to the current discussion. BK1-9 is usually catabolised to BK1-7, via ACE to a large extent. Alternatively, BK1-9 can be catabolised to BK1-8, usually via an arginine carboxypeptidase, an enzyme that is not specific for BK1-9, but is involved in the cleavage of a number of peptides. BK1-8 has a comparatively long half life in comparison to the other kinin peptides, being 4 to 12 times longer than BK1-9, depending on the species studied (Marceau 1995; Marceau, Hess et al. 1998). Furthermore, one of the major metabolic routes for BK1-8 is ACE cleavage to BK1-5. BK1-8 is unique among the breakdown products of BK1-9 in having significant activity as an agonist of the type-1 bradykinin receptor (B1) (Marceau 1995; Marceau, Hess et al. 1998). The B1-receptor is a G-protein that, when activated, increases turnover of phosphatidylinositol. Furthermore, the B1-receptor is inducible, being expressed in increased amounts in response to stress. While it has been shown to mediate vasoconstrictor effects on large conduit vessels, it has been clearly demonstrated to mediate vasodilatation in coronary vasculature, probably via release of prostanoids (Churchill and Ward 1986; Guimaraes, Vieira et al. 1986; Regoli, Dion et al. 1990; Persson and Andersson 1998). Thus, the appearance of increased levels of BK1-8 in the current experiment is of interest for two reasons. Firstly, it provides incremental evidence that BK1-9 catabolism via ACE is being inhibited, given that BK1-9 levels do not rise significantly; there presumably being a compensatory greater metabolism of BK1-9 via arginine carboxypeptidase. Secondly, the effects of BK1-8 as an agonist on the B1-receptor may mediate or contribute towards the beneficial coronary haemodynamic effects observed.
At this stage, only tentative comment can be made on the observation that effects of both ACE inhibitors on peptide levels are somewhat more pronounced during rapid pacing. It is possible that tachycardia may alter regional tissue perfusion (including that of the renal vascular bed) possibly affecting regional kinetics of ACE inhibition. However, this was not measured during the protocol. Alternatively, this apparent difference may be no more than artifact. It was consistently observed that the bradykinin peptide levels at baseline for the first tachycardia induction were higher than the secondary baseline taken immediately prior to drug administration for the uptake phase of the protocol. Although it is possible that drawing blood through a long catheter activated bradykinin, preliminary experiments were performed to exclude this possibility. Alternatively, siting of the coronary sinus catheter may have caused variable degrees of local trauma within the coronary sinus, resulting in activation of bradykinin. Subsequent tachycardia induction and measurement of coronary sinus flows by thermodilution would have 'washed out' this excess bradykinin, thus explaining the difference.

The most striking differences between these two drugs became apparent during examination of tachycardia induction. However, the interpretation of these results is somewhat modified due to the baseline differences between the two groups, there being a greater impairment of coronary vasodilator reserve in those patients receiving perindoprilat. This is of particular importance given that the impact of ACE inhibition on coronary blood flow is most marked when there is greater generation of bradykinin, more likely to occur in the setting of ischaemia (Houel, Su et al. 1997). Furthermore, cardiac index was not examined during the induction of tachycardia. It is possible that a greater negative inotropic effect of enalaprilat at basal heart rate may have translated into a significantly lower left ventricular stroke work during the induction of tachycardia thus resulting in the observed lack of coronary vasodilator, and tendency to coronary constrictor, effect. However, the relatively increased production of lactate, during tachycardia, following enalaprilat suggests that this may not be the entire explanation.
Perindoprilat increased coronary sinus blood flow and increased aerobic metabolism during induction of tachycardia whereas enalaprilat decreased coronary sinus blood flow and failed to impact on aerobic metabolism (as suggested by changes in oxygen extraction and lactate production). The most striking difference in effects on peptide levels was the lack of enalaprilat effect on coronary sinus bradykinin peptides, whereas perindoprilat significantly suppressed the BK1-7/BK1-9 ratio as well as increasing BK1-8 levels. This, together with the coronary haemodynamic and metabolic data, strongly suggests that bradykinin is closely involved in regulating coronary haemodynamics. Furthermore, the incremental ACE inhibition within the heart by perindoprilat suggests considerable impact via tissue ACE inhibition. The effects of perindoprilat on the coronary circulation have previously been investigated in patients with impaired coronary vasomotor reactivity. The intravenous administration of perindoprilat in these patients restored the normal responses to cold pressor test and flow-mediated coronary vasodilator reserve (Antony, Lerebours et al. 1995; Antony, Lerebours et al. 1996). While intracoronary infusion of enalaprilat has been demonstrated to increase coronary blood flow in patients with hypertrophic cardiomyopathy, it has also been shown to afford no protection to the heart during ischaemia compared to other ACE inhibitors such as captopril (Grover, Sleph et al. 1991; Kyriakidis, Triposkiadis et al. 1998).

The distribution of ACE within the heart has been studied in some detail (Johnston, Fabris et al. 1989). The highest density of tissue ACE expression occurs in heart valves, followed by right and left atria, and right and left ventricles. It is apparent from the current study that perindoprilat exerts a greater effect on cardiac tissue ACE activity than does enalaprilat. Despite similar negative inotropic effects, there is a trend towards greater uptake of perindoprilat into the heart and it remains to be determined whether this is an important factor or if, indeed, it is the differential effects of the two ACE inhibitors on tissue ACE that are responsible for the observed differences.
The data support a primary coronary vasodilator role for perindoprilat, particularly in the setting of tachycardia, this most likely being due to selective intracardiac effects on bradykinin metabolism. Comparatively, enalaprilat appears to have no similar effect. The hint of an adverse response to enalapril, particularly during tachycardia induction, is probably explained on the basis of a more marked negative inotropic effect. Chronic therapy with enalapril for patients with ischaemic heart disease has established it as a safe and highly effective drug (1991; 1992; Yusuf, Pepine et al. 1992). It is possible that chronic therapy with perindopril may have an added effect in reducing propensity to induction of myocardial ischaemia.
CHAPTER 3: ACUTE DRUG EFFECTS ON THE LEFT VENTRICAL FORCE-INTERVAL RELATIONSHIP

INTRODUCTION

The effects of acutely administered cardioactive drugs on the heart have been studied in detail previously. Despite a wealth of existing knowledge, there is a paucity of data concerning the interaction between acute drug effects and the left ventricular force-interval relationship. In patients with cardiovascular disease, symptoms usually occur in the presence of a relative tachycardia, either due to exercise, sympathetic stimulation due to ischaemia and/or heart failure, or acute cardiac events such as the onset of atrial fibrillation. It is therefore appropriate to have more detailed information regarding the interaction between acute myocardial drug effects and the force-interval relationship, as this may impact on the relative safety and efficacy of cardioactive drugs.

Determinants of contractile function

The systolic function of the human left ventricle, in vivo, is determined by a number of factors, including preload, afterload, contractility, heart rate and, in some cases, the shape and regional function of the ventricle (Mahler F 1975; Anderson, Manring et al. 1979; Manring and Anderson 1980). The accurate measurement of contractility in vivo is somewhat of a 'holy grail' largely because of the extensive interaction between these factors. Nevertheless, a range of invasive methodologies have been investigated and shown to be accurate, provided some variables are controlled for. There are 2 main indices of contractile function in routine invasive use. These are the maximal positive deflection of instantaneous left ventricular pressure, \( \text{LV+}dP/dt_{\text{max}} \), a parameter that is relatively independent of small changes in loading conditions across the physiological range, but is quite sensitive to changes in heart rate (a factor that actually makes it an attractive tool for measuring the interaction between these two variables), and left ventricular pressure-volume relationships which rely on
serial measures being performed in the presence of various loading conditions (Gleason WL 1962; Mahler F 1975; Barnes, Horwitz et al. 1979; Broughton and Korner 1980; Sasayama, Nonogi et al. 1984; Aroney, Herrmann et al. 1989; Kass 1992). While this latter technique may enhance accuracy, especially in the presence of marked changes in loading conditions, it is more time consuming to perform and limits the extent of meaningful additional haemodynamic measures that can be made.

The Left Ventricular Force-Interval Relationship

The force-interval relationship (FIR) was first described over 100 years ago (Bowditch 1871). Despite substantial advances in knowledge, the relative determinants of this response are not precisely defined. In the absence of marked changes in loading conditions, progressive increments in heart rate result in progressive increments in contractile force, for heart rates in the physiological range in normal ventricles (Gwathmey, Slawsky et al. 1990; Schmidt, Hajjar et al. 1995). This phenomenon can readily be reproduced both in vivo and in a broad range of in vitro circumstances making it an attractive methodology for examining contractile reserve (Schmidt, Hajjar et al. 1995).

An alternative methodology for examining the force-interval relationship is mechanical restitution. This refers to the recovery of contractile force following a non-steady state impulse. It involves a single 'test impulse' and, as such, can be used to examine recovery of contractile force at shorter cycle lengths than might be possible with sustained tachycardia (Ragnarsdottir, Wohlfart et al. 1982; Burkhoff, Yue et al. 1984; Freeman and Colston 1990; Zhou, Liu et al. 1991; Mesaeli and Juggi 1992; Ravens, Gath et al. 1997). It can also be used to examine intracellular calcium shifts within a single cardiac cycle in vitro (Wu, Shen et al. 1996; Oblonczek and Szymanski 1997; Vornanen and Shepherd 1997). The one limitation to the use of mechanical restitution measures in vivo in humans is the occurrence of spontaneous
depolarisations, limiting the examination of mechanical restitution to cycle lengths that are shorter than those occurring spontaneously.

A final facet that is linked to the FIR is the phenomenon of post extra-systolic potentiation (PESP). This refers to the increase in contractile strength that is observed in the first beat to occur following an extra-systole. This potentiation occurs independent of loading conditions and is inversely proportional to the cycle length of the extra-systolic beat (Sung, Mathur et al. 1980; Wisenbaugh, Nissen et al. 1986; Kuijer, van der Werf et al. 1990). While sympathetic activity may modulate this response, it is not fundamentally causal (Martin 1980; Geschwind, Lhoste et al. 1984). It is clear that calcium handling is intimately involved in the response and, furthermore, it is apparent that the sarcoplasmic reticulum (SR) is the major source of calcium responsible for the phenomenon. Ryanodine, an inhibitor of SR calcium release abolishes PESP and caffeine, which depletes this calcium store, also leads to loss of PESP (Henderson, Brutsaert et al. 1974; Sutko and Willerson 1980). Collection of data regarding PESP can readily be combined with mechanical restitution data collection, providing additional information regarding calcium handling and contractile reserve.

Factors modulating the force-interval relationship

The ability to modulate the force-interval relationship has important therapeutic implications. Perhaps the most important aspect of the left ventricular FIR is the abnormal response that occurs in heart failure. Patients with end-stage heart failure have a reversed FIR such that progressive increments in heart rate produce progressive reductions in contractile strength (Ezzaher, el Houda Bouanani et al. 1992; Mulieri, Hasenfuss et al. 1992; Eising, Hammond et al. 1994; Hasenfuss, Holubarsch et al. 1994; Hasenfuss, Reinecke et al. 1996). Therefore, the onset of tachycardia may be associated with the potential for haemodynamic deterioration or, alternatively, reductions in heart rate may be associated with beneficial effect on contractile function. This is particularly important in this patient group where
symptoms are largely experienced during exercise, likely to be associated with a relative tachycardia.

Rationale for examining the FIR and drug effects upon it

It has long been recognized that some cardioactive drugs may have variable inotropic and haemodynamic effects depending on heart rate (McCans, Lindenmayer et al. 1974; Artman, Graham et al. 1985; Bohm, Diet et al. 1988; Kambayashi, Miura et al. 1992; Holubarsch, Schneider et al. 1995; Ross, Miura et al. 1995). Investigation of β-agonists has revealed that the positive inotropic effects of these agents are attenuated at short cycle lengths (Kambayashi, Miura et al. 1992; Ross, Miura et al. 1995). The presence of heart failure, or impaired left ventricular systolic function, may have a further impact on these frequency-related drug effects. This has important implications for both the short and long term use of cardioactive drugs where there is the potential for tachycardia to occur, for example, during exercise.

To date, the majority of literature regarding the force-frequency relationship has been gathered in the in vitro setting. This has enabled isolation of the effects of heart rate on contractile function, in the absence of changing loading conditions or contractility. As a result, a clearer understanding of the cellular processes involved now exists. However, despite a number of in vitro, and a more limited number of in vivo studies, examining the force-frequency relationship in heart failure, there are significant limitations regarding interpretation of these data. Animal models of heart failure frequently rely on sustained tachycardia, due to rapid pacing, in order to induce heart failure (Vatner, Sato et al. 1994; Prabhu and Freeman 1995; Prabhu and Freeman 1995). This assumes commonality between the pathophysiology of tachycardia-mediated heart failure and that encountered clinically. A few studies have used purpose bred strains of animals with heart failure (Gwathmey and Morgan 1985). However, there have been no adequate studies using animal models of heart failure due to an ischaemic etiology, the situation most commonly
encountered clinically and the one most likely to require polypharmacy with drugs known to impact on inotropic function.

Attempts to examine human papillary muscle strips from patients with end-stage heart failure are limited due to concomitant drug therapy, in view of the fact that virtually all muscle strips are obtained from patients undergoing cardiac transplantation and, therefore, from a practical aspect, a drug free state cannot be achieved. This has also limited the opportunity to examine frequency-related drug effects in a human heart failure model.

*In vivo* data examining frequency dependent drug effects in humans are extremely limited in the setting of normal contractile function, but particularly so in the setting of heart failure (Feldman, Alderman et al. 1988; Hasenfuss, Holubarsch et al. 1994). Whilst it remains virtually impossible to carry out *in vivo* studies of drug effects on the force-frequency relationship, in man, in the setting of end-stage heart failure, due to the ethical problems of studying this condition in the drug-free state, it is possible to examine milder forms of heart failure, impaired left ventricular systolic function, or significant coronary artery disease in this way. Given that the underlying processes have the potential to progress to end-stage heart failure, it is likely that observed frequency related drug effects, in this group of patients would be predictive of effects in patients with end-stage heart failure.

**CELLULAR PROCESSES INVOLVED IN THE FORCE-INTERVAL RELATIONSHIP**

**Normal contractile physiology**

The contractile behaviour of a myocyte during a single contraction cycle depends on the amount of calcium delivered to the contractile apparatus, the sensitivity of the contractile apparatus to calcium and the subsequent clearance of calcium from the intracellular space in preparation for the next contraction cycle. There are a number of determinants for each of these 3 components, all of which have a number of modifiable factors, including stimulation frequency.
In broad terms, depolarization of the cell surface enables the entry of a small amount of calcium across the sarclemma, so called 'trigger' calcium. This not only is available for interaction with the contractile apparatus of the cell but, more importantly, signals the release of additional calcium from the sarcoplasmic reticulum (SR). The resulting surge in intracellular calcium increases calcium concentration in the vicinity of the myofilaments, which in turn triggers cross-bridge cycling. Coincident with this surge in intracellular calcium concentration is the process of calcium clearance from the intracellular compartment. This involves calcium re-uptake by the SR and, to a lesser extent, by mitochondria, and the removal of calcium to the extracellular space. All of these processes are energy requiring (Fabiato, Coraboeuf et al. 1970).

Interaction between contractile physiology and stimulation frequency

The simplistic description given above alludes to a number of important individual components to the contractile process. These factors will now be considered together with an assessment of how each can be modified by changes in stimulation frequency.

Source of intracellular calcium arriving at contractile apparatus

The surge in intracellular calcium concentration that occurs to activate cross bridge recycling has its origins from two major sources. These are transsarcolemmal calcium influx and calcium efflux from the SR. There is evidence to show that within the range of physiological stimulation frequencies, the relative contribution from each source can change quite dramatically. The major evidence in this regard comes from Borzak et al who performed frequency potentiation (rate staircase) experiments in rat papillary muscle (Borzak, Murphy et al. 1991). They used both verapamil, an L-type calcium channel antagonist that blocks the slow inward transsarcolemmal passage of calcium, and ryanodine which occupies low-affinity ryanodine binding sites on the calcium channels of SR-enriched microsomes, inhibiting calcium efflux through these channels. Verapamil produced a negative inotropic response that was highly significant at high stimulation frequencies (6 Hz) but not apparent at low
stimulation frequencies (1-2 Hz). Conversely, the administration of ryanodine produced a marked negative inotropic effect at low stimulation frequencies (1-2 Hz) with no impact evident at high stimulation frequencies (6 Hz). It is concluded from this evidence that the SR source of calcium is important at low stimulation frequencies but becomes progressively less important at higher stimulation with the reverse being true for the transsarcolemmal source of calcium i.e. cellular physiology per se is frequency dependent.

In addition to the two major sources of calcium described above, there are other intracellular organelles that can participate in calcium shifts, the most important of which are the mitochondria (Langer 1992). These behave in much the same way as the SR, effectively increasing the intracellular storage capacity for calcium. It is possible that mitochondrial respiration and energy output may be controlled by these activation dependent calcium transients.

High frequency-induced upregulation of calcium currents

If the data from Borzak et al (Borzak, Murphy et al. 1991) are representative of the human situation, the capacity to deliver calcium via the transsarcolemmal route must increase with increasing stimulation frequency. This has recently been shown to be the case (Piot, Lemaire et al. 1996). Increased stimulation frequency results in both an increase in the peak calcium current amplitude and a slowing of inactivation of the current, the combination resulting in a significant net increase in transsarcolemmal calcium flux. The steady state for this phenomenon is reached rapidly (within seconds), similar to the inotropic response. Furthermore, β-adrenergic stimulation enhances this effect over and above any positive chronotropic effect. This effect appears to be related to altered gating properties of the calcium channels (i.e. more sustained entry of calcium) rather than to an increase in the number of open channels, possibly involving cyclic adenosine monophosphate-dependent phosphorylation of the channels. This phenomenon may also be the basis for the observed hysteresis of contractile amplitude observed between step-wise
increases in stimulation frequency versus step-wise decreases during examination of the rate staircase (Borzak, Murphy et al. 1991).

**Sodium-calcium exchange**

Depolarization of the cell membrane not only induces calcium entry via L-type slow calcium channels but also via reverse-mode sodium-calcium exchange. Indeed this route of calcium entry into the cell has been shown to be sufficient to trigger SR calcium release, in the absence of L-type calcium current (Janvier, McMorn et al. 1997; Terracciano, Souza et al. 1998). At increased stimulation frequencies, the increased number of depolarizations/unit time results in a net increase in intracellular sodium concentration. This leads to increased activation of the sodium-calcium exchanger, increasing intracellular calcium concentration, the so-called sodium pump lag hypothesis. This is directly correlated with increased SR calcium content. A reduction in extracellular sodium concentration to half normal, by preventing diastolic calcium leak from the cell, increases peak twitch force considerably. However, at increased stimulation frequencies, a negative inotropic effect on peak twitch force occurs, without a corresponding significant decrease in SR calcium content, consistent with the extent of frequency potentiation of contractile force being related to the level of cell calcium loading (Flesch, Schwinger et al. 1996).

The interaction between diastolic calcium concentrations and contractile function has its roots in the control of calcium influx and efflux from the SR. Calcium induced calcium release from the SR occurs via a channel showing calcium and time dependent activation and inactivation. The calcium binding sites controlling activation have a lower affinity for calcium but a higher binding rate constant than those controlling inactivation. Therefore, while the peak intracellular calcium concentration remains unchanged, increased diastolic calcium concentrations (such as that occurring in heart failure) mean a reduced absolute flux in calcium, promoting relatively more uptake than release of SR calcium. This has the potential, at high stimulation frequencies, to overload SR calcium stores, exacerbating the
already elevated diastolic calcium concentration. As a result, SR calcium release is progressively inhibited (Glukhovsky, Adam et al. 1998).

**Interaction between SERCA and phospholamban**

The sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA2a) is the major, energy-requiring route for calcium uptake by the SR. Its importance is evidenced by the reduced levels of SERCA mRNA in human heart failure and the positive correlation between the optimum frequency of the force-interval relationship and the protein levels of SERCA (Linck, Boknik et al. 1996). Isoprenaline has been demonstrated to restore towards normal the force-interval relationship in human heart failure when administered at low doses (Schwinger, Bohm et al. 1993). This is thought to be due to phosphorylation of phospholamban, leading to enhanced uptake of calcium by the SR. The beneficial effects of isoprenaline are lost with incremental dosing, probably due to subsequent phosphorylation of L-type calcium channels, increasing calcium influx. More recently, the importance of the relative ratio between SERCA and phospholamban has been recognized as a major determinant of contractile behaviour (Koss, Grupp et al. 1997). This is likely to impact on the force-interval relationship as evidenced by the finding that inhibition of SERCA2a with cyclopiazonic acid causes the normal, positive force-interval relationship to be significantly reduced (Bavendiek, Brixius et al. 1996).

**Calcium sensitivity of the contractile apparatus**

It is apparent that a number of factors can independently impact on the sensitivity of the contractile apparatus to the presence of calcium. For example, for the same peak intracellular calcium content and flux in calcium, hypoxia induces a significant reduction in contractile force (MacKinnon, Gwathmey et al. 1987). Attempts have been made from a therapeutic aspect to modify calcium sensitivity in heart failure with some initially promising but ultimately disappointing results (Hajjar, Grossman et al. 1992; Hasenfuss, Pieske et al. 1995; Hasenfuss, Pieske et al. 1998). The extent to which this factor may vary with stimulation frequency is not known.
Theoretical bases for relating MRC and FP

Both of these phenomena have been used to describe the force-interval relationship. However, attempts to correlate them have not previously been made, largely because they are viewed as having separate physiological descriptors. Nevertheless, there are significant advantages in vivo to be gained from linking these parameters, particularly as regards the investigation of extreme tachycardia.

Not only has the FP response been shown to be abnormal in the setting of heart failure, but the parameter ‘c’, derived from computer curve fitting of incomplete mechanical restitution curves in humans, has been shown to be significantly correlated with left ventricular ejection fraction (Ritchie, Wuttke et al. 1995). From a theoretical standpoint, there is justification for examining the ability of MR to predict the FP response in humans in vivo. Rather than considering the systolic period of contraction, it is more prudent to consider the diastolic period. Following contraction, the myocyte restores its resting physiology in readiness for the next depolarization. An impairment of calcium clearance from the sarcoplasm may not necessarily lengthen the time for full mechanical restitution but will render the cell more vulnerable to an early depolarization. This will not only change the shape of the mechanical restitution curve but will also render the cell less able to cope with a series of extrasystoles, as characterized by tests of FP. Because the ability to clear calcium from the sarcoplasm is linked to SR function, it is readily apparent that disturbance of this process may also impact on PESP. It is important to make the distinction that even though SR calcium release plays a diminishing role during incremental FP, its role in the diastolic clearance of calcium nevertheless remains important.

Drug effects on the force-interval relationship

When one considers the complex cellular physiology underlying the left ventricular force-interval relationship and the frequency dependence of cellular physiology per se, it is not surprising to find evidence of cardioactive drugs with affects that vary depending on the cycle length of stimulation. The most widely investigated group of
drugs to date in this regard has been the β-agonists (Levy 1968; Kambayashi, Miura et al. 1992; Ross, Miura et al. 1995; Carpentier, Coleman et al. 1998; Money-Kyrle, Davies et al. 1998). There is now clear evidence to show that the positive inotropic response to the administration of a β-agonist is attenuated at shorter cycle lengths (Schwinger, Bohm et al. 1993). Verapamil has been shown to elicit incremental reductions in calcium channel current and contractile force in vitro with progressive increases in stimulation frequency (Ehara and Dauflmann 1978; Chappell, Henderson et al. 1985; Borzak, Murphy et al. 1991). However, similar effects by other calcium channel antagonists that do not interact with the slow channel in a frequency dependent manner suggest that this may not be the only important mechanism (Artman, Graham et al. 1985).

SCOPE OF THE PRESENT STUDY

The present investigation has focussed on five aspects of drug effects on the left ventricular force-interval relationship in vivo in humans.

(1) The effects of angiotensin converting enzyme inhibitors on the force-interval relationship have not previously been investigated. While there is some justification for expecting an inotropic impact, either through inhibition of angiotensin II or generation of nitric oxide, there is no certainty as to the expected impact of variations in stimulation frequency. This issue is of relevance given the significant role these agents play in the therapy of cardiac failure, a circumstance of significantly disturbed contractile behaviour and FIR.

(2) While the use-dependent negative inotropic effects of verapamil have previously been suggested in vitro, there has been no in vivo examination to confirm this. Furthermore, despite evidence of potentially variable effects of other cardioactive negatively inotropic agents, no clinically based haemodynamic comparison has previously been undertaken.

(3) There is a significant depth of knowledge regarding the effects of catecholamine positive inotropic agents on the FIR both in vitro and in vivo (Bohm, La Rosee et al. 1992; Schwinger, Bohm et al. 1993). However, there is
no analogous data available regarding the non-catecholamine positively inotropic agents. Improved understanding of the haemodynamic and inotropic responses to these agents as regards effects on the FIR is needed.

(4) The current series of experiments is based upon the construction of mechanical restitution curves, methodology that has demonstrated highly reproducible results in patients with ischaemic heart disease. However, comparison of drug effects on MRC may be limited by differential haemodynamic effects of drugs. In order to exclude a significant impact of modest changes in loading conditions on the fitted MRC, a series of experiments were performed with sodium nitroprusside infusion, in order to produce reductions in mean arterial and mean pulmonary capillary wedge pressure.

(5) Finally, it was prospectively determined that the complete database of patients investigated as regards MRC and FP experiments be analyzed to examine the determinants of FP.
METHODOLOGY

PATIENT SELECTION

Patients were selected from those presenting for elective cardiac catheterisation and coronary angiography for the investigation of chest pain. Patients were required to be clinically stable with all cardioactive medications withheld for at least five half lives prior to the research procedure. Exclusion criteria included:

- Unstable angina pectoris
- Q-wave myocardial infarction in the preceding three months
- Haemodynamically significant valvular heart disease or left main coronary artery stenosis (>50% reduction in luminal diameter)
- Atrial fibrillation
- PR prolongation or bundle branch block pattern on resting ECG

The protocol was approved by the Queen Elizabeth Hospital Ethics of Human Research Committee and written, informed consent was obtained prior to the procedure in all cases.

INSTRUMENTATION

Femoral arterial and femoral venous sheaths were inserted via the right groin (Barry, Levin et al. 1979). Routine coronary angiography was performed using non-ionic contrast media with a minimum of 15 minutes elapsing between the last injection of contrast and the commencement of the research protocol (Judkins 1968). A 7F Swan-Ganz catheter was positioned via the femoral venous sheath for serial determination of pulmonary capillary wedge pressure and cardiac output, utilizing the thermodilution method, the reading at each time point being the average of at least three recordings (Sorensen, Bille-Brahe et al. 1976). A 4F micromanometer-tipped catheter (Millar Instruments, Texas) was positioned in the left ventricle, via the femoral arterial sheath, for determination of instantaneous left ventricular pressure and its first derivative, dP/dt. A 7F bipolar pacing lead was positioned in the right
atrium via a femoral venous sheath, atrial stimulation being performed at twice threshold strength. Femoral arterial pressure was constantly monitored via the femoral arterial sheath. A 2-channel surface electrocardiograph was constantly recorded.

**EXPERIMENTAL PROTOCOL**

A series of baseline measurements, during atrial pacing at a rate just above that occurring spontaneously, were made prior to drug administration. These included, where possible, cardiac output, mean pulmonary capillary wedge pressure, electrocardiographic intervals, mean arterial pressure and the maximal first derivative of the integrated left ventricular pressure signal, $LV+dP/dt_{max}$. Frequency potentiation was examined by reducing the atrial pacing cycle length to about 60% of the resting cycle length (equal to 400 - 500 msec) (see below). Data were then gathered for later construction of incomplete mechanical restitution curves (MRC) (see below) also enabling examination of post extra-systolic potentiation (PESP).

Following the collection of baseline data, patients were serially assigned to receive a rapid intravenous bolus injection of either verapamil 4mg or digoxin 500mcg or to receive a continuous intravenous infusion of sodium nitroprusside 10 - 20 mcg/minute. Serial measures of haemodynamic, ECG and MRC data were performed, with rapid atrial pacing being performed at the time of peak haemodynamic effect. Utilizing information from previous acute myocardial drug uptake experiments for determining the timing of peak haemodynamic drug effects, rapid atrial pacing was performed 10 minutes following verapamil administration and 30 minutes following digoxin administration (Powell, Horowitz et al. 1990; Powell, Horowitz et al. 1990). Sodium nitroprusside was administered with the intention of causing a 10% fall in mean arterial pressure, repeat measures of all variables being made once this end point had been achieved. In the cases of perindoprilat and enalaprilat, acute drug uptake and haemodynamic studies were being performed concurrently, with rapid pacing being repeated presumptively 10-
15 minutes following drug administration. The procedure was well tolerated in all cases.

**Frequency Potentiation data recording and analysis**

FP was examined by reducing the atrial pacing cycle length abruptly to approximately 60% of the baseline cycle length while LV+dP/dt_{max} and mean arterial pressure were continuously recorded. Given that the induction of tachycardia in the planned cohort of patients had the potential to induce variable degrees of ischaemia and neurohumoral activation, it was prospectively decided to examine the FP responses at both 10 and 60 seconds following induction of tachycardia.

**Mechanical Restitution Curve Data Recording and Analysis**

The recording and analysis of MRC data has previously been described in some detail (Ritchie, Wuttke et al. 1995). In brief, during constant atrial pacing, at a rate just above that occurring spontaneously, programmed atrial stimulation is used to introduce atrial stimulation at a shorter cycle length every 8 beats. A progressively shorter cycle length for this atrial extrasystole is used until atrio-ventricular nodal refractoriness prevents impulse conduction to the ventricles. The post extra-systolic beat occurs at the same cycle length as the baseline paced cycle length, enabling later examination of effects on post extra-systolic potentiation. During the above pacing protocol, the 2-channel ECG, mean arterial pressure and left ventricular dP/dt are continuously recorded at a paper speed of 50-100 mm/sec.

The above data are subsequently graphically displayed with the RR’ interval, as a percentage of the baseline RR interval, being plotted on the x axis and LV+dP/dt_{max}, as a percentage of the baseline LV+dP/dt_{max}, being recorded on the y axis (Figure 3.1). The raw data for each time point are then fitted to one half of a rectangular hyperbola. The curve fitting was performed by the University of Adelaide Department of Mathematical Statistics using the SPLUS program. The curve fitted is

\[ y = a + \frac{b}{x - d}, \]
where \( y \) is the \( \% \text{LV}+\text{dP}/\text{dt}_{\text{max}} \), \( x \) is the RR' (as a \( \% \)), 'a' is the horizontal asymptote, 'd' is the vertical asymptote, and 'b' is a measure of the curvature of the hyperbola (Figure 3.2).

In order to enable simple comparisons between curves, the above equation can be re-written thus

\[
y = a - \frac{c(100 - d)(60 - d)}{40(x - d)}
\]

where 'c' is a measure of the reduction in \( \text{LV}+\text{dP}/\text{dt}_{\text{max}}(\%) \) that occurs with a reduction in cycle length to 60\% of baseline RR interval. The parameter 'c' is therefore a measure of individual sensitivity to reductions in cycle length and has been shown to be highly reproducible in humans.

**Post extra-systolic potentiation data recording and analysis**

The data for MRC construction were gathered without a compensatory pause occurring after the atrial extra-systole, this interval therefore remaining constant for each measurement (post extra-systolic interval = baseline paced cycle length). Therefore, by plotting the strength of the post extra-systolic beat against the RR' interval of the preceding beat, this relationship can be examined. A linear regression line was fitted to each data set and the slope of this line used for comparison of data pre and post drug administration.

**STATISTICAL ANALYSES**

All data are recorded as mean ± standard error of the mean. Drug effects on the force-interval relationship were examined at the time of peak haemodynamic effect and compared to baseline measures with Student’s \( t \) test. MRC curve fitting is described above. The 95\% confidence intervals for the residual standard deviations (a measure of the goodness of fit of the curve) are provided. FP data is analyzed with repeated measures analysis, using Dunnett’s correction for multiple comparisons when appropriate. The slopes of PESP relationship were compared with paired \( t \) tests. Non-paired \( t \) tests were used to compare baseline characteristics of the various groups studied. Significance was accepted at a \( p \) value of <0.05.
Figure 3.1  *MRC data collection – the contractile strength of the early beat is plotted against the RR interval of the early beat*  

Figure 3.2  *Mechanical Restitution Curve fitting to one half of a rectangular hyperbola*  

\[ y = a - \frac{c(100-d)(60-d)}{40(x-d)} \]
RESULTS

PERINDOPRILAT
A total of 12 patients were studied following the intravenous bolus administration of 1.25mg perindoprilat. The detailed myocardial drug uptake, haemodynamic, metabolic and biochemical effects have been presented in Chapter 2. Investigation of the acute effects of perindoprilat on the left ventricular force-interval relationship were examined in all of these patients. Results for frequency potentiation are available for 11 patients, post extra-systolic potentiation for 12 patients and MRC construction for 9 patients.

Data for the construction of MRC's were gathered at baseline and at 5 minute intervals for up to 20 minutes, data being available at 10 minutes in all cases. The frequency potentiation (FP) response was re-examined 14.6 ± 1.4 minutes following drug administration. The baseline characteristics of the 12 patients studied are presented in Table 3.1. An example of raw data fitted to an MRC is provided in Figure 3.3(a) with the pooled fitted curve data being presented in Figure 3.3(b). The time course of perindoprilat effects on LV+dP/dt_max and 'c' are presented in Figure 3.4. The effects of perindoprilat on the FP response are presented in Figure 3.5, including changes in mean arterial pressure during the induction of tachycardia. Finally, an example of raw data for post extra-systolic potentiation and the pooled data for all 12 patients are presented in Figure 3.6.
Table 3.1  Baseline characteristics of patients receiving intravenous perindoprilat and having examination of force-interval relationship

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PCWP = Mean Pulmonary Capillary Wedge Pressure  
CI = Cardiac Index  
LVEF = Left ventricular ejection fraction as measured on contrast ventriculography  
Coronary Disease = >50% luminal stenosis  
Cx = Circumflex, LAD = Left anterior descending, RCA = Right coronary artery
Figure 3.3  (a) Raw MRC data and fitted curves from a patient receiving perindoprilat and (b) pooled fitted curves pre and post drug

(a) Raw data and fitted curves pre and post perindoprilat (n=1)

(b) Perindoprilat effect on pooled MRC data
**Figure 3.4** Time course of effects of Perindoprilat on (a) $LV^+dP/dt_{max}$ and (b) $c'$
Figure 3.5  Perindoprilat effects on frequency potentiation response, including (a) Mean arterial pressure and (b) $LV+dP/dt_{max}$.
Figure 3.6  Effects of Perindoprilat on PESP with (a) raw and fitted data from a single patient and (b) pooled fitted data

(a) PESP raw data and regression lines pre and post perindoprilat

(b) Mean PESP regression data
Following the administration of perindoprilat 1.25mg as an intravenous bolus injection, the parameter ‘c’ increased from 30.8 ± 6.2 (range 8.3 to 62.1%) to 32.2 ± 5.0 (range 12.7 to 55.6%) within 5 minutes (p=ns) and at 10 minutes was 30.1 ± 4.5 (range 12.7 to 49.5%) (p=ns vs baseline or 5 minutes), there thus being no effect of perindoprilat upon MRC. The 95% confidence intervals for the residual standard deviations (a measure of the goodness of fit of the MR curve fitting) were 5.62 and 7.78.

During FP, LV+dP/dt_max increased by 14.1 ± 3.8% (range -3.6 to 40.1) within 10 seconds and by 16.0 ± 3.9% (range 1.4 to 38.1) within 60 seconds. Following the administration of perindoprilat, the increase in LV+dP/dt_max after 10 seconds was 11.7 ± 3.4% (range -1.4 to 28.3; p=ns vs baseline) and after 60 seconds was 16.2 ± 4.6% (range 0.6 to 46.2; p=ns vs baseline) i.e. perindoprilat did not significantly affect extent of FP. There was no effect of perindoprilat on mean arterial pressure during rapid atrial pacing.

At baseline, a reduction in the paced cycle length for a single beat resulted in a potentiated post extra-systolic beat. Reducing the cycle length of this beat from 100% to 60% of the baseline paced cycle length resulted in an increase of LV+dP/dt_max from 100.2 ± 0.9 to 120.3 ± 3.2% (p<0.001). Following the administration of perindoprilat, LV+dP/dt_max at baseline was unchanged at 96.5 ± 2.9%, but increased to 119.1 ± 5.0% (p<0.0001) with a reduction in the cycle length to 60% of baseline (p=0.065 vs PESP at baseline). These measures were made 10 minutes following perindoprilat administration with no incremental effects being evident at either 5 or 20 minutes.
ENALAPRILAT
A total of 10 patients were studied following the intravenous bolus administration of 2.5mg enalaprilat. The detailed myocardial drug uptake, haemodynamic, metabolic and biochemical effects have been presented in Chapter 2. Investigation of the acute effects of enalaprilat on the left ventricular force-interval relationship were examined in all of these patients. Results for frequency potentiation are available for 9 patients, post extra-systolic potentiation for 10 patients and MRC construction for 5 patients.

Data for the construction of MRC's were gathered at baseline and at 5 minute intervals for up to 20 minutes, data being available at 15 minutes in all cases. The frequency potentiation (FP) response was re-examined 13.0 ± 0.5 minutes following drug administration. The baseline characteristics of the 10 patients studied are presented in Table 3.2. An example of raw data fitted to an MRC is provided in Figure 3.7(a) with the pooled fitted curve data being presented in Figure 3.7(b). The time courses of enalaprilat effects on LV+dP/dt max and 'c' are presented in Figure 3.8. The effects of enalaprilat on the FP response are presented in Figure 3.9, including changes in mean arterial pressure during the induction of tachycardia. Finally, an example of raw data for post extra-systolic potentiation and the pooled data for all 10 patients are presented in Figure 3.10.
### Table 3.2  
**Baseline characteristics of patients receiving intravenous enalaprilat and having examination of force-interval relationship**

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LVEF = Left ventricular ejection fraction as measured on contrast ventriculography  
Coronary Disease = >50% luminal stenosis  
Cx = Circumflex, LAD = Left anterior descending, RCA = Right coronary artery
Figure 3.7  (a) Raw MRC data and fitted curves from a patient receiving enalaprilat and (b) pooled fitted curves pre and post drug

(a) Fitted MRC and raw data pre and post enalaprilat

(b) Mean fitted MRC data
Figure 3.8  Time course of effects of Enalaprilat on (a) $LV+dP/dt_{\text{max}}$ and (b) 'c'

(a)

\[ LV+dP/dt_{\text{max}}(\text{mmHg/sec}) \]

*\(p=0.03\) vs baseline

(b)

\[ \text{p=ns at all time points} \]
Figure 3.9  Enalaprilat effects on frequency potentiation response, including
(a) Mean arterial pressure and (b) LV+dP/dt_{max}

(a)

Mean Arterial Pressure (mmHg)

Baseline  Enalaprilat

p=ns

Seconds

(b)

LV+dP/dt_{max} (% baseline)

Baseline  Enalaprilat

p=ns

Seconds
Figure 3.10  Enalaprilat effects on PESP with (a) raw and fitted data for a single patient and (b) Pooled fitted data
Following the administration of enalaprilat 2.5mg as an intravenous bolus injection, which induced a significant progressive reduction in LV+dP/dt_{max} (Figure 3.8), the parameter 'c' did not vary significantly at any stage, with a value of 22.2 ± 3.8 (range 9.1 to 30.8%) at baseline and 25.3 ± 5.5 (range 13.5 to 44.5%) at 15 minutes. The 95% confidence intervals for the residual standard deviations (a measure of the goodness of fit of the MR curve fitting) were 3.28 and 4.95.

During FP, LV+dP/dt_{max} increased by 16.4 ± 6.1% (range -12.4 to 45.7) within 10 seconds and by 22.4 ± 4.8% (range 9.1 to 51.0) within 60 seconds. Following the administration of enalaprilat, the increase in LV+dP/dt_{max} after 10 seconds was 12.5 ± 6.3% (range -20.9 to 42.6; p=ns vs pre-enalaprilat) and after 60 seconds was 21.1 ± 3.3% (range 7.8 to 35.1; p=ns vs pre-enalaprilat). With the onset of rapid atrial pacing at baseline, there was an increase of 0.6 ± 3.6 mmHg (range -23 to 13) in mean arterial pressure at 10 seconds there being a decrease at 60 seconds of 2.4 ± 2.6 mmHg (range -14 to 8). Following enalaprilat, the induction of tachycardia resulted in a 4.7 ± 3.3 mmHg (range -23 to 6) decrease in mean arterial pressure at 10 seconds (p=ns), the decrease at 60 seconds being just 0.9 ± 1.4 mmHg (range -7 to 7).

At baseline, a reduction in the paced cycle length for a single beat resulted in a potentiated post extra-systolic beat. Reducing the cycle length of this beat from 100% to 60% of the baseline paced cycle length resulted in an increase of LV+dP/dt_{max} from 99.7 ± 0.5 to 117.4 ± 2.7% (p<0.001). Following the administration of enalaprilat, LV+dP/dt_{max} at baseline was unchanged at 97.5 ± 3.5%, but increased to 114.9 ± 5.5% (p<0.01) with a reduction in the cycle length to 60% of baseline (p=ns vs PESP pre-enalaprilat). These measures were made 10 minutes following enalaprilat administration with no incremental effects being evident at either 5 or 20 minutes.
VERAPAMIL
A total of 17 patients were studied following the intravenous bolus administration of 4mg verapamil. Investigation of the acute effects of verapamil on the left ventricular force-interval relationship were examined in all of these patients. Results for frequency potentiation are available for 12 patients, post extra-systolic potentiation for 12 patients and MRC construction for 17 patients.

Data for the construction of MRC's were gathered at baseline and at frequent intervals for 10 minutes, data being available at 10 minutes in all cases. The frequency potentiation (FP) response was re-examined 10 minutes following drug administration. The baseline characteristics of the 17 patients studied are presented in Table 3.3. An example of raw data fitted to an MRC is provided in Figure 3.11(a) with the pooled fitted curve data being presented in Figure 3.11(b). The time course of verapamil effects on LV+dP/dt_{max} and ‘c’ are presented in Figure 3.12. The effects of verapamil on the FP response are presented in Figure 3.13, including changes in mean arterial pressure during the induction of tachycardia. Finally, an example of raw data for post extra-systolic potentiation and the pooled data for all 17 patients are presented in Figure 3.14.

Verapamil is the third negatively inotropic agent to be studied in the laboratory and, in view of this, the opportunity has been taken to compare the effects of these 3 agents, with similar degrees of negative inotropic affect at baseline paced cycle lengths, on the left ventricular force-interval relationship.
### Table 3.3  
Baseline characteristics of patients receiving intravenous verapamil and having examination of force-interval relationship

<table>
<thead>
<tr>
<th>Number</th>
<th>Sex</th>
<th>Age</th>
<th>PCWP (mmHg)</th>
<th>CI (L/min/m²)</th>
<th>LVEF (%)</th>
<th>Coronary Disease</th>
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<td>Cx</td>
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<td>Mean</td>
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</tbody>
</table>

PCWP = Mean Pulmonary Capillary Wedge Pressure  
CI = Cardiac Index  
LVEF = Left ventricular ejection fraction as measured on contrast ventriculography  
Coronary Disease = >50% luminal stenosis  
Cx = Circumflex, LAD = Left anterior descending, RCA = Right coronary artery
Figure 3.11  (a) Raw MRC data and fitted curves from a patient receiving verapamil and (b) pooled fitted curves pre and post drug

(a) MRC fitted and raw data pre and post verapamil (n=1)

(b) Mean Fitted MRC Data

p<0.05 baseline vs 10 minutes
Figure 3.12  Time course of effects of Verapamil on (a) $LV+dP/dt_{max}$ and (b) ‘c’

(a)

\[
\begin{align*}
&\text{LV+dP/dt}_{\text{max}} \\
&\text{(mmHg/sec)}
\end{align*}
\]

Minutes

\[
\begin{align*}
0 & \quad * \\
5 & \quad ** \\
10 &
\end{align*}
\]

*p=0.003  **p<0.0001 vs baseline

(b)

\[
\begin{align*}
\text{‘c’} (%)
\end{align*}
\]

Minutes

\[
\begin{align*}
0 & \quad * \\
5 & \quad * \\
10 &
\end{align*}
\]

*p<0.05 vs baseline
Figure 3.13 Verapamil effects on frequency potentiation response, including (a) Mean arterial pressure and (b) $LV + dP/dt_{max}$

(a) Mean Arterial Pressure (mmHg)

- **Baseline**
- **Verapamil**

* $p=0.012$ for effect of verapamil

(b) $LV + dP/dt_{max}$ (% baseline)

- **Baseline**
- **Verapamil**

* $p<0.002$ for effect of verapamil
Figure 3.14  Verapamil effects on post extra-systolic potentiation for a typical patient, including raw data and (b) pooled mean data

(a) PESP raw and fitted data pre and post Verapamil (n=1)

(b) Mean PESP data pre and 10 minutes post Verapamil
Following the administration of verapamil 4mg as an intravenous bolus injection, the parameter 'c' increased from $17.9 \pm 3.9$ (range -1.8 to 57.8%) to $24.3 \pm 4.0$ (range 4.0 to 75.1%) within 5 minutes ($p=0.015$) and at 10 minutes was $24.1 \pm 4.3$ (range 2.8 to 66.1%) ($p=0.018$ vs baseline, $p=ns$ vs 5 minutes). The 95% confidence intervals for the residual standard deviations (a measure of the goodness of fit of the MR curve fitting) were 3.48 and 6.67.

During FP, $LV+\frac{dP}{dt_{max}}$ increased by $20.3 \pm 4.6\%$ (range -15.2 to 49.2) within 10 seconds and by $21.3 \pm 2.8\%$ (range 4.9 to 37.1) within 60 seconds. Following the administration of verapamil, the increase in $LV+\frac{dP}{dt_{max}}$ after 10 seconds was $0.8 \pm 3.9\%$ (range -35.4 to 17.0; $p<0.002$ vs baseline) and after 60 seconds was $9.9 \pm 3.7\%$ (range -10.3 to 32.4; $p=ns$ vs baseline). Mean arterial pressure increased by $5.1 \pm 3.5$ mmHg (range -21 to 22) within 10 seconds of the onset of rapid atrial pacing and by $4.9 \pm 2.8$ mmHg (range -16 to 17) after 60 seconds. Following the administration of verapamil, the induction of tachycardia resulted in a fall in mean arterial pressure of $3.2 \pm 3.2$ mmHg (range -20 to 18) within 10 seconds ($p=0.012$ vs baseline change), there being an overall increase by 60 seconds of $2.4 \pm 2.1$ mmHg (range -5 to 17) ($p=ns$).

Whilst verapamil exerted a significant negative inotropic effect at baseline paced cycle length and significantly accentuated this at short cycle lengths, it did not have any net positive or adverse effect on PESP. At baseline, a reduction in the paced cycle length for a single beat, to 60% of baseline, resulted in a $18.2 \pm 1.6\%$ (range -3.2 to 24.5) increase in the strength of the post extra-systolic beat. Following verapamil, the increase was $15.8 \pm 1.5\%$ (range 1.9 to 29.1; $p=ns$ vs baseline).
The above results for verapamil raised the possibility that the parameter ‘c’ was sensitive to modest changes in preload and, more importantly, afterload. In order to examine this issue further, 5 patients were investigated before and after receiving an infusion of sodium nitroprusside (SNP), at a rate sufficient to cause at least a 10% fall in mean arterial pressure (10-20 mcg/minute).

Of the 4 female and 1 male patients studied, 3 had significant coronary artery disease with a left ventricular ejection fraction of 65 ± 3%. Baseline pacing was performed at a cycle length of 710 ± 40 msec, the mean pulmonary capillary wedge pressure being 13 ± 1 mmHg. The haemodynamic and ECG effects of sodium nitroprusside at the baseline paced cycle length are displayed in Table 3.4.

Sodium nitroprusside, despite the intended significant decrease in MAP, did not significantly influence LV+dP/dtmax during baseline pacing (-5.0 ± 4.4% versus baseline, p = NS) or at shorter RR intervals, there being no significant fluctuation in the parameter ‘c’ (from 42.6 ± 5.8% to 43.0 ± 6.6%, p = NS) (Figure 3.15).

Table 3.4 Effects of sodium nitroprusside at baseline paced cycle length

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-SNP</th>
<th>Post-SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac index (L/min/m²)</td>
<td>2.9 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>117 ± 11</td>
<td>105 ± 9 *</td>
</tr>
<tr>
<td>PR interval (msec)</td>
<td>186 ± 19</td>
<td>182 ± 18</td>
</tr>
<tr>
<td>QT interval (msec)</td>
<td>370 ± 7</td>
<td>370 ± 9</td>
</tr>
<tr>
<td>LV+dP/dtmax (% baseline)</td>
<td>100 ± 0</td>
<td>95 ± 4</td>
</tr>
</tbody>
</table>

*p=0.003 vs baseline
COMPARISON OF VERAPAMIL, METOPROLOL AND SOTALOL EFFECTS ON THE LEFT VENTRICULAR FORCE-INTERVAL RELATIONSHIP

Previous data regarding the effects of two other negatively inotropic agents were available in the laboratory, these being metoprolol and sotalol. The opportunity was therefore taken to compare the effects of these 3 agents on the left ventricular force-interval relationship in humans. The comparison was limited to effects on FP and MRC.

The baseline characteristics of the 3 groups are recorded in Table 3.5 and the haemodynamic and ECG effects of the administered drugs at baseline paced cycle length recorded in Table 3.6. The groups were generally well matched, with predominantly normal left ventricular systolic function, and no significant differences in characteristics between the groups.
Table 3.5  **Baseline characteristics of all patients**

<table>
<thead>
<tr>
<th></th>
<th>Metoprolol (n=15)</th>
<th>Sotalol (n=15)</th>
<th>Verapamil (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>56 ± 2</td>
<td>54 ± 2</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>Sex: male/female</td>
<td>11 / 4</td>
<td>10 / 5</td>
<td>9 / 8</td>
</tr>
<tr>
<td>Coronary disease</td>
<td>10 (67%)</td>
<td>11 (73%)</td>
<td>12 (71%)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>63 ± 3</td>
<td>72 ± 2</td>
<td>69 ± 3</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Paced RR interval (msec)</td>
<td>800 ± 40</td>
<td>780 ± 30</td>
<td>730 ± 30</td>
</tr>
</tbody>
</table>

Coronary disease = greater than 50% stenosis in at least one coronary artery (left anterior descending, circumflex, or right coronary artery)

LVEF = left ventricular ejection fraction

PCWP = mean pulmonary capillary wedge pressure

---

**Table 3.6  Haemodynamic and ECG effects of negatively inotropic agents during baseline atrial pacing. Data shown at time of peak effect (10 minutes after injection of metoprolol, sotalol and verapamil)**

<table>
<thead>
<tr>
<th></th>
<th>Metoprolol 4mg</th>
<th>Sotalol 20mg</th>
<th>Verapamil 4mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Cardiac index (L/min/m²)</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>101 ± 4</td>
<td>103 ± 3</td>
<td>109 ± 3</td>
</tr>
<tr>
<td>PR interval (msec)</td>
<td>182 ± 6</td>
<td>190 ± 6*</td>
<td>193 ± 9</td>
</tr>
<tr>
<td>QT interval (msec)</td>
<td>367 ± 11</td>
<td>365 ± 10</td>
<td>379 ± 8</td>
</tr>
<tr>
<td>LV+dP/dt_max (%)</td>
<td>100 ± 0</td>
<td>88 ± 3*</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. pre-drug*
In all instances the drug was administered as a rapid intravenous bolus injection with no adverse effects being experienced by any patient. The three negatively inotropic drugs induced approximately a 10% reduction in LV+dP/dt\text{max}. There were no significant changes in cardiac index or QT intervals but there was some diversity of effect on other parameters. MAP was significantly reduced by verapamil but not by either metoprolol or sotalol. Both metoprolol and sotalol, but not verapamil, significantly prolonged the PR interval.

Whilst all three drugs exerted a similar negative inotropic effect during baseline pacing (Figure 3.16a), these effects were heterogeneous on MRC analysis. The negative inotropic effect of metoprolol during baseline pacing was described by a 12.3 ± 2.8% reduction in LV+dP/dt\text{max}. However, the extent of this effect became less marked at shorter RR intervals, such that it was completely abolished when the RR interval was reduced by 40%: LV+dP/dt\text{max} from the fitted MRC’s was 71.5 ± 3.9% and 70.2 ± 2.9% at an RR interval of 60% of baseline, before and after metoprolol respectively (Figure 3.17a). This convergence of the MRC’s was reflected by a significant reduction in ‘c’, from 28.4 ± 4.0 to 21.2 ± 2.7% (Figure 5; p < 0.005).

In the group of patients allocated to receive sotalol, a reduction of 10.5 ± 2.7% in LV+dP/dt\text{max} was observed during baseline pacing. However, as the RR interval decreased, the negative inotropic effect of sotalol was virtually unchanged on MRC analysis, resulting in the appearance of parallel MRC: LV+dP/dt\text{max} from the fitted MRC’s was 72.2 ± 6.1% and 58.5 ± 6.8% at an RR interval of 60% of baseline, before and after sotalol respectively (Figure 3.17b). Hence, ‘c’ did not fluctuate significantly from 28.9 ± 6.2 prior to injection to 31.3 ± 6.5% post sotalol (p = NS, Figure 3.16b).

In contrast to the observations with both metoprolol and sotalol, verapamil, as described earlier, induced significant divergence of MRC’s, i.e. the negative inotropic effect (a 7.5 ± 1.5% reduction in LV+dP/dt\text{max} at baseline) became progressively accentuated as the RR interval decreased. LV+dP/dt\text{max} from the fitted MRC’s was
81.3 ± 4.0% and 66.6 ± 5.1% at an RR interval of 60% of baseline, before and after verapamil respectively, indicative of a rate-dependent effect (Figure 3.17c) (p<0.05).

The 95% confidence intervals for the residual standard deviations of the model (the measure of goodness-of-fit) for the three negatively inotropic drugs were 3.34 and 6.39 for metoprolol, 3.48 and 6.67 for verapamil and 5.94 and 10.6 for sotalol. An example of curve fitting pre and post drug administration for each agent is given in Figure 3.18.

FP was examined in 10/15 patients receiving metoprolol, 11/15 patients receiving sotalol and 12/17 patients receiving verapamil. FP examination was not performed in patients with severe angina and was precluded by the development of AV block in some cases. Figure 3.19 illustrates the time course of LV+dP/dt_max during rapid pacing. Prior to drug administration, the effects of rapid pacing were comparable in the three treatment groups, exhibiting an initial increase of approximately 20% in LV+dP/dt_max, which was preserved for the full 60 sec. However, as with MRC analysis, the three negative inotropes exerted disparate effects on FP (Figure 3.20). Metoprolol did not appear to affect FP: pacing-induced increases in LV+dP/dt_max before and after the drug were 12.4 ± 4.5% and 9.3 ± 3.1% (p = NS) at 10 sec, and 12.5 ± 2.7% and 11.2 ± 2.6% (p = NS) at 60 sec of FP respectively. This did not appear to be the case for verapamil: the pacing-induced increase in LV+dP/dt_max decreased from 21.3 ± 2.8% to 0.8 ± 3.9% (p < 0.002) at 10 sec, suggesting FP had been abolished at this early time point. By 60 sec of rapid pacing the FP response after verapamil had been restored (from 20.3 ± 4.6% to 9.9 ± 3.7%, p = NS). A similar result was observed with sotalol at both time points, although this did not attain significance: pacing-induced increases in LV+dP/dt_max were 18.9 ± 3.5% and 11.4 ± 5.9% (p = 0.08) at 10 sec, and 20.0 ± 4.1% and 12.2 ± 4.6% (p = 0.09) at 60 sec of FP respectively.
Figure 3.16  Comparative time course of effects of metoprolol, sotalol and verapamil on (a) $LV+\frac{dP}{dt_{max}}$ and (b) ‘c’

(a)

* $p<0.05$

(b)

* $p<0.05$
Figure 3.17  Mean fitted MRC data for (a) Metoprolol (b) Sotalol and (c) Verapamil
Figure 3.18  An example of raw data and fitted curves for a representative patient from each group (a) metoprolol, (b) sotalol, and (c) verapamil
Figure 3.19  Pooled FP data for (a) Metoprolol, (b) Sotalol and (c) Verapamil

(a)

(b)

(c)
Figure 3.20  Pacing induced increases in \( LV^+dP/dt_{\text{max}} \) at 10 and 60 seconds following (a) Metoprolol, (b) Sotalol and (c) Verapamil.

(a)  
\[
\delta LV^+dP/dt_{\text{max}}(\%)
\]
- Baseline
- Metoprolol

(b)  
\[
\delta LV^+dP/dt_{\text{max}}(\%)
\]
- Baseline
- Sotalol

(c)  
\[
\delta LV^+dP/dt_{\text{max}}(\%)
\]
- Baseline
- Verapamil

*\( p<0.05 \)
DIGOXIN
A total of 14 patients were studied following the intravenous bolus administration of 0.5mg digoxin. Investigation of the acute effects of digoxin on the left ventricular force-interval relationship were examined in all of these patients. Results for frequency potentiation are available for 14 patients, post extra-systolic potentiation for 13 patients and MRC construction for 14 patients.

Data for the construction of MRC's were gathered at baseline and at frequent intervals for 30 minutes, data being available at 30 minutes in all cases. The frequency potentiation (FP) response was re-examined 30 minutes following drug administration. The baseline characteristics of the 14 patients studied are presented in Table 3.7. An example of raw data fitted to an MRC is provided in Figure 3.21(a) with the pooled fitted curve data being presented in Figure 3.21(b). The time courses of digoxin effects on LV+dP/dt_{max} and 'c' are presented in Figure 3.22. The effects of digoxin on the FP response are presented in Figure 3.23, including changes in mean arterial pressure during the induction of tachycardia. Finally, an example of raw data for post extra-systolic potentiation and the pooled data for all 14 patients are presented in Figure 3.24.

Digoxin is the second non-catecholamine positively inotropic agent to be studied in the laboratory and, in view of this, the opportunity has been taken to compare the effects of these 2 agents, with similar degrees of positive inotropic affect at baseline paced cycle lengths, on the left ventricular force-interval relationship.
Table 3.7  
**Baseline characteristics of patients receiving intravenous digoxin and having examination of force-interval relationship**

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<th>Number</th>
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<th>CI (L/min/m²)</th>
<th>LVEF (%)</th>
<th>Coronary Disease</th>
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<tbody>
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<td>7</td>
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<td>67</td>
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<td>2.98</td>
<td>82</td>
<td>Cx</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>65</td>
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<td>2.25</td>
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<tr>
<td>6</td>
<td>M</td>
<td>62</td>
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<td>2.5</td>
<td>71</td>
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<tr>
<td>7</td>
<td>F</td>
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<td>M</td>
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<td>51</td>
<td>11</td>
<td>2.47</td>
<td>84</td>
<td>Cx, RCA</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>71</td>
<td>12</td>
<td>2.19</td>
<td>70</td>
<td>Cx</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>57</td>
<td>8</td>
<td>2.55</td>
<td>71</td>
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<td>12</td>
<td>F</td>
<td>71</td>
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<td>2.64</td>
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<tr>
<td>13</td>
<td>M</td>
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<td>43</td>
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<tr>
<td>14</td>
<td>F</td>
<td>56</td>
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<td>3</td>
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<td>0.08</td>
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</tr>
</tbody>
</table>

PCWP = Mean Pulmonary Capillary Wedge Pressure  
CI = Cardiac Index  
LVEF = Left ventricular ejection fraction as measured on contrast ventriculography  
Coronary Disease = >50% luminal stenosis  
Cx = Circumflex, LAD = Left anterior descending, RCA = Right coronary artery
Figure 3.21  (a) Raw MRC data and fitted curves from a patient receiving digoxin and (b) pooled fitted curves pre and post drug

(a) MRC raw and fitted data pre and 30 mins post digoxin (n=1)

(b) Pooled MRC fitted data
Figure 3.22  Time course of effects of Digoxin on (a) \( LV+dP/dt_{max}\) and (b) ‘c’

(a)

<table>
<thead>
<tr>
<th>Minutes</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV+dP/dt_{max} (mmHg/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1200</td>
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<td>1200</td>
</tr>
<tr>
<td>30</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
<td>1200</td>
</tr>
</tbody>
</table>

*p*< 0.05  ANOVA *p*< 0.0005,  \( F=7.382 \)

(b)

<table>
<thead>
<tr>
<th>Minutes</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>'c' (%)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

p= ns
Figure 3.23  Digoxin effects on frequency potentiation response, including 
(a) Mean arterial pressure and (b) LV+dP/dt_max

(a)

Mean Arterial Pressure (mmHg)

Baseline  Digoxin

p=ns

Seconds

(b)

LV+dP/dt_max (% baseline)

Baseline  18.5±4.5  23.1±4.5
Digoxin  7.4±5.3  15.8±3.8

p=ns  p=ns

Seconds
Figure 3.24  Digoxin effects on post extra-systolic potentiation for a typical patient, including raw data and (b) pooled mean data

(a) PESP raw and fitted data pre and post digoxin (n=1)

(b) Mean Fitted PESP data
Following the administration of digoxin 0.5mg as an intravenous bolus injection, the parameter ‘c’ increased from 22.9 ± 3.2 (range 4.5 to 47.9%) to 27.7 ± 4.2 (range 0.4 to 53.0%) within 20 minutes (p=ns) and at 30 minutes was 26.5 ± 4.9 (range -2.7 to 51.7%) (p=ns vs baseline, 10 or 20 minutes). The 95% confidence intervals for the residual standard deviations (a measure of the goodness of fit of the MR curve fitting) were 5.14 and 8.85.

During FP, LV+dP/dt\text{max} increased by 18.5 ± 4.5% (range -6.9 to 48.1) within 10 seconds and by 23.1 ± 4.5% (range 0.0 to 49.5) within 60 seconds. Following the administration of digoxin, the increase in LV+dP/dt\text{max} after 10 seconds was 7.4 ± 5.3% (range -30.2 to 36.5; p=ns vs baseline) and after 60 seconds was 15.8 ± 3.8% (range -8.5 to 31.2; p=ns vs baseline). Mean arterial pressure decreased by 2.1 ± 4.1 mmHg (range -42 to 18) within 10 seconds of the onset of rapid atrial pacing and by -0.1 ± 2.1 mmHg (range -12 to 15) after 60 seconds. Following the administration of digoxin, the induction of tachycardia resulted in a fall in mean arterial pressure of 6.2 ± 4.4 mmHg (range -47 to 14) within 10 seconds (p=ns vs baseline change), the fall being 1.4 ± 3.2 mmHg (range -35 to 10) (p=ns vs baseline change) after 60 seconds.

Digoxin exerted a significant positive inotropic effect at baseline paced cycle length and this was unchanged at short cycle lengths. Similarly, it did not have any net positive or adverse effect on PESP. At baseline, a reduction in the paced cycle length for a single beat, to 60% of baseline, resulted in a 17.7 ± 2.8% (range -12.4 to 25.4) increase in the strength of the post extra-systolic beat. Following digoxin, the increase was 17.6 ± 3.3% (range -13.8 to 39.2; p=ns vs baseline). As a result, the strength of the potentiated beat at 60% of the baseline paced cycle length increased from 119.1 ± 2.1% to 128.8 ± 2.9% (p=0.0036) following the administration of digoxin.
Comparison of the effects of Digoxin and Milrinone on the Left Ventricular Force-Interval Relationship

As data was available for the phosphodiesterase III inhibitor, milrinone, in addition to the above data for digoxin, the opportunity was taken to compare these 2 non-catecholamine positive inotropes. The comparison was limited to effects on MRC and FP.

Of 26 patients studied, 14 received digoxin and 12 milrinone. The baseline characteristics of these patients are summarized in Table 3.8. The majority of patients had well preserved left ventricular systolic and diastolic function: 12/14 digoxin and 12/12 milrinone treated patients had a left ventricular ejection fraction of > 50 %. Eleven of the 26 patients had no haemodynamically significant coronary stenoses. There were no significant differences between groups as regards any haemodynamic parameters at baseline. The procedure was well tolerated in all patients.

The relative effects of digoxin and milrinone on resting haemodynamic and ECG parameters are recorded in Table 3.9. Both agents induced significant increases in LV+\(\frac{dP}{dt}\)\text{max} by 12.2 ± 1.3 % for digoxin and 11.4 ± 3.2 % for milrinone. Digoxin caused a small but significant increase in mean arterial pressure (MAP) but was without effect on either cardiac index (CI) or systemic vascular resistance index (SVRI). The QT interval was modestly shortened by digoxin, but resting spontaneous heart rate was unchanged (determined during transient interruption of baseline pacing). Conversely, milrinone decreased MAP (by 5.4 ± 1.2 mmHg, \(p < 0.001\)), consistent with its vasodilator properties. This was accompanied by decreases in both left ventricular end diastolic pressure (LVEDP) and SVRI, and an increase in spontaneous heart rate (by 4.7 ± 1.7 beats/min, \(p < 0.05\)).

The influence of both drugs on the force-interval relationship, in terms of both changes in FP and the MRC, is illustrated in Figures 3.25 and 3.26. The positive inotropic effect of digoxin was marginally attenuated at both 10 (FP\text{10 seconds}) (p=NS) and 60 seconds (FP\text{60 seconds}) (p=NS) after the commencement of rapid atrial pacing.
relative to baseline (Figure 3.25a). Similarly, MRC's obtained in the presence and absence of digoxin tended to remain parallel at progressively shorter cycle lengths (Figure 3.25b), as reflected by no significant change in the parameter 'c' ($\delta'c' = 3.6 \pm 3.7\%$, $p = \text{NS}$); thus the calculated positive inotropic effect of digoxin was $7.4 \pm 4.2\%$ at 60% of baseline cycle length ($p=0.1$); $p = \text{NS}$ vs digoxin effects at baseline cycle length.

In the case of milrinone, there was again a non-significant attenuation of positive inotropic effect during rapid atrial pacing (Figure 3.26a). During MRC construction, the positive inotropic effect was completely abolished at short cycle lengths (Figure 3.26b), with some suggestion of a net negative inotropic effect at the shortest RR intervals studied ($-6.8 \pm 5.9\%$; albeit nonsignificant). The parameter 'c' was increased by $19.5 \pm 7.6\%$ ($p < 0.05$), indicating a significant change in the shape of the MRC, the extent of inotropic effect of milrinone varying significantly and inversely with cycle length ($-17.5 \pm 7.9\%$; $p=0.05$). There was no correlation between the change in 'c' following the administration of milrinone and any baseline haemodynamic parameters measured, or the effects of the drug on MAP or SVRI.

The residual standard deviations of the MRC model (the measure of goodness-of-fit) for the two drugs studied were 7.00 (95%CI; 5.14 to 8.85) and 7.89 (95%CI; 3.86 to 11.91) for digoxin and milrinone respectively.
Table 3.8  Baseline characteristics of patients receiving positive inotropes

<table>
<thead>
<tr>
<th></th>
<th>Digoxin (n=14)</th>
<th>Milrinone (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>63 ± 3</td>
<td>58 ± 3</td>
</tr>
<tr>
<td><strong>Male/Female</strong></td>
<td>6/8</td>
<td>12/0</td>
</tr>
<tr>
<td><strong>Coronary disease</strong></td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>LV ejection fraction (%)</strong></td>
<td>68 ± 4</td>
<td>65 ± 2</td>
</tr>
<tr>
<td><strong>Mean PCWP</strong></td>
<td>7.8 ± 1.2</td>
<td>7.7 ± 1.0</td>
</tr>
</tbody>
</table>

Coronary disease = number of major epicardial vessels with >50% luminal stenosis

Table 3.9  Haemodynamic and ECG effects of digoxin and milrinone at baseline paced cycle length

<table>
<thead>
<tr>
<th></th>
<th>Digoxin (n=14)</th>
<th>Milrinone (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>109 ± 5</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>Post</td>
<td>115 ± 4†</td>
<td>101 ± 3†</td>
</tr>
<tr>
<td><strong>LV+dP/dt_{max}(mmHg/s)</strong></td>
<td>1550 ± 70</td>
<td>1480 ± 90</td>
</tr>
<tr>
<td>Pre</td>
<td>1740 ± 80‡</td>
<td>1670 ± 130**</td>
</tr>
<tr>
<td>Post</td>
<td>1480 ± 90</td>
<td>1670 ± 130**</td>
</tr>
<tr>
<td><strong>LVEDP (mmHg)</strong></td>
<td>13 ± 2</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Pre</td>
<td>14 ± 2</td>
<td>13 ± 2*</td>
</tr>
<tr>
<td>Post</td>
<td>14 ± 2</td>
<td>13 ± 2*</td>
</tr>
<tr>
<td><strong>CI (L/min/m²)</strong></td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>Pre</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Post</td>
<td>2.7 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td><strong>SVRI(dynes.sec.cm⁻⁵/m²)</strong></td>
<td>3460 ± 230</td>
<td>3440 ± 300</td>
</tr>
<tr>
<td>Pre</td>
<td>3580 ± 250</td>
<td>3030 ± 220**</td>
</tr>
<tr>
<td>Post</td>
<td>3440 ± 300</td>
<td>3030 ± 220**</td>
</tr>
<tr>
<td><strong>PR interval (msec)</strong></td>
<td>185 ± 5</td>
<td>215 ± 5</td>
</tr>
<tr>
<td>Pre</td>
<td>190 ± 5</td>
<td>210 ± 5</td>
</tr>
<tr>
<td>Post</td>
<td>190 ± 5</td>
<td>210 ± 5</td>
</tr>
<tr>
<td><strong>QT interval (msec)</strong></td>
<td>395 ± 10</td>
<td>375 ± 5</td>
</tr>
<tr>
<td>Pre</td>
<td>385 ± 10**</td>
<td>375 ± 5</td>
</tr>
<tr>
<td>Post</td>
<td>385 ± 10**</td>
<td>375 ± 5</td>
</tr>
<tr>
<td><strong>Cycle length (msec)</strong></td>
<td>895 ± 45</td>
<td>870 ± 45</td>
</tr>
<tr>
<td>Pre</td>
<td>885 ± 45</td>
<td>815 ± 45*</td>
</tr>
<tr>
<td>Post</td>
<td>885 ± 45</td>
<td>815 ± 45*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, †p<0.001, ‡p<0.000001 vs pre-drug
Figure 3.25  Digoxin effects on (a) FP and (b) MRC

(a)

\[
\text{LV+\text{dP}/\text{d}t_{\text{max}}}(\% \text{baseline})
\]

- **Baseline**
  - \(FP_{10 \text{ sec}}\%\): 18.5 ± 4.5
  - \(FP_{60 \text{ sec}}\%\): 23.1 ± 4.5

- **Digoxin**
  - \(FP_{10 \text{ sec}}\%\): 7.4 ± 5.3
  - \(FP_{60 \text{ sec}}\%\): 15.8 ± 3.8

\(p = \text{ns}\)

(b)

**Pooled MRC fitted data**

- **Baseline**
  - \(c' = 22.9 \pm 3.2\)
  - \(p = \text{ns}\)

- **Digoxin**
  - \(c' = 26.5 \pm 4.9\)
  - \(p = \text{ns}\)

\[
\text{RR Interval (\% baseline)}
\]
Figure 3.26 Milrinone effects on (a) FP and (b) MRC

(a)

![Graph showing FP effects](image)

(b)

![Graph showing MRC effects](image)
DETERMINANTS OF EXTENT OF FREQUENCY POTENTIATION

It is evident from the extent of data presented thus far that a significant number of patients have had the left ventricular force-interval relationship investigated in terms of both frequency potentiation and mechanical restitution curve construction. This database has been used to investigate the determinants of extent of frequency potentiation experienced with the onset of pacing induced tachycardia.

Complete data were available for 50 patients and the characteristics of this group are displayed in Table 3.10. Univariate analysis was performed in order to identify baseline characteristics that correlated with the extent of FP at either 10 or 60 seconds. Multivariate analysis was then performed to determine which factors, if any, were independent predictors of extent of FP.

Table 3.10  Patient Characteristics prior to FP determination

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Coronary Disease</td>
<td>1.0 ± 0.1</td>
<td>0 to 3</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>68.7 ± 1.6</td>
<td>36 to 93</td>
</tr>
<tr>
<td>Cardiac Index (L/min/m²)</td>
<td>2.70 ± 0.08</td>
<td>1.35 to 4.27</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>106 ± 2</td>
<td>68 to 135</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>7.9 ± 0.5</td>
<td>1 to 18</td>
</tr>
<tr>
<td>LV+dP/dtₘₐₓ (mmHg/sec)</td>
<td>1600 ± 50</td>
<td>940 to 2800</td>
</tr>
<tr>
<td>Rapid Pace Cycle Length(%)</td>
<td>65.2 ± 1.0</td>
<td>55.6 to 87.3</td>
</tr>
<tr>
<td>'c' (%)</td>
<td>23.4 ± 2.2</td>
<td>-1.8 to 57.8</td>
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</tbody>
</table>

Coronary Disease = Number of major epicardial vessels with >50% luminal stenosis
LVEF = Left ventricular ejection fraction
MAP = Mean arterial pressure
PCWP = Mean pulmonary capillary wedge pressure
Rapid Pace Cycle Length = Percentage of baseline paced cycle length
The onset of rapid atrial pacing produced an increment in LV+dP/dt_max of 18.1 ± 2.0% (range -14.6 to 48.1; p<0.0001) within 10 seconds, the increment being 20.4 ± 2.2% (range -15.2 to 52.5; p<0.0001) at 60 seconds (p=ns vs 10 seconds). Univariate analysis to determine correlates of FP_{10} and FP_{60} revealed that there were 2 determinants at 10 seconds and 3 at 60 seconds (Figures 3.27 – 3.29).

Figure 3.27  Significant correlates of FP_{10} (a) Coronary Disease and (b) ‘c’

![Coronary Disease](image1)

![‘c’](image2)
Figure 3.28  Significant correlates of FP60 (a) Coronary Disease and (b) ‘c’

(a) 

Coronary Disease

FP60 (%)

r = -0.339  
p = 0.017

(b) 

'c' (%)

FP60 (%)

r = -0.354  
p = 0.012
Increasing values of both ‘c’ and extent of fixed coronary artery disease significantly decreased the FP response at both 10 and 60 seconds following the onset of tachycardia. An elevated pulmonary capillary wedge pressure also reduced the response to tachycardia but only after 60 seconds. Left ventricular ejection fraction was not a predictor.

Multivariate analysis was then performed in order to determine factors that were independently predictive of response to tachycardia. Dependent variables included in the analysis were; left ventricular ejection fraction, cardiac index, mean arterial pressure, pulmonary capillary wedge pressure, extent of fixed coronary disease and ‘c’. Analysis revealed that the only significant predictor under these circumstances was ‘c’, for both $FP_{10}$ (beta = -0.367, p = 0.019) and $FP_{60}$ (beta = -0.307, p = 0.047). No other variables approached significance, indicating that of all available patient characteristics, ‘c’ is the only factor to predict the subsequent response to the induction of tachycardia.
SUMMARY OF MAJOR FINDINGS
Acute drug effects on the left ventricular force-interval relationship were investigated for a wide range of commonly used cardioactive drugs. In brief, the major findings from these investigations were:

(1) Perindoprilat exerts no significant effect on mechanical restitution or frequency potentiation but reveals a borderline trend towards increasing the potentiation of post extrasystolic beats.

(2) Enalaprilat exerts no significant effect on mechanical restitution, frequency potentiation or post extrasystolic potentiation

(3) Verapamil markedly accentuates the negative inotropic effect observed at baseline paced cycle length, at short cycle lengths, as evidenced by both MRC construction and FP. There was no effect of verapamil on PESP.

(4) Comparison of verapamil, sotalol and metoprolol reveals that despite these agents having similar negative inotropic effects at baseline paced cycle lengths, they exhibit widely disparate effects on the FIR. Data gathered for sodium nitroprusside confirm that this observation is not due to differential effects on loading conditions.

(5) Digoxin induces no significant change in MRC or FP (although there was a modest, but non-significant decrease in LV+dp/dt_max at 10 seconds) and no change in PESP. This indicates that the significant positive inotropic effect of digoxin observed at baseline paced cycle length, is maintained at short cycle lengths.

(6) Comparison of digoxin and milrinone effects on the FIR reveal that haemodynamic effects cannot always be predicted on the basis of changes observed at rest.

(7) Incomplete mechanical restitution curve construction *in vivo* in humans is a significant and independent predictor of the FP response.
DISCUSSION

NEGATIVELY INOTROPIC AGENTS

Of the 6 agents examined, 5 revealed negative inotropic effects in the absence of tachycardia, the exception being sodium nitroprusside. Both perindoprilat and enalaprilat exerted significant negative inotropic effects as evidenced by the range of haemodynamic changes induced. Verapamil, metoprolol and sotalol also all induced significant negative inotropic effects at rest, this being clearly evident as reductions in LV+dP/dt_{max}.

Ace Inhibitors

Neither perindoprilat nor enalaprilat was observed to have any significant impact on the left ventricular force-interval relationship. Although the major haemodynamic effect of these agents was negative inotropy, there was no significant additional effect on the force-interval relationship.

Perindoprilat exerted a borderline effect on PESP, tending to increase this response, whereas enalaprilat had no effect at all. Clearly this is not an effect with a large magnitude and additional numbers of subjects may not necessarily see this effect become significant. However, it is appropriate to pause and consider whether the local effects of perindoprilat on bradykinin catabolism within the heart are exerting a small positive inotropic effect via nitric oxide. This effect could well be frequency dependent given that it is via cyclic GMP and most likely to exert its effect via the sarcoplasmic reticulum (Finkel, Oddis et al. 1995). Another possible interpretation of this borderline increase in PESP response would be that it reflects abolition of underlying myocardial ischaemia by perindoprilat. This cannot be ascertained in the current model, but is relatively unlikely given that most patients had no biochemical evidence on coronary myocardial metabolic data for myocardial ischaemia under conditions of baseline pacing.
Verapamil

The interaction between the negative inotropic effects of verapamil and the left ventricular FIR were examined in 17 patients. On MRC analysis, the negative inotropic effect of verapamil was accentuated at shorter RR intervals. The rate-dependent nature of the negative inotropic effects of verapamil have been clearly defined in animal models in vitro (Ehara and Daufmann 1978; Chappell, Henderson et al. 1985; Borzak, Murphy et al. 1991). For example, the extent of slow calcium channel blockade by verapamil is markedly increased at faster stimulation frequencies in voltage clamp studies (Ehara and Daufmann 1978). However, there are no previously available data of this type for verapamil or any other calcium antagonist in humans. Clinical experience with the acute administration of verapamil has suggested the potential for acute hypotensive and negative inotropic sequelae (Buxton, Marchlinski et al. 1987; Rankin, Rae et al. 1987; Sharma, Purves et al. 1990), a complication that is rarely observed in the absence of tachyarrhythmias.

The effects of verapamil on PESP and during FP were also examined. During FP, the incremental negative inotropic effects of verapamil were again confirmed, although after 60 seconds of pacing this difference was no longer statistically significant, possibly due to progressive neurohumoral activation or variable induction of ischaemia. Verapamil did not induce any changes in the extent of PESP. This is consistent with the fact that the extent of PESP is largely determined by SR calcium content and release. Even in the presence of L-type calcium channel blockade, activator calcium via the sodium-calcium exchanger is able to trigger SR calcium release. The observation that PESP is independent of verapamil effects is therefore consistent with this.

Verapamil vs Metoprolol and Sotalol

The impact of verapamil on the left ventricular FIR was compared to that of two other agents with similar degrees of negative inotropy at resting heart rates. The results demonstrate that the negative inotropic effects, during tachycardia, of a
pharmacologically heterogeneous group of cardioactive drugs cannot be predicted on the basis of observed effects in the resting state. Metoprolol, sotalol and verapamil exerted markedly different negative inotropic effects at shorter RR intervals, as determined from both MRC construction and FP analysis. The negative inotropic effects of verapamil were potently and directly rate-dependent, while the effects of sotalol appeared relatively independent of RR interval, on both MRC and FP analysis. The negative inotropic effects of metoprolol varied inversely with rate on MRC and were unchanged on FP analysis. The finding that metoprolol exerts negative inotropic effects which are largely abolished at short cycle lengths is of considerable importance, and reflects an area which has not been investigated extensively in the past. The data in the current large series of patients are consistent with those previously examined in a preliminary study of metoprolol effects (Ritchie, Wuttke et al. 1995), while more recently Anderson et al have provided additional data to the effect that metoprolol (during chronic therapy) is likely to improve the force-interval relationship in patients with impaired left ventricular systolic function (Andersson, Stromblad et al. 1999). In the later study, acute effects of metoprolol on the force-interval relationship were not examined. Furthermore, previous studies with β-adrenoceptor agonists have raised the possibility of reverse rate-dependent positive inotropic effects, again consistent with the findings for metoprolol in the current study (Schwinger, Bohm et al. 1993).

The fact that sotalol’s effects were not identical with those of metoprolol is most likely to reflect additional interactions between its d-enantiomer (responsible for prolongation of repolarization) and the process of mechanical restitution. While reverse rate-dependent effects of sotalol on cellular electrophysiology have been described (Kovoor, Byth et al. 1996), no previous investigations analogous with the current study have been reported, and it has been assumed generally that fluctuations in K⁺-channel activity are associated with minimal changes in inotropic state. This important issue would best be delineated by performing separate investigations with both d-sotalol (to minimize concomitant β-adrenoceptor
blockade) and possibly also with K⁺-channel opening drugs, in order to see whether these exert opposite effects to those of d-sotalol.

The doses of metoprolol and sotalol utilized in the present study induced 12.3 ± 0.75% and 10.5 ± 2.7% reductions in LV+dP/dt max respectively during baseline pacing, consistent with previous investigations in humans (Hutton, Lorimer et al. 1972; Bourdillon, Canepa-Anson et al. 1979; Dell'Italia and Walsh 1989; Ritchie, Wuttke et al. 1994; Ritchie, Sallustio et al. 1998). However, the negative inotropic effect of metoprolol is reverse rate-dependent on MRC analysis. Conversely, sotalol did not significantly influence MRC: indeed a nonsignificant trend for ‘c’ to increase was observed (p = 0.075). Data regarding the influence of agents that interact with β-adrenoceptors (agonists or antagonists) on the force-interval relationship are limited, although there is some suggestion that β-adrenoceptor agonists exhibit reverse rate-dependent positive inotropic effects (Anderson, Rankin et al. 1976; Anderson, Manring et al. 1977; Pidgeon, Lab et al. 1980; Schwinger, Bohm et al. 1993; Mizutani, Kobayashi et al. 1994). Furthermore, reverse rate-related electrophysiological effects of d,l-sotalol have been described (1989; Lathrop, Varro et al. 1989; Huikuri and Yli-Mayry 1992; Kovoor, Byth et al. 1996).

Clinically, metoprolol is well-tolerated in the setting of tachycardia, can be safely administered after acute myocardial infarction, and there is now strong evidence for haemodynamic safety of β-adrenoceptor antagonists in patients with significant impairment of left ventricular systolic function, the group most prone to haemodynamic compromise during tachycardia (Boyle, Barber et al. 1983; Ryden, Ariniego et al. 1983; 1985). Sotalol is used primarily in the management of arrhythmias (atrial/ventricular) in patients with ischaemic heart disease, and has been relatively well-tolerated in such patients (Lloyd, Charles et al. 1988; 1989; Mason 1993). However, the recent finding of increased mortality in patients with symptomatic impairment of left ventricular systolic function associated with d-sotalol administration (Waldo, Camm et al. 1995) has raised residual mechanistic
issues. It is possible that arrhythmogenesis and/or haemodynamic deterioration contributed to this result.

The second quantitative measure of drug effects on the force-interval relationship was the comparison of pacing-induced increase in \( LV+\text{d}P/\text{d}t_{\text{max}} \) before and after drug administration in each patient, at 10 and 60 sec. At 10 sec after the induction of rapid pacing, there were widely disparate effects of the agents studied on this ratio. Metoprolol had no detectable effect, while verapamil significantly (and sotalol to a lesser, nonsignificant extent) impaired the FP response. This was no longer significant after 60 sec, probably as a result of neurohumoral activation (a protective countermeasure) (Twidale, Rayner et al. 1993) and/or progressive induction of ischaemia in some patients (Linhart, Hildner et al. 1969). Thus the results of the frequency potentiation experiments are consistent with those of MRC construction, with use-dependent negative inotropic effects demonstrated only for verapamil. On the other hand, it is recognised that data from the FP experiments are complex mechanistically (Wood, Allen et al. 1975), with elements of mechanical restitution partially obscured by the positive staircase phenomenon. Furthermore, in the majority of patients currently studied, there was a significant risk of induction of ischaemia. For these reasons, data from FP experiments probably reflect a less 'pure' representation of the rate-related pharmacological effects of the drugs examined. Their importance is largely confined to the demonstration of net changes in cardiac performance, occurring with sustained tachycardia, in the presence of the agents utilized, while recognising the multifactorial determinants of this performance.

As regards the basic cellular mechanisms reflected in the observed changes in MRC and FP, transsarcolemmal calcium flux appears to become increasingly important for contractile performance at increased stimulation frequencies: calcium derived from the sarcoplasmic reticulum (demonstrated by sensitivity to ryanodine) is more critical at lower stimulation frequencies. This hypothesis has resulted from studies utilizing predominantly \textit{in vitro} animal models (Chappell, Henderson et al. 1985; Wier and Yue 1986; Cooper and Fry 1990; Borzak, Murphy et al. 1991). More recently
it has been suggested that such a frequency-dependent regulation of cardiac contractility may be attributable to upregulation of calcium entry through the sarcolemmal voltage-gated calcium channels at increased stimulation frequencies in human cardiomyocytes (Piot, Lemaire et al. 1996). Thus, the diverse effects of metoprolol and verapamil at shorter RR intervals observed in the current study may simply reflect different sites of action on frequency-dependent cellular physiology. The interaction between verapamil and the L-type calcium channel per se is frequency-dependent: the drug binds preferentially to its receptor when the channel is in the open state (Chappell, Henderson et al. 1985). No analogous cellular phenomenon has been observed for metoprolol, other β-adrenoceptor antagonists, or indeed β-adrenoceptor agonists. Possibly, the basis for differences in effects of metoprolol and sotalol in the present study is related to the latter’s modulation of the outward delayed rectifier potassium current (Carmeliet 1985; Varro, Nanasi et al. 1991; Connors, Gill et al. 1992), although no information is available regarding the potential for d- and l-sotalol isomers to exert differential effects on intracellular calcium metabolism. However, it is also possible that metoprolol and sotalol may vary as regards their antagonism of β3-adrenoceptor receptors in myocardium, a process that may considerably modify the net negative inotropic effects of β-adrenoceptor antagonists (Gauthier, Tavernier et al. 1996).

Recent data from normal guinea-pig ventricular myocytes (Money-Kyrle, Davies et al. 1998) have suggested that changes in cyclic AMP concentrations induced by variation in β-adrenoceptor stimulation may have a relatively minor effect on the force-interval relationship in the absence of cardiomyopathic changes, the main determinant of the positive staircase being likely to be activity of sarcoplasmic reticulum ATPase (SERCA 2a) controlling uptake of calcium into sarcoplasmic reticulum. The results of the current studies with metoprolol could in theory reflect not only decreases in cAMP concentrations but also secondary changes at the level of SERCA 2a/phospholamban activity. This cannot be delineated in the absence of specific probes for sarcoplasmic reticulum calcium kinetics, which have not been utilized in human experiments.
Sodium Nitroprusside

Sodium nitroprusside exerted no significant effects on MRC, suggesting that the parameter 'c' is relatively independent of small changes in preload and afterload. Although the number of patients studied in this regard was small, the lack of any variation in 'c' despite a 10% fall in mean arterial pressure and a modest reduction in mean pulmonary capillary wedge pressure, indicate that there are no substantial effects, although a less marked impact can not be excluded on the basis of these data.

POSITIVELY INOTROPIC AGENTS

Digoxin

In the case of digoxin, no significant change in inotropic effect with variations in cycle length was found. Digoxin binds to an inhibitory site on the α subunit of sodium, potassium adenosine triphosphatase (Na,K-ATPase) and, by decreasing sodium-calcium exchange, increases intracellular calcium, resulting in positive inotropy. Previous studies have indicated that the positive inotropic effect of another cardiac glycoside, ouabain, is somewhat attenuated with decreasing cycle length in rabbit papillary muscle in vitro (Morner and Wohlfart 1990). However, the interaction of the sodium-calcium exchange mechanism with fluctuation in cycle length has not been specifically examined. Furthermore, our results suggest that the positive inotropic effect of digoxin is essentially independent of cycle length in patients with well-preserved systolic function. This has been determined using all three methodologies employed.

This 'negative' finding is of key importance. Digoxin is an established therapy for patients with heart failure and has been shown to exert significant improvement in morbidity for this condition (although there has been no impact on mortality), when added to conventional therapy, inclusive of an ACE inhibitor (1997). Given that symptoms in heart failure usually arise either during exercise or during cardiac crises, the ability of digoxin to maintain its positive inotropic effect during tachycardia is consistent with this observation. Thus, either the sodium-calcium
exchange mechanism is equally important at short and long stimulation intervals, or alternative positive inotropic mechanisms may exist, such as increased calcium entry through slow calcium channels (Anderson, Manring et al. 1977).

Digoxin vs Milrinone

The interaction of digoxin with the left ventricular FIR was compared with milrinone, an inhibitor of phosphodiesterase III, which is known to produce a similar positive inotropic effect at basal heart rate. The finding of the current investigation, that the positive inotropic effect of milrinone can be shown to be attenuated at short cycle lengths, has not been anticipated on the basis of previous clinical observations. However, in vitro studies provide both a potential rationale for these observations, and indeed partial supportive evidence. The positive inotropic effect of milrinone is mediated through increased levels of cyclic 3,5,-adenosine monophosphate (cAMP), via inhibition of phosphodiesterase III. This results in protein kinase A-dependent phosphorylation of phospholamban, increasing both sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA)2a affinity for calcium and the maximum velocity of calcium uptake (Kargacin, Ali et al. 1998). Whilst the sarcoplasmic reticulum is an important store for the clearance of calcium during diastole, its role in releasing calcium to the contractile apparatus during systole is relatively reduced, compared to transsarcolemmal calcium, at short stimulation intervals (Borzak, Murphy et al. 1991): this is consistent with the progressive attenuation of the positive inotropic effect of milrinone at short cycle lengths. Furthermore, the positive inotropic effects of both milrinone, and its structurally similar predecessor amrinone, become less marked with reductions in cycle length in isolated guinea pig papillary muscles (Alousi and Johnson 1986; Morner and Wohlfart 1990). The data with milrinone should also be compared with the analogous results for metoprolol. In both cases, the results suggest that cAMP-mediated inotropic effects are most important at relatively long cycle lengths in the 'normal' human heart, irrespective of secondary changes in SERCA 2a activity. These results are slightly at odds with those of Money-Kyrle et al in guinea-pig myocytes.
(Money-Kyrle, Davies et al. 1998). One possibility is that the interaction of cAMP with SERCA 2a activity may be (effectively) frequency dependent. This should be explored in appropriate in vitro models.

The observed interaction between the positive inotropic effects of milrinone and the left ventricular FIR is consistent with the experience for this agent during chronic therapy for heart failure, which has been disappointing (Packer, Carver et al. 1991). Indeed the adverse long term effects of milrinone therapy for heart failure were not anticipated on the basis of observations made during acute administration of the drug, although these investigations involved examination at resting heart rates only. While the effects of milrinone on the FIR may be responsible for reduced efficacy of this agent during chronic therapy, the mechanism of adverse effects has not been investigated and may potentially include such factors as arrhythmogenesis.

The current investigation of milrinone was performed in patients with largely well preserved systolic function. It has been proposed that the loss of the positive inotropic effect of milrinone at short cycle lengths is due to progressive loss of SR calcium importance with reducing cycle lengths of stimulation. It is not therefore possible to predict the effect in patients with heart failure, either acute or chronic, given that this is a situation of relative SR failure. Indeed the inotropic effects of milrinone in the heart failure population have been disappointing, largely due to a deficiency of cyclicAMP in this group, a factor not corrected by the administration of milrinone (Perreault, Shannon et al. 1992).

DETERMINANTS OF FP

MRC construction was the primary methodology utilized in the current study for the quantitative examination of drug effects on the force-interval relationship. It has previously been demonstrated that this yields highly reproducible data. Significant determinants of the parameter 'c' are RR interval (held constant in all investigations) and left ventricular ejection fraction. Where possible, sustained tachycardia was also
examined in these patients with the intention of seeking the determinants of FP. This component of the experimental protocol was not performed in patients with severe angina and was precluded by the development of atrio-ventricular block in some patients.

The examination of sustained tachycardia in this type of patient population is limited by the potential for neurohumoral activation and variable induction of ischaemia. However, in the current investigation, no difference between the extent of FP at 10 and 60 seconds was demonstrated (2.2 ± 1.6% increase), although this may be consistent with the variable nature of these factors. The examination of sustained tachycardia in other populations is also potentially limited. For example, in patients with significant impairment of systolic function the FIR may be reversed, giving potential for significant haemodynamic deterioration during tachycardia. It would therefore be useful to have an alternative methodology for examining the left ventricular FIR that avoided induction of these potentially confounding factors. On the basis of MRC construction being highly reproducible in human subjects and the minimal disturbance in homeostasis induced by data collection, potential determinants of FP were sought.

The FP response at 10 seconds was significantly correlated with both extent of coronary disease and ‘c’, both of these parameters remaining correlated at 60 seconds, with the addition of a weak but significant correlation of pulmonary capillary wedge pressure. However, multivariate analysis revealed that ‘c’ was the only parameter to remain significantly predictive of the FP response at both 10 and 60 seconds. This finding is of potential clinical importance as regards assessment of the FIR, offering a safe tool for examining this parameter in any patient undergoing invasive haemodynamic assessment.

The cellular physiology underlying MRC and FP while having different descriptions do share some similarities. They can be considered as distinct measures of the ability to tolerate either a single or a series of extrasystoles. The finding that ‘c’ is an
independent correlate of the FP response suggests that this may provide a sensitive measure of the FIR. This coupled with its high reproducibility makes it an ideal tool for examining acute drug effects on the FIR. Indeed, the acute data gathered in the current investigations, when compared with the existing knowledge regarding the tolerability of the various agents during chronic therapy suggest that MRC may be a more sensitive index of effects on the FIR than FP.
CHAPTER 4: CONCLUSIONS, RECOMMENDATIONS AND FUTURE STUDIES

This series of studies has yielded a number of important results. First and foremost, it again highlights the importance of gaining detailed in vivo data in humans via invasive testing. Although these procedures are difficult and labour intensive, the information obtained is novel and apparently clinically relevant. Despite this, there remain a number of limitations that must be considered in interpreting the data presented below.

CONCLUSIONS

Chapter 2

Acute ACE inhibitor administration is associated with negative inotropic effects which are small in patients with good left ventricular systolic function, as well as with inhibition of intracardiac ACE and, with perindoprilat, augmentation of vasodilator reserve.

Chapter 3

Of the negatively inotropic agents examined (perindoprilat, enalaprilat, verapamil, metoprolol and sotalol), verapamil and metoprolol exhibited rate-related effects (which were contrasting).

This was also the case with the positive inotrope milrinone, but probably not digoxin.

Sodium nitroprusside exerted no significant inotropic effects at spontaneous or rapid heart rates.

The MRC was correlated with extent of FP. Extent of FP also reflects severity of coronary artery disease and mean pulmonary capillary wedge pressure.

These conclusions may be impacted upon by a number of potential limitations.
LIMITATIONS

The acute myocardial uptake of the drugs studied in the current series of experiments was examined only for perindoprilat and enalaprilat. While there are reliable previous data regarding the uptake of the other drugs studied, it has been assumed that the current patient population is similar to those previously reported (Powell, Horowitz et al. 1990; Powell, Horowitz et al. 1990). It is apparent that the haemodynamic observations made are very similar to those reported during the original acute uptake experiments and this is likely to reflect similar pharmacodynamics. The period of observation of acute drug effects is limited by the invasive nature of the experiments. In some cases e.g. digoxin, peak haemodynamic effects appear at the end of the study period. It is not known whether this represents peak drug effect or if incremental action would be apparent with increased duration of experimentation i.e. what is the extent of hysteresis. Furthermore, if an adverse effect is uncovered e.g. verapamil effects on the force-interval relationship, it is not clear if this represents a transient or sustained effect.

None of the currently reported studies had any formal sizing calculations performed. This raises the issue that some of the experiments may have been negative via type II error. For example, the lack of significant reduction in LV+dP/dt_{max} with perindoprilat, while all other perindoprilat and enalaprilat data indicate negative inotropy.

A number of haemodynamic, metabolic and biochemical observations were made following the administration of perindoprilat and enalaprilat. Of necessity, these observations were made without recourse to the acute uptake of these agents or knowledge of the hysteresis of effects. The induction of tachycardia was repeated 12 to 15 minutes following drug administration in both cases. The peak haemodynamic effects of perindoprilat were apparent at 4 to 6 minutes whereas those of enalaprilat were apparent at 15 minutes. The induction of tachycardia was therefore performed at a time of maximal effect for enalaprilat but not necessarily for perindoprilat,
although the early haemodynamic effects observed for perindoprilat did tend to persist. Serial examination of induction of tachycardia following drug administration was not performed.

Coronary arterial blood flow was not measured, rather using the surrogate measure of coronary sinus blood flow. While this provides highly reproducible measures of flow (the cv for replicate measures in the current study being 4.3%), it remains dependent on maintaining catheter position and assumes that regional coronary blood flow remains proportional to total coronary blood flow throughout the range of experimental conditions. It must be emphasized in this regard that the techniques utilized for measuring myocardial drug uptake are inadequate for determination of regional heterogeneity of uptake, such as has previously been reported in association with myocardial ischaemia (Siegel, Fealy et al. 1987; Rehr, Fuhs et al. 1991). Furthermore, the studies evaluated both uptake and effects during stable heart rates. It has previously been shown that uptake is markedly increased for most drugs in the presence of tachycardia both in tissue models and in man (Busse, Lullman et al. 1979; Horowitz and Powell 1986): this might also have enhanced acute drug effects which would be particularly relevant for negatively inotropic agents.

The index of contractility for the current study was peak left ventricular isovolumic contraction LV+\(\frac{dP}{dt_{\text{max}}}.\) This marker is relatively insensitive to small changes in afterload but is highly responsive to variations in heart rate (Mahler F 1975). This latter aspect was controlled for by performing all experiments under conditions of atrial pacing at a fixed cycle length.

The collection of blood samples through a long catheter such as the coronary sinus catheter has the potential to cause activation of bradykinin. Furthermore, the siting of the coronary sinus catheter has the potential to cause localized trauma within the coronary sinus, again giving rise to potential activation of bradykinin, artifactualy elevating levels in comparison to the femoral artery. In recognition of these factors, a number of preliminary experiments were performed to exclude the potential for
these problems to occur. No significant generation of bradykinin was caused by the withdrawal of blood samples through a long catheter versus a short catheter (femoral arterial sheath). However, initial coronary sinus bradykinin levels were increased compared to right atrial samples and tended to fall over time. It became apparent that the measurement of a coronary sinus flow, which involved the infusion of room temperature 5% Dextrose into the coronary sinus, rapidly and permanently restored bradykinin levels to 'normal'. Therefore, sampling for bradykinin peptides from the coronary sinus was always performed following the initial measurements of coronary sinus blood flow. Nevertheless, an impact of artefactual bradykinin generation cannot be excluded. However, the fact that drug effects on bradykinin peptides during the uptake phase of the experiment were identical to effects observed during tachycardia (the initial tachycardia data being those most susceptible to artifact) suggests that the effects observed are representative of the true situation.

There remains a possibility that some haemodynamic effects were due to the experimental procedure itself. All experiments were performed with the patient having fasted for some hours. Furthermore, the protocol called for the sampling of in excess of 200mls of blood. These factors may have combined to induce a greater extent of counter-regulatory responses than might otherwise be observed in a well hydrated patient. However, the techniques employed in performing the various haemodynamic measures, including thermodilution flow measurements, resulted in the administration of approximately 500 mls of fluid, minimising the potential for a significant impact.

The experiments performed on the force-interval relationship involved a single decrease in cycle length for examination of frequency potentiation, rather than construction of a full 'staircase'. This was necessary due to the population of ischaemic patients being studied. Extreme tachycardia could not therefore be examined.
While there was some diversity amongst the patient cohort, this was essentially a study of patients with intact left ventricular systolic function. It is possible that a cohort of patients with impaired systolic function (and therefore likely to have impaired baseline force-interval relationships and counter-regulatory responses) would produce different results with respect to the robustness of the relationship between MRC and FP data. Such patients may exhibit an underlying defect of intracellular calcium availability, the consequences of which would increase progressively as heart rate increased (Hasenfuss, Holubarsch et al. 1994; Pieske, Sutterlin et al. 1996; Hasenfuss 1998). Furthermore, although it has been postulated that progressive induction of ischaemia and counter-regulatory neurohumoral activation is the basis for the observed attenuation of FP effects between 10 and 60 sec following the onset of rapid pacing, the actual mechanisms underlying this observation was not investigated.

The current study did not enable the examination of potential correlation between changes in the force-interval relationship and simultaneous evaluation of cellular electrophysiology: this additional information is difficult to accurately obtain in vivo in humans. Nevertheless, it is apparent that this information might provide additional insight.

**RECOMMENDATIONS FOR FUTURE STUDIES**

**Force-interval relationship**

The investigation of the left ventricular force-interval relationship utilizing MRC construction is ready to evolve to the next level. This methodology should be prospectively applied to the investigation of new agents coming to the market. This will allow the more detailed evaluation of the implications of inotropically active treatment before large mortality studies are conducted in tachycardia prone individuals. Subsequent long term studies, if considered safe, could then be
performed to test the correlation between acute observations and those made during longer term therapy.

The findings regarding drug effects on the force-interval relationship have implications also for bradycardia-prone individuals, such as with sick sinus syndrome. It is possible that the usual beneficial effects of some agents, e.g. metoprolol, may be compromised by the presence of exaggerated negative inotropic effects at very slow heart rates. This may have implications for the choice of therapy for such conditions as tachy-brady syndrome i.e. perhaps digoxin would be preferable to metoprolol.

The MRC methodology has a potential role to play in the assessment of impaired left ventricular function. The force-interval relationship is markedly distorted in the setting of heart failure, thereby giving a large signal to be measured. This may, for example, offer an additional mechanism for evaluating patients with impaired systolic function and significant valvular heart disease, to help in predicting the response to valve surgery. The major imperative at present, as regards MRC methodology, is to apply these studies in a larger cohort of patients with impaired systolic function. This is not only important in testing whether the robustness of the relationship between MRC and FP is maintained in this group of patients but also in establishing the reproducibility of observed drug effects to date in this group of patients. Once this methodology is established for this group of patients, it can be used to evaluate the relative safety of new agents such as AT₁ antagonists and endothelin antagonists.

The results of the current investigation herald a re-evaluation of acute ACE inhibitor therapy in the setting of congestive cardiac failure. There may be benefit in a slower approach to dose titration or, more importantly, an avoidance of full dose ACE inhibition being given at the outset of treatment.
Myocardial Drug Uptake

A number of issues remain to be resolved with this methodology. For example, the studies to date have involved patients with largely normal left ventricular systolic function. It is not certain whether patients with impaired cardiac function, possibly evident as congestive cardiac failure and/or undue tachycardia at rest would experience similar findings during acute uptake experimentation. Furthermore, it is not known whether variation in extent of uptake would have any impact on the resultant effects of the drugs.

Given the somewhat different haemodynamic effects observed following acute bolus administration of ACE inhibitors, compared to previous data utilizing predominantly slow infusions, it remains to be established that slower administration of intravenous ACE inhibitors permits a greater component of peripheral vasodilatation versus negative inotropic effect. This may further unmask variable cardioselectivity for various agents. Furthermore, it remains to be established whether the coronary vasomotor effects are maintained in the presence of a slower administration, potentially in the absence of a significant measurable negative inotropic effect.

ACE Inhibition

The findings in the current study are best explained by differential effects of ACE inhibitors on intracardiac bradykinin metabolism. This finding has greatest potential implications for therapy with inhibitors of the angiotensin II receptor, where no or minimal effects on bradykinin metabolism would be anticipated. Examination of such agents using the same methodology would also provide additional support to the observations already made. Alternatively, follow-up experimentation with co-administration of perindoprilat and an antagonist of the bradykinin receptors would also address this issue.
Improved technology

Advances in various invasive and imaging techniques are offering new opportunities for gathering further data. For example, the improved technology of conductance catheters now enables the use of these devices to study drugs with marked effects on loading conditions, providing for significant benefits over the use of $\frac{\text{LV}+\text{dP}}{\text{dt}_{\text{max}}}$ in this situation (Kass, Midei et al. 1988; Kass 1992; Feldman, Pak et al. 1996).

The current study has used coronary sinus blood flow as a surrogate for coronary arterial blood flow, the advantage being the simultaneous ability to sample coronary sinus blood. However, the use of intracoronary Doppler flow wires in these types of experiments would enable validation of regional coronary vasomotor effects.

Finally, improved technology and reduced imaging times are rapidly making magnetic resonance imaging a potentially useful tool for the evaluation of changes in myocardial energetics.
BIBLIOGRAPHY


ADDENDA AND CORRIGENDA

The following grammatical corrections to this thesis are to be noted.

CHAPTER 1

Page 18, Paragraph 2, Lines 11-12

Such effects as those described above may begin rapidly, distorting the apparent correlation between myocardial drug uptake and haemodynamic and other effects.

Page 26, Line 3

Recently, the construction of incomplete mechanical restitution curves \textit{in vivo} in humans has been reported.

CHAPTER 2

Page 30, Paragraph 2, Line 5

There is a distribution of chymase within the human heart which is recognised as being able to cleave angiotensin I to form angiotensin II and, indeed, it has been proposed that this is the major route for angiotensin II formation in humans.

Page 31, Paragraph 2, Line 9

The current study was funded by Servier laboratories and the perindoprilat was provided as a gift.

Page 31, Paragraph 3, Line 5

The need for metabolism results in peak haemodynamic effects being delayed for at least one hour following acute administration of the pro-drug.

Page 35, Paragraph 1, Line 4

The protocol was approved by the Queen Elizabeth Hospital Ethics of Human Research Committee and written, informed consent was obtained from the subject prior to the procedure in all cases.

Page 137, Paragraph 2, Line 2

Following intracoronary administration of enalaprilat, during baseline atrial pacing at a rate 5.9 ± 2.3% above spontaneous heart rate, haemodynamic variables were monitored for up to 20 minutes.
The perindoprilat group had a significantly higher level of both bradykinin 1-7 and bradykinin 1-8 at baseline, in comparison to the enalaprilat group (Table 2.18).

The effects of enalaprilat and perindoprilat on haemodynamic parameters during baseline atrial pacing shared both some similarities and some differences (Table 2.19).

The acute myocardial drug uptake of either perindoprilat or enalaprilat was studied in 21 patients. The performance of the assay utilized was very good with the coefficient variation for both duplicate and triplicate samples being very low. At first glance, a significant variability in the measured concentration of quality control samples (QC's) is noted.

CHAPTER 3

In patients with cardiovascular disease, symptoms often occur in the presence of a relative tachycardia,

Theoretical bases for relating Mechanical Restitution Curves and Frequency Potentiation

Mechanical restitution curve (MRC) construction uses a single beat measure of the contractile response to a non steady state beat to describe the interaction between stimulation frequency and contractile performance whereas frequency potentiation (FP) uses a sustained increase in stimulation frequency to directly examine this relationship.

it is not surprising to find evidence of cardioactive drugs with effects that vary depending on the cycle length of stimulation.
Finally, the relationship between MRC and FP was examined. It was prospectively determined that the complete database of patients, in our laboratory, who were investigated as regards MRC and FP experiments would be analyzed in order to examine the determinants of FP.

Page 229, Paragraph 2, Line 9
This convergence of the MRC’s was reflected by a significant reduction in ‘c’, from 28.4 ± 4.0 to 21.2 ± 2.7% (Figure 3.11b; p < 0.005).

Page 254, Paragraph 1, Line 8
However, preliminary experiments examining the effect of verapamil on mechanical restitution curves had been described (PhD thesis of Anne Powell).

CHAPTER 4

Page 264, Paragraph 2
Acute ACE inhibitor administration is associated with negative inotropic effects which are small in patients with good left ventricular systolic function. Furthermore, acute ACE inhibitor administration is associated with inhibition of intracardiac ACE and, in the case of perindoprilat, augmentation of vasodilator reserve.

Page 264, Paragraph 3, Line 4
Similarly, the positive inotrope, milrinone, exerted rate-related effects, but these were not apparent with digoxin.

Page 265, Paragraph 2, Line 3
For example, the lack of a significant reduction in LV+dP/dt_max with perindoprilat is likely due to small numbers, given that all other perindoprilat and enalaprilat data indicate negative inotropy.

The following additional comments are provided for clarification of some sections.

CHAPTER 2
A series of experiments were performed in which patients being investigated for chest pain received either an intravenous or intracoronary dose of either
perindoprilat or enalaprilat. As part of the determination of drug effects, examinations were made both at resting heart rate and during a pacing induced tachycardia. This tachycardia was maintained for a period of two minutes, the intention being to induce myocardial ischaemia where possible. Whilst such methodology has been employed previously, it does not guarantee the induction of ischaemia in individual patients and, indeed, subsequent examination of measured trans-cardiac gradients of lactate production would tend to confirm this. Nevertheless, ethical constraints prevent the use of substantially greater (potential) induction of myocardial ischaemia.

The responses to a pacing induced tachycardia were examined on two separate occasions; once prior to the administration of any drug and the second time at approximately 14 minutes following drug (perindoprilat or enalaprilat) administration. In both cases, the haemodynamic, biochemical and metabolic measures used as the baseline or rested state observation were made immediately prior to the induction of tachycardia ie. in the case of post drug experiments, the observations of resting state were made at approximately 13 minutes.

The series of experiments examining the acute effects of parenteral administration of angiotensin converting enzyme inhibitors included only a single patient with impairment of left ventricular systolic function. It is quite possible that examination of a larger cohort of such patients may have yielded different results for the effects of these agents. In particular, previous studies examining the acute effects of angiotensin converting enzyme inhibitors in patients with heart failure have demonstrated systemic vasodilatation, reductions in filling pressures and a consequent increase in stroke volume (Acampora, Melendez et al. 1989; MacFadyen, Lees et al. 1993; Varriale, David et al. 1993; Tohmo, Karanko et al. 1994; Evans, Burnett et al. 1995; Annane, Bellissant et al. 1996). It is likely that these haemodynamic effects would camouflage any associated negative inotropic effect. However, it is noted that in the one patient with impaired systolic function, systemic vascular resistance was increased following the administration of perindoprilat.
CHAPTER 3
The construction of mechanical restitution curves was first described by Ritchie et al (Ritchie, Wuttke et al. 1995). Included in this publication was the equation
\[ Y = a - \frac{c(100-d)(60-d)}{40(x-d)} \]
It is this publication that first demonstrated that the parameter 'c' is a measure of individual sensitivity to reductions in cycle length and is highly reproducible (Ritchie, Wuttke et al. 1995).

CHAPTER 4
The conclusions for chapters 2 and 3 are re-stated here

Chapter 2
In patients undergoing investigation for chest pain of presumed ischaemic origin and with relatively well preserved left ventricular systolic function, acute administration of an ACE inhibitor is associated with negative inotropic effects in the absence of any associated systemic vasodilatatory response. There was significant evidence of inhibition of both peripheral and intracardiac ACE activity and, in the cases of perindoprilat only, augmentation of vasodilator reserve.

Chapter 3
A total of seven different drugs were examined for evidence of rate-related inotropic effects. Verapamil exhibited direct use dependent negative inotropic effects whilst metoprolol exhibited reverse use-dependent effects. Sodium nitroprusside exerted no significant inotropic and/or rate related inotropic effects at spontaneous or rapid heart rates, confirming that the results obtained for verapamil and metoprolol were not simply due to altered loading conditions.

The positively inotropic agent, milrinone, demonstrated reverse use-dependent effects with the possible appearance of negative inotropy at short cycle lengths. In
contrast, digoxin administration did not reveal any substantial evidence of rate-related effects.

On multivariate analysis, the parameter 'c' was the only haemodynamic factor significantly correlated with extent of increase in \( \text{LV+}\frac{dP}{dt_{\text{max}}} \) during sustained tachycardia.
BIBLIOGRAPHY ADDENDUM


Rehr, R. B., B. E. Fuhs, et al. (1991). “Effect of brief regional ischemia followed by reperfusion with or without superoxide dismutase and catalase administration on
myocardial sarcoplasmic reticulum and contractile function.” Am Heart J 122(5): 1257-69.


