The systemic and cerebrovascular effects of catecholamines under inhalational and intravenous anaesthesia

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For

Gill, Kate and Ashy

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Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any university or tertiary institution.

To the best of my knowledge and belief, it contains no material previously published or written by any other person, except where due reference has been made in the text.

I give my consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

John A Myburgh

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Abstract

Adrenaline, noradrenaline and dopamine are widely used in intensive care medicine and anaesthesia. Isoflurane is used as an anaesthetic and propofol as an anaesthetic as well as a sedative in intensive care. There may be interactions between these drugs that could adversely effect cardiovascular and cerebrovascular function.

In order to study these interactions, established methods in sheep were used whereby mean arterial pressure, cerebral blood flow and intracranial pressure were continuously measured. Cardiac output was measured intermittently and blood samples form arterial, pulmonary artery and sagittal sinus catheters were taken for determination of systemic and cerebral oxygen consumption.

In awake sheep, infusions of adrenaline, noradrenaline and produced significant and equivalent increases in mean arterial pressure and cardiac output. Systemic vascular resistance and oxygen consumption were not significantly changed. Dopamine increased cerebral blood flow and oxygen consumption during induced systemic hypertension.

Propofol was associated with a 55% reduction in cerebral blood flow whereas isoflurane did not significantly affect cerebral blood flow. Cerebrovascular reactivity under both propofol and isoflurane was significantly different to that in awake sheep, but was similar for both agents.

During steady-state propofol anaesthesia, catecholamine induced increases in cardiac output were associated with increased propofol clearance and reversal of anaesthesia.

Under propofol and isoflurane anaesthesia, all three catecholamines significantly increased cerebral blood flow, with dopamine demonstrating the most pronounced effects, particularly under propofol. Anaesthesia was associated with a reduction in cerebral oxygen consumption, suggesting preservation of flow metabolism coupling.

There was no difference between the slopes of the autoregulatory curves between cerebral blood flow and mean arterial pressure between the awake sheep and those under propofol and isoflurane anaesthesia. There were differences between the intercepts of these curves, consistent with the overall reduction in cerebral blood flow induced by propofol and, to a lesser extent, by isoflurane. The concomitant administration catecholamines, propofol or isoflurane was not associated with altered autoregulatory function.

Chapter 1. Introduction and literature review

1.1 INTRODUCTION

1.1.1 Defence of the circulation

The pharmacological defence of the circulation is a cornerstone of the disciplines of intensive care medicine and anaesthesia. Drugs such as the endogenous catecholamines, adrenaline, noradrenaline and dopamine and synthetic derivatives such as dobutamine, isoprenaline and dopexamine have an established place in clinical practice.

The principle aim of these drugs is to restore inadequate systemic and regional perfusion to physiological levels in conditions of circulatory failure. These conditions include acute myocardial infarction, cardiogenic shock, cardiopulmonary resuscitation, anaphylaxis and septic shock. In these conditions, the prompt restoration and maintenance of appropriate systemic and regional perfusion has been attributed to improved outcomes.

Manipulation of blood pressure has been extended to situations where the circulation is ostensibly normal, with the specific aim of increasing or improving regional perfusion. This strategy has been used for many years and has been directed at the prevention or amelioration of acute renal failure, hepatic insufficiency and acute brain syndromes, particularly traumatic brain injury and aneurysmal subarachnoid haemorrhage. In patients receiving anaesthesia or sedation that may be associated with a degree of circulatory depression, low doses of vasoactive agents are increasingly used to maintain systemic and regional perfusion.

The clinical use and prescription of catecholamines is based on clinical experience and dogma derived from the purported pharmacological and physiological attributes of each drug. Despite their established use, there is little scientific evidence upon which to base clinical practice. Many of the early physiological studies were based on small numbers of volunteers using crude measurements, whilst pathophysiological studies are limited by heterogeneous study populations and a variety of measurements and primary endpoints for efficacy or clinical outcomes. Indeed, there are no controlled studies that show any benefit of one or any combination of catecholamine(s) over another.

Equally, there is a paucity of scientific and clinical data analysing the specific effects that catecholamines have on regional circulations. Many of these studies are limited by measurement techniques that employ surrogate or indirect measurements. The extrapolation of the results to the clinical scenario has to be made with circumspection.

The associated effects of pathophysiological processes and drug interactions on the pharmacokinetics and pharmacodynamics of catecholamines is a further confounding variable. Most experimental models use highly controlled preparations to minimise the signal:noise ratio, and whilst insights may be obtained from these studies, the clinical expression of these results may be variable.

Despite intensive research into the physiology of the adrenergic system over the last 60 years, the established and effective place of catecholamines in clinical practice has resulted primarily from an intention-to-treat basis. This has limited rigorous clinical research in this area.

1.1.2 Defence of the cerebral circulation

The increasing use of catecholamines to augment the cerebral circulation in conditions of absolute or relative cerebral hypoperfusion prompted the interest that lead to this thesis.

Cerebral hypoperfusion is directly attributed to adverse outcomes in traumatic brain injury (1-3) and aneurysmal subarachnoid haemorrhage (4). Catecholamines are increasingly used to augment cerebral perfusion pressure and have an established role in clinical practice (5). However, this has developed in the absence of rigorously conducted clinical trials, or indeed, conclusive animal or basic science studies. There is an increasing body of evidence that suggests that the indiscriminate use of catecholamines in acute brain injury may be associated with adverse outcomes, potentially negating the benefits of restoring cerebral blood flow (6).

The effects of catecholamines on cerebrovascular mechanics (viz cerebral blood flow, intracranial pressure, cerebral perfusion pressure, cerebrovascular resistance), cerebral oxygen consumption and autoregulation remains contentious. This applies equally to physiological and pathophysiological conditions.

Many patients with acute brain injury receive anaesthesia or sedation with intravenous or inhalational anaesthetics and concomitant infusions of catecholamines. Potential and real catecholamine:anaesthetic interactions and the subsequent effects on regional (e.g. cerebral) and systemic circulation are essentially unknown.

The following literature review will summarise and integrate the current state of knowledge of cerebrovascular function, catecholamine physiology and intravenous and inhalational anaesthesia.

1.2 CEREBROVASCULAR FUNCTION

1.2.1 The cerebral circulation

1.2.1.1 Anatomical considerations

In health, the human brain receives 20% of cardiac output. Although the arterial circulation is configured as the Circle of Willis, 80% of the arterial circulation is delivered via the anterior circulation through the internal carotid artery and 20% via the vertebro-basilar system. Venous drainage is via the transverse and inferior petrosal sinuses, with 70% of venous drainage from the ipsilateral side (7).

In sheep, the anatomy follows a similar pattern (8). The principle difference relates to the arterial circulation, where carotid arterial blood passes through the rête mirabile, a vascular bed responsible for ovine thermoregulation, before entering the brain (9). Intracranially, capillary blood drains through cerebral veins and into cerebral sinuses that have walls composed of fibrous dura mater lined with endothelium. These sinuses pass out of the skull to become veins and, shortly afterwards, are usually joined by veins draining the soft tissues of the head and face (10).

1.2.1.2 The blood-brain barrier

The cerebral vasculature is surrounded by the blood-brain barrier. This constitutes an anatomical barrier characterised by inter-astrocyte "tight junctions" that regulates molecular transfer by molecular weight. (Figure 1.1).

A "metabolic" blood-brain barrier maintains regulation of molecular transfer by enzymatic metabolism within endovascular endothelium. An example is the inability of L-3,4-dihydroxyphenylalanine (L-dopa) to access the brain from the systemic circulation. L-dopa crosses the capillary luminal membrane of the endothelium by active diffusion using large neutral amino acid transport systems. Once it crosses the blood-brain barrier, it is decarboxylated to dopamine and dihydroxyphenylacetic acid (dopac) by endothelial monoamine oxidase at the nigrostriatal nerve terminals, thereby preventing passage of dopa into the brain.

Access to the brain across the blood-brain barrier is further regulated by limited diffusion and active transport mechanisms, limiting transfer to highly lipid soluble molecules according to the oil:water partition coefficient (12,13).

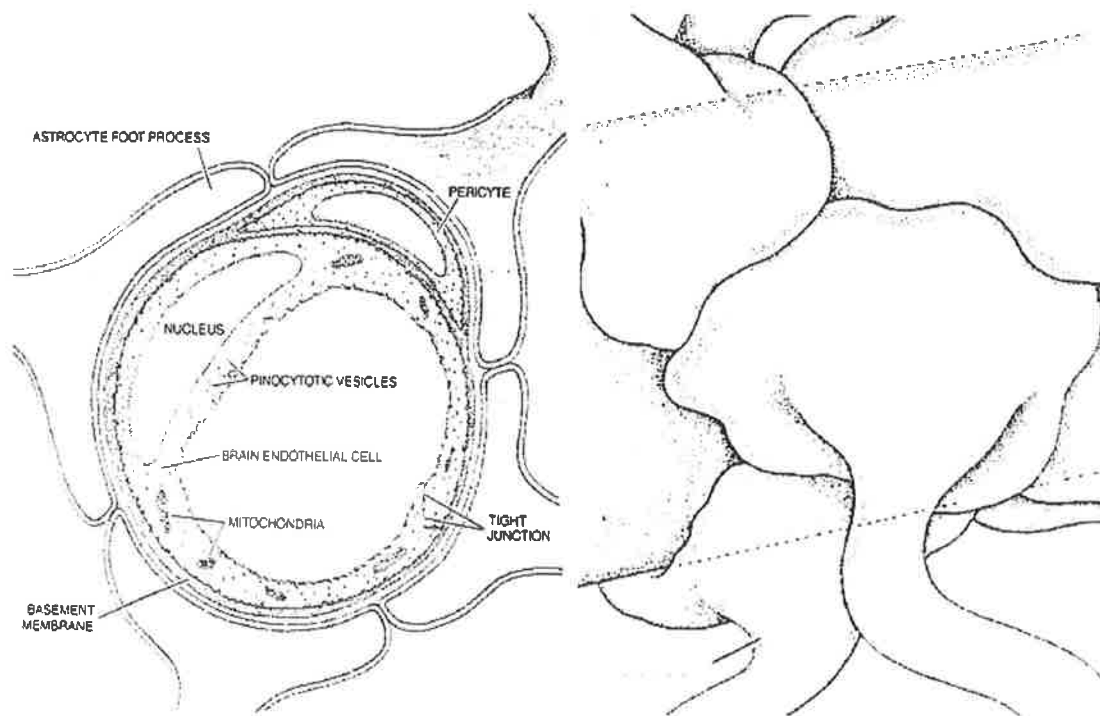


Figure 1.1
Schematic diagram of the anatomical blood-brain barrier. (Adapted from Goldstein and Betz (11))

Blood-brain barrier permeability is maintained by complex neurohumoral mechanisms. Of these, adrenergic innervation of the cerebral vasculature and modulation of active transport mechanisms (such as noradenergic mediated Na^+ / K^+ -ATPase) is an important element (14,15).

The blood-brain barrier is not a homogenous anatomical and physiological structure. Naturally occurring deficits in the blood-brain barrier exist in the posterior hypothalamus, peripineal regions and area postrema of the medulla.

The anatomic and metabolic blood-brain barrier systems are integrally involved with cerebral vasoregulation and autoregulation. However, blood-brain barrier permeability may be altered by physiological perturbations, such as induced hypertension, and pathological states such as malignant hypertension, traumatic brain injury and subarachnoid haemorrhage.

1.2.2 Intracranial elastance

In 1783, the "Monro-Kelly doctrine", outlining the asymptotic relationship between intracranial pressure and volume was defined (Figure 1.2). The doctrine states that due to the non-compliant skull and dura, small increases in intracranial volume result in sharp increases in intracranial pressure.

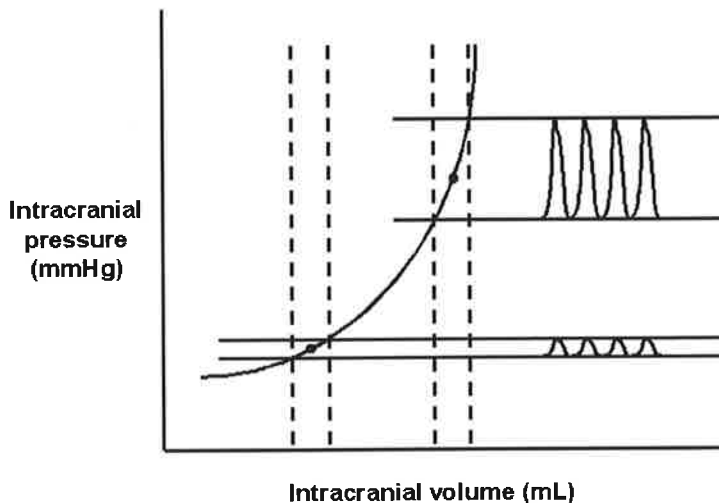


Figure 1.2
The Monro-Kelly doctrine.

Fifty years ago, pioneering work by Guillaume and Janny identified intracranial hypertension as the prime pathophysiological entity in traumatic head injury (16). In 1960, Lundberg described continuous measurement of intracranial pressure and ventricular drainage of cerebrospinal fluid in patients with head injury (17).

The unique anatomical relationship of the skull and brain are important when determining the effects of changes in cerebral blood flow. The elastant reserve of the brain to accommodate increases in intracranial volume is limited. Cerebral blood flow is maintained at a constant rate in the presence of changing systemic blood pressures. Under physiological conditions, increases in cerebral blood flow are minimised by these autoregulatory processes. Physiological increases in cerebral blood flow that are usually associated with transient rises in sympathetic activity are accommodated by decreases in intracranial volume by recirculation of cerebrospinal fluid and venoconstriction. Regulation of cerebrovascular volume is under intense neurohumoral control (18).

Pathological increases in cerebral blood flow (hyperaemia), or interstitial/cellular oedema will rapidly exhaust elastant reserve if it continues unabated. In pathological conditions such as traumatic brain injury, increases in intracranial volume are multifactorial and involve vascular and non-vascular mechanisms.

1.2.3 Cerebral autoregulation

The brain is a highly efficient autoregulator. Normally, cerebral blood flow is maintained at a constant rate over a range of systemic pressures. (Figure 1.3). Autoregulatory systems are complex and involve a number of myogenic (pressure) and metabolic systems (18,19).

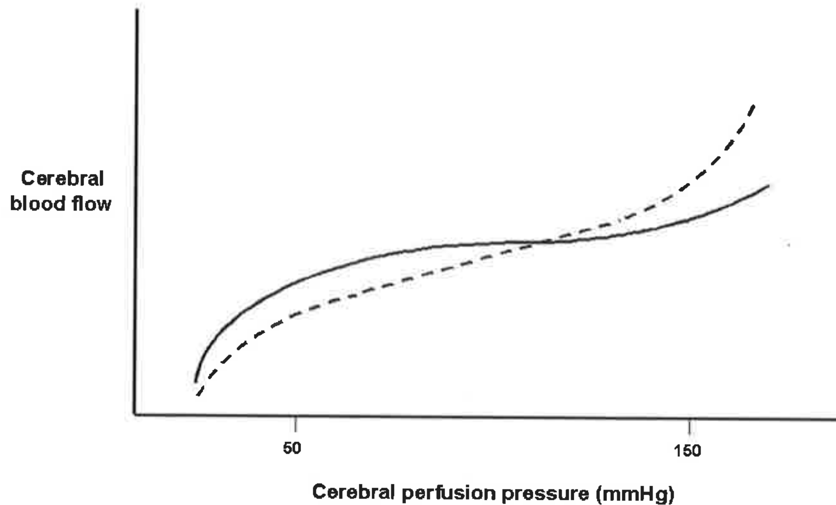


Figure 1.3
Idealised autoregulatory relationship between cerebral blood flow and cerebral perfusion pressure showing range of constant flow under physiological conditions (solid line) and following traumatic brain injury (dashed line).

1.2.3.1 Myogenic autoregulation

Myogenic autoregulation is the term used to describe changes in cerebrovascular transmural pressure in response to fluctuations in mean arterial pressure. This is mediated through adrenergic stimulation of vascular smooth muscle and microfluxes of endogenous vasodilators (nitric oxide) and vasoconstrictors (e.g. endothelin). In cerebrovascular terms, myogenic autoregulation is regarded as the relationship between cerebral blood flow and cerebral perfusion pressure (or mean arterial pressure in the absence of intracranial hypertension). Under physiological conditions, cerebral blood flow is maintained at a constant rate until autoregulatory thresholds (both upper and lower) are exceeded and cerebral blood flow becomes "pressure passive". The "break points" where this occurs vary between individuals, but is traditionally recorded in standard physiology textbooks as 60 and 160 mmHg.

1.2.3.2 Metabolic autoregulation

Metabolic autoregulation is the term used to define non-myogenic mechanisms of cerebral vasoregulation. Of these, reactivity of the cerebral vasculature to systemic and local changes in arterial carbon dioxide tensions ($PaCO_2$) is regarded as the basis of metabolic autoregulation. Under

physiological conditions, cerebral blood flow and $PaCO_2$ have a pseudo-linear relationship: hypercapnia causes cerebral vasodilation, whilst hypocapnia causes vasoconstriction. This mechanism is due to carbon dioxide induced changes in cerebral perivascular pH resulting in changes in cerebrovascular tone (20). However, this is an oversimplification. Cerebrovascular reactivity is also influenced by cerebral tissue oxygenation (21), calcium and potassium fluxes, nitric oxide, endothelin, eicosanoids, vasopressin, endogenous neuropeptides (18) and drugs such as volatile (22,23) and intravenous anaesthetics (24,25).

Although cerebrovascular carbon dioxide reactivity is frequently used as a surrogate index, several mechanisms determine metabolic cerebral autoregulation.

In practical terms, cerebral autoregulation primarily relates to the cerebral blood flow / cerebral perfusion pressure relationship. Studies analysing the effects of drugs, physiological and pathological processes on cerebral autoregulation are directed at this relationship. There are numerous methods of measurement and quantification of this relationship. These will be discussed subsequently. The extents to which the various components of autoregulation are impaired or altered by physiological perturbations or pathological processes vary considerably.

1.2.4 Measurement

The validity and interpretation of studies of cerebrovascular function is dependent on the accuracy of physiological measurement. Of these, the measurement of cerebral blood flow and quantification of cerebral autoregulatory relationships are the most critical.

1.2.4.1 Cerebral blood flow

The accurate, real-time measurement of cerebral blood flow is essential for studies of cerebrovascular function. To date, no ideal measurement system exists, either in the basic science milieu or in clinical medicine.

Measurement has proven difficult due, in part, to the relative inaccessibility of the brain and the complexity of the cerebrovascular anatomy. The recognition that global changes in cerebral blood flow may not represent regional or cellular changes is another confounding variable. A diverse range of methods to measure cerebral blood flow have been devised, each with particular advantages and limitations. Some of the most popular methods are described below.

1.2.4.1.1 Indicator methods

These may be defined as methods in which the rate of delivery or removal of a substance to or from the brain by the circulation is measured. These

substances may be categorised into those that readily diffuse in and out of the brain, and those that remain confined to the cerebral vasculature.

All of these methods are limited by the intermittent nature of measurement. The measurement interval is dependent on the rate of uptake and elution of the indicator and requires a constant flow rate during the measurement period. Consequently, these techniques are not suitable for studies where rapid measurements or dynamic changes are required, such as those conducted in this thesis. However, the principles are important, particularly when making comparisons with the results of other studies.

1.2.4.1.1.1 Diffusible indicators

These methods are based on the Fick principle that states that "if the quantity of a tracer increases or decreases during the passage through a vascular bed, the blood flow can be calculated by dividing the amount taken up or added to the blood in a given time by the arterio-venous difference" (26).

Kety modified this to measure cerebral blood flow, stating that the rate of change of the indicator can be measured directly in the brain (27). Ideally, the indicator should be neither metabolised nor pharmacologically active, should have a short half-life and equilibrate rapidly between blood and brain tissue.

Nitrous oxide was one of the first indicators used (28) and is often regarded as the benchmark for indicator methodology. However, nitrous oxide may exert independent effects on cerebral blood flow (29) that may affect measurement. Furthermore, incomplete pseudo-equilibrium within the brain after considerably longer than 10 minutes, has been described (30).

Hydrogen clearance is an established method of cerebral blood flow measurement (31,32). This is performed by the implantation of platinum electrodes, using polarography to determine hydrogen concentrations. Whilst this has the advantage of obtaining an index of local flow around the implanted electrodes, multiple sites are required to determine global blood flow. Implantation haematoma may cause false negatives and if large enough, may result in post-traumatic changes in regional and global cerebral blood flow (33).

Xenon is an inert, freely diffusible gas and used to measure cerebral blood flow. This may be done using the radiolabelled isotope (Xe^{133}) and scintillation counters or with the non-radioactive form in conjunction with computerised tomography (34,35). These techniques provide reproducible, repeated intermittent measurements and are useful in mapping cerebral blood flow changes (36,37). However, the equipment required for isotope detection limits its application in physiological animal preparations. Furthermore, xenon may have direct cerebrovascular effects (38).

1.2.4.1.1.2 Non-diffusible indicators

Cerebral blood flow may be measured by injecting a known quantity of an indicator that remains intravascular and measuring the time-course of its appearance in the cerebral venous blood. This is the principle that is commonly used in dye or thermodilution measurement of cardiac output.

These measurements are made intermittently and are subject to errors associated with incomplete indicator mixing, variability in rate of injection and recirculation.

Radiolabelled microspheres of appropriate diameter may be injected that become trapped in capillary beds. Scintillography over the region is performed and the degree of radioactivity is compared to another region for which the blood flow is known. These techniques were originally used to measure cerebral blood flow in 1970 (39), and have since been used and validated extensively (40-42). Limitations with this method include the intermittent nature of measurement, species-specific selection of microsphere size, expense, and limitation by the number of available isotopes and potential inaccuracy under pathophysiological states.

1.2.4.1.2 Flow meter methods

Most of these techniques utilise the Doppler principle which states that sound reflected from a moving target will be shifted in frequency by an amount proportional to the target velocity (43).

Electromagnetic and ultrasonic Doppler flow meters may be directly implanted on arteries or veins to measure blood velocity. This is used an index of flow and is dependent on vessel diameter. Doppler methods have the advantage of providing a continuous and accurate measurement of velocities.

The anatomy of the arteries supplying the brain varies between species and is frequently via multiple vessels. In addition, the proportion of total flow in any single vessel may not be constant, and flow redistribution between major cerebral vessels may occur (7). Therefore, accurate measurement of global cerebral blood flow cannot be assured unless simultaneous flow in all major vessels is measured.

In sheep, cattle and goats, measurement of arterial inflow is rendered even more difficult by the arterial rête mirabile (9,44).

Flow in cerebral veins or sinuses may be used as an alternative to arterial inflow methods. The anatomical features of the sinuses remove one of the problems associated with the use of flow meters on cerebral arteries. Flow meters measure blood velocity which is proportional to flow when the cross-sectional area and pattern of flow remains constant. The lack of smooth muscle in the cerebral sinuses and their rigid walls result in a constant vessel

diameter over a wide range of intraluminal pressures, such as those induced by drugs or physiological changes in smooth muscle tone. Blood flow velocity therefore remains proportional to total flow, if the flow profile remains constant.

Probe placement becomes critical because of the distribution of cerebral blood flow and the risk of measuring extra-cerebral blood flow as well, due to variations in cerebral venous anatomy. In humans, cerebral venous blood drains via the cerebral sinuses to reach the jugular veins and is joined by significant quantities of extra-cerebral blood from the facial veins. Sampling from the proximal internal jugular vein results in between 3-5% contamination with extra-cerebral blood, although this method predominantly measures ipsilateral cerebral blood flow (45).

In animals, access to veins or sinuses containing relatively pure cerebral blood is more difficult due to the influx of extra-cerebral blood from subcutaneous tissues (8). Ligation of veins containing extra-cerebral blood has allowed successful measurements of cerebral blood flow in particular species.

The relevant cerebral venous anatomy of the sheep has been described. Using dissection and microsphere flow measurement techniques, flow in dorsal sagittal sinus represents approximately 75% of cerebral venous blood. This blood drains from both hemispheres and is almost completely free of extra-cerebral blood provided measurements or sampling from the transverse sinuses are avoided (46,47). This technique however, does require a craniotomy for placement of Doppler probes or sampling catheters.

1.2.4.1.2.1 Implanted Doppler flow meters

Technological advances have resulted in the ability to place Doppler transducers onto arteries or veins, regardless of diameter. These may be implanted for chronic use, thereby allowing continuous measurements.

Vessel selection is critical as local vessel factors may influence the accuracy of measurement such that the vessel may not represent other areas of the brain. Although velocity measurements are highly accurate using this type of method, because the relationship between velocity and flow depends on the cross-sectional area, calculations of flow measurements assume no change in vessel diameter. For this reason, cerebral arteries cannot be used due to the presence of smooth muscle, which are under the influence of numerous vasoregulatory factors (18). Comparative studies of Doppler recorded velocities and cerebral blood flow have frequently demonstrated discrepancies between the two variables. Assessing cerebral blood flow changes in response to changes in $PaCO_2$ in a lapine model, Doppler flow measurements of the basilar arteries were significantly lower than corresponding values of cerebral blood flow measured by hydrogen

clearance methods (48). This difference was attributed to velocity measurements underestimating flow changes as a result of increases in vessel cross sectional area. These observations were consistent with those from other animal studies (49,50).

1.2.4.1.2.2 Transcranial Doppler flow meters

Transcranial Doppler ultrasonography with a 2MHz pulsed Doppler probe allows non-invasive, intermittent or continuous assessment of the velocity of blood flow through large cerebral vessels. Insonation through a naturally occurring acoustic window such as the trans-temporal approach allows assessment of flow through the anterior, middle and posterior cerebral arteries, terminal internal carotid artery and anterior and posterior communicating arteries (51). This technique is now widely used as a clinical, qualitative assessment of cerebral blood flow velocity.

Despite widespread use, transcranial Doppler has the same limitations as implantable arterial Doppler transducers with respect to the difference between blood velocity and flow. The technique is highly operator dependent and marked inter-individual differences have been described. An operator error rate of 15-30% has been described, although this may be reduced by continuous transcranial Doppler measurement techniques (52).

Measured indices of flow include systolic, mean and diastolic flow velocities. Distinct patterns associated with normal, hyperaemic, vasospastic and absent flow are recognised. Derived indices such as the Gosling pulsatility index (systolic/diastolic difference divided by the mean velocity) and Lindegaard ratio (between middle cerebral and extracranial internal carotid artery velocities) may assist in differentiating these flow patterns (53,54). These derived indices are used to indirectly assess the state of cerebrovascular resistance and to improve the accuracy of the recorded signal. However, the relationship between velocity and flow will always be subject to error without a real-time assessment of vessel diameter.

Ultrasonic Doppler techniques therefore provide a semi-quantitative measure of changes in cerebral blood flow. Accurate knowledge of changes in vessel diameter is essential. An alternative solution is to use vessels with little or no vascular smooth muscle, such as cerebral veins or sinuses.

Cerebral venous Doppler measurements have not been routinely used in humans. A report from Ohsumi described the use of continuous internal jugular bulb velocity measurements in patients undergoing cardiopulmonary bypass (55). These measurements were compared with measurements obtained by the argon (Kety Schmidt) method, demonstrating significant correlations (r^2=0.87). Further utility of this promising technique is awaited.

1.2.4.1.2.3 Laser Doppler flowmetry

Using the Doppler principle of detecting a frequency shift in reflections from moving objects, laser Doppler flowmetry is used to examine the microcirculation. Coherent light at wavelengths of 600-800 nm is delivered to the underlying tissues via a fibre optic cable. The magnitude and frequency shifts of the reflected light relate to the number and velocity of tissue red blood cells (56,57). This method measures frequency shifts due to red cell movement in a small region of tissue (e.g. cerebral). This diameter may be as small as 1mm. Although measurement is continuous, there is an assumption that flow in this region is representative of that in the rest of the brain. Unlike hydrogen clearance, this technique is relatively non-invasive with no effect on cerebral blood flow. Further developments of this technique include scanning laser Doppler flowmetry, where regional cerebral blood flow maps are recorded (58).

Evaluation of this method in a variety of tissue types has demonstrated acceptable agreement with flow measured by other techniques such as hydrogen clearance (59-61). As with other Doppler techniques, alterations in vessel calibre will affect measurement. Local concentrations of vasoreactive substances may influence measurements. A lapine study compared observed pial diameter with changes in laser Doppler flowmetry in response to changes in $PaCO_2$. Topical applications of adenosine and bradykinin produced markedly different results, suggesting a variability in the penetration of these substances and the subsequent flows (62,63).

This technique is primarily applicable to continuous measurement of local blood flows in discrete regions.

1.2.4.1.2.4 Thermal diffusion flow measurement

Quantitative, continuous measurements of cerebral blood flow by thermal diffusion have been developed using thermocouples placed directly into tissue. Changes in temperature between two points measured by the thermocouples are proportional to flow. Early techniques used a Peltier stack, which was limited by the size of the probe. Modifications of this technique have been developed using smaller gold plates, suitable for use in small animals (64,65). Good agreement with measurements using hydrogen clearance has been demonstrated. As with laser Doppler flowmetry, this technique has an application in the measurement of regional blood flows.

1.2.4.2 Cerebral metabolism

1.2.4.2.1 Substrate measurements

Measurement of the rate of metabolism of biological systems is most commonly achieved by the rates of consumption of metabolic substrates or production of metabolic products.

This may be calculated from global or regional changes in fluxes of substrates such as oxygen (indirect calorimetry), glucose or heat (direct calorimetry). This assumes that substrate utilisation (consumption and production) remains constant, yet it may under- or overestimate metabolic activity if substrate consumption changes, or if there are significant regional differences.

Oxygen consumption may be “indirectly” calculated from carbon dioxide production and the estimated respiratory quotient (RQ):

$$VO_2 = \frac{VCO_2}{RQ}$$

where VO_2 is oxygen consumption, and VCO_2 is carbon dioxide production. This provides a reasonable estimate of whole body oxygen consumption (66).

Indirect calorimetric methods are usually conducted using “metabolic carts”. By collecting expired air through a closed circuit, minute ventilation, inspired and expired oxygen and carbon dioxide concentrations are measured with pneumotachographs, paramagnetic oxygen analysers and pH electrodes. In these systems, oxygen consumption is calculated from the following equation:

$$VO_2 = \frac{V_I \times FiO}{V_E \times FeO_2}$$

where VO_2 is oxygen consumption, V_I is inspired minute volume, FiO_2 is fractional inspired oxygen concentration, V_E is expired minute volume and FeO_2 fractional expired oxygen concentration.

The Fick principle is described by the relationship between flow and the difference between arterial and venous oxygen consumption in the following equation:

$$VO_2 = Q \times (CaO_2 - CvO_2)$$

where VO_2 is oxygen consumption, Q is cardiac output and CaO_2 and CvO_2 are arterial and venous blood oxygen contents, respectively. Calculation of oxygen consumption from this equation, using thermodilution (pulmonary artery catheter) measurements of cardiac output will underestimate whole body oxygen consumption, as the additional contribution of pulmonary oxygen consumption may be as much as 15-20%. This should be recognised if Fick based measurements are used (67).

Many metabolic measurement systems incorporate flow as a derived endpoint of metabolism, if the afferent and efferent concentrations of substrate are known. This may be extrapolated to determine global or total body consumption, or regional (e.g. cerebral) values, substituting cerebral

blood flow for cardiac output and arterial and jugular venous oxygen contents accordingly:

$$CMRO_2 = CBF \times (CaO_2 - CcvO_2)$$

where $CMRO_2$ is the cerebral oxygen consumption, CBF is cerebral blood flow and $CcvO_2$ is cerebral venous oxygen content .

Determination of cerebral oxygen consumption using the Fick principle is therefore dependent on an accurate measurement of cerebral blood flow, which is discussed in 1.2.2.1. This measurement of cerebral metabolic rate has been used extensively in humans (68). As discussed in the section above, extra-cerebral blood may contaminate cerebral venous blood, thereby falsely elevating venous oxygen content, although this is minimal when sampling is done from high in the jugular bulb (3-5%) (45).

More commonly, a "reverse Fick" principle is used to determine an indirect measurement of cerebral blood flow for a given cerebral oxygen consumption using the arterio-jugular oxygen content difference. This is the principle of jugular bulb oximetry (45), and has been used extensively in human traumatic brain injury (69-72).

In animal studies, the dorsal sagittal sinus is a suitable vessel for sampling blood for measuring cerebral venous oxygen content without contamination. In dogs, the dorsal sagittal sinus has been used for cerebral venous oxygen content sampling after the ligation of extra-cranial vessels (73). In sheep, minimal extracranial contamination of the dorsal sagittal sinus makes this vessel ideal for sampling measurement of oxygen content (74).

Substrate extraction or utilisation may be calculated from the ratio of substrate consumption to delivery. Convective oxygen delivery (DO_2) is determined by:

$$DO_2 = Q \times CaO_2$$

Oxygen extraction is then calculated by VO_2 / DO_2. Similarly, this may be applied to global or regional (cerebral) conditions. Despite being commonly used as an index of metabolic activity, this calculated relationship is inherently flawed by mathematical coupling (75-77). Common factors (e.g. cardiac output or cerebral blood flow) in both sides of the equation will guarantee a positive association. In order to minimise this, one of the variables, usually oxygen consumption, is measured by expired gas analysis, using a metabolic cart.

1.2.4.2.2 Imaging methods

Metabolic activity may also be assessed by direct measurement of substrate concentrations. Autoradiographic measurement of cerebral uptake of labelled glucose has been used as an index of cerebral metabolic rate (78). These

techniques provide accurate regional and global metabolic maps, but are limited by the need to conduct post-mortem autoradiographic counting. This principle led to imaging techniques using radiolabelled glucose, such as positron emission tomography (79). This allows real-time measurement of glucose consumption without anatomical dissection, but is limited by the intermittent nature of measurement and the capital outlay required for the equipment. Further modifications of nuclear medical techniques include technetium labelled hexamethylpropyleneamineoxime (^{99}Tm-HMPAO) in conjunction with computerised tomography or magnetic resonance imaging (80-82).

These techniques are increasingly being used to map cerebral metabolism in conditions such as traumatic brain injury and cerebrovascular or degenerative disease (83,84). The applications are similarly limited by capital costs.

1.2.4.3 Intracranial pressure measurement

Intracranial pressure monitoring has an established place in clinical neurosurgical and neurointensive care practice (85). The recognition that raised intracranial pressure is associated with adverse outcome led to the measurement of this parameter in order to quantify the degree of injury and to assess the response to treatments directed at reducing intracranial pressure (86).

Despite being used for more than 60 years and the recent promulgation of evidence based medicine guidelines, there are wide variations in the usage, interpretation and treatment thresholds. Many of these variations are due to practical, logistic and commercial reasons, but are also due to an appreciation that intracranial pressure is primarily an index of intracranial elastance. The relationship between cerebral blood flow and intracranial pressure is highly regulated under physiological conditions but variable under pathological states.

Measurement of intracranial pressure (87) with an intraventricular catheter is the most accurate and clinically useful method. It has the advantages of zero calibration, cerebrospinal fluid drainage for raised intracranial pressure and allows dynamic testing of pressure volume relationships. Disadvantages include technical difficulty with insertion, particularly in patients with cerebral oedema and compression of the lateral ventricles, and an increased incidence of infection. These systems allow the measurement of absolute values.

Solid state systems such as fibreoptic (e.g. Camino®) or strain gauge tipped catheters (e.g. Codman®) may be placed subdurally, intraparenchymally or intraventricularly (88,89). These systems transduce intracranial pressure to provide high fidelity waveforms. They have the advantage of being of small

calibre. Insertion is relatively atraumatically performed through a small craniotomy. Disadvantages include inability to perform zero calibration after insertion and baseline drift that may be clinically significant after 5 days (90). However, this may be obviated in the research situation by measuring qualitative changes from baseline, rather than absolute quantitative changes.

The values derived are used to calculate cerebral perfusion pressure: mean arterial pressure minus intracranial pressure. For this calculation, both measurements should be referenced to the external auditory meatus (equivalent to the circle of Willis).

Research applications of intracranial pressure monitoring include cerebrovascular pressure transmission where fast Fourier analysis of systemic and intracranial pressure waveform are integrated to determine the phase shift of pressure across the cerebrovascular bed (91). This analysis has been used to determine patterns of intracranial hypertension in traumatic brain injury models in animals.

Other applications include the determination of intracranial pressure:volume relationships. The pressure volume index is defined as the response in intracranial pressure after the injection of 1ml fluid into an intraventricular catheter over one second. A change of >3mmHg/ml is regarded as an index of reduced intracranial elastance. This calculation requires the placement of an intraventricular catheter and may be complicated by infection and secondary pressure waves following intraventricular injection.

Intracranial pressure monitoring in animal preparations have used all of the above measurement systems. Most of these studies have measured intracranial pressure in response to traumatic brain injury models using percussive injury or cryoprobe techniques (92-94). The insertion of an intracranial pressure monitor is incorporated with the craniotomy, followed by the series of studies and euthanasia. These studies are limited by finite periods for studies, but allow accurate measurement of intracranial pressure.

Implantation trauma by fibreoptic intraparenchymal catheters has been assessed in dogs (95). Variable degrees of haemorrhage and mechanical brain damage were observed focally around the catheter site in brains from dogs immediately sacrificed after insertion, especially when the cable entered through a sulcus. This appeared to resolve over time (to a total of 30 days). This study suggests that catheter associated trauma may result in local damage and potential increases in intracranial pressure.

1.2.4.4 Cerebrovascular resistance

In haemodynamic systems, the relationship between flow and pressure is often expressed in terms of Ohm's Law. This doctrine applies to electrical current flowing through a conductor of uniform length and diameter. It is expressed as:

$$V = I \times R$$

where V is the potential difference between two points along the conductor, I is the current flowing through the conductor and R is the universal resistance. In haemodynamic terms, this is modified to:

$$P = Q \times R$$

where P is pressure, Q is cardiac output and R is vascular resistance. As flow and pressure are readily measured variables in clinical and research practice, vascular resistance is used as an index of vascular reactivity. For the cerebral circulation, cerebrovascular resistance (CVR) is represented as:

$$CVR = \frac{MAP - ICP}{CBF}$$

where the difference between mean arterial pressure (MAP) and intracranial pressure (ICP) is defined as cerebral perfusion pressure. This calculation is frequently used in studies of cerebrovascular reactivity in conditions such as subarachnoid haemorrhage, pre-eclampsia, stroke and traumatic brain injury (96).(97)

However, cerebrovascular resistance is derived from cerebral blood flow and cerebral perfusion pressure. This derivation is therefore calculated from a non-biological system that assumes linear flow through a uniform conductor down a pressure gradient. This does not apply to non-Newtonian systems such as a human or animal haemodynamics. The use of cerebrovascular resistance as an index is therefore of limited validity. Despite this, it continues to be used as a surrogate index of vascular reactivity and for determination of autoregulatory function in a number of clinical and research studies (98-102).

The potential for error and limitation of interpretation increases if techniques of cerebral blood flow measurement are themselves limited, such as using velocity changes with transcranial Doppler as an index. Consequently, interpretation of studies using cerebrovascular resistance as a primary endpoint demands considerable circumspection. The systemic equivalent (systemic vascular resistance) is discussed below.

1.2.4.5 Blood-brain barrier permeability

As outlined above, the blood-brain barrier has both an anatomical and metabolic basis. The permeability of the blood-brain barrier to solutes and drugs under physiological and pathological conditions has been extensively studied.

The integrity of blood-brain barrier has been determined by anatomical and imaging techniques. Early studies used labelled dextran (^{3}H-dextran), Evans blue-albumin complexes (103), ^{125}I-albumin (104) and horseradish peroxidase tracers (105). Normally, these molecules do not cross the

anatomical blood-brain barrier due to molecular size, but may cross under conditions of altered permeability. Findings using early animal models of alterations in blood-brain barrier permeability to changes in systemic blood pressure were important in defining these mechanisms. The findings demonstrated in spontaneously hypertensive rats, using Evans blue labelled albumin, were particularly important (106,107).

Changes in blood-brain barrier permeability have been demonstrated with systemic hypertension (108-110), following osmotic disruption with urea and arabinose (111,112), systemic catecholamines (105,113,114), epilepsy (115,116), hypothermia (117) and anaesthetic agents (118-120). Many of these studies provided the basis for the determination of the pathogenesis of malignant hypertension (121).

Most of these studies require anatomical verification of alterations in blood-brain barrier permeability. Although potential for non-invasive assessment of blood-brain barrier permeability may develop with imaging techniques that link metabolism to flow (e.g. positron emission tomography, single photon emission with computerised tomography or magnetic resonance techniques), none have been published to date.

1.2.4.6 Autoregulation

As outlined in section 1.2.1.4, the principle factor defining cerebral autoregulation is the relationship between cerebral blood flow and cerebral perfusion pressure.

The assessment and quantification of cerebral autoregulation is limited by the accuracy of cerebral blood flow measurement. Ideally, continuous real-time measurements are required to determine dynamic changes of cerebral blood flow over a range of cerebral perfusion pressures.

The perturbations that are used to alter cerebral perfusion pressure or cerebral blood flow need to be reproducible and representative of the autoregulatory spectrum. The upper autoregulatory threshold may be determined by induced hypertension and the lower by induced hypotension. In conditions of intracranial hypertension, the component that increases intracranial pressure to reduce cerebral perfusion pressure needs to be standardised. In physiological preparations without intracranial hypertension, mean arterial pressure may be regarded as the main determinant of cerebral perfusion pressure. Alternatively, manipulations of cerebral blood flow may be conducted.

1.2.4.6.1 Perturbations

There is no standard method to characterise myogenic autoregulation. Selection of one of the following techniques will depend on the experimental

model used and measurement facilities available. Consequently, there are differing results in studies of autoregulatory function.

Systemic hypertension may be induced by administration of vasoactive agents such as phenylephrine (122), noradrenaline, dopamine, adrenaline (123) or angiotensin II (124). Agents such as catecholamines have the advantage of short half-lives and may easily be titrated by altering the rates of continuous infusion.

Hypotensive perturbations may be induced by administration of drugs such as β blockers (e.g. esmolol) or ganglionic blockers (e.g. trimetaphan). These agents have short half-lives and may be titrated by altering their infusion rates. Vasodilator agents, such as sodium nitroprusside are not usually used due to concomitant vasodilatory effects on cerebral blood flow.

Mechanical methods for inducing changes in systemic blood pressure include inflation and deflation of intravascular (arterial or venous) balloons (101), pneumatic cuffs (mainly used in clinical research) (122) and controlled haemorrhage (125).

Reduction in cerebral perfusion pressure may also be induced by elevation in cerebrospinal fluid pressure by cisternal infusion of water (52,125)

Alterations in cerebral blood flow have been produced by manipulations of $PaCO_2$ (126). Hypocapnia causes cerebral vasoconstriction and hypercapnia cerebral vasodilation. However, under physiological conditions, carbon dioxide induced changes in cerebral blood flow will be maintained within autoregulatory limits by metabolic mechanisms.

1.2.4.6.2 Measurements

The relationship between cerebral blood flow and cerebral perfusion pressure may be determined by a number of statistical methods. There does not appear to be a standard method of measurement. The conclusions of many studies are influenced by the method of cerebral blood flow measurement.

Most commonly, and probably most accurately, autoregulatory relationships have been determined by regression analysis between mean arterial pressure and cerebral blood flow (92,127). This provides a statistically appropriate method of determining the response of the dependent variable (i.e. cerebral blood flow) on the abscissa (pressure). The slope of the regression line is primarily used as the index of autoregulatory function. Other methods of measurements have used derived cerebrovascular resistance (128) from changes in flow and pressure. For the reasons outlined above, this is not an accurate representation of vascular reactivity.

Patterns of autoregulatory responses have been proposed. Static autoregulatory responses are defined as relative blood flow changes in response to steady-state changes in the blood pressure. Dynamic methods

assess the response to a rapid change in blood pressure. These two methods were compared in humans with both intact and impaired autoregulatory capacity (102). Cerebral blood flow was assessed using transcranial Doppler insonation of bilateral middle cerebral artery velocities. Static autoregulation was determined by analysing the response to a phenylephrine-induced rise in blood pressure, whereas rapid deflation of a blood pressure cuff around one thigh was used as a stimulus for testing dynamic autoregulation. Patients acted as their own controls under propofol and high dose isoflurane anaesthesia, which was used to impair autoregulation. No differences between static and dynamic autoregulatory measurements were demonstrated under high dose isoflurane anaesthesia. This study concluded that measurements of dynamic autoregulation yielded similar results to static testing of intact and pharmacologically impaired autoregulation.

Other Doppler derived indices used to quantify autoregulatory function include the autoregulatory index (129) and Doppler waveform component analysis. Autoregulatory index is calculated from second order linear differential equations calculating the best fit for a transcranial Doppler velocity profile to one of ten hypothetical cerebral blood flow velocity autoregulatory curves. The slope of the autoregulatory curve increases the speed of the autoregulatory response, thereby elevating the derived autoregulatory index. This technique has been used to compare the effects of intravenous (remifentanil and propofol) and inhalational anaesthetics (isoflurane) (128,129).

A further modification of the autoregulatory index has been described by Strebel (122) using transcranial Doppler cerebral blood flow velocities. The dynamic rate of regulation was assessed using computer modelling from the rate of change in calculated cerebrovascular resistance when the blood pressure was decreased. Static rates of regulation were assessed from the change in middle cerebral artery velocities with changes in mean arterial pressure.

Doppler waveform component analysis uses running linear regression analyses of the systolic and diastolic components of the insonated waves. In an ovine traumatic brain injury model, Lewis determined that systolic and diastolic Doppler waveforms became divergent (associated with an increased pulsatility index) as cerebral blood flow became pressure dependent (52). Reductions in cerebral blood flow were induced by controlled haemorrhage and cisternal infusion. This continuous technique in an animal preparation was validated against simultaneous sagittal sinus Doppler measurements.

1.2.4.7 Carbon dioxide reactivity

Carbon dioxide reactivity is frequently used as an index of "metabolic" cerebral autoregulation. As outlined in 1.2.1.4.2, this represents an oversimplification of a complex neurohumoral system. In interpreting measurements of carbon dioxide reactivity, other factors that may influence cerebral metabolic function should be considered. These include oxygenation, cerebral blood volume, intracranial pressure and cerebral metabolic rate, each of which may be influenced by drugs such as anaesthetic agents (flow-metabolism coupling) and by a variety of pathophysiological states (40).

Estimation of carbon dioxide reactivity requires validated measurements of cerebral blood flow and arterial carbon dioxide tension. Some studies have used end-tidal carbon dioxide concentrations as an index of arterial carbon dioxide tensions. However, arterial to end-tidal gradients may exist in conditions of altered systemic perfusion and mechanical ventilation, which may over- or under-estimate $PaCO_2$. Accordingly, arterial measurements are preferable.

Clinical studies of carbon dioxide reactivity have used end-tidal carbon dioxide concentrations and transcranial Doppler velocities as the primary measurements (130,131). For the reason outlined above, interpretation of the results of these studies requires considerable circumspection due to methodological limitations.

Recent advances in intraparenchymally placed electrodes allow highly localised measurements of brain tissue carbon dioxide, pH and oxygen. These direct measurements have the advantage of producing tissue-based measurements that are independent of arterial concentrations and provide information about regional or local metabolic activity. However, they do not reflect global cerebral metabolic function (21,132).

1.3 CATECHOLAMINES

The sympathomimetic catecholamines are the most commonly used vasoactive agents in clinical medicine. The naturally occurring amines, adrenaline, noradrenaline and dopamine, have well-established roles in critical care medicine and anaesthesia.

The introduction of synthetic agents such as dobutamine, isoprenaline and dopexamine has generally been received with variable enthusiasm, largely based on pharmaceutical marketing rather than objective scientific evidence.

The following literature review will concentrate on the biological, systemic and regional, specifically cerebrovascular, effects of the endogenous catecholamines. These are the drugs that were studied for this thesis.

The adrenergic system, of which the catecholamines are the effector agents, has been intensely studied for the last 110 years. Indeed, the Nobel Prize for medicine and physiology has been awarded to scientists of neurobiology thirteen times since inception (133). Specifically, advances in the physiology of the adrenergic system been awarded the Prize six times. In 1936, Dale and Loewi were awarded the Prize for defining the chemical transmission of nerve impulses. This was followed by Hess (1950) and Katz, von Euler and Axelrod (1970) for work on neurotransmitters and the mechanism for their storage, release and inactivation (the concept of synaptic transmission). The identification of G proteins received the prize in 1994, and the discovery of nitric oxide as the predominant vasodilator mediator was awarded the prize in 1998. Further work on signal transduction in the nervous system by Carlsson, Greengard and Kandel received the award in 2000.

However, the findings of much of the innovative basic from these studies has not been integrated into the clinical milieu.

1.3.1 Biosynthesis

The biosynthesis and chemical structures of the naturally occurring catecholamines are shown in Figure 1.4.

Catecholamines consist of an aromatic ring attached to a terminal amine by a carbon chain. The configuration of each drug is important for determining affinity to various receptors. The length of the amine chain influences β receptor affinity, whilst the presence of an ammonium compound at the end of the β chain confers α receptor affinity.

Dopamine is hydroxylated to form noradrenaline, which is the predominant peripheral sympathetic chemotransmitter in man, acting at all adrenergic receptors. The release of noradrenaline from sympathetic terminals is controlled by re-uptake mechanisms mediated via α_2 receptors and augmented by adrenaline released from the adrenal gland at times of stress. Noradrenaline is converted to form adrenaline that is subsequently metabolised in liver and lung (134,135).

All catecholamines have very short biological half-lives (40-90 seconds) and a steady-state plasma concentration is achieved within 5 -10 min after the start of a constant infusion. This allows rapid titration of drug to a clinical endpoint such as mean arterial pressure.

Adrenaline and noradrenaline infusions produce blood concentrations similar to those produced endogenously in shock states, whereas dopamine infusions produce much higher concentrations than those naturally encountered. Dopamine may exert much of its effect by being converted to noradrenaline, thus bypassing the rate-limiting (tyrosine hydroxylase) step in catecholamine synthesis.

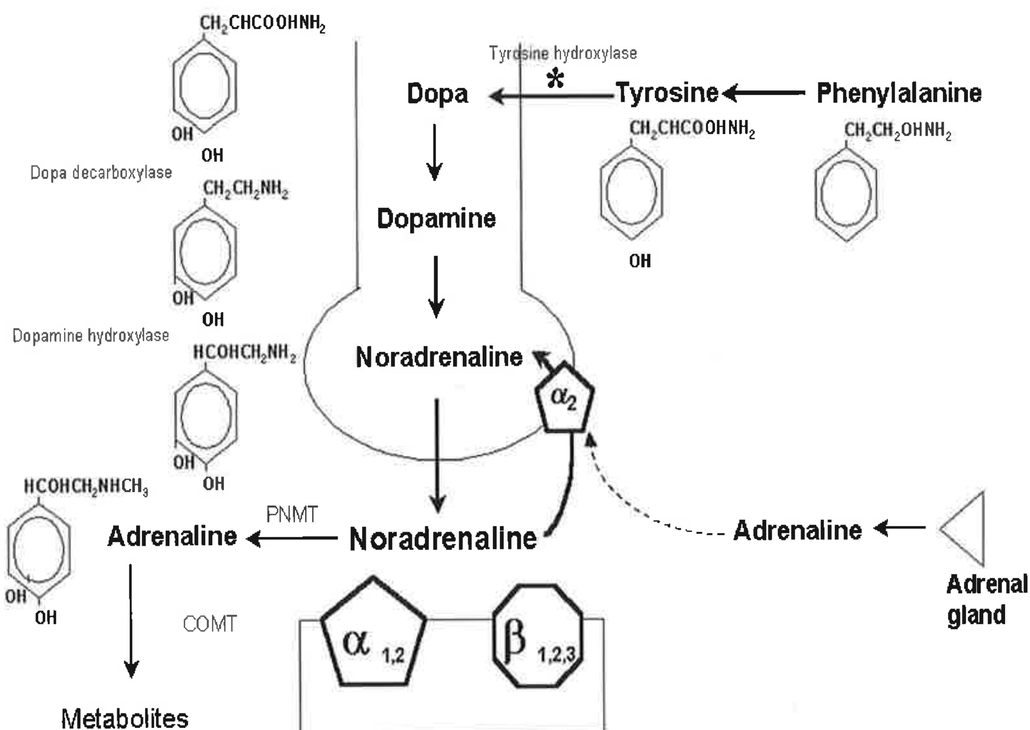


Figure 1.4
*Biosynthesis and structure of endogenous catecholamines. * refers to rate limiting step of tyrosine hydroxylase, PNMT = phenethanolamine-N-methyltransferase, COMT = catechol-o-methyl-transferase.*

The synthetic catecholamines are derivatives of dopamine. These agents are characterised by increased length of the carbon chain, which confers affinity for β receptors. Dobutamine is a synthetic derivative of isoprenaline. These agents have relatively little affinity for α receptors due to the configuration of the terminal amine, which differ from the endogenous catecholamines.

Adrenaline, noradrenaline and isoprenaline all have hydroxyl groups on the β carbon atom of the side chain, and this is associated with 100 fold greater potency than dopamine or dobutamine. (134)

1.3.2 Receptor biology

1.3.2.1 Physiological

Agonists bind to populations of adrenergic receptors, largely divided into α and β subgroups. Further subgroups of α (α_{1A}, α_{1B}, α_{2A}, α_{2B}, α_{2C}) and β receptors (β_1, β_2 and β_3) have been identified (136).

Signal transduction from agonist-receptor occupation to the effector cell is modulated by conformational changes in G proteins associated with these receptors. Under the additional influence of second messengers such as nitric oxide, endothelin and eicosanoids, these conformational changes promote the release of calcium from intracellular stores and increase

membrane calcium permeability. Subsequent phosphorylation of substrate proteins via protein kinases act as third messengers to trigger cascades of events which lead to specific effects, including those on the cardiovascular system (136).

Beta-receptor occupancy predominantly activates adenyl cyclase to increase the conversion of adenosine triphosphate to cyclic adenosine monophosphate (cAMP). Alpha-receptor occupancy acts independently of cAMP by activation of phospholipase C which increases inositol phosphates (IP_3 and IP_4) and diacyl glycerol.

These complex agonist-receptor-effector relationships are responsible for homeostatic mechanisms such as the physiological responses to stress and autoregulation of circulatory beds.

1.3.2.2 Pathophysiological

The activity and function of this system is dynamic and may be markedly influenced by pathological states. This may result in qualitative changes in the agonist-receptor-effector relationship (desensitisation) where receptors no longer respond to physiological or pharmacological sympathetic stimulation to the same extent. Receptor desensitisation has been associated with pro-inflammatory mediators of sepsis e.g. interleukin 6 (IL-6) and tumour necrosis factor alpha (TNFα) (137).

Quantitative changes such as reduced receptor density, receptor sequestration and enzymatic uncoupling (downregulation) may also result in impaired responses (138-140). Prolonged exposure to catecholamine infusions, myocardial ischaemia, hypertension and cardiac failure are associated with receptor downregulation (140,141).

The adrenergic neurovascular system is therefore complex, with many levels of agonist-receptor-interface-transmission-effector interactions.

1.3.3 Pharmacodynamic effects

1.3.3.1 Cardiovascular effects

There are wide and varied opinions about the effects of the catecholamines on the cardiovascular system. The classification of sympathomimetic agents into α and β agonists, based on the above structure/function relationships, is only a crude predictor of systemic effects. However, this remains the standard method of classification in most standard physiology and pharmacology textbooks, albeit some 30 years behind current physiological understanding. Consequently, there are many tables and classifications about the action and affinity of the catecholamines on receptor subtypes. Most of these classifications are not substantiated by controlled physiological

or clinical studies. What is consistent is the inconsistency of these texts and their faithful propagation from edition to edition.

It could be argued teleologically that as noradrenaline is the ubiquitous, naturally occurring endogenous catecholamine, dopamine is essentially a noradrenaline precursor, whilst adrenaline acts primarily as a noradrenaline precursor or regulator. A rationale may be made that the drug of choice in clinical situations of circulatory failure should be noradrenaline. Exogenous infusions are used to replace or augment failing endogenous systems.

The systemic effects of any of these agents will vary greatly between individuals and within individuals at different times. Adequacy of response is often unpredictable and depends on the aetiology of circulatory failure and systemic co-morbidities. In some patients, dramatic responses to small doses may occur, whilst in others, large doses of inotropes may be required to support the failing circulation (135).

From an agonist-receptor-effector perspective, adrenaline, noradrenaline and dopamine are all predominantly β agonists at low doses, with increasing α effects becoming evident as the dose is increased. Alpha-adrenergic mechanisms have an increasingly important role in the failing, or downregulated circulation (142). This applies at both myocardial and peripheral vascular levels.

Isoprenaline increases cardiac output predominantly by increasing heart rate and by moderate inotropy. This occurs with variable effects on blood pressure due to predominant β_2 receptor induced veno- and vasodilatation. Dobutamine is an isomer of isoprenaline. Both of these agents are characterised by long β amine chains. The profile of dobutamine is similar to isoprenaline, although increases in heart rate are not as pronounced. Both of these agents tend to decrease mean arterial pressure, particularly in the presence of inadequate circulating blood volume, due to reduced venous return caused by venodilation. The adverse effects of dobutamine and isoprenaline on heart rate and mean arterial pressure may compromise patients with ischaemic heart disease. However, the vasodilatory effects of dobutamine may be useful in selected patients with predominant systolic heart failure as a means of reducing afterload.

Dopexamine is a dopamine analogue, which has been marketed as a gut-specific synthetic catecholamine, although its profile is essentially similar to dobutamine.

From the above perspective, the cardiovascular effects of the catecholamines are summarised in Table 1.

Agent	β_1 effects + chronotropy + dromotropy + inotropy	β_2 effects + inotropy vasodilatation bronchodilatation	α_1 effects + inotropy vasoconstriction	α_2 effects + inotropy vasoconstriction
Noradrenaline Adrenaline Dopamine	β effects predominate at low dose; α effects predominate at high dose			
Dobutamine	+	+	(+)	-
Isoprenaline	+	(+)	-	-
Dopexamine	+	+	-	-

Table 1.1
Cardiovascular effects of the catecholamines. + = stimulation; (+) = mild effect; - = no effect

The predominant cardiovascular effects of adrenaline, noradrenaline and dopamine are essentially similar. All increase systemic blood pressure, cardiac output and venous return. This is achieved by increasing stroke volume of the ejecting ventricle by increased myocardial contractility and associated increase in cardiac output. The latter may be further increased by associated increases in heart rate. The effects on the peripheral vasculature are similar with all agents increasing venous return, without significant changes in calculated systemic vascular resistance.

Cardiac output is controlled by the peripheral vasculature that is as energetic at returning blood to the heart as the heart is at pumping blood to the periphery (143-145). Vascular responsiveness is mediated via adrenergic receptors: α mechanisms predominantly cause vasoconstriction (both arterial and venous), β mechanisms, specifically β_2 receptors, mediate vasodilatation.

Right atrial pressure, as a surrogate of mean systemic pressure, is a more accurate measurement of venous return and tone than systemic vascular resistance (145). The effects of vasoactive agents on the peripheral vasculature are predominantly on the venous circulation, augmenting and increasing return from the capacitance venous system, rather than specific arteriolar vasoconstriction. Both the arterial and venous systems are integrated under complex neurohormonal influences.

Catecholamines have a significant effect on the venous circulation. These drugs primarily restore or maintain "stressed volumes" of the capacitance vessels under pathological conditions, thereby maintaining or increasing cardiac output and mean arterial pressure. This is important in "vasoplegic" states such as septic shock (146,147).

In clinically used doses, intravenously administered catecholamines have minimal direct vasoconstrictive effects on conducting arterial vessels. Consequently, derived indices such as systemic vascular resistance do not reliably reflect the effect of catecholamines on the peripheral vasculature.

As outlined in section 1.2.2.4, the cardiovascular system is not a homogenous pump driven system through conduits of uniform length and resistance. Consequently, the extrapolation of Ohm's Law to describe or assess haemodynamic function is flawed. The use of derived indices such as systemic vascular resistance to assess the effects of a particular drug on vascular function is dubious. Furthermore, the principle of using systemic vascular resistance as an endpoint to titrate the haemodynamic effect of an exogenous catecholamine is physiologically invalid.

Calculated global oxygen delivery and consumption measurements, using the Fick principle, are frequently used as surrogate indices of global metabolic activity. Calculated oxygen delivery and consumption are surrogate measurements of cardiac output and arterial oxygen tension. Oxygen extraction ratio, using derived oxygen delivery and consumption, are invalidated by mathematical coupling (77,148).

1.3.3.2 Regional vascular effects

1.3.3.2.1 Renal

Many of the effects that the catecholamines exert on regional vascular systems relate to the primary effect on systemic blood pressure and cardiac output. This phenomenon is highlighted in the debate of "renal protection" in patients at risk for developing acute renal failure. "Low dose" dopamine (2-4 μg/kg/min) has been used for 20 years to increase renal blood flow, based on the identification of dopaminergic receptors in the kidney. Dopamine at this dose does indeed induce a natriuesis, which is due to inhibition of adenyl cyclase in the renal tubular cell. Modest increases in cardiac output and blood pressure are also associated with this dose. Similar effects on urine output and blood pressure were demonstrated with dobutamine, adrenaline and noradrenaline (149-151). Increasingly, the recognition of maintaining appropriate blood pressure rather than a specific dopaminergic effect led to many clinicians increasingly questioning the role of "low dose" dopamine. A large randomised, controlled trial recently demonstrated no significant difference between dopamine and placebo in patients with early renal dysfunction (152).

1.3.3.2.2 Splanchnic

The splanchnic circulation is more dependent on mean arterial pressure and the duality of the mesenteric and portal systems, as autoregulation is not as robust as in the brain and kidney. Potentially, the gut is more vulnerable to

ischaemia in shock states, although this remains contentious as diversion of blood away from the splanchnic circulation is part of the stress response (153). Splanchnic metabolic rate is significantly lower than other "vital" organs, thereby tolerating lower perfusion pressures.

Concerns about catecholamine induced splanchnic vasoconstriction with mesenteric and hepatic ischaemia have been raised for many years, but remain unfounded. (153)

Dopamine and dopexamine have been promoted as selective splanchnic vasodilators, but there are no conclusive studies indicating a significant benefit over noradrenaline or adrenaline. Many studies have used gastric intramucosal pH (pHi), a surrogate measurement of splanchnic blood flow, as the primary endpoint (154,155). However, as pHi remains an unvalidated measurement, the results of many of the comparative trials of the different sympathomimetics are inconclusive (156,157). It would appear that all catecholamines are equally effective in increasing splanchnic perfusion by improving cardiac output and mean arterial pressure.

1.3.3.3 Vasoregulation

In addition to adrenergic regulation, neurohumoral influences have a "permissive" or regulatory role in maintaining vasomotor tone. These are mediated through the renin-aldosterone-angiotensin axis and locally acting mediators such as vasopressin, corticosteroids, nitric oxide and endothelin.

The response of the whole neurohumoral system may become blunted in conditions such as severe sepsis, where qualitative and quantitative changes may occur. In this context, failure of vasomotor responsiveness may be considered as part of multiple organ failure.

1.3.3.3.1 Vasopressin

Specific vasopressinergic receptors (V_1, V_2) have been identified in association with sympathetic terminals. Vasopressin is a naturally occurring peptide secreted by the posterior pituitary gland. Reduced serum levels of vasopressin have been demonstrated in septic shock (158) and following cardiopulmonary bypass (159), suggesting an inflammatory mediated mechanism. Levels are maintained during cardiogenic shock (158).

A proportion of patients with severe septic shock, requiring high levels of catecholamines to support the circulation, will respond to low doses of infused vasopressin (0.04u/hr) by significantly reducing doses of infused catecholamine (160). This phenomenon appears to be independent of any directly attributable vasopressor effect, rather as a supplemental "catecholamine sparing" strategy (161). However, the impact on mortality has not been determined in conclusive clinical trials.

1.3.3.3.2 Corticosteroids

The role of steroid supplementation in circulatory failure has been studied for many years. Immunosuppressive or anti-inflammatory doses of corticosteroids have been shown to be ineffective, particularly in septic shock. Low dose steroids, administered as replacement of "stress response" doses (approximately 100-200 mg hydrocortisone per day) have been shown to improve vasoresponsiveness to catecholamine infusions in patients with refractory shock (162).

Patients who respond to low doses of steroids may have biochemical evidence of hypoadrenalism. This may be defined by a low serum cortisol level and/or a blunted response to intravenous adrenocorticotrophin, or functional hypoadrenalism as part of the multiple organ failure syndrome (163,164).

1.3.3.3.3 Nitric oxide synthetase inhibitors

Nitric oxide is a ubiquitous molecule that has an important role in regulation of vasomotor tone, particularly vasodilatation. "Vasoplegic" states, such as those occurring in septic shock may be associated with pathologically high levels of nitric oxide activity.

Inhibition of nitric oxide synthetase, the predominant enzyme in nitric oxide synthesis, with compounds such as methylene blue, N^G-mono-methyl-l-arginine (L-NMMA) or N^ω-nitro-l-arginine methyl ester (L-NAME), is associated with a transient pressor response. However, clinical trials, particularly in septic shock, have not shown improvement is organ failure resolution and may be associated with increased mortality (165,166).

1.3.3.4 Non-vascular effects

A large number of catecholamine mediated metabolic, immunological and systemic effects have been identified.

In brief, catecholamines have been implicated in the regulation of alveolar water clearance in acute pulmonary oedema, immunomodulation and T-cell activity and bacterial clearance (167-169).

1.3.3.5 Metabolic effects

Catecholamine mediated β-stimulation may result in hyperglycaemia, hypokalaemia and hypophosphataemia which may need monitoring and correcting.

Adrenaline is associated with the development of lactic acidosis, due to inhibition of pyruvate dehydrogenase (170). Whilst pH may fall to levels around 7.2, the acidosis is not associated with impaired tissue perfusion or hypoxia. In most patients who are haemodynamically stable, this is a self-limiting phenomenon and is not associated with adverse outcomes (171).

This may become an issue in severe sepsis with the development of a marked acidosis (172).

1.3.4 Studies in pathological states

There have been few controlled physiological studies comparing the effects of catecholamines over the last 50 years since early studies in volunteer medical students. This may be due to the established role that these drugs have in practice. Most of the research has been in pathological states, particularly septic and cardiogenic shock. Many of these studies have been predicated on the purported action of particular drugs, based on little or no comparative physiological data. Furthermore, research into the clinical use of catecholamines has been markedly influenced by pharmaceutical company driven research.

Given a degree of pharmacological equivalence between the endogenous catecholamines in the septic shock literature, it is tempting to apply these data to patients with normal cardiovascular systems who are receiving vasoactive agents to augment systemic blood pressure. There are no human studies addressing this issue and the answer has to be provided by extrapolation from animal studies, in spite of the obvious limitations of this approach.

1.3.4.1 Cardiopulmonary resuscitation

Adrenaline has been used for circulatory collapse at least since 1907. The International Liaison Committee on Resuscitation guidelines recommend adrenaline as first line inotrope/vasopressor in cardiopulmonary resuscitation (173). The use of "high dose" adrenaline (5mg), noradrenaline or phenylephrine in cardiopulmonary resuscitation has not been demonstrated to improve return of spontaneous circulation or survival (174).

1.3.4.2 Cardiogenic shock

Theoretically, catecholamine infusions may confer some advantages in cardiogenic shock, particularly in association with acute myocardial infarction (175). In patients with systolic heart failure, adrenaline, noradrenaline, dopamine and dobutamine have been shown to cause satisfactory short-term effects. This may allow the myocardium time to recover from post ischaemic "stunning," particularly after revascularisation. However, no increased long term survival due to their use has been demonstrated.

Noradrenaline is increasingly being used as a first line drug in patients with cardiogenic shock, although dobutamine has traditionally been used in this situation.

1.3.4.3 Separation from cardiopulmonary bypass

Numerous combinations of catecholamines have been used successfully to wean patients from cardiopulmonary bypass. However, there are no definitive studies demonstrating significant benefits of one catecholamine over another (176). Similarly, the question as to whether mechanical support devices, such as intra-aortic counterpulsation, offer a significant advantage over catecholamines following cardiac surgery remains unanswered (177).

Adrenaline, noradrenaline and/or dopamine have been found to increase cardiac output with little increase in heart rate or afterload and are often regarded as first line drugs. At higher doses (>40μg/kg/min), dopamine has been shown to cause more tachycardia than adrenaline and noradrenaline. Dobutamine may be associated with vasodilatation and hypotension.

There is no conclusive evidence that the catecholamines, including noradrenaline, cause vasospasm of arterial conduits in clinically used doses.

Phosphodiesterase inhibitors, such as milrinone, either as sole agents or in conjunction with adrenaline or noradrenaline have been used with success. These may have a role following mitral valve replacement in patients with pulmonary hypertension or pre-operative diastolic failure.

Cardiopulmonary bypass may be associated with a systemic inflammatory response syndrome characterised by a hyperdynamic, vasodilated "low systemic vascular resistance" state (178,179). Noradrenaline is frequently advocated as a "pressor" agent in this context to restore mean arterial pressure. This condition is usually self-limiting with a nadir eight hours post bypass.

1.3.4.4 Right ventricular failure

Right ventricular infarction and major pulmonary embolism may be associated with acute right ventricular failure. Right ventricular depression may also occur in severe sepsis (180). Restoration of preload is critical in these conditions, as the failing right ventricle is particularly susceptible to reductions in preload (181).

Inotropes such as noradrenaline and adrenaline are regarded as first line drugs in these situations in order to maintain adequate mean arterial pressure so that right coronary artery perfusion, that occurs throughout the cardiac cycle, is maintained.

Concerns about pulmonary artery vasoconstriction and increased right ventricular afterload by noradrenaline and adrenaline appear to be unfounded. Consequently, these drugs have superseded the use of traditional vasodilators such as isoprenaline in acute right ventricular failure.

1.3.4.5 Septic shock

The cardiovascular effects of septic shock are complex and range from a hyperdynamic, vasodilated state to one of increasing myocardial failure and paralysis of the peripheral vasculature (vasoplegia) (182-184) The latter represents inability of the venous circulation to respond to endogenous or exogenous catecholamines with resultant venous pooling. Noradrenaline was considered a potent vasoconstrictor agent and used to reverse vasoplegia, but potentially caused renal and splanchnic ischaemia. However, an increasing body of literature now supports the use of noradrenaline and adrenaline as first line agents in the septic syndrome and septic shock by effectively defending cardiac output, mean arterial pressure and thereby tissue perfusion (185-189).

Systemic vascular resistance was not demonstrated to be significantly altered by catecholamine infusions in septic shock (190). Despite widespread recent use, the efficacy of dobutamine and isoprenaline in septic shock is questionable and appears to add little to the efficacy of noradrenaline or adrenaline when used in combination (191).

The use of noradrenaline has changed significantly over this period and is now regarded by most clinicians as a first line drug for the defence of blood pressure (186,187). This change in practice has occurred in the absence of controlled trials, due rather to the recognition of augmenting failing endogenous systems, by using exogenous infusions of the predominant endogenous catecholamine. However, it is likely that adrenaline is equi-effective with a similar profile, although there are no studies to affirm this.

In summary, it appears that under physiological conditions, the endogenous catecholamines produce equivalent systemic haemodynamic effects, predominantly increasing systemic blood pressure. Under pathological conditions, a similar profile is probable, although there are no outcome-based studies that attribute benefit to one vasoactive agent over another.

1.3.5 Cerebrovascular effects

Aspects of the cerebral circulation are discussed in section 1.2.1. The physiological effects of catecholamines on the cerebral circulation and under altered conditions of autoregulation and blood-brain barrier permeability are the focus of this thesis. These interactions are important given the increasing use of catecholamines to augment the cerebral circulation in conditions of absolute and relative cerebral hypoperfusion.

1.3.5.1 Catecholamines and the blood-brain barrier

Under physiological conditions, catecholamines do not normally cross the blood-brain barrier (12).

The integrity of the endothelial cell lining of the cerebrovascular bed constitutes a morphological blood-brain barrier mechanism to neurotransmitter monoamines. Circulating monoamines are prevented from entering the brain primarily at the luminal membrane of the endothelial lining. The small percentage of amines that may pass this membrane are deaminated within the endothelial cells, pericytes and smooth muscle layers of brain microvessels where O-methylation occurs (12,192). In the choroid plexus a corresponding deamination and O-methylation occurs in the epithelial cells. The presence of these enzymes constitutes a further, enzymatic, blood-brain barrier in the brain vessels for these monoamines. The monoamine precursors L-dopa and L-5-hydroxytryptophan readily pass from the luminal endothelial cell membrane but are trapped by another enzymatic barrier mechanism. Within the endothelial cells and pericytes of the microvasculature, these compounds are decarboxylated to their corresponding amines and then immediately deaminated. A clinical implication of these enzymatic barrier mechanisms is the use of decarboxylase and monoamine oxidase inhibitors as adjuncts to L-dopa treatment of Parkinson disease. These substances facilitate the entry of L-dopa into brain and thus increase the amount of dopamine available at receptor sites.

However, there is evidence that dopamine may still exert a direct effect on the cerebral circulation in the presence of an intact blood-brain barrier. Selective transmission of dopamine may occur across the natural defects in the blood-brain barrier in areas such as the posterior pituitary gland or pineal gland that have specific dopaminergic receptors, or via non-adrenergic central neural mechanisms (193,194). This may be compounded by high circulating concentrations of catecholamines (either endogenous or exogenous) which may also open the morphological barrier, by inducing an acute rise in systemic blood pressure (12,114).

1.3.5.2 Hypertension and blood-brain barrier function.

A brief hypertensive or hypertonic stimulus can transiently open the blood-brain barrier through an effect on endothelial cell linings. High circulating concentrations of monoamines can also open the morphological barrier, but probably only indirectly by inducing an acute rise in systemic blood pressure. Once the barrier is open, systemically administered monoamines enter the brain parenchyma, where they can induce pronounced changes in cerebral blood flow and metabolism.

In animal models, catecholamine induced hypertension has been demonstrated to alter the morphology of the blood-brain barrier.

In a conscious rodent model, Sokrab used intravenous adrenaline to induce acute hypertension and determined the effect on blood-brain barrier

permeability with Evan's blue and horseradish peroxidase tracers (195). Multifocal sites of extravasation of the tracers and endogenous plasma albumin, fibrinogen and fibronectin were identified by immunohistochemistry in the cortex, hippocampus, thalamus and basal ganglia within 24 hours. A study by Abdul-Rahman correlated adrenaline induced increases in cerebral blood flow with associated alterations in blood-brain barrier permeability (109). Similar findings were demonstrated in a feline model using angiotensin (108).

Tuor examined the relationship between catecholamine-induced hypertension and cerebral blood flow, cerebral glucose metabolism and blood-brain barrier permeability in a rodent model (114). Blood-brain barrier permeability, assessed using an autoradiographic technique, was increased during moderate hypertension induced by dopamine but not when induced by noradrenaline.

The mechanism of catecholamine induced hypertensive changes in blood-brain barrier permeability has been attributed to increased pinocytotic activity of endothelial cells. This was demonstrated with noradrenaline in a rodent model by Saramento, but is probably common to all catecholamines (196). This mechanism has been implicated in the pathogenesis of hypertensive encephalopathy (107,121).

1.3.5.3 Osmolality and blood-brain barrier function

Changes in serum osmolality, or the delivery of high concentration of hypertonic solutions to the brain will disrupt blood-brain barrier permeability. This is a technique that has been used to facilitate delivery of chemotherapeutic drugs such as melphalan and etoposide into the brain (111,197). These studies have particular importance to interactions with intravenous and inhalational anaesthetics with may potentiate osmotic disruption of the blood-brain barrier. Potentially, the concomitant use of catecholamines with these hypertonic solutions or under conditions of hyperosmolality may result in direct effects of catecholamines on the cerebral vasculature.

1.3.5.4 Catecholamines and cerebral blood flow

1.3.5.4.1 Physiological studies

The cerebral circulation is under intense neurohumoral regulation. The role of central adrenergic neurones (194) and adrenal medullary catecholamines in regulating regional cerebral blood flow has been elucidated (198). These systems are regulated in the dorsal medullary reticular formation and may be responsible for blood-brain barrier permeability regulation and cerebral blood flow autoregulation.

Under physiological conditions, in the presence of an intact blood-brain barrier, catecholamines should not directly effect cerebrovascular mechanics. Provided catecholamine induced hypertension is confined within upper autoregulatory thresholds, this statement is probably true.

The effect of hypertension on cerebral blood flow has been the subject of some studies, with the majority using pharmacological agents to increase blood pressure. Hypertension induced with metaraminol has previously been shown to have no effect on cerebral blood flow, despite significant increases in mean arterial pressure, as long as the systemic pressure is kept below approximately 150 mmHg (199,200). Similar results have been found with other vasopressors such as phenylephrine and noradrenaline (201-203). As none of these agents were considered to have a significant direct effect on the cerebrovascular smooth muscle, these findings were believed to reflect the normal autoregulatory mechanisms of the cerebral circulation.

There are few studies quantifying and comparing the effects of catecholamines on cerebrovascular mechanics in normal subjects. In an early study, King et al compared the effects of adrenaline (using doses of 6-22 μg/min) and noradrenaline (19-73 μg/min) on cerebral blood flow, measured using the nitrous oxide (Kety Schmidt) method, in awake human volunteers (204). Induced hypertension with adrenaline was associated with increased cerebral blood flow that was attributed to increased cerebral metabolism. Noradrenaline was associated with decreased cerebral blood flow, attributed to increased (calculated) cerebrovascular resistance in the absence of demonstrable changes in metabolism (determined by using the Fick equation). Indeed, although this study has provided much of the "evidence" about catecholamines and cerebral blood flow that appears in standard physiology textbooks, it is limited by the use of disparate doses of adrenaline and noradrenaline in different individuals, and by using an intermittent measurement of cerebral blood flow.

Tuor examined the relationship between catecholamine-induced hypertension and cerebral blood flow, cerebral glucose metabolism and blood-brain barrier permeability in a rodent model (114). Direct effects of dopamine and noradrenaline on regional and global cerebral blood flow were demonstrated for dopamine, but not for noradrenaline.

There are few published studies analysing the effects of catecholamines on intracranial pressure or autoregulation in normal subjects. Most of the studies analysing these parameters have been conducted in patients with traumatic brain injury.

1.3.5.4.2 Pathological studies

Although there is a relative paucity of physiological studies on the cerebrovascular effects of catecholamines, these drugs are widely used in

pathological conditions. Most of the conditions where catecholamines are used to augment cerebral blood flow and cerebral perfusion pressure are associated with alterations in blood-brain barrier permeability. These include traumatic brain injury and aneurysmal subarachnoid haemorrhage. The demonstrable direct effects of catecholamines on cerebrovascular function in these circumstances should prompt caution or at least invite systematic study.

The Brain Trauma Foundation published evidence based medicine guidelines for the management of traumatic brain injury. In these aggressive defence of cerebral perfusion pressure is recommended, using “vasopressors” once hypovolaemia has been corrected (5,205). The evidence level for this recommendation is Class III – i.e. case series and expert opinion. The broad application of these guidelines has now raised concerns about the open-ended use of catecholamines in this situation (6).

Augmentation of cerebral blood flow by induced hypertension, hypervolaemia and haemodilution (“HHH” therapy) to treat cerebral vasospasm associated with aneurysmal subarachnoid haemorrhage is a strategy that has been advocated for 20 years. However, there is little evidence that “HHH” therapy either reverses vasospasm or improves outcome for aneurysmal subarachnoid haemorrhage (206). Indeed, the use of vasoactive agents to increase cerebral blood flow in these patients may be associated with adverse outcomes due to cerebral hyperaemia and ischaemia, particularly with dopamine (207). Furthermore, “HHH” therapy is associated with an increased rate of medical complications that may be in part due to the injudicious use of inotropes in these patients (208).

Studies comparing the effects of catecholamines in these conditions are few, those that have been done have significant methodological limitations.

A small study by Biestro et al compared the effects of dopamine, noradrenaline, methoxamine and dopamine plus methoxamine on cerebral perfusion pressure and intracranial pressure in a series of head injured patients (209). Noradrenaline and dopamine plus methoxamine were equally effective in increasing cerebral perfusion pressure without demonstrable increases in intracranial pressure.

In a recent study, Ract compared the effects of dopamine and noradrenaline, using a cross-over design, in nineteen head injured patients (210). For the same mean arterial pressure, intracranial pressure was significantly greater with dopamine than with noradrenaline. This was not associated with changes in indirect measurements of cerebral blood flow using jugular venous saturation or transcranial Doppler. This small human study is in accordance with the physiological studies outlined, suggesting that, in traumatic brain injury which is associated with altered cerebral autoregulation

and blood-brain barrier permeability, dopamine has more pronounced, potentially adverse effects on the cerebral circulation.

1.4 PROPOFOL

Propofol is a commonly used intravenous anaesthetic. In modern anaesthesia, its use has superseded that of thiopentone for induction of general anaesthesia. Increasingly, propofol is used as a sedative agent (often for prolonged periods) in critically ill patients managed in the Intensive Care Unit. Many of these patients concomitantly receive catecholamines as part of their clinical management.

The potential for catecholamine:anaesthetic interactions is high in these clinical situations. There is little data analysing these interactions. The following is a summary of the pharmacology of propofol, with reference to the cerebrovascular effects.

1.4.1 Pharmacology

Propofol is 2,6-di-isopropyl-phenol, a member of the group of hindered alkyl phenols.

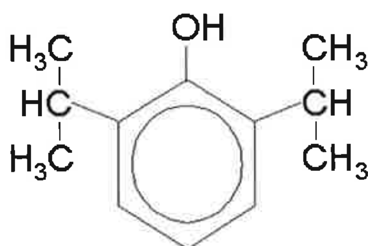


Figure 1.5
Chemical structure of propofol.

Propofol was introduced to clinical anaesthesia in1977. It is poorly soluble in water and was initially solubilised in cremofor EL, but this was withdrawn due to anaphylactoid reactions. It has subsequently been reformulated with an emulsion of lipid (Intralipid®).

1.4.2 Pharmacokinetics

Intravenous administration of propofol results in rapid loss of consciousness, with a rapid recovery.

The pharmacokinetics of propofol have been analysed by examination of the time-course of concentrations in either systemic arterial or venous blood and fitting the data to conventional compartmental models. The findings of a number of studies indicate that, even across species, the time-course of blood concentrations fit a three-compartment model (211,212).

Propofol is assumed to instantaneously distribute in a central compartment with a calculated mean volume of distribution in man of approximately 0.2 L/kg (0.1-0.7 L/kg). There is an initial rapid decrease in drug concentrations in the blood, thought to represent distribution from blood to well-perfused tissues, with a measured half-life of between 2-4 minutes. Following this, there is a slower decrease, thought to represent metabolic clearance, with an elimination half-life of between 25-60 minutes. The third phase of prolonged slow decrease is believed to represent exchange between blood and fat and has a half-life between 150-800 minutes.

Based on these "three-compartment" analyses, predictions of the time course of propofol concentrations during anaesthesia have been made (213).

The pharmacokinetics of propofol have been extensively studied in our sheep laboratory (214-217). Compartmental pharmacokinetic analysis of propofol concentrations from the brain and arterial circulation poorly describe the time course of brain and arterial concentrations of propofol during rapid administration. These standardised pharmacokinetic analyses are inadequate as a basis for accurate dose regimens. Brain concentrations of propofol are relatively independent of the administration rate, although slight dose sparing with slow administration can occur (214,216). Very rapid administration may increase the risk of hypotension without significantly affecting the depth or rate of onset of anaesthesia.

The consistent relationship between brain concentrations and depth of anaesthesia over time has also been shown to support the concept of acute tolerance during induction of anaesthesia.

Distribution to the brain is dependent on cardiac output, rather than conventional factors such as body mass (218).

1.4.3 Pharmacodynamics

1.4.3.1 Systemic

Apart from induction of anaesthesia, the principle pharmacodynamic effect of propofol is reduction in mean arterial pressure. This effect has been implicated in documented examples of mortality and morbidity (219). The exact mechanism of the haemodynamic effect of propofol remains unclear. Propofol has an effect on the peripheral vasculature, probably by ablation of sympathetic tone and reduction in venous return (220). Other mechanisms implicated in this reduction in preload include blockade of calcium channels and endothelial derived tone (221,222).

Propofol has an effect on myocardial contractility. A negative inotropic effect on isolated myocardial muscle has been demonstrated *in vitro* through mechanisms that are different to those seen with volatile anaesthetic agents (223). Propofol administered to both pigs and dogs was found to induce

systemic hypotension and dose-dependent depression in myocardial contractility (220,224).

In vivo studies have demonstrated that propofol induced hypotension is multifactorial with variable contributions of myocardial depression, reduced venous return and vasodilation (225,226). A further factor relates to central nervous system depression, reduction in sympathetic tone and arterial baroreceptor reflexes. These may reduce normal homeostatic responses to hypotension and it is in this context that exogenous catecholamines are frequently used to defend systemic blood pressure in patients with ostensibly normal circulations. These effects may become more pronounced in patients with associated cardiac disease on cardiac medication such as β blockers or angiotensin converting enzyme inhibitors.

1.4.3.2 Cerebrovascular pharmacodynamics

Anaesthetic agents have variable effects on cerebral blood flow. Volatile agents generally produce cerebral vasodilation, particularly at high doses, producing gradual relative increases in cerebral blood flow as cerebral metabolic rate decreases (227,228). In contrast, intravenous anaesthetic agents produce parallel dose-dependent decreases in both cerebral blood flow and cerebral metabolism. Coupling of flow and metabolism are considered to be preserved with agents such as propofol, barbiturates and benzodiazepines, with the observed decreases in cerebral blood flow considered to be secondary to drug induced decreases in cerebral metabolism.

After administration of single doses, decreases in cerebral blood flow of 51% and 27% were recorded in man in some of the first studies of cerebral blood flow and propofol (229,230). In these studies however, reductions in cerebral blood flow exceeded associated reductions in cerebral metabolism. Propofol-induced reductions in cerebral blood flow have also been demonstrated in canine and lapine preparations, with maximal decreases of 60%. Greater reductions in cerebral blood flow may be due to associated systemic hypotension.

The effects of propofol on cerebral blood flow and autoregulation have been studied using a number of methods. Using a porcine preparation, Lagerkranser studied the effects of propofol on the cerebral circulation over a range of mean arterial pressures (101). Changes induced under propofol anaesthesia were compared to a "control" group receiving low dose isoflurane and nitrous oxide anaesthesia, where autoregulation was assumed to be well preserved. Propofol caused a significant reduction in cerebral blood flow and did not significantly alter the slope of the regression line of regional cerebrovascular resistance versus mean arterial pressure, although inter-individual differences were observed.

In a primate preparation, van Hemelrijck compared the effects of propofol in increasing doses on cerebral blood flow and autoregulation (231). Dose-dependent reductions in cerebral blood flow were demonstrated that were considered to be coupled to cerebral oxygen consumption. In this preparation, physiological responsiveness to alterations in mean arterial pressure was preserved.

These animal studies suggest that cerebral autoregulation is reasonably well preserved under propofol anaesthesia.

There are few controlled studies of the effects of propofol on autoregulation in humans. Strebel assessed alterations in dynamic autoregulation from the response of middle cerebral artery blood flow velocities in anaesthetised patients (122). Transient step increases and decreases in mean arterial pressure were induced by infusions of phenylephrine and rapid inflation/deflation of thigh cuffs respectively. Patients receiving low or high dose propofol infusions were compared to "baseline" anaesthesia with fentanyl and nitrous oxide. This study demonstrated that neither static nor dynamic rates of regulation were altered by propofol. Other studies by Engelhard comparing the effects of combination anaesthesia with remifentanil/propofol with isoflurane demonstrated that intravenous anaesthesia did not significantly alter physiological cerebrovascular responses to changes in mean arterial pressure (128).

A brief report about sudden cardiovascular death in a series of head injured patients receiving infusions of noradrenaline and propofol highlighted the potential interaction between propofol and catecholamines in conditions of impaired brain function (232). There is a paucity of data on these interactions and this forms the basis of the studies for this thesis.

1.5 Isoflurane

Isoflurane is the most commonly used volatile anaesthetic agent in neuroanaesthesia. This is primarily due to a wide safety profile with preservation of cerebral blood flow. The following is a summary of the pharmacology of isoflurane with reference to its cerebrovascular effects.

1.5.1 Pharmacology

Isoflurane is 2,2,2-triflouro-1-chloroethyl-difluoromethyl-ether.

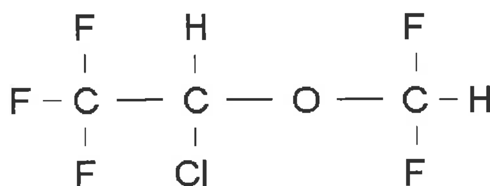


Figure 1.6
Chemical structure of isoflurane.

Isoflurane is a halogenated ether. It was introduced into clinical anaesthesia in 1981. It has a minimum alveolar concentration (MAC) of 1.15% (0.5% in 70% nitrous oxide/oxygen). It is lipid soluble with an oil:gas solubility coefficient of 0.99, and a saturated vapour pressure of 250 mmHg (20°C).

1.5.2 Pharmacokinetics

The uptake and distribution of inhalational anaesthetic agents requires the transfer of the agent from the anaesthetic delivery system to the brain. The depth of anaesthesia produced by these agents is dependent on the tension produced in the brain. Factors that determine uptake from the delivery system to the alveoli include the inspired gas concentration and alveolar ventilation. Transfer from alveoli to arterial blood is dependent upon the blood:gas partition coefficient, cardiac output and alveoli:venous pressure difference. Transfer from arterial blood to the brain tissue is dependent upon the tissue:blood partition coefficient, cerebral blood flow the arterial:tissue pressure difference.

Elimination is by reversal of the uptake process. Isoflurane is essentially eliminated unchanged, as it undergoes almost no biotransformation (0.2%).

Dosage is prescribed according to MAC. This is a concept that was introduced to quantitate and compare the potencies of volatile anaesthetic agents and to allow correlation between the administered dose of an inhalational anaesthetic agent and its expected effects. MAC is defined as the concentration in the alveoli (at atmospheric pressure at steady-state) that prevents a motor response to a standard surgical skin incision in 50% of a population. A further extension of this concept is MAC_{bar}, which is the concentration at which any autonomic response to such a stimulus is prevented in 50% of the population (233).

Whilst MAC has an established place in clinical anaesthesia and is a useful method of comparing relative potencies of anaesthetic agents, it has limitations when used in pharmacokinetic or pharmacodynamic studies. A surgical incision is a difficult stimulus to standardise and there are cross species differences in animal studies. Such pharmacodynamic studies may

be standardised by establishing a depth of anaesthesia that may be assessed by reproducible stimuli, such as with analgesiometry, or by performing studies at variable levels of steady-state concentration (234). These concentrations may be determined using end-tidal expired gas concentrations of the volatile agent in question with specific expired gas analysers.

1.5.3 Pharmacodynamics

1.5.3.1 Systemic pharmacodynamics

Isoflurane produces dose-dependent reductions in mean arterial pressure and cardiac output. This is primarily due to a reduction in venous return due to reduction of sympathetic tone. Direct myocardial depression has been attributed to calcium channel inhibition (223), which has an equivalent vasodilatory effect. However, cardiac output is usually well maintained, without significant changes in heart rate (235). Isoflurane in clinically used concentrations produces haemodynamically stable anaesthesia (236).

1.5.3.2 Cerebrovascular pharmacodynamics

Volatile agents generally produce cerebral vasodilation, particularly at high doses, producing gradual relative increases in cerebral blood flow as cerebral metabolic rate decreases (227,236).

An early study by Boarini compared the cerebrovascular effects of isoflurane and halothane in a canine model (236). Isoflurane was administered in doses of 1.5% and 2%. Mean arterial pressure and heart rate were significantly higher than that produced under 1% and 1.3% halothane respectively. Cerebral blood flow was initially maintained at baseline levels, but progressively decreased over time. However, marked regional blood flow changes were demonstrated, with increased cerebral blood flow demonstrated in the posterior fossa, cerebellum and brain stem. Other studies have demonstrated a relative increase in cerebral blood flow by an uncoupling of flow and metabolism (124,237).

Studies on the effects of isoflurane on cerebral autoregulation have demonstrated dose-dependent changes in autoregulatory thresholds. In a series of patients undergoing spinal surgery (124), Olsen concluded that autoregulation was disrupted at 2 MAC, but not during 1 MAC isoflurane anaesthesia.

Strebel assessed alterations in dynamic autoregulation from the responses of middle cerebral artery blood flow velocities in patients anaesthetised with low (0.5 MAC) and high (1.5 MAC) dose isoflurane (122). The study demonstrated that low dose isoflurane delayed autoregulatory responses to changes in systemic blood pressure, whilst high dose isoflurane ablated autoregulation.

Engelhard compared the effects of 1.5 MAC isoflurane anaesthesia on dynamic cerebrovascular autoregulation compared to awake humans (128). Autoregulatory responses were delayed under isoflurane anaesthesia compared to the awake subjects.

These effects may be attributed to isoflurane induced changes in cerebral blood flow and blood-brain barrier permeability (119,124,236).

There are little data about specific isoflurane:catecholamine interactions in studies where autoregulatory thresholds were determined by induced hypertension. In a lapine preparation, Patel studied the effects of induced hypertension by angiotensin II, noradrenaline and phenylephrine on global and regional cerebral blood flows (measured by radioactive microspheres) during 1.0 MAC isoflurane anaesthesia (41). The slopes of pressure/flow curves produced by noradrenaline and phenylephrine were significantly steeper than that produced by angiotensin II in all cerebral blood flow regions. Patel concluded that noradrenaline and phenylephrine caused indirect cerebral vasodilation, whilst angiotensin II caused vasoconstriction during 1.0 MAC isoflurane.

Chapter 2. Description and validation of a method of cerebral blood flow measurement

In the previous chapter, the principles and pitfalls of cerebral blood flow measurement in various research preparations were reviewed. Many studies analysing dynamic changes in cerebrovascular mechanisms in response to fluctuations in systemic blood pressure were limited by intermittent and indirect measurements of cerebral blood flow. Many of the studies conducted under anaesthesia compared the changes under one method of anaesthesia with another. Very few studies had physiological control groups with baseline stability with which interventions could be compared.

2.1 Principles of measurement

In order to conduct studies of dynamic changes in cerebrovascular mechanics, the following characteristics of cerebral blood flow measurement are considered ideal:

1. Baseline stability
2. Continuous (real-time) measurements
3. Accurate frequency response over a range of pressures and flow
4. Anatomical site that is representative of global or targeted regional flow
5. Stable flow patterns that are independent of vessel calibre
6. A method validated against quantitative measurements
7. A method of measurement does not have direct or indirect effects on cerebral blood flow

Similar principles apply to the measurement of systemic and intracranial pressures, particularly if autoregulatory function is to be determined.

The performance of instruments for high fidelity pressure measurement in terms of frequency response and transduction of pressure waves have significantly improved with technological advances. These include high frequency transducers that act on a fluid interface, fibreoptic pressure transducers (88) and strain gauge tipped solid state systems (89). All of these systems allow continuous measurement of pressures and have been extensively validated. Furthermore, access to systemic and intracranial pressures is relatively easy by accessing the arterial circulation, or the intracranial space respectively.

Estimating or measuring the rate of cerebral oxygen consumption using the Fick principle requires access to arterial and venous blood draining the brain.

This may be achieved by placement of catheters in an artery and in the jugular bulb or sagittal sinus. Previous studies in the sheep have demonstrated that arterial and organ-specific venous blood can be frequently sampled in the awake or anaesthetised sheep through chronically implanted catheters (215,238).

2.2 DOPPLER FLOW METHODOLOGY

The principles of Doppler measurements were reviewed in section 1.2.4.1.2.

Doppler flow meters on cerebral arteries cannot be used to measure cerebral blood flow in sheep because multiple arteries supply blood to the brain and major flow redistribution can occur between these vessels (7). Measurement at only one arterial site could therefore lead to inaccuracies in flow estimation. Furthermore, changes in vessel diameter that may occur under physiological or pathophysiological circumstances can alter vessel diameter and hence the relationship between flow and blood velocity (49).

The sagittal sinus in sheep is a fixed structure that is not subject to fluctuations in vessel calibre and is representative of global cerebral blood flow. Using dissection and microsphere flow measurement techniques, blood in the dorsal sagittal sinus represents approximately 75% of cerebral venous blood. This blood drains from both hemispheres and is almost completely free of extra-cerebral blood provided measurements or sampling from the transverse sinuses are avoided (46,47).

2.3 THE UNIVERSITY OF ADELAIDE PREPARATION

A system for measuring cerebral blood flow and sampling cerebral venous blood, developed at the Department of Anaesthesia and Intensive Care at the University of Adelaide, has evolved over the last 15 years. This system involves the placement of a Doppler flow meter onto the sagittal sinus through a craniotomy and selective cannulation of the sinus as part of a chronically catheterised ovine preparation.

This has been applied to a number of pharmacokinetic and pharmacodynamic studies of cerebral drug disposition (215,217,238-242). These studies have included descriptions of validation studies of this preparation.

The following is a summary of validation studies of this preparation that were used for the cerebral blood flow measurements and cerebral venous sampling studies conducted in this thesis.

Development and validation of this method involved two separate processes: identification of a suitable location for probe and catheter placement, and validation of the flow method.

2.3.1 Identification of a suitable anatomical site

2.3.1.1 Anatomy

This is reviewed in section 1.2.1.1. In sheep, the anatomy has been described in detail (8). Intracranially, capillary blood drains through cerebral veins and into cerebral sinuses that have walls composed of fibrous dura mater and lined with endothelium. These sinuses pass out of the skull to become veins and, shortly afterwards, are usually joined by veins draining the soft tissues of the head and face (10). Therefore, a site proximal to this confluence of extra- and intra-cerebral veins must be accessed if pure cerebral effluent blood is to be collected, and only the flow of cerebral blood is to be measured.

Measurement of flows in the jugular bulb have been used to measure cerebral blood flow, but are not considered suitable because this collects blood predominantly from only one hemisphere in sheep. Furthermore, in a chronically catheterised preparation, neck movement during chronic implantation could dislodge the catheter, and changes in vessel diameter with changes in venous pressure could alter the blood velocity-blood flow relationship.

2.3.1.2 Validation studies

Early reports suggested that the dorsal sagittal sinus may be a suitable site (74). The anatomical validation studies were conducted in the University of Adelaide sheep laboratory. Of these, *in vivo* digital subtraction angiography and retrograde dye studies were conducted by Ludbrook *et al* (217,243).

In the angiographic studies, the arterial phase of passage of contrast revealed multiple branches of the carotid artery with a relatively small fraction of blood flow going to the brain. Examination of the parenchymal and venous phases revealed that the predominant drainage of blood (approximately 75%) was through cerebral veins draining into the sagittal sinus along the dorsal aspect of the brain, then to the two transverse sinuses. Extra-cerebral blood flow joining the transverse sinus ("downstream" from the sagittal sinus) represented less than 5% of the blood flow at this point.

The area drained by the sagittal sinus was examined post-mortem by retrograde injection of India ink. Dye was extensively distributed throughout the brain parenchyma, with the exception of the inferior aspect of the cerebral hemispheres, the cerebellum and the brainstem. Minimal extracranial dye (i.e. in soft tissue) was detected. The distribution of dye was consistent with the findings on angiography.

These studies concluded that measurement of flow in extra-cerebral arteries supplying the brain is impractical in sheep. Exposure of the sagittal sinus at the point of junction of the frontal and parietal bones would access

approximately 75% of cerebral blood, with minimal contamination from extra-cerebral blood draining into the transverse sinus. These findings were consistent with those previously described in sheep (8,47), but reassuring given that venous anatomy can be variable between species and possibly breeds. For example, in the dog, contamination from facial blood is much more significant and ligation of facial veins is necessary before accurate measurements of cerebral blood flow can be made using sagittal sinus blood (244).

2.3.2 Validation of the Doppler flow probe method

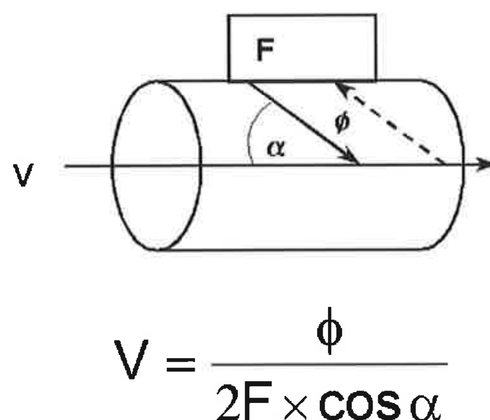
2.3.2.1 Equipment

The Doppler flow meter used to measure flow in the sagittal sinus in this preparation is a pulsed Doppler flow probe, which measures the Doppler shift of ultrasonic pulses when reflected from moving particles (245). It is more accurately termed a velocity probe (Figure 2.1).

The flow meter is a combined 545C-4 directional pulsed Doppler flow meter (Bioengineering, University of Iowa, Iowa, USA) (246) and "suture down" style 1 mm diameter 20 MHz piezoelectric transducers mounted on a cloth patch (Tritonics Medical Instruments, Iowa City, Iowa, USA).

The Doppler flow meter and the probe sends bursts of 20 MHz ultrasound of microsecond duration at a pulse repetition frequency of 62.5 kHz into the blood at an angle of approximately 45° to the vessel wall.

The resulting echoes from the vessel walls and blood cells are received by the same transducer and amplified by the Doppler flow meter. The emitted ultrasound is pulsed so that the returning echoes are separated in time according to the distances travelled.



$$V = \frac{\phi}{2F \times \cos\alpha}$$

Figure 2.1
Doppler principles relating to the pulsed Doppler flow probe used in the studies for this thesis. V is the flow through the target vessel, ϕ is the frequency shift between the emitted and received signal, F is the intensity of the emitted signal and α the angle between the emitted signal and flow at the centre of the vessel.

Functionally, “time-gating” of the returning ultrasound echoes can be used to determine the distance into the vessel that the flow meter is measuring velocity. This is controlled by the range of the Doppler flow meter, which is adjusted until the velocity signal from the flow meter is maximal.

This corresponds to the maximum velocity of the blood in the centre of the vessel. By recording the changes in velocity with changes in the value of the range it is possible to characterise the velocity profile of the blood at various depths from one side of the vessel to the other. This technique is used to determine the influence of cerebral blood flow on vessel diameter.

The output from the flow meter is recorded using an analogue to digital card (Metrabyte DAS 16-G2) and a personal computer (Microbits 486-based IBM compatible). For most studies, the mean velocity was recorded at a sampling rate of 1Hz.

Advantages of the chosen probe are its small size and the fact that access to only one side of the vessel is needed; both important considerations for implantation in a small enclosed area such as the extradural space.

2.3.2.2 Validation studies

A constant vessel cross-sectional area, a constant flow profile, and a constant angle of attack to the vessel are all necessary if changes in flow are to be accurately measured. Upton *et al* conducted validation studies of the probe to determine four aspects of performance (217,243).

2.3.2.2.1 Vessel diameter over a range of flows

The consistency of sagittal sinus diameter and flow profile over a range of cerebral blood flow values was evaluated under inhalational (halothane) anaesthesia. Under hypocarbic conditions (end tidal carbon dioxide concentrations approximately 25-30 mmHg), the range was altered to define the velocity profile in the vessel and its diameter as described above when the Doppler velocity had reached its minimum. Under hypercarbia (end tidal carbon dioxide concentrations 65-70mmHg), measurements were repeated once the maximum Doppler velocity was reached.

The average increase in velocity from low to high flows was 135%. No statistical difference between the radius at low and high flows was demonstrated, showing that there were no significant changes in vessel dimensions with changes in flow.

2.3.2.2.2 The relationship between sagittal sinus flow and Doppler velocity

The linearity of the relationship between actual flow over a range of cerebral blood flow values and Doppler output was determined.

Actual cerebral blood flows were determined using a timed, direct venous outflow method. Blood was drained from the sagittal sinus and the actual flow rate expressed as mL/minute using a stop-watch and measuring cylinder. This was correlated with the corresponding mean sagittal sinus Doppler velocity recorded over each 30-second period. A range of sagittal sinus flow rates were produced by manipulating end-tidal carbon dioxide as described above.

Highly significant correlations between Doppler velocities and actual flow were demonstrated (r^2 = 0.91-0.99). Although every animal showed an excellent linear relationship between velocity and flow, the slope and intercept of the line-of-best-fit varied between animals. This suggests that that every animal must be individually calibrated if actual flow values are required.

2.3.2.2.3 The time-course of cerebral blood flow

Normal ranges and intra-sheep variability in cerebral blood flow on different days were determined. Sagittal sinus blood velocity (as Doppler shift in kHz) was measured in six sheep for a 40 minute period, whilst they remained in their metabolic crates. The sheep were supported in a sling to prevent them from lying down, thereby minimising posture-related systemic haemodynamic changes. During this period, the animals were allowed access to food and water.

The time-course of cerebral blood flow was generally constant. Measured velocities were dependent on the level of arousal of the animal. For consistent measurements it was necessary to acclimatise the sheep to the experimental conditions and to ensure that they were not startled (such as by someone walking into the room) during a study. There was little obvious relationship between cerebral blood flow and head movement.

Absolute cerebral blood flow measurements were determined by taking measurements over a 3-minute period at approximately the same time on three different days. These values were subsequently calibrated by the timed venous outflow methods described above to give cerebral blood flow in mL/min. The coefficient of variation of the control cerebral blood flow for the 3 minute recording periods was between 5 and 15%.

Following determination of brain weight at autopsy, absolute cerebral blood flow values of approximately 65 mL/min/100g were determined at rest. This was calculated assuming the percentage of the brain drained by the dorsal sagittal sinus under the Doppler probe to be 75%.

These values are in accordance with values commonly found across other species (247,248). Values of 63 $\pm$ 4.5 mL/min/100g and 70 $\pm$ 4.9 ml/min/100g measured using a microsphere methods for the whole sheep brain on separate occasions have been demonstrated (46).

2.3.2.2.4 Response to physiological perturbations

Pilot studies of the response of the sagittal sinus Doppler to changes in arterial carbon dioxide tension (carbon dioxide reactivity) and systemic blood pressure were conducted in awake sheep and under halothane anaesthesia.

2.3.2.2.4.1 Carbon dioxide reactivity

Changes in sagittal sinus flow velocities were correlated against changes in $PaCO_2$ induced by a re-breathing circuit in the awake sheep and by variations in mechanical ventilation under anaesthesia.

As expected, these data showed a sigmoid relationship between end-tidal carbon dioxide concentrations and cerebral blood flow with r^2 values ranging from 0.75-0.99. Inter-individual variations in baseline were demonstrated in the awake cohort, which were minimised by normalising carbon dioxide values and pooling data. This allowed comparisons with the anaesthetised cohort, which demonstrated no statistically significantly difference between regression lines. Subsequent analyses of the curves showed that the maximum and minimum cerebral blood flows occurred at an end-tidal carbon dioxide concentrations of approximately 65 mmHg and 30 mmHg, respectively. The relationship between the two parameters was approximately linear over the end tidal range of 40-60 mmHg. This carbon dioxide response curve is similar to those described in the literature (247).

2.3.2.2.4.2 Responses to hyper- and hypotension

The physiological responses of the sagittal sinus Doppler to changes in systemic blood pressure were determined. Hypotension was induced with an intravenous infusion of sodium nitroprusside (0.4 mg/min for 10 minutes) and hypertension with metaraminol (0.5 mg/min for 5 minutes).

Sodium nitroprusside produced a significant, gradual 50% reduction in mean arterial pressure. During this period, cerebral blood flow decreased by a maximum of 20% despite a reduction in mean arterial pressure.

Metaraminol produced a significant 30% increase in mean arterial pressure, but cerebral blood flow did not change significantly.

These cerebral blood flow responses to drug-induced changes in blood pressure recorded in this study are also consistent with reported behaviour of the cerebral circulation. The manipulations of systemic blood pressure in these studies were within the predicted autoregulatory range.

2.3.2.2.5 Agreement with nitrous oxide method

A direct comparison between the indirect Fick nitrous oxide equilibration method for measurement of cerebral blood flow and the direct ultrasonic Doppler index of venous outflow was determined (249).

Using simultaneous measurements, high and low flow states of cerebral blood flow were determined by manipulation of carbon dioxide under anaesthesia. Four different sets of calculations were used to make the nitrous oxide (Kety Schmidt) estimations: arterial-venous nitrous oxide concentration differences during uptake or elution of the indicator; and with or without extrapolation of arterial-venous differences to infinity.

No significant differences between cerebral blood flow estimates using the Kety-Schmidt method and Doppler measurements were demonstrated. Kety-Schmidt estimates based on nitrous oxide uptake correlated more strongly with the Doppler method than estimates based on nitrous oxide elution. In high-flow states, cerebral blood flow estimates based on nitrous oxide uptake, but not those based on elution, distinguished between rapid and slow blood:tissue equilibration of nitrous oxide.

These studies provided validation of the Doppler method against the widely used nitrous oxide method.

2.4 Conclusions

Whilst many methods of cerebral blood flow measurement exist, none is regarded as a gold standard.

Selection of a method of measurement is based on the intended endpoints of a particular study. These may be global vs regional and continuous vs intermittent measurements. Logistical factors are important and include resources, expertise in surgical techniques and species specificity in animal preparations.

The Doppler flow meter model developed in the University of Adelaide sheep laboratory provides a continuous, accurate measurement of approximately 75% of cerebral blood flow with minimal contamination from other vascular beds.

The published validation studies discussed above confirm that the dorsal sagittal sinus is an anatomical site that is not subject to potential confounding variables that may affect Doppler based measurements. Of these, stability of vessel calibre, linearity between sagittal sinus flow and Doppler velocities and stability of cerebral blood flow time course measurements were demonstrated.

Furthermore, placement of a catheter in the sinus would allow sampling of almost pure cerebral venous blood. This has applications for the measurement of cerebral drug elution and for assessment of cerebral metabolic rate.

It is important to recognise that these validation studies were conducted under physiological conditions. The hypercarbia induced in these studies was

unlikely to induce major changes in intracranial pressure as changes occurred relatively slowly and normal homeostatic mechanisms such as shunting of cerebrospinal fluid would be expected to be intact. Because of the rigid structure of the skull, large increases in intracranial pressure may compress the sinus and alter the flow-velocity relationship, leading to inaccuracies in flow measurement. The performance of this Doppler flow meter under conditions of intracranial hypertension and exhausted intracranial elastance remain unknown.

Advances in flow probe technology have allowed chronic implantation of these devices at multiple sites in many species and successful measurement of blood flow over prolonged periods (246,250). Stability of vessel diameter remains paramount and calibrated flow probes that rely on placement around a vessel have been developed. The use of such probes is not feasible in the sagittal sinus of sheep. However, as the walls of the cerebral sinuses are comprised of dura and contain no smooth muscle, no active change in diameter occurs (10). Consequently, these flow meters have been chronically implanted into sheep for repeated measurements of cerebral blood flow.

Chapter 3. Aims of research

There has been extensive research into the neurophysiology and quantification of cerebrovascular dynamics. Much of this research is dependent on an accurate, validated measurement of cerebral blood flow.

With such a measurement, insights may be obtained into the effects of vasoactive agents, such as catecholamines, on these dynamics.

To date, there is a paucity of controlled physiological and applied physiological studies assessing the effects that catecholamines have on parameters such as cerebral blood flow, intracranial pressure, cerebral oxygen consumption and cerebrovascular resistance.

Catecholamines are first line agents in clinical intensive care. However, the applications of these drugs are based on early physiological studies that, whilst intrinsically valid, do not take account of current advances in the understanding of the complexities of the adrenergic system.

The effects of these drugs under physiological conditions may be significantly different from those under pathological conditions where cerebral autoregulation and blood-brain barrier permeability may be altered. Despite these deficiencies in current knowledge, these drugs are increasingly used to augment the cerebral circulation in conditions of absolute and relative cerebral hypoperfusion.

The use of propofol in intensive care medicine and anaesthesia as a sedative and/or anaesthetic agent has become standard practice. Catecholamine:anaesthetic interactions have not been clearly defined, particularly in conditions of altered cerebral autoregulation and blood-brain barrier permeability.

The sheep laboratory in the Department of Anaesthesia and Intensive Care at the University of Adelaide has thirteen years experience in regional blood flow measurement and pharmacokinetic modelling. A method of cerebral blood flow measurement has been developed and extensively validated. The application of this model to conduct pharmacodynamic studies of the systemic and cerebrovascular effects of catecholamines is an extension of the work that has preceded this thesis.

The aims of this thesis were to modify the current animal preparation to determine the following pharmacodynamic studies:

1. Quantification and comparison of the effects of infusions of exogenous catecholamines on systemic haemodynamics in awake animals.

2. Quantification and comparison of the effects of infusions of exogenous catecholamines on cerebrovascular variables in awake animals.
3. Determination of the above variables under anaesthesia using intravenous anaesthesia with propofol, and inhalational anaesthesia with isoflurane.
4. Comparisons of the effects that catecholamines and anaesthesia have on cerebral autoregulation, with comparisons between the cohorts.

These studies were conducted using drugs commonly used in clinical practice. These included the catecholamines adrenaline, noradrenaline and dopamine and the anaesthetic agents propofol and isoflurane.

These drugs were administered in clinically applicable doses. With the exception of cerebral blood flow, physiological measurements were those that commonly used in clinical practice.

A secondary aim of this thesis was to provide information from a validated animal model that may have applications to clinical practice, potentially providing new insights and an analysis of physiological effects and drug interactions.

Chapter 4. Methods and materials for studies in sheep

This chapter will describe the general methods and materials that were used in the sheep studies conducted in this thesis.

The studies were conducted in the animal laboratory of the Department of Anaesthesia and Intensive Care at the University of Adelaide. This laboratory has over thirteen years experience in handling sheep. A chronically catheterised sheep preparation has been the platform for many studies conducted in this laboratory. The original preparation was developed by Runciman and described in 1984 (238,239). Adaptations of this preparation have been used in numerous pharmacokinetic, drug disposition, pharmacodynamic and regional blood flow studies (214-218,240,241,251-264).

4.1 Ethics statement

The Animal Ethics Committee of the University of Adelaide approved the studies. Animals were handled in accordance with the 1997 edition of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes issued by the National Health and Medical Research Council of Australia.

4.2 Animal selection and handling

Adult sheep were used in these studies because of their ready availability and tolerance to repeated handling and chronic instrumentation. In addition, their size meant that many physiological parameters were quantitatively similar to those found in man, and their blood volume allowed repeated blood sampling over time without risk of anaemia or hypovolaemia.

The sheep used in these studies readily adapted to being housed in metabolic crates for periods of up to three months. Sheep recovered uneventfully from anaesthesia and surgery for implantation of flow probes and catheters, with rapid return of normal behaviour patterns, typically within an hour.

Female Merino sheep (Ovis Aries) were purchased from the Institute of Medical and Veterinary Science in Adelaide. They were selected from a single flock and were well matched for age and weight. The average weight of each animal was 50kg.

Only sheep with haemoglobin type A, determined using electrophoresis were used in these studies. Ovine type A haemoglobin and human adult

haemoglobin have similar affinity for oxygen, thereby resulting in values of haemoglobin saturation and oxygen content similar to man (265,266).

Within the animal house, sheep lived in mobile metabolic crates (Figure 4.1). In these crates they had free access to lucerne chaff and water at all times, except during studies.

Sheep were inspected at least once a day. Faeces and urine were collected in a container beneath the crate. Food and water consumption was measured daily. At least two sheep were housed in the room at any given time to avoid isolation stress. Sheep were allowed a minimum of five days to adapt to their environment before surgery was performed.

Figure 4.1
Sheep housed in metabolic crates at the Animal House, University of Adelaide.

4.3 ANAESTHESIA AND INSTRUMENTATION

4.3.1 Anaesthesia

One week before interventional studies, sheep underwent general anaesthesia for all procedures involving implantation of catheters and flow probes.

Anaesthesia was induced by the direct, rapid intravenous injection of sodium thiopentone (Abbott Australasia, Kernell, NSW, Australia), 30 mg/kg, into the left internal jugular vein. Following loss of consciousness, the animal was turned supine and underwent endotracheal intubation with a size 10 cuffed endotracheal tube using direct laryngoscopy. Correct placement of the tube

was confirmed by direct visualisation of the tube passing through the vocal cords, misting of the tube on expiration and subsequently by capnography.

Ventilation was controlled using a volume-controlled ventilator (7000 Ventilator, Ohmeda, Madison, WI, USA) with 100% oxygen. End-tidal CO_2 was measured with an infrared analyser (Capnomac, Datex Instrumentarium Corp, Helsinki, Finland) and mechanical ventilation was adjusted to maintain end-tidal CO_2 at 40 mmHg.

Anaesthesia was maintained with 2% halothane (Zeneca, Cheshire, England, UK) in oxygen, delivered via a vaporiser (Fluotec 3, Ohmeda BOC Group, UK and a circle system.

Temperature was maintained by using a heat and moisture exchanger in the ventilator circuit (Ultipore 100 breathing system filter, Pall Biomedical, Portsmouth, UK). Hydration was maintained by the infusion of 1L of normal saline per hour intravenously after placement of the venous catheters.

4.3.2 Instrumentation

Following anaesthesia, a two-stage instrumentation procedure was performed. Intravascular catheters were placed in the femoral artery and vein with the animal in the supine position. Thereafter, the animal was turned prone to the "sphinx" position for the "head preparation," comprising the placement of a Doppler flow probe, sagittal sinus catheter and intracranial pressure monitor.

All procedures were conducted under strict aseptic conditions – full surgical scrub, gowns, gloves and masks. The skin was shaved and prepared with povidone iodine antiseptic solution at the operative sites. An intramuscular injection of procaine 1.25g penicillin and 1.25g streptomycin (Penstrep®, Troy Laboratories, Smithfield, NSW, Australia) was administered prior to the first skin incision as antibiotic prophylaxis.

4.3.2.1 Vascular preparation

The femoral triangle was cleaned and prepared as outlined above. A sterile field using surgical drapes was prepared. (Figure 4.2).

The femoral artery and vein were exposed through a transverse incision and mobilised using stay sutures. Using a Seldinger technique, flexible guidewires were inserted through a 12G introducer needle (Cook Incorporated, Bloomington, USA) into the femoral artery. A 6F pulmonary artery catheter introducer sheath with side port and self sealing valve (Cook Incorporated, Bloomington, USA) was inserted into the femoral artery and secured. The same procedure was then performed on the femoral vein.

A 7F catheter (Multipurpose A1 catheter, Cordis Corporation, Miami, USA) was inserted through the femoral artery sheath to the level of the diaphragm,

measured using the guidewire length. In conjunction with the side port of the introducer sheath, these two arterial catheters allowed measurement of arterial blood pressure and sampling of arterial blood.

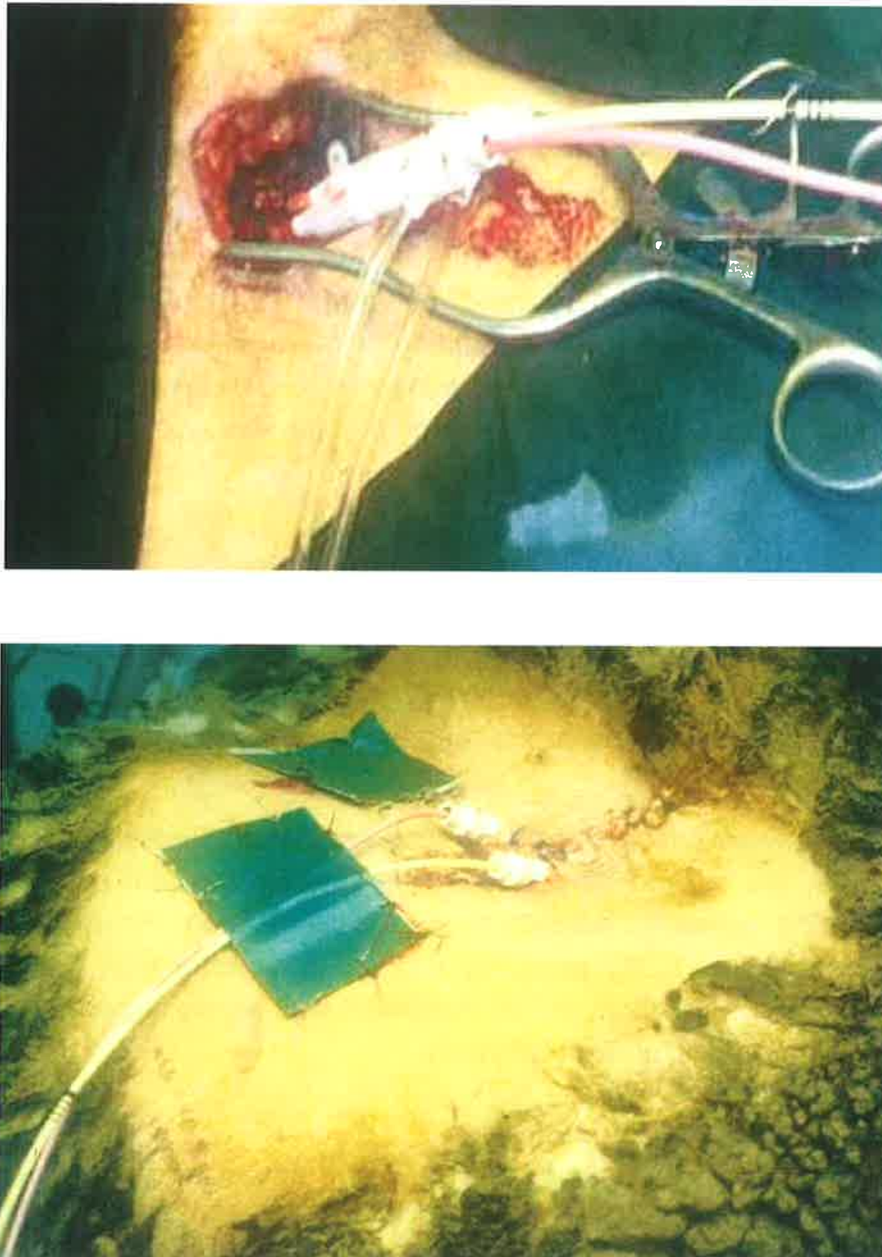


Figure 4.2
Top panel: Insertion of vascular catheters into the femoral artery and vein through introducer sheaths. Bottom panel: Completed vascular preparation showing secure catheters.

A three-lumen thermodilution pulmonary artery catheter (Model TD1755H, Biosensors International, Singapore) was inserted through the femoral vein sheath and positioned in the pulmonary artery by observation of the pressure waveforms. Pulmonary arterial and right atrial pressures were measured through the respective lumens of the pulmonary artery catheter; drug

infusions were administered through the infusion line of the pulmonary artery catheter. Fluids were administered through the introducer sheath side port.

Catheter patency was maintained using intraluminal heparin locks (10 i.u/mL) after flushing the catheters with heparinised saline (0.9% saline with 5 i.u/mL of sodium heparin: David Bull Laboratories, Melbourne, Australia).

Catheters were then secured with non-occlusive clamps to a base plate that was sutured to underlying muscle. Skin was closed in a single layer.

Catheters were then secured to the skin using fixation tape, and tied to the wool on the animal's back. The distance of the catheters from the animal's head ensured animal comfort and minimised catheter damage.

4.3.2.2 Brain preparation

Following the vascular preparation, the animal was turned to the sphinx position. (Figure 4.3).

The head was shaved and skin prepared as above.

A midline longitudinal incision approximately 8 cm in length was made in the scalp, and the periosteum reflected. A 2 cm frontal craniotomy was performed anterior to the trifurcation of the frontal and parietal sutures using a 19 mm trephine. The bone plug was removed to expose the dorsal sagittal sinus and dura.

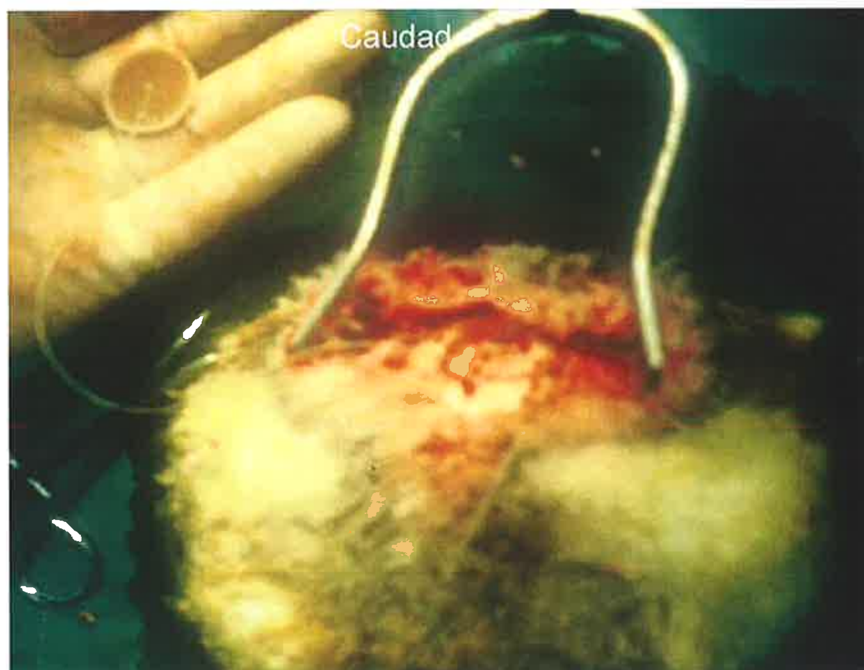


Figure 4.3
Doppler flow meter and transducer mounted on cloth backing (prior to trimming and placement over the sagittal sinus).

A 2 mm drill hole was made 20 mm laterally from the midline at the rostral edge of the trephine hole. The wire of a range gated Doppler transducer (Tritonics Medical Instruments, Iowa, USA) was fed from the trephine hole, between the skull and the dura, and the wire pulled though the drill hole. The cloth backing of the probe was then trimmed to a size whereby the leading edge could be pushed in between the dura and the skull, with the transducer situated directly over the sagittal sinus. The transducer was moved laterally until maximum output from the probe was recorded.

Using a Seldinger wire through a 20G introducer needle, a 4F polyethylene catheter (Cook Incorporated, Bloomington, USA) was inserted into the sagittal sinus and advanced 2 cm. Patency was determined by free aspiration of blood from the catheter.

A solid state, strain-gauge tipped intracranial pressure monitor (Microsensor ICP Transducer, Codman, Randolph MA, USA) was placed into the subdural space and advanced 2 cm. The subdural space was selected after two sheep developed paraplegia following intraparenchymal placement, necessitating euthanasia. Equivalent waveforms were obtained from both sites.

A notch was cut in the bone plug to accommodate the sagittal sinus catheter and intracranial pressure monitor wire. This was replaced and secured using a titanium plate and stainless steel screws. The Doppler transducer wire was secured in a single loop to the plate and externalised. Prior to skin closure, output from the Doppler flow meter and the ability to aspirate blood from the sagittal sinus catheter was confirmed. The periosteum and scalp were then closed.

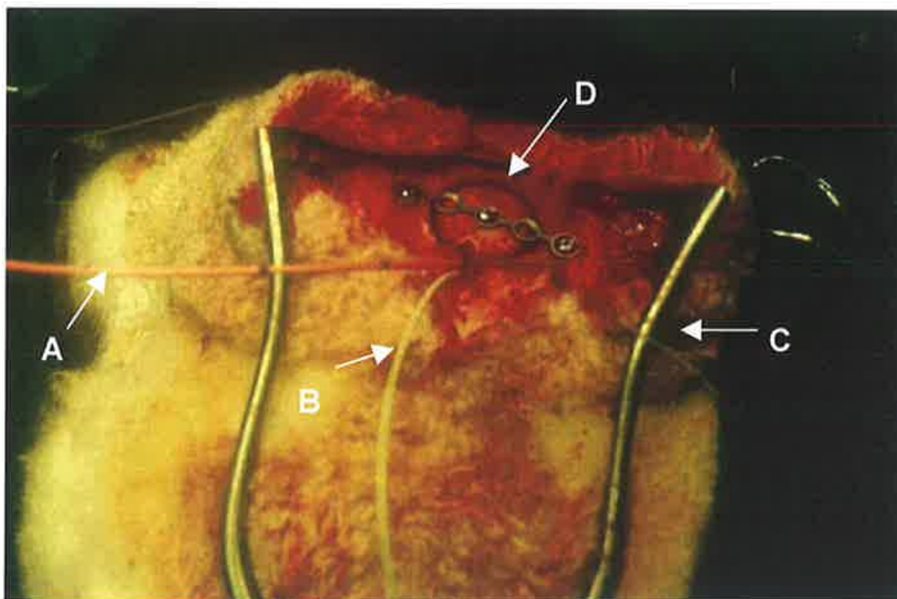


Figure 4.4
Completed brain preparation prior to skin closure. A: Sagittal sinus catheter; B: intracranial pressure monitor; C: Doppler flow meter wire; D: craniotomy plug secured with plate and screws.

Sheep were then allowed to recover from anaesthesia and were transferred to their metabolic crates. Postoperatively, sheep were able to stand and were eating within 3-4 hours of surgery. Analgesia was provided with methadone 10 mg intramuscularly (Parnell Laboratories) or xylazine 2.5-5 mg intramuscularly (Troy Laboratories, Smithfield, NSW, Australia), as necessary.

A period of 5-10 days elapsed between insertion and measurements to allow a fibrous scar to develop around the flow meter and the sagittal sinus. This ensured minimal movement between the two and a constant angle between the ultrasonic beam and the direction of blood flow.

4.3.3 Maintenance of instrumented sheep

Most catheters remained patent for the duration of catheterisation. If a catheter was found to be blocked, it was repeatedly flushed with sterile heparinised saline (0.9% saline with 5 iu/mL of sodium heparin: David Bull Laboratories, Melbourne, Australia) to remove foreign material from the tip. If this failed, the catheter was withdrawn by 2-4 mm to reverse any impaction on the vessel wall. If this failed, a guide wire (TSF-38-145-X, Cook Inc., Bloomington, IN, USA) was inserted down the lumen to a distance of approximately 1-2 cm beyond the catheter tip using an aseptic technique, in an attempt to unblock the catheter. If these techniques failed, the sheep was no longer used for studies and was killed by the intravenous administration of pentobarbitone (Sigma Chemical Company, Castle Hill, NSW, Australia).

If an animal became ill, as evidenced by a sustained decrease in food or water intake or urine output, lethargic appearance, or a blood temperature over 40°C, antibiotic therapy was immediately commenced using intravenous gentamicin (Deltra West, Bentley, WA, Australia) at a dose of 160 mg twice daily. If sheep did not rapidly improve, they were killed by the intravenous administration of pentobarbitone.

4.4 INTERVENTIONAL STUDIES

4.4.1 Animal preparation

On the day of measurement, the animal was moved from the metabolic crate holding area to a specific study laboratory.

Figure 4.5
Interventional study in an awake, chronically catheterised sheep model. Note supportive canvas sling for minimisation of movement.

4.4.1.1 Studies in awake sheep

The animal was supported in its crate by a canvas sling that allowed it to partially weightbear on its hind limbs and a strap was placed horizontally under the mandible.

This prevented excessive movements, limiting head movement to 5 cm in awake sheep or immediately before onset of anaesthesia. Extraneous noise and light was minimised to reduce changes in cerebral blood flow and haemodynamic parameters induced by startling. Transportation and placement in the sling was well tolerated by the animals.

Monitoring lines were connected and the animal was allowed to settle so that a period of baseline stability was achieved before commencement of infusions.

4.4.1.2 Studies under anaesthesia

Prior to anaesthesia, the output of the Doppler probe and mean arterial pressure transducer was recorded with the animal in an awake, calm state. Anaesthesia was induced with either thiopentone or propofol, depending on the protocol. Intubation, ventilation and maintenance of anaesthesia proceeded as described in section 4.3.1.

The animal was turned prone and placed in the left lateral position to prevent lower limb neuropraxia.

4.4.2 Measurements

4.4.2.1 Cerebral blood flow

Once a period of baseline stability was established, Doppler frequencies were expressed as a percentage of the reading obtained during this baseline period. The Doppler output was sampled at 1Hz using an analogue to digital card (Metrabyte DAS 16-G2) and a personal computer (Microbits 486-based IBM compatible) and recorded digitally on computer disc.

4.4.2.2 Mean arterial pressure

Mean arterial pressure was recorded using a standard transducer and amplifier (78342A, Hewlett Packard Company, USA) and recorded through the same computerised acquisition system used for cerebral blood flow. Following electronic zero calibration, mean arterial pressure was recorded as mmHg and averaged over two minute epochs when required.

For data analysis, absolute changes in mmHg were converted to the relative percentage change of baseline.

4.4.2.3 Right atrial pressure

Right atrial pressure was measured intermittently at nominated intervals from the proximal lumen of the pulmonary artery catheter after zero calibration and expressed as mmHg.

4.4.2.4 Intracranial pressure

Intracranial pressure was measured directly from the subdural pressure monitor (Microsensor ICP Transducer, Codman, Randolph MA, USA) and recorded through the same computerised acquisition system.

As these pressures were not zero-calibrated, pressures were expressed as a percentage of the reading obtained in the baseline period. This obviated problems associated with zero drift that have been demonstrated with these systems.

4.4.2.5 Cardiac output

Cardiac output was measured by the intermittent thermodilution method using injections of iced saline (0-2°C). Three injections were delivered throughout the respiratory cycle and values were rejected if there was >10% deviation from the previous values.

4.4.2.6 Blood gas analysis

Blood was sampled for blood gas analysis from the femoral arterial and sagittal sinus catheters and measured using a standard blood gas analyser, (ABL 625, Radiometer Medical, Copenhagen, Denmark). Samples were stored at 0°C and analysed within 30 minutes.

Oxygen tension and saturation, carbon dioxide tension and pH were recorded directly from the blood gas analyser.

Sheep specific haemoglobin coefficients were used to correct haemoglobins measured with the blood gas analyser (266). These were calculated by the equation:

$$Hb^* = (0.926 \times SO_2) + 0.0299$$

where Hb* is the corrected haemoglobin (g/100mL) and SO_2 is the corresponding oxygen saturation (%).

Oxygen contents were then calculated from a standard calculation:

$$O_2C = 1.39 \times Hb^* \times SO_2$$

where O_2C is oxygen content for arterial, venous or sagittal sinus blood (mL /100mL).

4.4.2.7 Derived indices

Indices for systemic and cerebrovascular resistance, and global and cerebral oxygen delivery and consumption were derived from standard equations.

4.4.2.7.1 Systemic vascular resistance (SVR):

$$SVR = \frac{MAP - RAP}{Q}$$

where MAP = mean arterial pressure (mmHg), RAP = right atrial pressure (mmHg), Q = cardiac output (L/min). This was expressed as resistance units (dyne.sec/cm^5).

4.4.2.7.2 Systemic oxygen consumption (VO_2):

$$VO_2 = Q \times (CaO_2 - CvO_2)$$

where CaO_2 and CvO_2 are arterial and venous oxygen contents respectively. This was expressed as mL/min.

4.4.2.7.3 Systemic oxygen delivery (DO_2):

$$DO_2 = Q \times CaO_2$$

This was expressed as mL/min.

4.4.2.7.4 Cerebrovascular resistance (CVR):

$$CVR = \frac{MAP - ICP}{CBF}$$

where ICP = intracranial pressure (% baseline), CBF = cerebral blood flow (% baseline). This was expressed as resistance units (dyne.sec/cm^5).

4.4.2.7.5 Cerebral oxygen consumption ($CMRO_2$):

$$CMRO_2 = CBF \times (CaO_2 - CcvO_2)$$

where $CMRO_2$ is the cerebral oxygen consumption, CBF is cerebral blood flow and $CcvO_2$ is cerebral venous oxygen content. This was expressed as mL/min/% baseline flow.

4.4.2.7.6 Cerebral oxygen delivery (CDO_2):

$$CDO_2 = CBF \times CaO_2$$

This was expressed as mL/% baseline flow

4.4.2.8 Temperature

This was measured directly from the pulmonary artery catheter thermistor.

4.4.2.9 Volatile agent monitoring

In the isoflurane studies, end tidal volatile agent monitoring was measured with a volatile agent detector (Capnomac, Datex Instrumentarium Corp, Helsinki, Finland).

4.4.3 Drug administration and dosage

4.4.3.1 Drug administration

Drugs were administered using a 50 mL syringe (Becton Dickinson, Dublin, Ireland). This was attached to the infusion lumen (right atrial) of the pulmonary artery catheter by a 75 cm extension line (Tuta Laboratories, Lane Cove, NSW, Australia) and a 3-way stopcock. The volume of dead space in the catheter-stopcock-extension system was determined by aspiration of blood before each study. The dead space was then filled with study drug prior to commencement of each study.

All drugs were administered by constant rate infusion using a calibrated syringe driver (Model 33, Harvard Apparatus Ltd, Kent, England).

4.4.3.2 Drugs

4.4.3.2.1 Catecholamines

The following catecholamines were used: noradrenaline (Abbott Australasia, Sydney, Australia), adrenaline (Astra Pharmaceuticals, Sydney, Australia) and dopamine (David Bull Laboratories, Melbourne, Australia).

Noradrenaline and adrenaline were made up as 3 mg/50 mL 0.9% saline. Using a syringe driver, a delivery rate of 1 mL/hr is equivalent to 1 μg/minute.

Dopamine was constituted as 200 mg/50 mL 0.9% saline. A delivery rate in mL/hr is approximately equivalent to 1 μg/kg/min for a 50 kg sheep.

Doses were reported as mL/hr representing μg/(kg)/min.

Catecholamines were administered as ramped infusions in 5 minute intervals through the infusion lumen of the pulmonary artery catheter. Each animal acted as its own control. Infusions of adrenaline, noradrenaline and dopamine in doses of 10, 20, 40, 60 mL/hr were administered. On reaching the target dose, infusions were ceased and a 20 minute (4x5 minute) drug elimination period was recorded. On completion of the study, one hour lapsed before the following catecholamine infusion to ensure clearance of the preceding drug, as manifested by restoration of cardiovascular parameters to baseline values.

4.4.3.2.2 Isoflurane

Isoflurane (Ohmeda, New Jersey, USA) was delivered by a vaporiser (Isotec 3, Ohmeda BOC Group, UK) to maintain an expired concentration of 2%, measured by a volatile agent detector (Capnomac, Datex Instrumentarium Corp, Helsinki, Finland).

4.4.3.2.3 Propofol

Propofol (AstraZeneca Pharmaceuticals, Sydney Australia) in a 1% (10mg/mL) emulsion was administered via a syringe driver (Model 33, Harvard Apparatus Ltd, Kent, England). Following induction of anaesthesia, propofol was administered at a constant rate of 15mg/min.

4.4.3.3 Drug randomisation

In the catecholamine studies, infusions of catecholamines were administered in random order. This was done before each study using a random number generator (StatMate®, GraphPad Software, San Diego, USA).

4.5 Data management

4.5.1 Data acquisition and compression

Output from the computerised acquisition system using an analogue to digital card (Metrabyte DAS 16-G2) and a personal computer (Microbits 486-based IBM compatible) was recorded digitally on computer disc.

Measurements of cerebral blood flow, mean arterial pressure and intracranial pressure that were recorded digitally at 1 Hz output were transferred in a packaged analogue format. A customised averaging software program was developed by Mr Cliff Grant using the Python programming language (http://www.python.org). This is an interactive, object-oriented programming language with a clear syntax and support for extensions such as graphical toolkits and databases. It runs on various UNIX platforms, Windows, DOS, OS/2, Macintosh and Amiga. Averaged data for nominated time points were then transferred to a commercial spreadsheet program (Excel 97 SR-2, Microsoft Corporation, USA).

Other variables such as cardiac output, right atrial pressure and blood gas analysis where entered manually into the same spreadsheet. This spreadsheet was used to manipulate data and calculate derived variables.

4.5.2 Statistical analysis and presentation

Statistical analysis was made using a commercial statistics package program (GraphPad Prism, GraphPad Software, San Diego, California USA).

The specific methods of analysis used are outlined in the relevant studies.

4.5.2.1 Sample size determination

The sample size required to determine baseline stability within and between cohorts and reproducibility of each intervention was determined from previous studies using our experimental preparation (214,216,240,256). Due to the stability of the preparation, six studies for each intervention (i.e. catecholamine infusion) were considered appropriate. Stability and reproducibility were evaluated by determining normality of distribution and 95% confidence interval analysis and corrected using degree of freedoms.

4.5.2.2 Presentation software

Graphing of data was done using the same statistics program. These were imported into a commercial word processing program (Word 97 SR-2, Microsoft Corporation, USA).

References were added to the text using a commercial reference program (Reference Manager 9, Research Information Systems, USA).

Chapter 5. Pilot studies on catecholamine dose responses

5.1 INTRODUCTION

Catecholamine physiology and pharmacology are outlined in Chapter 1. The studies conducted for this thesis were directed at determining the systemic and cerebrovascular effects of catecholamines in awake sheep and under propofol and isoflurane anaesthesia.

It was considered that these studies should be representative of clinical practice – i.e. drugs were chosen that are used in everyday intensive care and anaesthesia practice.

Of these, the endogenous catecholamines, noradrenaline and adrenaline, are used most commonly in Australasia, South Africa and the United Kingdom. Dopamine is widely used in the United States. None of these agents has been shown to be more effective than another, alone or in combination. The synthetic drugs, dobutamine and isoprenaline, were not studied due to their limited role in clinical practice, particularly for the defence of systemic blood pressure. Given their predominantly vasodilatory properties, they are contraindicated when there is concern about cerebral perfusion.

As outlined in Chapter 1, adrenaline acts primarily by displacing noradrenaline from presynaptic stores, particularly under times of stress. Dopamine is an endogenous noradrenaline precursor and probably exerts most of its effects by *in vivo* metabolism to noradrenaline.

This suggests the hypothesis that, at a cellular level, the catecholamines are functionally equivalent, with noradrenaline as the final effector. This hypothesis has not been evaluated in pharmacokinetic or cellular biological models. There is an increasing body of indirect clinical evidence that these agents are functionally equivalent. This has been highlighted in a number of studies analysing the effects of catecholamines on "renal protection" (149-151), septic (185-189), and cardiogenic shock (175,177) with no drug demonstrating an advantage over another.

This experience in the clinical research literature has not translated to everyday clinical practice. There is marked variation in prescribing practices with respect to catecholamines. Drugs are usually selected according to Unit protocols that are essentially based on clinician opinion and dogma, preference and experience. The pharmacological basis for the prescription of

these drugs is usually derived from standard pharmacology textbooks and pharmaceutical texts that are poorly referenced.

There has been a shift away from prescribing catecholamines according to a weight (i.e. μg/kg) basis to one of titration against effect, usually to target mean arterial pressure. This has evolved with the recognition of marked inter- and intra-individual variability in the response of patients to these drugs. In some instances such as postoperative recovery or resuscitation after trauma, patients will respond to small incremental doses (e.g. 1 – 10 μg/min of noradrenaline). In others, such as anaphylaxis, decompensated septic or cardiogenic shock, very large doses may be required (e.g. 20 – 100 μg/min of noradrenaline). In the latter scenarios, increasing doses of catecholamines are regarded as exogenous supplementation of failing endogenous systems. Patient survival is primarily dependent on reversal of the precipitating cause and recovery of host response. In these situations, "catecholamine sparing" strategies, such as those outlined in section 1.3.3.3 may be employed.

5.2 Dose selection

In accordance with the above principles and observations, adrenaline, noradrenaline and dopamine were selected as the interventional catecholamines for this thesis.

A dosing profile designed to produce a significant physiological response was selected. Principally, this was directed at significantly increasing mean arterial pressure.

As adrenaline and noradrenaline have hydroxyl groups on the β carbon atom of the side chain, this is associated with 100 fold greater potency than dopamine (134).

Accordingly, equivalent doses of the three catecholamines were required. Using the solutions and concentrations described in section 4.4.3.2.1., a range of 0-60 mL/hr was used.

The application of these dose selections were then tested in the sheep preparation.

5.3 Aim

A pilot study was designed to determine whether infusions of noradrenaline and adrenaline (3mg/50mL 5%dextrose water) and dopamine (200mg/100 mL 5% dextrose water) produced expected and equivalent haemodynamic response in a chronically catheterised sheep preparation.

The effect of induced hypertension on cerebral blood flow was also determined.

5.4 METHOD

5.4.1 Animal preparation

One week prior to the study, the animals were prepared under thiopentone and halothane anaesthesia as described in Chapter 4.3.

In brief, through a frontal craniotomy an ultrasonic, range gated Doppler transducer was placed on the dorsal sagittal sinus and secured under the replaced bone plug.

The animal was then turned supine and the femoral triangle exposed. A 7F catheter was inserted into the femoral artery for measurement of mean arterial pressure and into the femoral vein for drug and fluid delivery.

5.4.2 Interventions

On consecutive days, each animal received a randomly allocated ramped intravenous infusion of noradrenaline, adrenaline or dopamine through the femoral venous catheter. Each animal acted as its own control. Infusions of adrenaline, noradrenaline and dopamine (10,20,40,60 mL/hr) were administered at 5-minute intervals. On reaching the maximum concentration, infusions were ceased. (Figure 5.1).

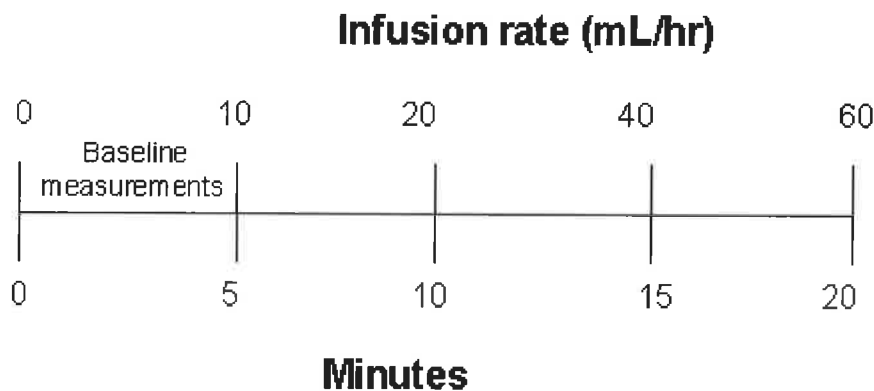


Figure 5.1
Intervention timeline diagram for the pilot studies. Measurements were made continuously during the infusion period.

5.4.3 Measurements

Changes in cerebral blood flow were inferred from changes in the outputs from the Doppler probe. Doppler frequencies were expressed as a percentage of the reading obtained in the baseline period.

Mean arterial pressure was recorded using a standard transducer and amplifier and recorded through the same computerised acquisition system. Following electronic zero calibration, mean arterial pressure was recorded as

mmHg and averaged over two minutes after the change in catecholamine infusion rate.

The method of data capture and recording is explained in detail in Chapter 4.5

5.4.4 Statistical analysis

Comparison of the effects of catecholamines on cerebral blood flow and mean arterial pressure from baseline was determined using two-way analysis of variance. Significance was determined by 95% confidence intervals, assuming a t-distribution. A p-value of <0.05 was considered to be statistically significant.

Data were reported as means where the aim was to define the behaviour of the average animal.

5.5 Results

Studies were conducted in three sheep.

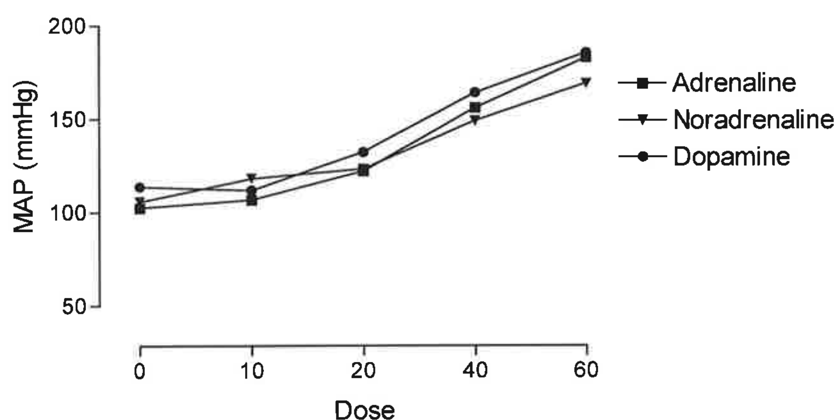


Figure 5.2
Effect of ramped infusions of adrenaline, noradrenaline and dopamine (0-60 mL/hr) on mean arterial pressure (MAP) in mmHg. Mean values are shown.

Baseline equivalence of mean arterial pressure and cerebral blood flow was demonstrated before commencement of infusions.

Infusions of adrenaline, noradrenaline and dopamine resulted in dose-dependent increases in mean arterial pressure from baseline (Figure 5.2).

Adrenaline increased mean arterial pressure from 102 to 180 mmHg (+56%), noradrenaline from 105 to 169 mmHg (+62.12%) and dopamine from 113 to 185 mmHg (+61%).

The effects on cerebral blood flow are shown in Fig 5.3. No significant effects on cerebral blood flow were demonstrated, although there was a trend for dopamine to increase cerebral blood flow from baseline (104 –198 % baseline).

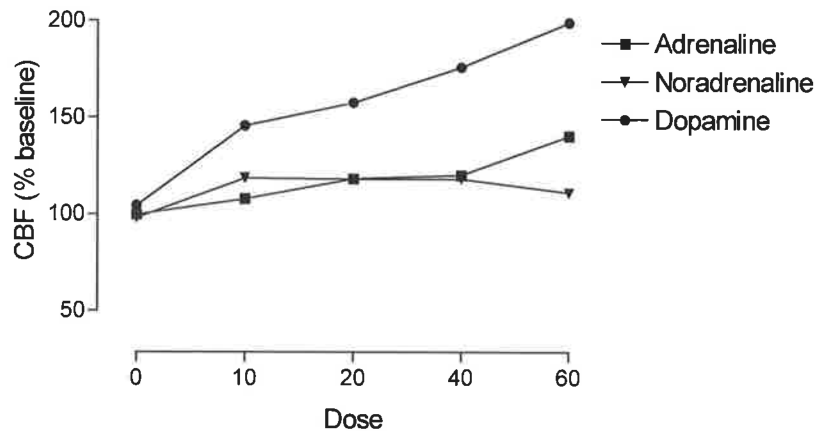


Figure 5.3
Effect of ramped infusions of adrenaline, noradrenaline and dopamine (0-60 mL/hr) on cerebral blood flow (CBF) in % change from baseline.

Due to the small sample size of these studies, none of the changes achieved statistical significance; nor were any significant differences demonstrated between catecholamines.

5.6 Discussion

This pilot study was designed to test whether the selected dose range of catecholamines resulted in expected changes in mean arterial pressure.

All three catecholamines increased mean arterial pressure from baseline in an equivalent manner suggesting that there was pharmacodynamic equivalence over the nominated dose range in this ovine preparation. The effects on cerebral blood flow suggested a different effect of dopamine in the presence of equivalent increases in mean arterial pressure compared to those of adrenaline and noradrenaline. It was concluded that cerebral autoregulation remained intact with noradrenaline and adrenaline induced hypertension, but may have been altered by dopamine. It was speculated whether this could have been due to a direct cerebrovascular effect of dopamine or induced hypertension that exceeded upper autoregulatory thresholds. These results prompted the studies that follow.

The results of this pilot study were similar to those done during the validation studies for the Doppler flow meter (See chapter 2.3.2.2.4.2). In these studies, hypertension was induced with metaraminol (a direct acting α_2 agonist with selective venous and arterial vasoconstrictor activity). Metaraminol was associated with a 30% increase in mean arterial pressure, and cerebral blood flow did not change significantly. Other studies demonstrated that vasopressor induced hypertension (with metaraminol, phenylephrine and noradrenaline) had no effect on cerebral blood flow, despite significant increases in mean arterial pressure, as long as the systemic pressure is kept below approximately 150 mmHg (199-203). The levels of mean arterial pressure exceeded this threshold in this pilot study.

In conclusion, in the dose ranges selected, adrenaline, noradrenaline and dopamine appeared to have equivalent systemic haemodynamic effects. This study laid the platform for more definitive studies on systemic and cerebrovascular effects.

Chapter 6. Systemic and cerebrovascular effects of catecholamines

As outlined in Chapter 1, despite intensive research on the adrenergic system, the use catecholamines in clinical practice is largely empirical.

There have been few studies in which the effects of catecholamines on different global and regional circulations have been compared. Few, if any, have compared the effects of catecholamines against a standardised baseline.

Most studies have been conducted in pathological states, on heterogeneous study populations, using different doses and a range of end points. Outcome based studies are invariably underpowered. Deductions about the effects of vasoactive agents in conditions such as septic and cardiogenic shock are made from subset analyses or secondary endpoints (176,177,185-189).

The animal preparation described was used in this conduct a series of physiological studies to compare the systemic and cerebrovascular effects of infusions of exogenous catecholamines on standard haemodynamic and metabolic variables. Each of the catecholamines, adrenaline, noradrenaline and dopamine, could then be directly compared to baseline physiological conditions and the effects of each catecholamine compared with the other two.

6.1 Aim

The aim of the studies in this chapter were to compare the systemic cardiovascular and cerebrovascular effects of adrenaline, noradrenaline and dopamine.

6.2 Methods

The general methods used in this preparation are presented in detail in Chapter 4.

6.2.1 Animal preparation

One week prior to the study, the animals were instrumented under thiopentone and halothane anaesthesia as described in Chapter 4.3.

Through a frontal craniotomy an ultrasonic Doppler transducer was placed on the dorsal sagittal sinus.

Using a Seldinger technique, a 4F polyethylene was inserted into the sagittal sinus and advanced to a distance of 1 cm.

A solid state, strain-gauge tipped intracranial pressure monitor was placed into the subdural space and advanced to a distance of 2 cm.

Through a transverse incision over the femoral triangle, a 7F vascular catheter was inserted through a pulmonary artery catheter introducer sheath into the femoral artery.

The same procedure was then performed on the femoral vein and a three-lumened thermodilution pulmonary artery catheter was inserted through the femoral vein sheath and positioned into the pulmonary artery under waveform imaging.

The animal was then recovered and returned to housing crates and allowed free access to food and water.

6.2.2 Interventions

In random order, each animal received three ramped intravenous infusions of noradrenaline, adrenaline or dopamine through the infusion lumen of the pulmonary artery catheter. Each animal acted as its own control. Infusions of adrenaline, noradrenaline and dopamine (0-60mL/hr) were administered at 5-minute intervals, followed by an elimination period of 20 minutes. (Figure 6.1)

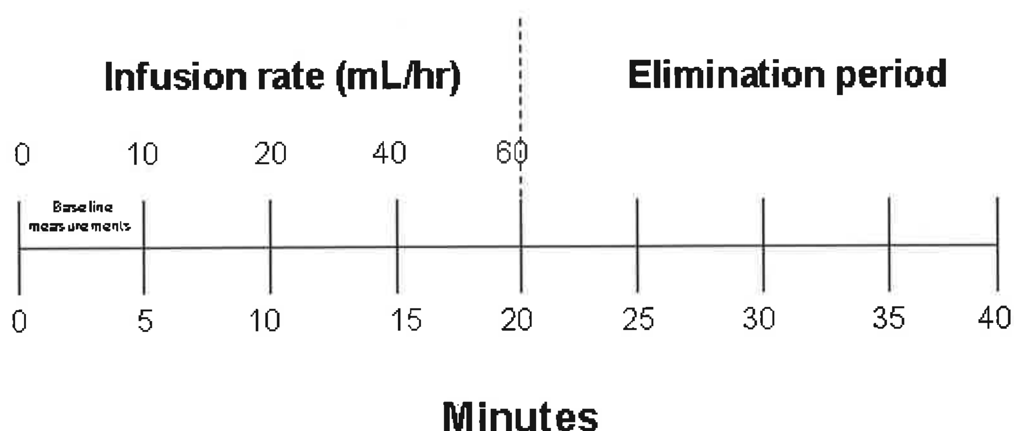


Figure 6.1
Intervention timeline diagram for the studies. Measurements and blood samples were taken at each time point.

One hour was allowed to elapse between completion of each study to ensure clearance of each catecholamine and restoration of baseline values.

6.2.3 Measurements

The methods of measurement are outlined in detail in section 4.4.2.

Mean arterial pressure was measured continuously. Measurements of cardiac output and right atrial pressure were made at 5 minute intervals, after the start of the catecholamine infusion and every five minutes during the

elimination period. At the same time, blood samples were taken from the arterial and pulmonary artery catheters for measurements of arterial and mixed venous blood gases.

Cerebral blood flow and intracranial pressure were continuously measured. Intermittent blood samples were taken from the sagittal sinus catheter for measurement of cerebral venous blood gas analysis.

Following the studies, systemic and cerebrovascular vascular resistances and systemic and cerebral oxygen consumption and delivery were calculated according to the calculations outlined in section 4.4.2.7.

6.2.4 Statistical analysis

Normal distribution of datapoints before parametric analyses was determined using the using the Kolmogorov-Smirnov test.

Comparison of the effects of catecholamines on all parameters were made using two-way analysis of variance and Bonferroni corrections for multiple time points. Significance was determined by 95% confidence intervals, assuming a t-distribution. A p-value of <0.05 was considered to be statistically significant.

6.3 Results

Studies of each catecholamine (adrenaline, noradrenaline and dopamine) were conducted in six animals.

6.3.1 Systemic haemodynamic effects

All three drugs significantly increased mean arterial pressure from baseline. The effects of each catecholamine on mean arterial pressure are shown in Figure 6.2. Data are expressed as mean ± standard error of the mean (sem).

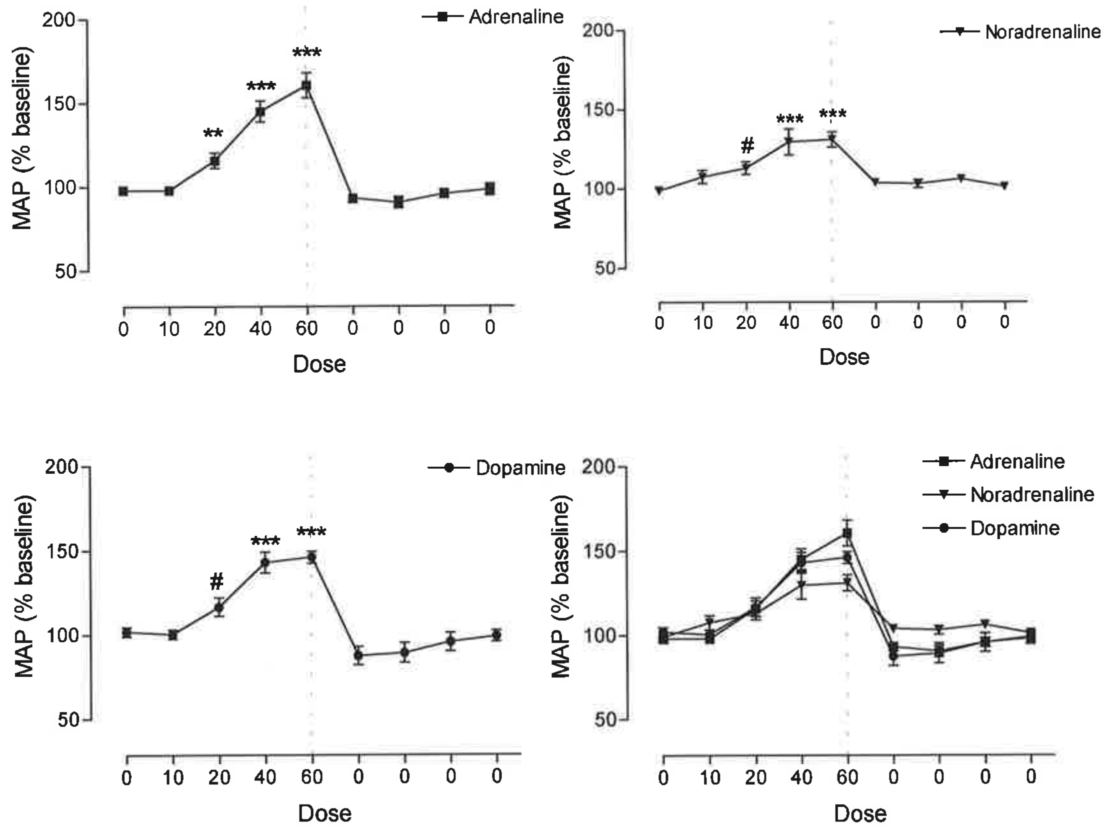


Figure 6.2
*Effects of adrenaline (top left panel), noradrenaline (top right) and dopamine (bottom left) on mean arterial pressure (MAP, expressed as % baseline). Comparisons of the three drugs are shown in bottom right panel. Dose is expressed as mL/hr. # = p<0.05, *** = p<0.001. Data are expressed as mean±sem.*

There was a 30% difference between adrenaline and noradrenaline on the maximal effect on mean arterial pressure (95% confidence intervals –46 to –13; $p<0.001$). No differences between adrenaline and dopamine or dopamine and noradrenaline were demonstrated.

The effects of catecholamines on cardiac output are shown in Figure 6.3. All three significantly increased cardiac output from baseline, although this was least pronounced with noradrenaline ($p<0.05$). No differences between adrenaline, noradrenaline and dopamine were demonstrated.

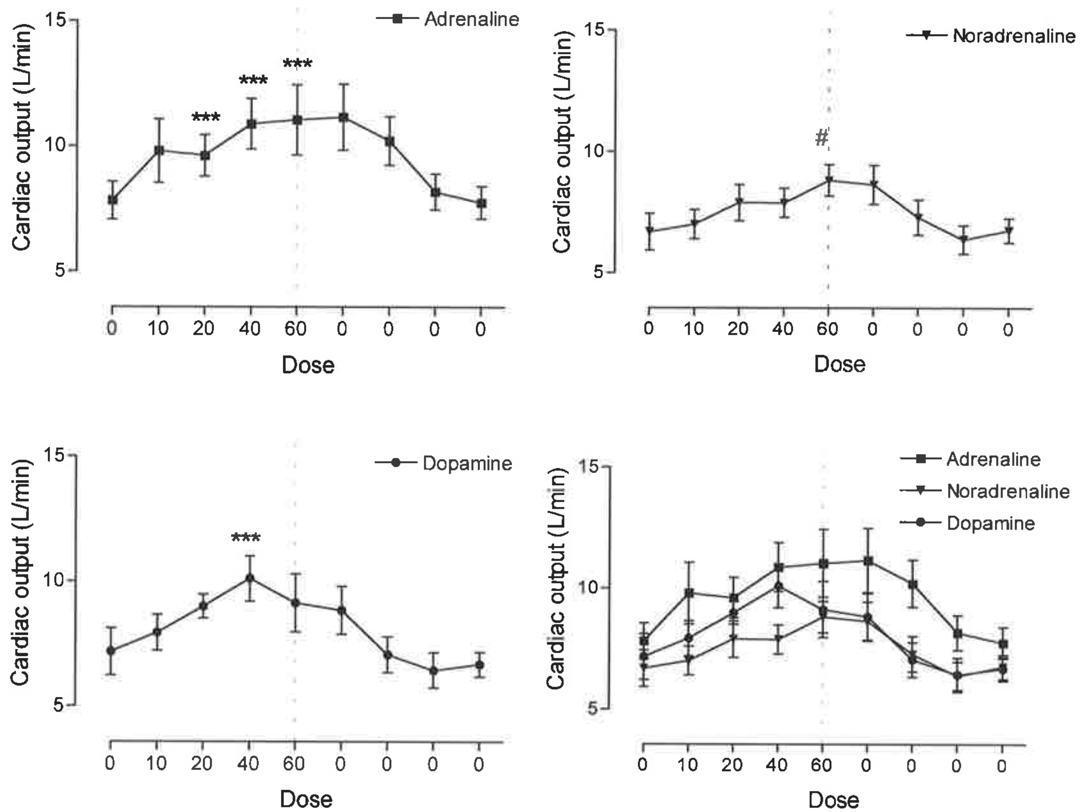


Figure 6.3
*Effects of adrenaline (top left panel), noradrenaline (top right) and dopamine (bottom left) on cardiac output (L/min). Comparisons of the three drugs are shown in bottom right panel. Dose is expressed as mL/hr. #= p<0.05, *** = p<0.001. Data are expressed as mean±sem.*

The changes in right atrial pressure are shown in Figure 6.4. Although adrenaline and dopamine increased right atrial pressure from baseline, these changes did not achieve statistical significance (p>0.05). There was no difference between adrenaline, noradrenaline and dopamine, although noradrenaline appeared to have the least effect on right atrial pressure.

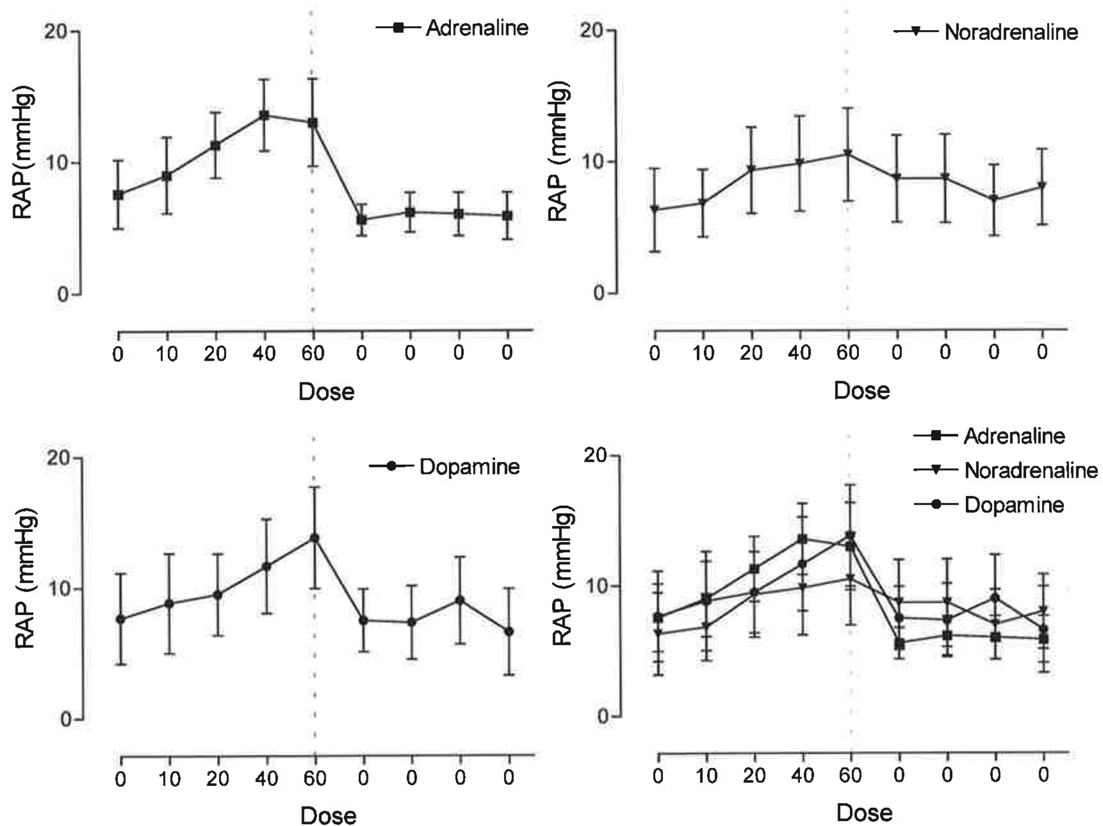


Figure 6.4
Effects of adrenaline (top left panel), noradrenaline (top right) and dopamine (bottom left) on right atrial pressure (mmHg). Comparisons of the three drugs are shown in bottom right panel. Dose is expressed as mL/hr. Data are expressed as mean±sem.

The effects on calculated systemic vascular resistance are shown in Figure 6.5. None of the catecholamines produced significant increases in systemic vascular resistance from baseline. Adrenaline produced an initial dose-dependent decrease in systemic vascular resistance from baseline from 0 – 40 mL/hr (1400→1035 Units, mean difference 365, 95% CI –598 to –130, $p<0.001$). Thereafter systemic vascular resistance returned to baseline levels as doses were increased. This was a phenomenon common with all catecholamines. Noradrenaline appeared to have the least effect on calculated systemic vascular resistance.

Following cessation of infusions, a significant reduction in systemic vascular resistance occurred with all drugs. This was followed by a prompt return to baseline values within 10 minutes.

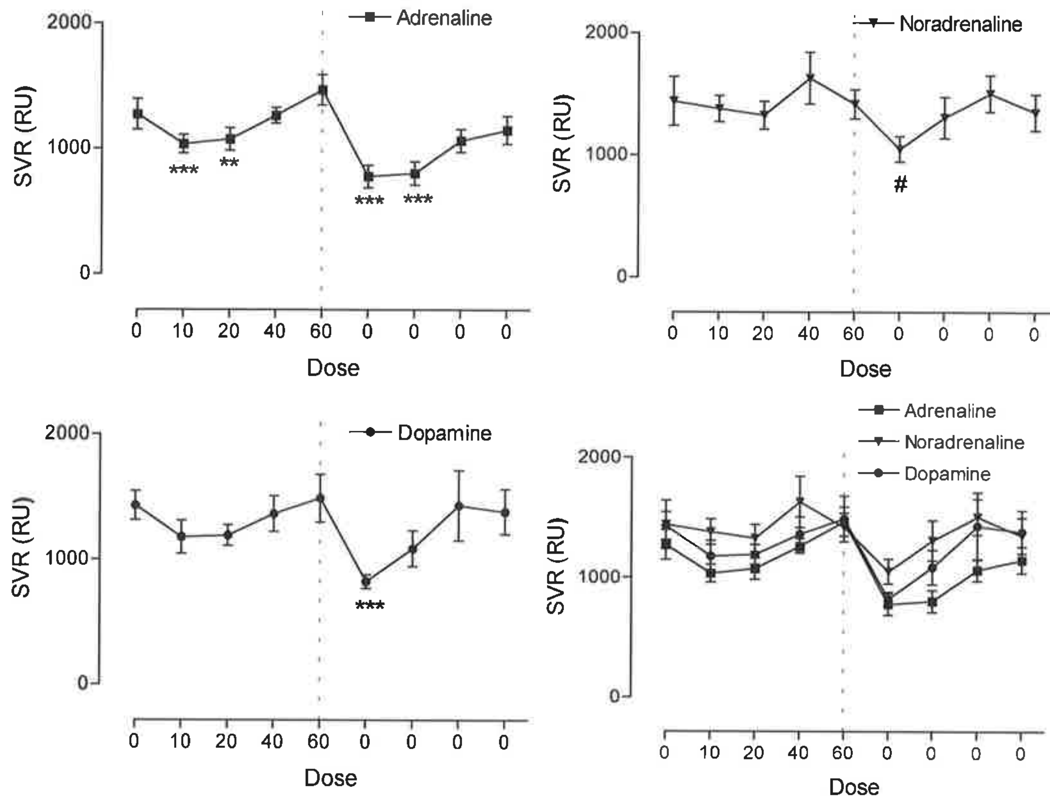


Figure 6.5
*Effects of adrenaline (top left panel), noradrenaline (top right) and dopamine (bottom left) on systemic vascular resistance (Resistance Units) Comparisons of the three drugs are shown in bottom right panel. Dose is expressed as mL/hr. # = p<0.05; *** = p<0.001. Data are expressed as mean±sem.*

6.3.2 Systemic metabolic effects

Metabolic effects were expressed as the effects on systemic oxygen consumption and arterial and mixed venous pH.

The effects on systemic oxygen consumption and delivery are shown in Figure 6.6. No significant change from baseline oxygen consumption was demonstrated apart from a transient increase in oxygen consumption by adrenaline at 40 mL/hr ($p<0.01$). No significant difference between the catecholamines were demonstrated. The effects on arteriovenous oxygen content difference mirrored those demonstrated with systemic oxygen consumption.

The changes in oxygen delivery were more pronounced than those on cardiac output. Significant increases in oxygen delivery from baseline occurred with the three drugs, the most pronounced effects wereseen with adrenaline and dopamine ($p<0.001$ for both agents). Noradrenaline was associated with similar significant increases in oxygen delivery ($p<0.05$) albeit at higher concentrations.

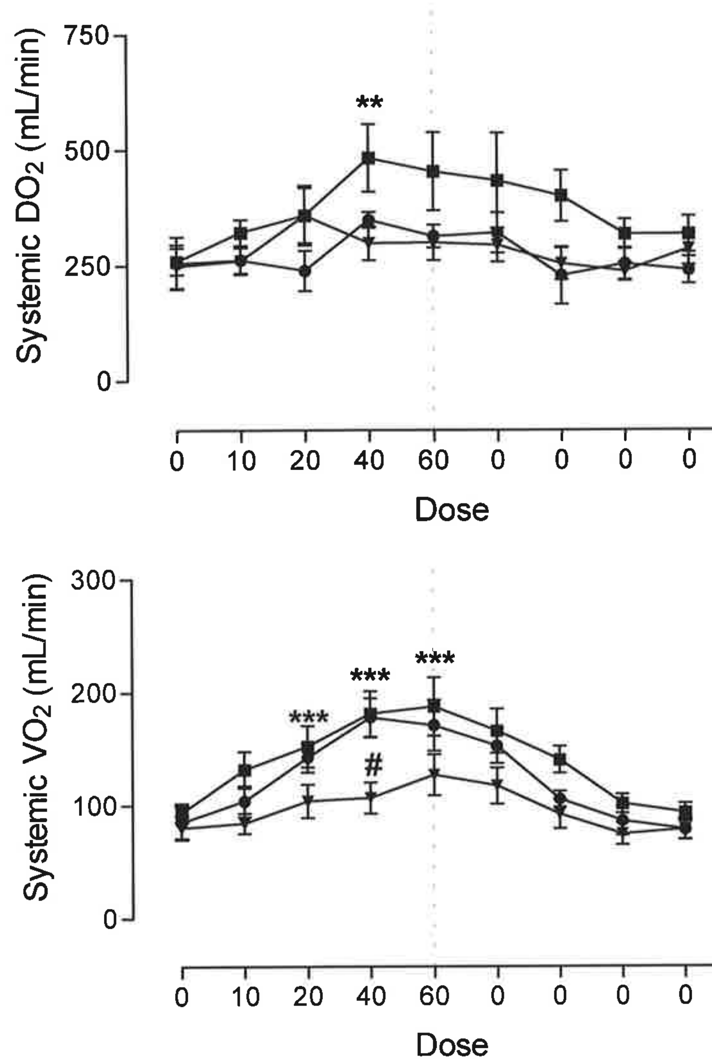


Figure 6.6
*Effects of adrenaline, noradrenaline and dopamine on systemic oxygen delivery (DO_2) (mL/min) (top panel) and systemic oxygen consumption (VO_2) (mL/min) (bottom panel). Dose is expressed as mL/hr. # = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$. Data are expressed as mean±sem.*

The concomitant effects on arterial and venous pH are shown in Figure 6.7. Dopamine was associated with dose-dependent reductions in arterial and venous pH, but these were of modest magnitude and did not significantly differ from baseline. No significant difference between the three catecholamines were demonstrated. In order to determine whether this was a metabolic or respiratory phenomenon, the effects on arterial and venous $PaCO_2$ are shown in Figure 6.7. Dopamine significantly increased $PaCO_2$ from baseline that was significantly different to the other two drugs. During the high doses of dopamine, the animals became slightly agitated with demonstrably increased respiratory rates.

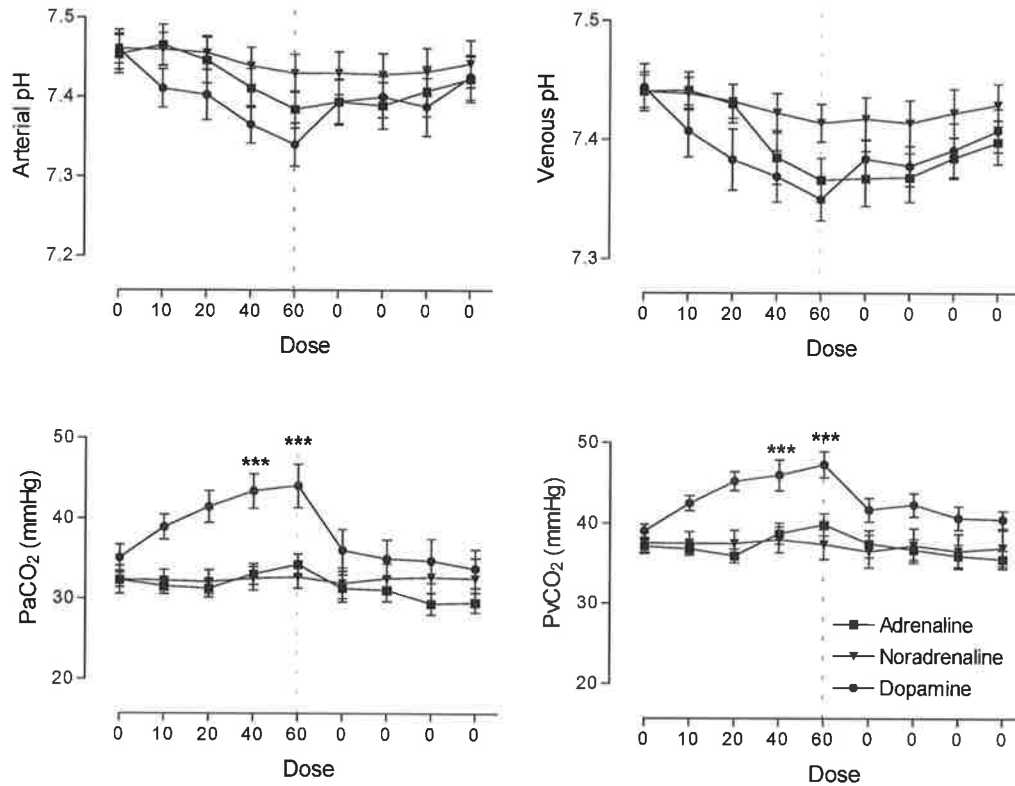


Figure 6.7
*Effects of adrenaline, noradrenaline and dopamine on arterial and venous pH (top panels) and associated arterial ($PaCO_2$) and venous carbon dioxide tensions ($PvCO_2$) (mmHg). . Dose is expressed as mL/hr. *** = p<0.001. Data are expressed as mean±sem*

6.3.3 Cerebrovascular effects

The effects of adrenaline, noradrenaline and dopamine on cerebral blood flow are shown in Figure 6.8.

Dopamine and adrenaline produced dose-dependent increases in cerebral blood flow, with significant increases (dopamine $p<0.01$; adrenaline $p<0.05$) from baseline at the maximal dose. Noradrenaline did not significantly change cerebral blood flow from baseline. There were no significant difference between the catecholamines.

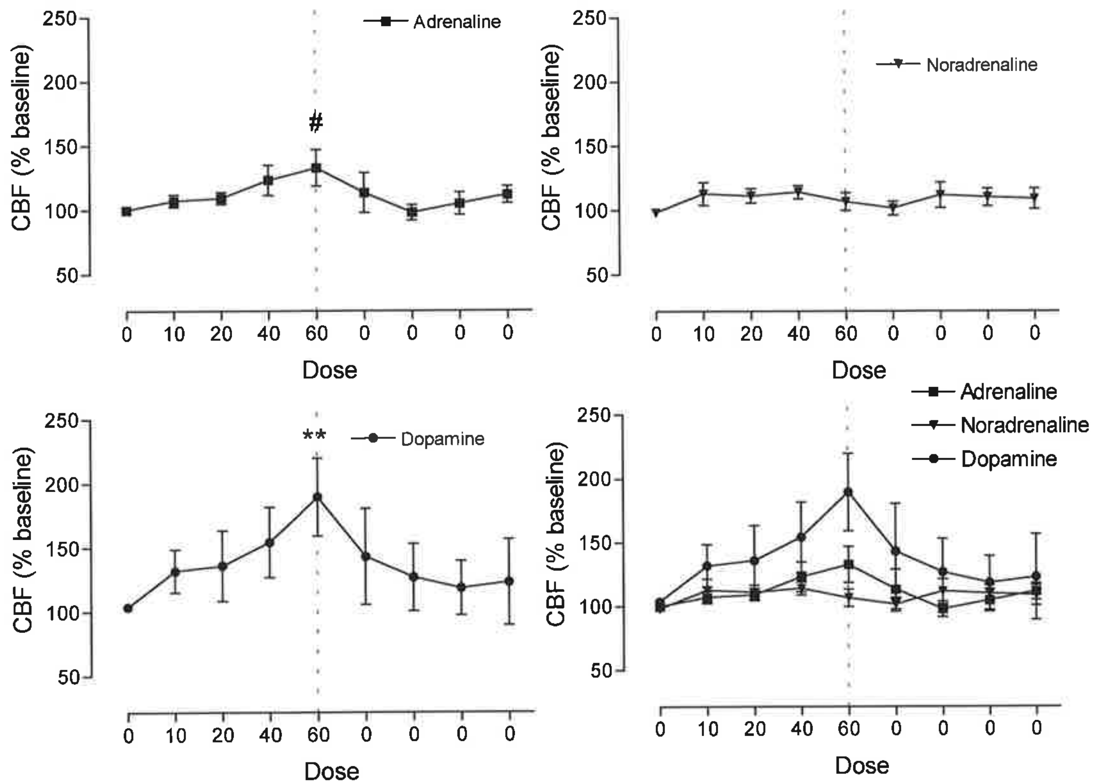


Figure 6.8
*Effects of adrenaline (top left panel), noradrenaline (top right) and dopamine (bottom left) on cerebral blood flow (CBF: % baseline). Comparisons of the three drugs are shown in bottom right panel. . Dose is expressed as mL/hr. # = p<0.05; ** = p<0.01. Data are expressed as mean±sem.*

The effects on intracranial pressure are shown in Figure 6.9. Dopamine was associated with a dose-dependent, significant increase in intracranial pressure (p<0.001). Adrenaline produced an increase in intracranial pressure at the maximal dose (p<0.001), whilst noradrenaline had no significant effects on intracranial pressure.

There was a 73% difference in intracranial pressure between dopamine and adrenaline at the 30mL/hr (95% confidence intervals 30.1 to 115; p<0.001); and a 80% difference between dopamine and noradrenaline at the maximal dose (95% confidence intervals 40.1 to 121; p<0.001). The difference between adrenaline and noradrenaline (41.5%, 95% confidence intervals (-78.5 to –4.5, p<0.05) also achieved statistical significance.

The effects on cerebrovascular resistance are shown in Figure 6.10.o significant change from baseline cerebrovascular resistance was demonstrated with any of the catecholamines; nor were there significant differences between adrenaline, noradrenaline and dopamine.

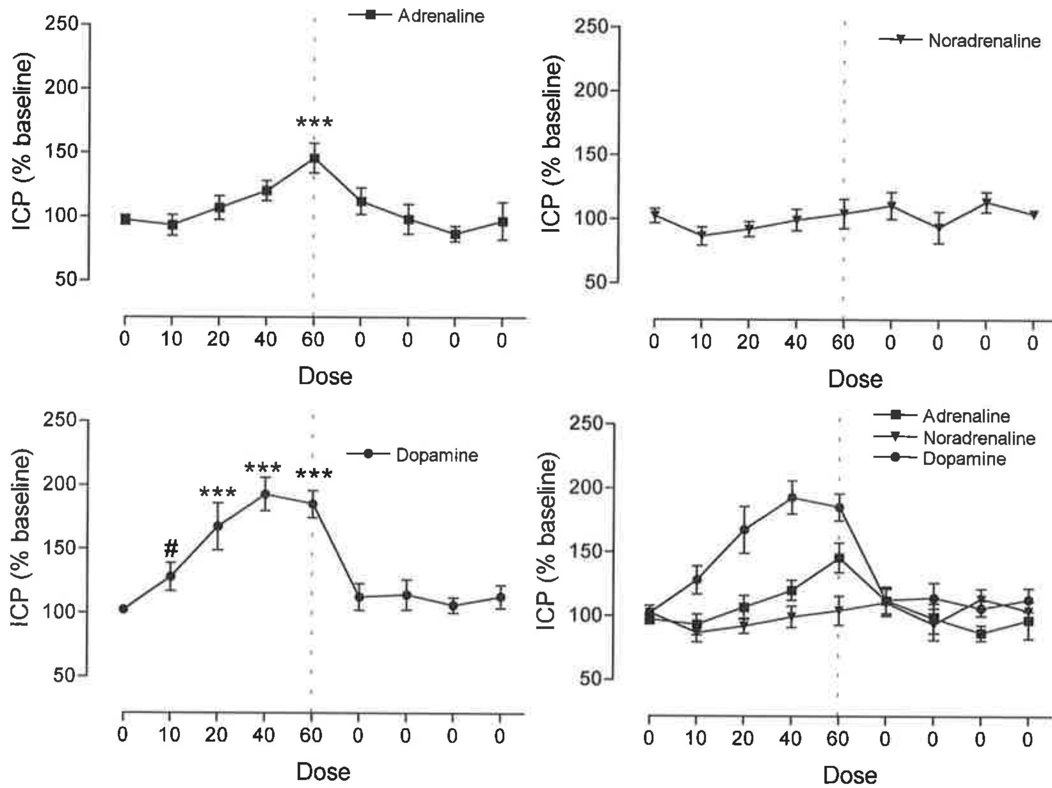


Figure 6.9
*Effects of adrenaline (top left panel), noradrenaline (top right) and dopamine (bottom left) on intracranial pressure (ICP : % baseline). Comparisons of the three drugs are shown in bottom right panel. . Dose is expressed as mL/hr. # = p<0.05; *** = p<0.001. Data are expressed as mean±sem.*

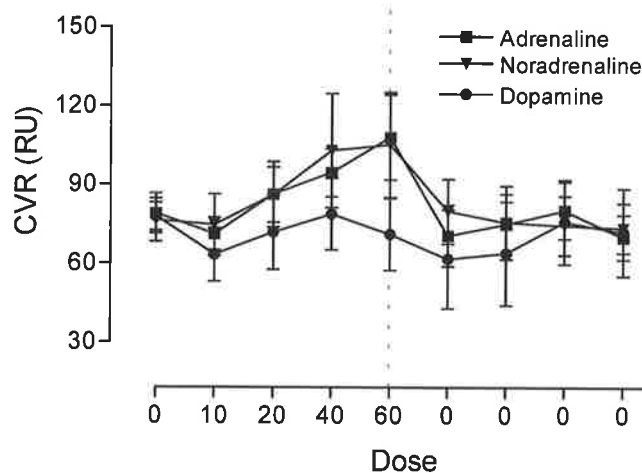


Figure 6.10
Comparisons of adrenaline, noradrenaline and dopamine on cerebrovascular resistance (CVR: resistance units). Dose is expressed as mL/hr. Data are expressed as mean±sem.

6.3.4 Cerebral metabolic effects

The effects of adrenaline, noradrenaline and dopamine on cerebral oxygen consumption and arterio-sagittal sinus oxygen content differences are shown in Figure 6.11

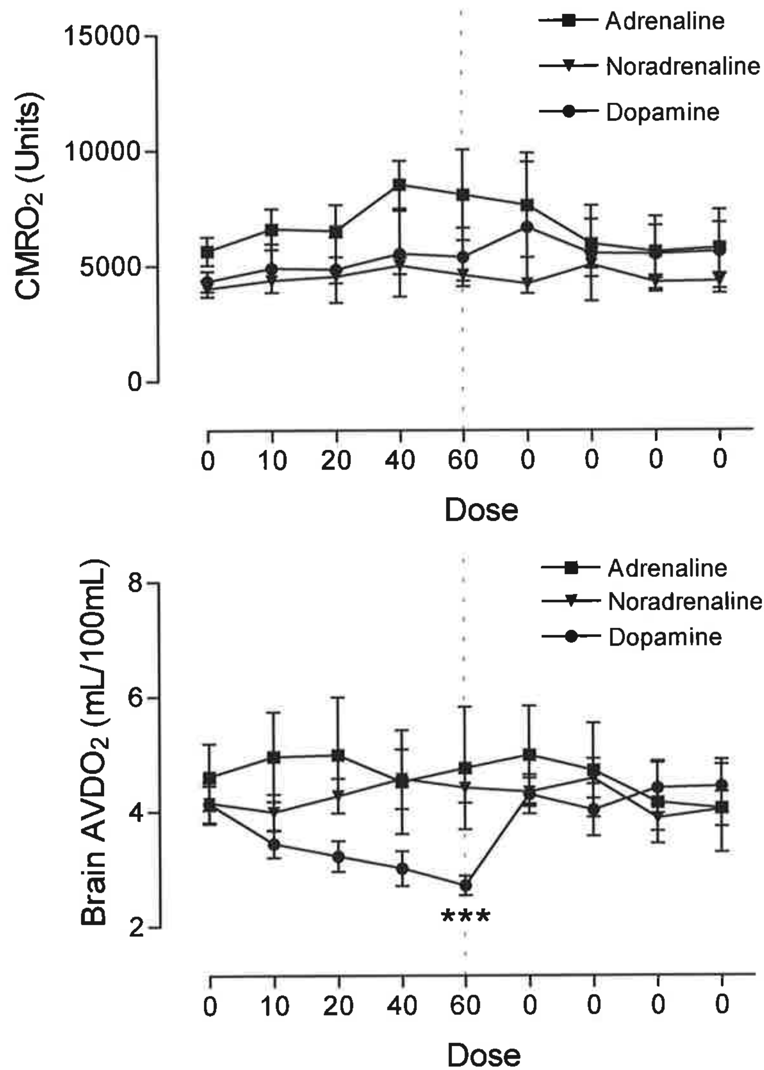


Figure 6.11
*Comparisons of adrenaline, noradrenaline and dopamine on cerebral oxygen consumption ($CMRO_2$ units) (top panel); and cerebral arterio-sagittal sinus oxygen content difference (Brain $AVDO_2$: mL/100mL) (bottom panel). . Dose is expressed as mL/hr. *** = $p<0.001$.*

No significant change from baseline cerebral oxygen consumption was evident for adrenaline, noradrenaline or dopamine. However, dopamine produced a dose-dependent, significant decrease in arterio-sagittal sinus oxygen content difference from baseline (mean difference 1.79 g/100mL; 95% confidence intervals –2.8 to –0.8; $p<0.001$). The changes in

arteriovenous contents mirrored those changes induced on cerebral blood flow.

The effects of catecholamines on cerebral venous pH are shown in Figure 6.12

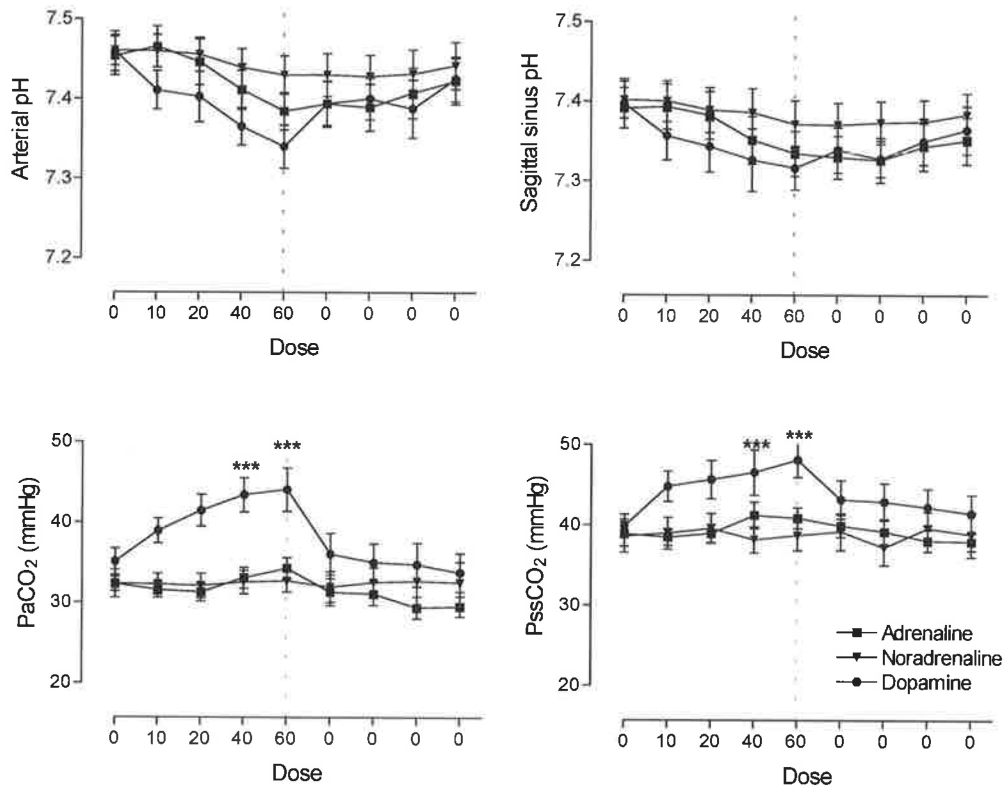


Figure 6.12
*Effects of adrenaline, noradrenaline and dopamine on arterial and sagittal sinus pH (top panels) and associated arterial ($PaCO_2$) and sagittal sinus carbon dioxide tensions ($PssCO_2$) (mmHg). Dose is expressed as mL/hr. *** = $p<0.001$. Data are expressed as mean±sem.*

6.4 Discussion

The studies in this chapter analysed the systemic and cerebrovascular haemodynamic and metabolic effects of infusions of adrenaline, noradrenaline and dopamine.

6.4.1 Systemic effects

The systemic effects of adrenaline, noradrenaline and dopamine on mean arterial pressure and cardiac output were consistent with those observed in humans and other species. These drugs produced significant and equivalent increases in these two parameters, affirming their efficacy as positive inotropic agents. The effects were immediate and corresponded to their half-lives, both in producing the observed physiological effects and following

cessation of the infusions. Despite modest statistical differences in the degree of magnitude of effects in mean arterial pressure and cardiac output, the three drugs produced clinically equivalent "pressor" (i.e. augmentation of blood pressure) effects. Of the three catecholamines, adrenaline appeared to have the most positive effect on mean arterial pressure and noradrenaline the least. This is contrary to conventional dogma that regards noradrenaline as a predominantly vasoconstrictive inotrope.

Cardiac output was uniformly and equivalently increased. Catecholamines increase cardiac output by two mechanisms. Stroke volume is augmented by increased contractility (velocity of cardiac contraction). Increases in ventricular stroke volume are matched by associated increases in venous return. (143-145).

The three catecholamines produced dose-dependent increases in right atrial pressure and did not significantly alter calculated systemic vascular resistance over the infusion range. This suggests that calculated systemic vascular resistance does not represent vascular reactivity in responses to changes in mean arterial pressure or cardiac output.

From these data, it may be inferred that the increases in cardiac output, without concomitant alterations in calculated systemic vascular resistance, were responsible for increases in mean arterial pressure.

Calculated systemic oxygen consumption was not significantly changed during the catecholamine infusions. The use of calculated indices to measure changes in whole-body oxygen consumption are unlikely to produce significant changes in the absence of major physiological or pathological perturbations.

In this preparation, the disparity between the effects on oxygen delivery and cardiac output may be explained by a species-specific phenomenon. Under conditions of stress, sheep produce sympathetically mediated splenic constriction, which effectively results in an autotransfusion. The additional haemoglobin "injected" into the circulation results in an increase in real and calculated oxygen delivery (267).

Modest, dose-dependent decreases in arterial and mixed venous pH were observed. Of the three drugs, this effect was most pronounced for dopamine, where progressive, significant increases in arterial and mixed carbon dioxide tensions were demonstrated. The cause of this phenomenon is unclear. In humans, adrenaline has been implicated in producing a specific metabolic (lactic) acidosis, attributable to specific inhibition of pyruvate dehydrogenase (170). The observed effects on pH by dopamine, but not adrenaline in sheep may be is may be a real or species specific phenomenon or both.

6.4.2 Cerebrovascular effects

This study demonstrated that, in this preparation, dopamine appeared to have specific cerebrovascular effects that were different to those produced by adrenaline and noradrenaline.

Cerebral blood flow was significantly increased from baseline in a dose-dependent manner, but this only achieved statistical significance at the maximal dose range. This was significantly different to the effects caused by adrenaline, which similarly increased cerebral blood flow, but to a lesser extent. Noradrenaline did not increase cerebral blood flow.

The hyperaemic effect by dopamine was accompanied by a significant increase in intracranial pressure that was significantly different to the effects produced by adrenaline and noradrenaline. This hyperaemic response was also indicated by the significant changes in arterio-sagittal sinus oxygen content difference. Decreases in this difference are associated with cerebral hyperaemia or reductions in cerebral metabolism (68). Given that this was a physiologically intact preparation, it may be concluded that decreased cerebral arteriovenous oxygen content differences indirectly represent cerebral hyperaemia.

This is contrasted by the lack of demonstrable change in calculated cerebrovascular resistance, despite these changes in the cerebral circulation. This highlights the relative insensitivity of using cerebrovascular resistance as an index of cerebrovascular reactivity. These findings question the interpretation of studies of cerebral autoregulation that use changes in cerebrovascular resistance as a primary endpoint (102,122).

Cerebral oxygen consumption did not significantly change during infusion of the catecholamines. The changes in sagittal sinus pH and $PaCO_2$ were similar to systemic pH responses, although they these appeared to be of a lesser magnitude.

The progressive increases in arterial and cerebral carbon dioxide tensions for dopamine may have resulted in increased cerebral blood flow and intracranial pressure. Although the animals remained fully conscious throughout the studies, they did become agitated with increasing doses of dopamine. It is not possible from these results to determine what is cause and what is effect.

6.5 Conclusions and Future Directions

This study demonstrated that ramped infusions of adrenaline, noradrenaline and dopamine produced equivalent systemic haemodynamic effects. Mean arterial pressure and cardiac output were significantly increased from baseline by the three catecholamines.

Catecholamine induced hypertension produced variable effects on the cerebral circulation. Dopamine produced cerebral hyperaemia that was associated with increased intracranial pressure.

Under physiological conditions, catecholamines do not cross the blood-brain barrier. The results of this study are consistent with other studies where brief hypertensive stimuli, transiently open the blood-brain barrier, thereby inducing direct changes in cerebral blood flow and metabolism (12,108,109,114,195). The mechanism of catecholamine induced hypertensive changes in blood-brain barrier permeability has been attributed to increased pinocytotic activity of endothelial cells. This has been demonstrated with noradrenaline in a rodent model, but is probably common to all catecholamines (196). This is a likely mechanism to explain the effects of dopamine seen in this study.

A study in a rodent model produced the closest results to this study (114). Blood-brain barrier permeability was increased during moderate hypertension induced by dopamine but not when it was induced by noradrenaline.

Dopamine may also exert a direct dose-dependent effect on the cerebral circulation in the presence of an intact blood-brain barrier through selective transmission of dopamine across naturally occurring defects in the blood-brain barrier (193,194). However, as this study did not include quantification of blood-brain barrier permeability, this is a speculative mechanism of action.

It was concluded that, in this preparation, dopamine exerts a specific, dose-dependent cerebral hyperaemic response in the presence of induced systemic hypertension.

On the basis of these results, the next phase of this thesis was planned.

This was to include a repeat of comparative studies of adrenaline, noradrenaline and dopamine on systemic and cerebrovascular haemodynamics, but under conditions of reproducible changes in cerebral function - specifically, these induced by altered cerebral blood flow, autoregulation and blood-brain barrier permeability.

To this end, volatile anaesthesia using isoflurane, and intravenous anaesthesia using propofol were selected as perturbations.

Aspects of the studies in this chapter were published in the following article:

Myburgh JA, Upton RN, Grant C, Martinez A. (1998) A comparison of the effects of norepinephrine, epinephrine and dopamine on cerebral blood flow and oxygen utilisation. Acta Neurochirurgica [Suppl] 71:19-21.

Chapter 7. The effects of anaesthesia on cerebral blood flow and carbon dioxide reactivity

Before commencing the studies of adrenaline, noradrenaline and dopamine on cerebrovascular function under anaesthesia, the effects of anaesthesia on the cerebral circulation were quantified.

The effects of steady-state isoflurane and propofol anaesthesia on cerebral blood flow and cerebrovascular carbon dioxide reactivity were compared to those values in awake sheep.

7.1 Introduction

Under physiological conditions, cerebral blood flow and arterial carbon dioxide tensions ($PaCO_2$) have a pseudo-linear relationship: hypercapnia causes cerebral vasodilation, whilst hypocapnia causes vasoconstriction. This mechanism is due to carbon dioxide induced changes in perivascular cerebral pH, resulting in changes in cerebrovascular tone (20).

Cerebrovascular reactivity is also influenced by cerebral tissue oxygenation (21), calcium and potassium fluxes and drugs such as volatile (22,23) and intravenous anaesthetics (24,25).

Anaesthetic agents have variable effects on cerebral blood flow. Volatile agents generally produce cerebral vasodilation, particularly at high doses, producing gradual relative increases in cerebral blood flow as cerebral metabolic rate decreases (227,228). In contrast, intravenous anaesthetic agents produce parallel dose-dependent decreases in both cerebral blood flow and cerebral metabolism. Coupling of flow and metabolism are preserved with agents such as propofol, barbiturates and benzodiazepines, with the observed decreases in cerebral blood flow considered to be secondary to drug induced decreases in cerebral metabolism.

These factors may influence carbon dioxide reactivity, thereby affecting cerebral metabolic autoregulation (flow-metabolism coupling). For example, during propofol anaesthesia, cerebrovascular reactivity of blood flow and blood volume is maintained during hypercapnia but is markedly diminished during hypocapnia (40).

There are few studies that define these effects. A number of physiological studies used a variety of subjects and methods of measurement (22,40,268-270). The array of methods of measurement and study subjects has resulted in conflicting opinions about the effects of these anaesthetics on carbon dioxide reactivity. These discrepancies highlighted further in studies using

pathophysiological models (132,271,272). Few studies have directly compared the effects of anaesthesia with those in awake subjects, using a consistent measurement of cerebral blood flow.

Preliminary studies of cerebrovascular carbon dioxide reactivity in this preparation in awake sheep with those under halothane anaesthesia are described in section 2.3.2.2.4.2. These studies demonstrated that the relationship between cerebral blood flow and carbon dioxide tensions in awake sheep with those under anaesthesia were not statistically different, demonstrating an approximately linear relationship over an end tidal carbon dioxide range of 40-60 mmHg. Maximum and minimum cerebral blood flows occurred at an end-tidal carbon dioxide concentration of approximately 65 mmHg and 30 mmHg respectively.

In the context of the planned studies of catecholamines on cerebrovascular function under anaesthesia, precise knowledge of the effects of the selected anaesthetic agents on cerebral blood flow and carbon dioxide reactivity were considered to be essential.

7.2 Aims

The aim of the studies in this chapter were to:

1. Quantify the effects of isoflurane and propofol anaesthesia on mean arterial pressure, cerebral blood flow and intracranial pressure by comparisons with those values in awake sheep.
2. Determine and compare cerebrovascular carbon dioxide reactivity in awake sheep with those under isoflurane and propofol anaesthesia.

7.3 Method

The general methods used in this preparation are presented in detail in Chapter 4.

7.3.1 Animal preparation

One to two weeks prior to the study, the animals were instrumented under thiopentone and halothane anaesthesia as described in Chapter 4.3.

For these studies, a "head preparation" using the sagittal sinus Doppler transducer and intracranial pressure monitor was used.

The complete vascular preparation (arterial, venous and pulmonary artery catheters) was used.

7.3.2 Dose selection

Anaesthesia was induced with propofol (4mg/kg) for both anaesthetised cohorts. For the propofol cohort, anaesthesia was maintained by constant

infusion; for isoflurane, the volatile agent was administered to achieve a steady-state end tidal concentration.

7.3.2.1 Propofol

Propofol infusion rates were selected based on previously published studies from our laboratory analysing the cerebral pharmacokinetics of propofol in sheep. Previous work had shown dysequilibrium between the arterial blood and brain concentrations of propofol following rapid administration to sheep (215,216,242). The extent of dysequilibrium was examined following slower administration as a constant rate infusion. Pharmacokinetic models were subsequently established from these studies (214,216), from which infusion rates that induced a constant depth of anaesthesia and brain effluent concentrations of propofol were determined (259).

Based on these studies, an infusion of 15mg/min was chosen.

7.3.2.2 Isoflurane

Steady-state 2% isoflurane was used and confirmed by maintaining end-tidal isoflurane concentrations. This dose was based on previously published animal studies using isoflurane that demonstrated adequacy of anaesthesia and stability of cerebrovascular volumes over a range of $PaCO_2$ (228).(124). The effects of isoflurane demonstrated in the carbon dioxide reactivity studies described in the previous chapter were consistent with other studies (236) where isoflurane was administered in doses of 1.5% and 2%.

Based on this information, an end tidal concentration of 2% was chosen.

7.3.3 Interventions

Studies were performed in three cohorts of adult female merino sheep: awake or under steady-state propofol or isoflurane anaesthesia.

7.3.3.1 Studies in awake sheep

After a period to allow the animal to settle, monitors were attached and calibrated before a period of measurement to ensure baseline stability.

Following baseline measurements, 100% oxygen at 20 L/min was administered via a sealed soft plastic mask attached to a semi-open breathing circuit.

Carbon dioxide was then introduced into the circuit in increments, while oxygen flow rates were reduced to maintain total flow at 20 L/min. Following each increase in inspired carbon dioxide concentration and when cerebral blood flow had reached a new plateau, an arterial blood sample was taken for blood gas analysis.

The inspired concentration of carbon dioxide was manipulated to keep arterial carbon dioxide tension within a range of 35-60 mmHg.

7.3.3.2 Studies under anaesthesia

Prior to anaesthesia, the output of the Doppler probe was recorded with the animal in an awake, calm state.

Anaesthesia was then induced with 200mg propofol and endotracheal intubation was performed with the sheep in the supine position. Thereafter, the studies were performed in the lateral position.

For the isoflurane cohort, an end tidal concentration of 2% isoflurane was maintained. For the propofol cohort, propofol was at a constant infusion rate of 15mg/min.

For both cohorts, the animals were mechanically ventilated using a volume controlled ventilator and 100% oxygen. Tidal volume, expired gas analysis of end tidal carbon dioxide and volatile agent (for isoflurane) were measured. The ventilator was adjusted to maintain an end tidal carbon dioxide concentration of 40 mmHg.

After one hour of continuous intravenous anaesthesia, a pseudo-steady-state was assumed to have been established based on previous studies of mathematical modelling of propofol disposition (218,261). Isoflurane anaesthesia was allowed to stabilise for one hour.

Thereafter, manipulations in carbon dioxide were performed. Initial measurements were made with the $PaCO_2$ titrated to 35 mmHg. This allowed direct comparisons with pre-anaesthesia cerebral blood flow values to determine the effect of each anaesthetic agent on cerebral blood flow at normocarbia.

Subsequently, a range of carbon dioxide levels were induced by randomly increasing or decreasing minute ventilation in a ramped manner by adjusting the tidal volume and respiratory rates on the mechanical ventilator. The minimum value of $PaCO_2$ was that achieved by maximal hyperventilation of the animal; the maximum was that achieved by hypoventilating the animal to the point where spontaneous respiration occurred.

Typically, six discrete levels of end tidal carbon dioxide were possible, with each characterised by cerebral blood flow reaching a plateau within approximately five minutes. At this point, an arterial blood sample was taken from the femoral arterial catheter for blood gas analysis.

Temperature was monitored via the pulmonary artery catheter and maintained at baseline levels via humidification of inspired gases using a heat and moisture exchanger.

Following the studies, the sheep were allowed to recover from anaesthesia and transferred to their holding crates where they were allowed free access to food and water.

7.3.4 Measurements

Changes in cerebral blood flow were inferred from changes in the outputs from the Doppler probe.

Mean arterial and intracranial pressures were recorded as percentage changes from baseline values and averaged over thirty seconds when end-tidal carbon dioxide values stabilised at a discrete level.

Arterial blood gases for measurement of $PaCO_2$ and pH where taken from the arterial catheter at the intervals outlined above and measured using a standard blood gas analyser.

7.3.5 Data analysis

The effects of anaesthesia on cerebral blood flow (pre-anaesthesia vs 1 hour post anaesthesia) and mean arterial pressure were examined using paired t-tests.

Relationships between changes in cerebral blood flow and $PaCO_2$ were determined using linear regression, with data normalised to the cerebral blood flow at a $PaCO_2$ of 35mmHg. Accordingly, the ordinate of the regression lines represents the percentage increases in cerebral blood flow from the baseline value. Regression was performed on the individual data from each animal for the three cohorts, and also on the pooled data for the propofol and isoflurane cohorts.

Pooled data were expressed as means and 95% confidence intervals. Comparison between cohorts were made by two-way analysis of covariance (273).

7.4 RESULTS

Studies in awake sheep were performed on one occasion in each of six different sheep (n=6). Studies under anaesthesia were conducted in three different animals, each animal undergoing studies on separate days under either propofol (n=8) or isoflurane (n=6) anaesthesia. Analysis of variance was used to test for differences between individual sheep for these data.

7.4.1 Effects on cerebral blood flow and mean arterial pressure

Propofol anaesthesia was characterised by a substantial, statistically significant decrease in cerebral blood flow (55% of baseline, $p=0.001$) and a significant increase in mean arterial pressure (132% baseline, $p=0.04$).

Isoflurane anaesthesia did not significantly change cerebral blood flow (88% of baseline, $p=0.39$) or mean arterial pressure (98% of baseline, $p=0.76$) (Figure 7.1).

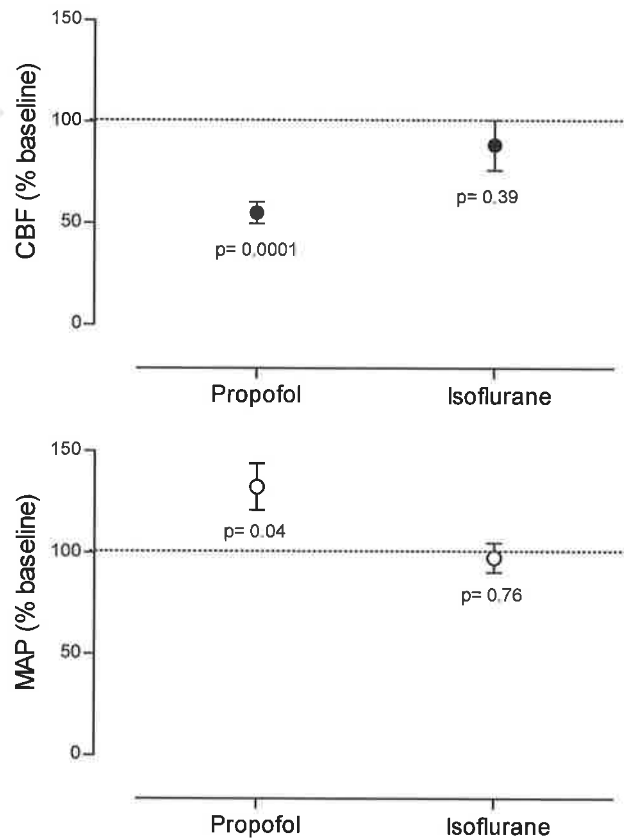


Figure 7.1
The effect of propofol (15mg/min) and 2% isoflurane anaesthesia on cerebral blood flow (CBF: solid circles) and mean arterial pressure (MAP: open circles) expressed as % baseline compared to the baseline awake state (dashed line at 100%). Data are expressed as mean ± sem.

7.4.2 Carbon dioxide reactivity

The ranges of $PaCO_2$ achieved for the awake, propofol and isoflurane cohorts were 32-63, 18-51 and 21-52 mmHg, respectively.

Blood pressure, intracranial pressure and temperature did not significantly change from baseline through the period of carbon dioxide manipulation in all cohorts ($p>0.05$).

The regression lines between mean arterial pressure and $PaCO_2$ for the awake ($r^2=0.46$), isoflurane ($r^2=0.46$) and propofol ($r^2=0.43$) cohorts are shown in Figure 7.2. Regression lines between intracranial pressure and $PaCO_2$ for the isoflurane ($r^2=0.56$) and propofol ($r^2=0.51$) cohorts are shown in Figure 7.3.

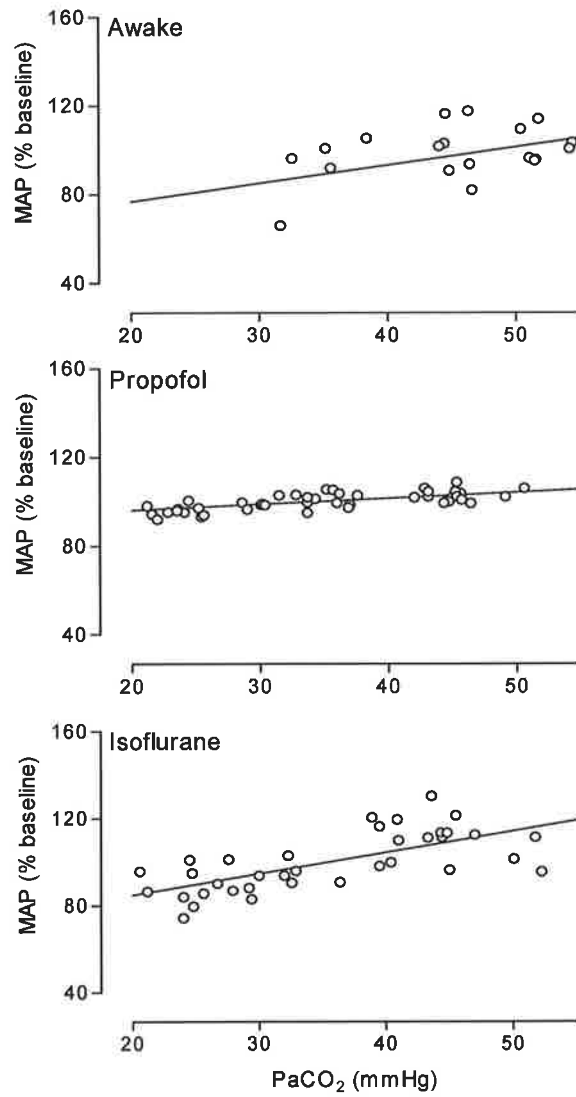


Figure 7.2
Relationships between mean arterial pressure (MAP: expressed as % baseline) and arterial carbon dioxide tension ($PaCO_2$: mmHg) in the awake (r^2=0.46); propofol (r^2=0.43) and isoflurane (r^2=0.46).

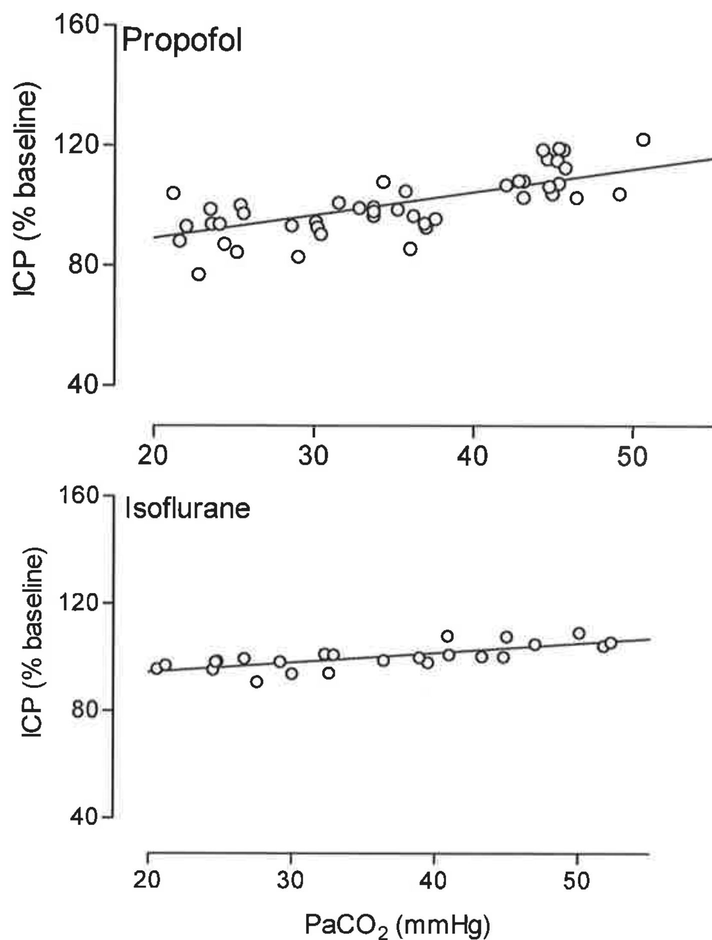


Figure 7.3
Relationships between intracranial pressure (ICP: expressed as % baseline) and arterial carbon dioxide tension ($PaCO_2$: mmHg) in the isoflurane (r^2=0.56) and propofol (r^2= 0.51) groups.

Significant linear relationships between cerebral blood flow and $PaCO_2$ were found in all individuals (Figure 7.4). For the anaesthetised cohorts, the analysis of variance showed no effect of sheep number on the slope of the regressions (p = 0.46 for propofol, 0.38 for isoflurane), suggesting that intra-animal variability in the data was comparable to inter-animal variability.

In the awake cohort, regression lines demonstrated increased cerebral blood flow for carbon dioxide although reactivity was highly variable between animals. In contrast, the reactivity under anaesthesia was more consistent. The pooled data and their regression lines are shown for propofol and isoflurane in Figure 7.5.

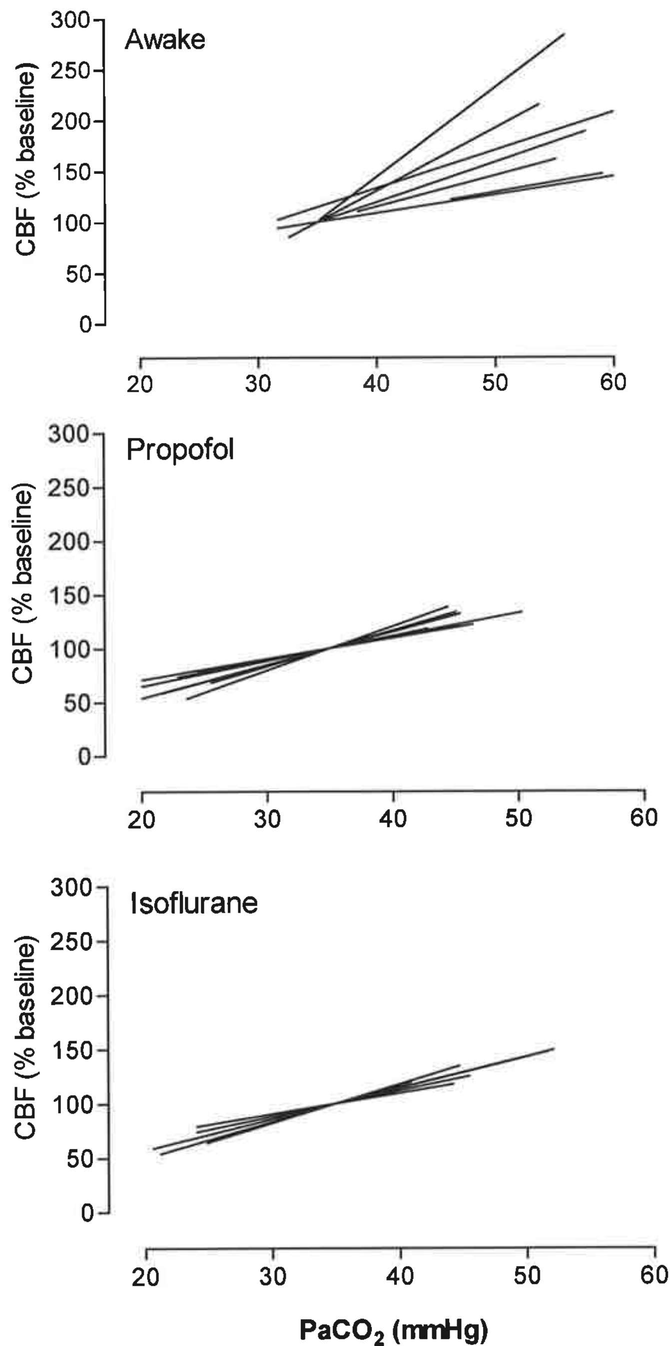


Figure 7.4

Regression lines of the percent change in cerebral blood flow (CBF) versus arterial carbon dioxide tension ($PaCO_2$: mmHg) for each individual study in the awake, propofol and isoflurane cohorts. The r^2 values ranged from 0.72 to 0.99 - only the regression lines are shown for clarity. A summary of the regression coefficients is shown in Table 7.1. Cerebral blood flow is expressed as percentage change from flow at a $PaCO_2$ of 35 mmHg.

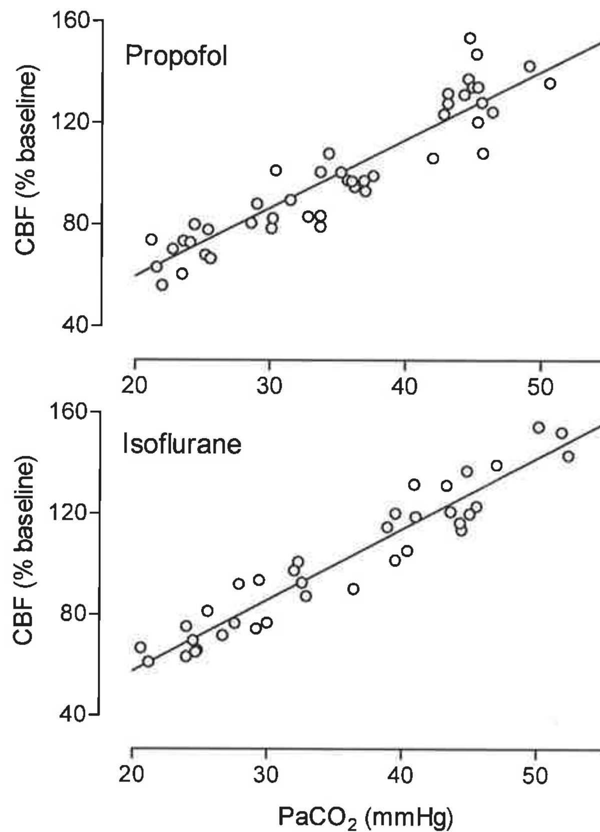


Figure 7.5
Regression lines for pooled data on cerebral blood flow (CBF) and arterial carbon dioxide tension ($PaCO_2$: mmHg) for the propofol (r^2=0.87, $p<0.0001$) and isoflurane (r^2=0.91, $p<0.0001$) cohorts. CBF is expressed as percentage change from flow at 35 mmHg.

By confidence interval analysis (Table 7.1), there was no statistically significant difference between the slopes of the regression lines between the awake and anaesthetised cohorts (p = 0.21 for awake vs propofol, 0.25 for awake vs isoflurane). There was no statistically significant difference between the slopes of propofol and isoflurane anaesthesia (p = 0.68).

	r2	Slope	Intercept
Awake	0.79 (0.57 to1.00)	4.194 (1.186 to 6.49)	-44.68 (-125.7 to 36.35)
Propofol	0.90 (0.85 to 0.96)	2.78 (2.154 to 3.41)	2.689 (-19.24 to 24.62)
Isoflurane	0.92 (0.87 to 0.97)	2.69 (2.09 to 3.29)	3.452 (-15.01 to 21.92)

Table 7.1
Summary of the linear regression coefficients for individual cerebral blood flow / carbon dioxide reactivity curves. Data are mean and (lower to upper 95% confidence intervals).

Changes in $PaCO_2$ were associated with statistically significant inverse changes in pH: in the isoflurane cohort, the relationship had an $r^2 = 0.95$ ($p<0.0001$), and in the propofol cohort $r^2=0.86$, ($p<0.0001$). Statistically significant correlations were demonstrated between changes in cerebral blood flow and pH in the isoflurane ($r^2=0.88$, $p<0.0001$) and propofol ($r^2=0.70$, $p<0.0001$) cohorts, and are shown in Figure 7.6.

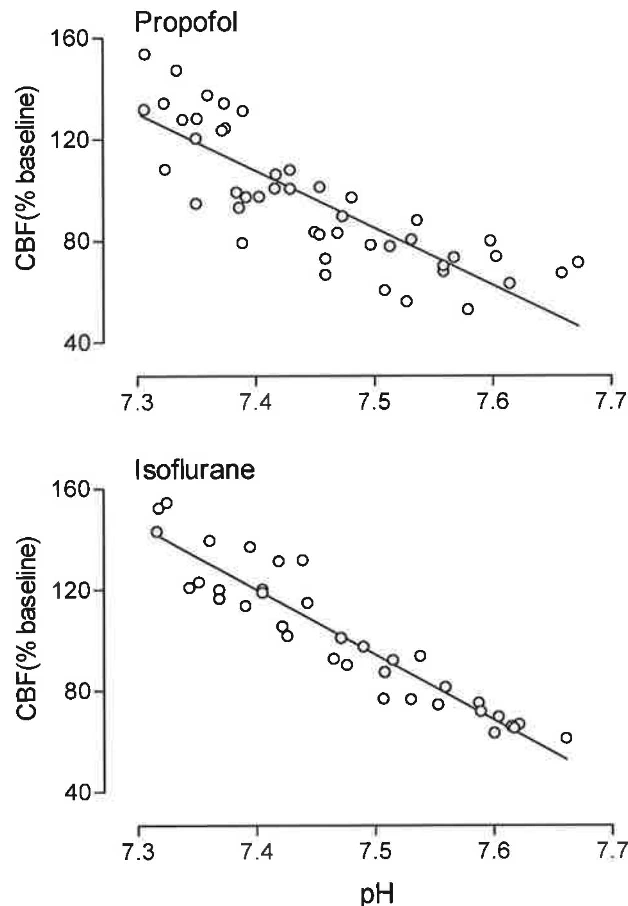


Figure 7.6
Relationships between cerebral blood flow (CBF expressed as % baseline) and arterial pH in the isoflurane (r^2=0.88, p<0.0001) and propofol (r^2=0.70, p<0.0001) groups.

7.5 Discussion

The responsiveness of the cerebral vasculature to changes in arterial carbon dioxide tensions may influence metabolic autoregulation, cerebral volume regulation and cerebral oxygen delivery. These homeostatic mechanisms are influenced by anaesthesia.

There have been many studies examining the relationship between cerebral blood flow and $PaCO_2$. A variety of methods of measurement of cerebral blood flow have been used, such as transcranial Doppler (130,274) or microsphere (40) studies. Many of these techniques assume that cerebral

blood flow is in a state of equilibrium during such measurements. The results and conclusions drawn from such experiments have been remarkably consistent, especially when one considers the variety of species tested and the different experimental conditions used. The (pseudo) linear relationship of cerebral blood flow to $PaCO_2$ over a range of approximately 30-70mmHg is described in many standard texts of physiology.

Interactions with other factors such as mean arterial pressure and anaesthetic agents are less clear. Anaesthetic agents have variable effects on cerebral blood flow. Nitrous oxide causes an absolute increase in cerebral blood flow; halothane and isoflurane cause a relative increase by uncoupling cerebral blood flow and metabolism; whilst propofol, thiopentone and benzodiazepines decrease cerebral blood flow (231,236,237,269). However, as these agents are frequently administered simultaneously in clinical situations, the effect of an individual agent on cerebral blood flow and carbon dioxide reactivity is difficult to predict or measure.

There are numerous studies analysing carbon dioxide reactivity under anaesthesia to determine the degree of alteration of cerebral metabolic autoregulation (130,228,269,270). Most of these studies have been conducted using a number of anaesthetic techniques for a range of surgical procedures, using varied techniques for cerebral blood flow measurement. In studies using patients undergoing cardiopulmonary bypass, factors such as blood pressure and temperature are confounding variables (100).

Many studies have used end-tidal carbon dioxide measurements to estimate $PaCO_2$, which may be inaccurate in ventilated or hypotensive subjects. Not surprisingly, there are conflicting data about the degree and magnitude of cerebral carbon dioxide responses under anaesthesia.

An important source of conflict arises from the method of reporting changes in cerebral blood flow in response to carbon dioxide. Absolute changes in cerebral blood flow (usually expressed as mL/100g/minute/mmHg) may differ from relative changes (expressed as % of baseline). This was highlighted in a study analysing carbon dioxide reactivity during propofol-induced electroencephalographic suppression (24), where absolute carbon dioxide reactivity was reduced whilst relative carbon dioxide reactivity was maintained within normal limits within the same subject. In this situation, cerebral blood flow and carbon dioxide tensions preserve a normal relationship, albeit from a different baseline level. By comparison, in the studies in this chapter, the effects on cerebral blood flow under propofol and isoflurane anaesthesia were compared to those in awake sheep beforehand. Conclusions about the comparative effects of anaesthesia were then made in this context.

The data from our study demonstrate that in awake sheep, there was a linear relationship between cerebral blood flow and $PaCO_2$, but that the slope of this relationship was highly variable between animals. There is a neurogenic component to the regulation of cerebral blood flow, which would be active when awake, but not under anaesthesia, mediated partly by the sympathetic response to hypercapnia. This was evident during in awake sheep when they became visibly agitated as $PaCO_2$ increased. It was speculated that this contributed to the variability in the awake sheep.

Hypercapnia was induced by different mechanisms in the awake and anaesthetised cohorts – i.e. carbon dioxide administration compared to alterations in mechanical ventilation. It is unlikely that this had a significant effect on carbon dioxide reactivity, as the ranges of carbon dioxide tensions produced were not significantly different between cohorts. The effects of mechanical ventilation on cerebral blood flow were minimised by maintaining an euvolaemic state and avoiding positive end-expiratory pressure. The changes in cerebral blood flow under anaesthesia were consistent with other published studies that did not utilise mechanical ventilation (236,237).

While the mean slopes of the reactivity curves did not differ between the awake sheep and under anaesthesia, this may have been a function of the high variability of the data observed in awake sheep. The trend in the data suggests that reactivity in awake sheep is generally higher (i.e.. higher slope) than under anaesthesia. What is clear is that the slopes of the lines for isoflurane and propofol were determined with high precision, and that there was no statistically significant difference between the carbon dioxide reactivity curves for these anaesthetic agents. This suggests that anaesthesia per se, irrespective of the agent used, is the main determinant of altered of carbon dioxide reactivity.

In this preparation, induction of anaesthesia with propofol was not associated with reductions in mean arterial pressure from baseline. Indeed there was a statistically significant increase in mean arterial pressure that was a reproducible phenomenon. This mechanism of this effect is unclear and may be a species-specific phenomenon and is consistent with other studies published by our laboratory (258). No significant changes in mean arterial pressure, intracranial pressure or temperature were observed during these studies over the hypercapnic or hypocapnic ranges.

It is acknowledged that the fractional inspired oxygen concentration was different between awake sheep (0.21) to those under isoflurane and propofol anaesthesia (1.0). The range of arterial oxygen tensions ranged from 92 mmHg (awake sheep) to 374 mmHg (anaesthetised sheep). The effect of arterial oxygen tensions on cerebrovascular reactivity has been studied under hyperoxic, normoxic and hypoxic conditions. In a human study, Fortune demonstrated no statistically significant evidence of an interaction of

oxygen and carbon dioxide cerebrovascular responses under normoxic conditions over a range of $PaCO_2$. Under hypoxic conditions, carbon dioxide changes altered cerebral blood flow simultaneously and additively (275). Under normobaric hyperoxic (PaO_2>200mmHg) and hypoxic (50mmHg) conditions, Vovk demonstrated similar cerebral blood flow responses and suggested that oxygen dependent responses were mediated through a common neural pathway (276). Hyperbaric, hyperoxic condition may result in alterations in cerebral blood flow, although patterns of cerebrovascular reactivity have not been conclusively studied (277,278).

Given the stability of the model, accuracy of cerebral blood flow and $PaCO_2$ measurements, and steady-state conditions of anaesthesia, the observed effect of these agents on carbon dioxide reactivity may reasonably be attributed to the agent alone.

The linearity of the reactivity response was maintained over the range of $PaCO_2$ studied. This observation makes this study at variance to others that have suggested that cerebral blood flow responses to carbon dioxide plateau outside of the range 25 to 70 mmHg (279). However, as the range of $PaCO_2$ produced represented a range of 18-63 mmHg, conclusions about the shape of the reactivity curve at carbon dioxide ranges above or below this range cannot be made with certainty. Flattening of cerebral blood flow response is expected at $PaCO_2$ levels < 20mmHg: cerebral blood flow responses below this threshold will need to be studied, specifically to exclude a species-specific phenomenon. The mechanism for this phenomenon remains unclear, but has been attributed to pH mediated changes in cerebrovascular responsiveness. The strong association between cerebral blood flow and pH demonstrated in Figure 7.6 is supportive of this hypothesis. Other mechanisms suggested include coupled changes in cerebral blood flow and metabolism and alterations in cerebral blood volumes.

This study used a global measurement of cerebral blood flow in a physiologically intact preparation. It therefore does not provide information about regional changes in cerebral blood flow that may be significant, particularly in situations where pathological processes impair cerebral autoregulation. Similarly, the cumulative effects of multiple anaesthetic agents in individual patients may have significant effects of cerebral autoregulation. Cerebral autoregulation is a complex process involving myogenic and metabolic systems, and varies under pathophysiological conditions (274). Any deduction about the effect of one aspect of this process on overall autoregulatory function must be made with circumspection.

7.6 CONCLUSIONS AND FUTURE DIRECTIONS

Induction of propofol anaesthesia in this preparation resulted in a significant decrease in cerebral blood flow. This occurred without a significant reduction in mean arterial pressure.

Isoflurane anaesthesia did not significantly change cerebral blood flow or mean arterial pressure.

Cerebral blood flow was shown to always increase in a linear manner with increases in $PaCO_2$.

Isoflurane and propofol anaesthesia produced more predictable, but significantly different, carbon dioxide reactivity curves when compared to awake sheep, which tended to produce steeper reactivity curves (possibly due to an additional reactive autonomic component to cerebral blood flow). However, there was no significant difference between these anaesthetic agents with respect to the carbon dioxide reactivity of cerebral blood flow.

These results suggest that although metabolic autoregulation under propofol and isoflurane anaesthesia may be different than in awake sheep, there is no demonstrable difference between the two anaesthetics.

The relationship between changes in cerebral blood flow and metabolism (flow-metabolism coupling) and myogenic autoregulation in response to infusions of catecholamines formed the basis of subsequent studies in this thesis.

Aspects of the studies in this chapter were published in the following article:

Myburgh JA, Upton RN, Ludbrook GL, Grant C, Martinez A. (2002). Cerebrovascular carbon dioxide reactivity: effects of propofol or isoflurane anaesthesia. Anaesthesia and Intensive Care 40: 413-422.

Chapter 8. Effects of catecholamines on propofol pharmacokinetics

As outlined at the end of the previous chapter, the next phase was to analyse the effects of infusions of adrenaline, noradrenaline and dopamine on systemic and cerebrovascular haemodynamic and metabolic function under intravenous and inhalational anaesthesia.

Propofol and isoflurane were selected as the intravenous and inhalational anaesthetics, respectively, because of their widespread use in neuroanaesthesia. Also, propofol is increasingly used as a sedative agent in the Intensive Care Unit in neurosurgical and as well as other patients.

In preparation for these studies, an appropriate and validated dose regimen for the anaesthetic agents and catecholamines was necessary.

8.1 PILOT STUDIES OF CATECHOLAMINES AND ANAESTHETICS

8.1.1 Dose selection

8.1.1.1 Catecholamines

The selection of catecholamine doses has been discussed in sections 4.4.3.2.1 and 5.2.

In Chapters 5 and 6, the doses of adrenaline, noradrenaline and dopamine administered to awake sheep (0-60mL/hr) demonstrated equivalent systemic effects on mean arterial pressure and cardiac output.

As these studies were done in the awake animal, the dose responses of the catecholamines under propofol and isoflurane anaesthesia needed to be determined before comparative studies were conducted in a larger cohort of animals.

It was hypothesised that the previously used doses of catecholamines would result in the same systemic haemodynamic responses as to those observed in awake sheep. This would be assessed in a pilot study.

8.1.1.2 Propofol

The selection of propofol doses is discussed in Chapter 7.3.2.1.

A constant infusion of 15 mg/min was used in this study.

8.1.1.3 Isoflurane

The selection of isoflurane doses is discussed in Chapter 7.3.2.2.

An end tidal concentration of 2% isoflurane was used in this study.

8.1.2 Pharmacodynamic pilot studies

A pilot study of the systemic effects of adrenaline, noradrenaline and dopamine on mean arterial pressure and cerebral blood flow was conducted. The aim of this study was to assess the reproducibility of the systemic response to catecholamine infusions and the animals' responses under anaesthesia.

8.1.2.1 Animal preparation and interventions.

The same pilot preparation described in Section 5.4.1 was used.

Prior to anaesthesia, the output of the Doppler probe and mean arterial pressure catheter was recorded. Anaesthesia was induced with 200mg propofol and the animal intubated and ventilated as described in section 4.3.1.

After 1 hour of anaesthesia, ramped infusions of 0-60 mL/hr of adrenaline, noradrenaline and dopamine were administered. One hour elapsed between each catecholamine study.

8.1.2.2 Measurements

Changes in cerebral blood flow were inferred from changes in the outputs from the Doppler probe. Mean arterial pressure was continuously recorded.

8.1.2.3 Results

8.1.2.3.1 Propofol

A total of three studies under propofol anaesthesia were conducted on separate days: two studies in one animal, one in another.

Adrenaline, noradrenaline and dopamine produced significant, equivalent increases in mean arterial pressure. Adrenaline increased mean arterial pressure by 63%, noradrenaline by 57% and dopamine by 61%. This was associated with similar increases in cerebral blood flow.

Two other phenomena were observed as the doses of the catecholamines reached the maximum level (60 mL/hr).

1. In two of the studies, the animal developed dysrhythmias (one with noradrenaline and one with dopamine) as the dose increased. One of these animals (dopamine) developed a profound bradycardia, necessitating cessation of the infusion. In the third study, transient arrhythmias developed as the dose increased from 40 to 60 mL/hr of dopamine, but promptly stopped when the infusion was reduced to 40 mL/hr. As we did not have electrocardiographic monitoring *in situ*, it was not possible to ascertain whether the arrhythmias were supraventricular or ventricular in origin.

2. During the maximum infusion rates (60mL/hr), the animals appeared to emerge from anaesthesia. This was despite nearly two hours of steady-state propofol anaesthesia, during which time the animals appeared deeply anaesthetised. This was observed with all three catecholamines.

8.1.2.3.2 Isoflurane

Two studies were conducted under isoflurane anaesthesia in one animal.

Similar effects on mean arterial pressure and cerebral blood flow were observed to those under propofol anaesthesia.

Increasing the catecholamine infusion rate under isoflurane was not associated with significant dysrhythmias, although transient ectopics at 60mL /hr of adrenaline were observed in one study.

No apparent reversal of isoflurane anaesthesia was evident at the maximal catecholamine infusion rates.

8.1.2.4 Conclusion

The animals did not appear to tolerate higher doses of catecholamines under anaesthesia. This was particularly evident under propofol anaesthesia, manifesting as cardiac irritability. Infusion rates to 40 mL/hr were well tolerated and produced significant, reproducible and equivalent changes in mean arterial pressure. For this reason, it was decided to conduct the subsequent studies using a reduced infusion range to those done in the awake animals.

Secondly, the mechanism by which catecholamines apparently reversed propofol anaesthesia warranted further investigation.

8.2 THE EFFECTS OF CARDIAC OUTPUT ON PROPOFOL PHARMACOKINETICS

The observations in the above pilot studies drew attention to a possible pharmacodynamic interaction between catecholamine-induced increases in cardiac output and the pharmacokinetics of propofol.

Relatively little attention has been given to catecholamine:drug interactions. Catecholamines may influence the pharmacokinetics of other drugs by a number of potential mechanisms. These include altered clearance and volumes of distribution secondary to changes in regional blood flow, metabolic activity, protein binding and changes in pH (280).

Previous studies from our laboratory described an inverse relationship between cardiac output and propofol concentrations after short infusions (218). Using the same animal preparation, the cardiac output dependence of the initial kinetics of propofol were determined in low, medium and high cardiac output states.

A previously validated recirculatory model of propofol disposition in sheep was used to determine the kinetic profile of propofol under these conditions (214,216). The initial arterial concentrations of propofol after intravenous administration were shown to be inversely related to cardiac output. This implies that cardiac output may be a determinant of the induction of anaesthesia with propofol.

On this basis, it was hypothesised that this effect may also occur during longer propofol infusions when cardiac output was altered by catecholamine infusions.

A study analysing the effect of catecholamine induced increases in cardiac output on propofol anaesthesia was conducted.

8.2.1 Aim

Two aims of this study were identified:

1. To document the effect of increasing doses of adrenaline, noradrenaline and dopamine on cardiac output, depth of anaesthesia and the concentrations of propofol in arterial and effluent blood from the brain under steady-state propofol anaesthesia.
2. To provide insight into the mechanisms involved, observed effects were compared to a recirculatory model of propofol disposition in sheep.

8.2.2 Methods

The general methods used in this preparation are presented in detail in Chapter 4.

8.2.2.1 Animal preparation

One to two weeks prior to the study, the animals were instrumented under thiopentone and halothane anaesthesia as described in Chapter 4.3.

For these studies, a "head preparation" using the sagittal sinus Doppler transducer and sagittal sinus catheter was used.

The complete vascular preparation (arterial, venous and pulmonary artery catheters) was used.

8.2.2.2 Interventions

On the day of measurement, the animal was moved to a specific study laboratory.

Anaesthesia was induced with 200mg propofol and endotracheal intubation performed (supine position). Thereafter, the studies were performed in the lateral position.

Propofol was delivered via a syringe driver to maintain a constant infusion rate of 15mg/min.

The animals were mechanically ventilated using a volume control ventilator and 100% oxygen. End tidal carbon dioxide was measured using an end tidal analyser and the ventilator adjusted to maintain an end tidal carbon dioxide concentration of 40 mmHg.

Temperature and hydration were maintained throughout the experiment at baseline levels with passive insulation and intermittent infusions of saline according to central venous pressure, respectively.

No muscle relaxant was used.

After one hour of continuous intravenous anaesthesia, a pseudo-steady-state was assumed based on mathematical modelling of propofol disposition (218,261).

Thereafter, in random order, each animal received three ramped intravenous infusions of noradrenaline, adrenaline or dopamine through the infusion lumen of the pulmonary artery catheter. (Figure 8.1)

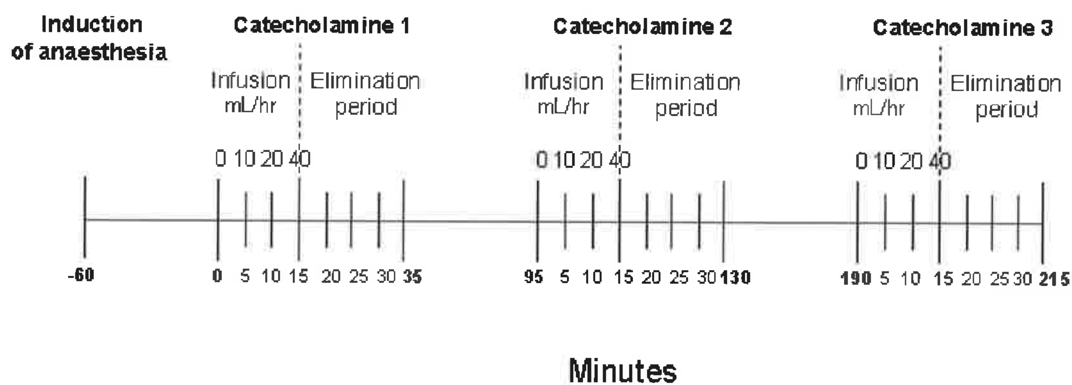


Figure 8.1
Intervention timeline for studies under propofol and isoflurane anaesthesia. Measurements were made at each time interval. Catecholamines were administered in random order in each animal.

8.2.3 Measurements

The following intermittent measurements were made at time intervals shown in Figure 8.1.

8.2.3.1 Cardiac output

Cardiac output was measured by intermittent thermodilution method using injections of iced saline (0-2°C).

8.2.3.2 Blood samples and propofol assays

8.2.3.2.1 Sampling method

Blood samples from the sagittal sinus and femoral arterial catheters were collected through two 3-way stopcocks connected in series to the appropriate catheters.

At the time of sampling, 5 mL of blood was withdrawn into a syringe attached to the stopcock distal to the sheep to account for the catheter and stopcock dead-space.

Before each study, 10 mL of arterial "blank" blood (drug free blood) was taken prior to commencement of the study.

During the studies, blood was withdrawn into a separate 1 mL syringe attached to the proximal stopcock. This was transferred into 1.5 mL microtubes (Micro Test-tubes 3810, Eppendorf, Hamburg, Germany) which contained 25 μl of heparin (1000 IU/mL) for drug assay.

After blood samples were taken, dead space blood and heparin was returned to the animal and the catheter flushed with 5mL of heparinised saline and sealed until the next samples were taken.

At the end of each study, samples were stored at -5°C along with the 10 mL of "blank" blood until thawed for assay.

8.2.3.2.2 Propofol assays

Propofol concentrations in whole blood were assayed using a modification of a previously described high-performance liquid chromatographic and fluorescence detection technique (281).

Samples were frozen to -5°C within 45 minutes of collection, were stored for an average of 72 hours and then thawed at room temperature for assay. Previous studies from our laboratory have confirmed storage stability of samples at this temperature for up to one month following assay (217,242).

In brief, the method of assay was as follows:

For solvent extraction, an internal standard (25 μL of thymol solution), 200 μL of 1M KH_2PO_4 and 200 μL of n-hexane were added to the 1 mL sample. This was vortex-mixed (Thermolyne Maximix, Thermolyne Corp., Iowa, USA) and centrifuged (Model 5412, Eppendorf, Hamburg, Germany).

An aliquot of the supernatant n-hexane layer was transferred to glass vials and the n-hexane evaporated at 40°C in a partial vacuum. The residue containing the propofol and thymol was reconstituted in the acetonitrile-water-acetic acid (45-55-0.5) mobile phase (100 μL). Ten μL samples were then injected into the chromatograph using an autoinjector (Perkin-Elmer IS100, Beaconsfield, Buckinghamshire, England, UK).

For chromatography, a liquid chromatography pump (Perkin-Elmer Series 410) was connected in series with a high-performance liquid chromatography column (Activon Goldpak ODS E18, Sydney, Australia) and fluorescence detector (Perkin-Elmer LS40). An excitation wavelength of 210 nm and emission wavelength of 320 nm was used.

Standard curves were prepared by adding known amounts of propofol to the sheep "blank" blood samples taken prior to drug administration. In all cases linear regression of a five-point standard curve covering the range of drug concentrations encountered in the studies produced a r^2 value of 0.995 or greater. The limit of sensitivity was approximately 0.02 μg/ml.

The mean intra-assay coefficient of variation over the range 1.25 to 10 mg/L was 8.8%

8.2.3.3 Consciousness index

Qualitative, clinically based observations of the depth of anaesthesia were expressed as a consciousness index.

Assessments of the extent of spontaneous limb or trunk movement and the degree of swallowing or gagging on the endotracheal tube were made. These parameters were then assigned a score out of 3, where 0 represented no movement and 3 movement consistent with an awake animal. These two scores were summed to give the consciousness index, where a maximum of 6 corresponded to an awake animal and 0 represented complete anaesthesia.

8.2.4 Comparison with pharmacokinetic model

A previously published recirculatory model of propofol disposition in sheep was used to simulate the experimental conditions (261). This model has been extensively validated against *in vivo* data sets, and differs from conventional compartmental models of propofol kinetics in that it accounts for the effect of both cardiac output and initial vascular mixing on initial drug concentrations. For this experimental study, the propofol dose was set as a 250mg intravenous bolus, and an infusion of 15 mg/min for 210 min.

In one simulation, the time-courses of the resultant arterial and sagittal sinus propofol concentrations were predicted assuming the cardiac output remained at the measured baseline value throughout the study.

A second simulation was identical to the first except that cardiac output was transiently altered to the values measured during the periods of catecholamine infusions. Due to restrictions imposed by the modelling software, these changes were assumed to be step changes that corresponded to the baseline and peak cardiac output changes observed during the catecholamine infusions.

8.2.5 Statistical Analysis

Normal distribution of datapoints before parametric analyses was determined using the using the Kolmogorov-Smirnov test.

Comparison of the effects of catecholamines on all parameters from baseline values were determined using two-way analysis of variance and Bonferroni corrections for multiple time points. Significance was determined by 95% confidence intervals, assuming a t-distribution (273). A p-value of <0.05 was considered to be statistically significant.

Relationships between changes in cardiac output and drug concentration were determined using linear regression analysis.

8.2.6 Results

Five studies were performed in four sheep.

Adrenaline ($p<0.001$), noradrenaline ($p<0.01$) and dopamine ($p<0.001$) increased cardiac output from baseline in a dose-dependent fashion, returning to baseline values within 15 minutes. (Figure 8.2)

Baseline mean arterial propofol concentrations were not statistically different prior to each catecholamine infusion (range 5.1–5.8 μg/mL). Consciousness indexes were 0 for all cohorts prior the commencement of infusions.

The three catecholamines produced significant reductions in mean arterial propofol concentrations (Figure 8.3). Adrenaline reduced arterial propofol concentrations to 41.8% of baseline, ($p<0.01$), noradrenaline to 63% ($p<0.05$) and dopamine to 52.9% ($p<0.01$). There was no statistically significant difference between the catecholamines in this respect.

There were similar reductions in mean sagittal sinus propofol concentrations from a baseline concentration range of 3.7-4.6 μg/mL. Adrenaline reduced propofol concentrations to 60% ($p<0.05$) of baseline, noradrenaline to 70% ($p<0.05$), and dopamine to 48% (4 to 123 CI); the latter did not achieve statistical significance ($p>0.05$) (Figure 8.4).

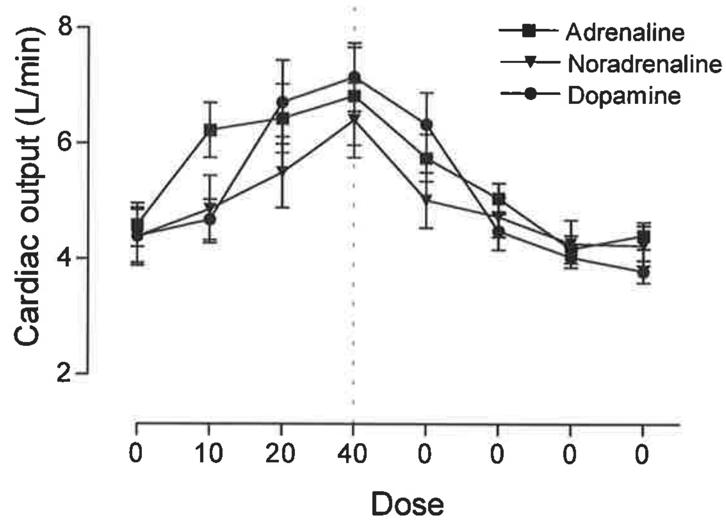


Figure 8.2
Effects of adrenaline, noradrenaline and dopamine on cardiac output (L/min). Dose is expressed as mL/hr. Data are expressed as mean±sem.

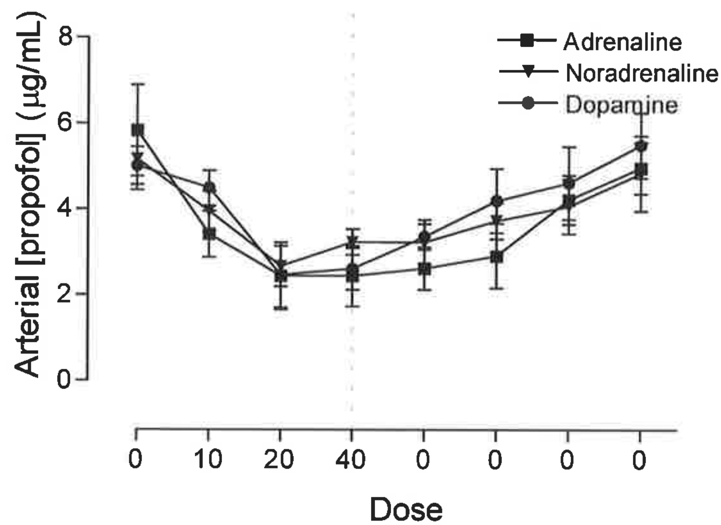


Figure 8.3
Effects of adrenaline, noradrenaline and dopamine on mean arterial propofol concentrations (μg/mL). Dose is expressed as mL/hr. Data are expressed as mean±sem.

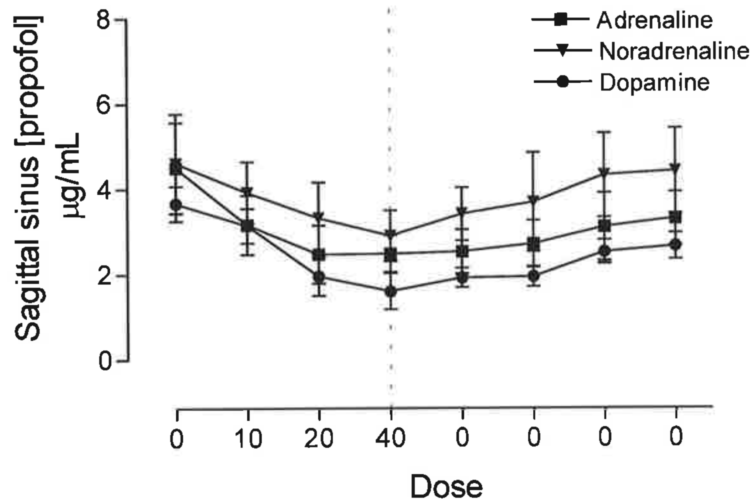


Figure 8.4
Effects of adrenaline, noradrenaline and dopamine on mean sagittal sinus propofol concentrations (µg/mL). Dose is expressed as mL/hr. Data are expressed as mean±sem.

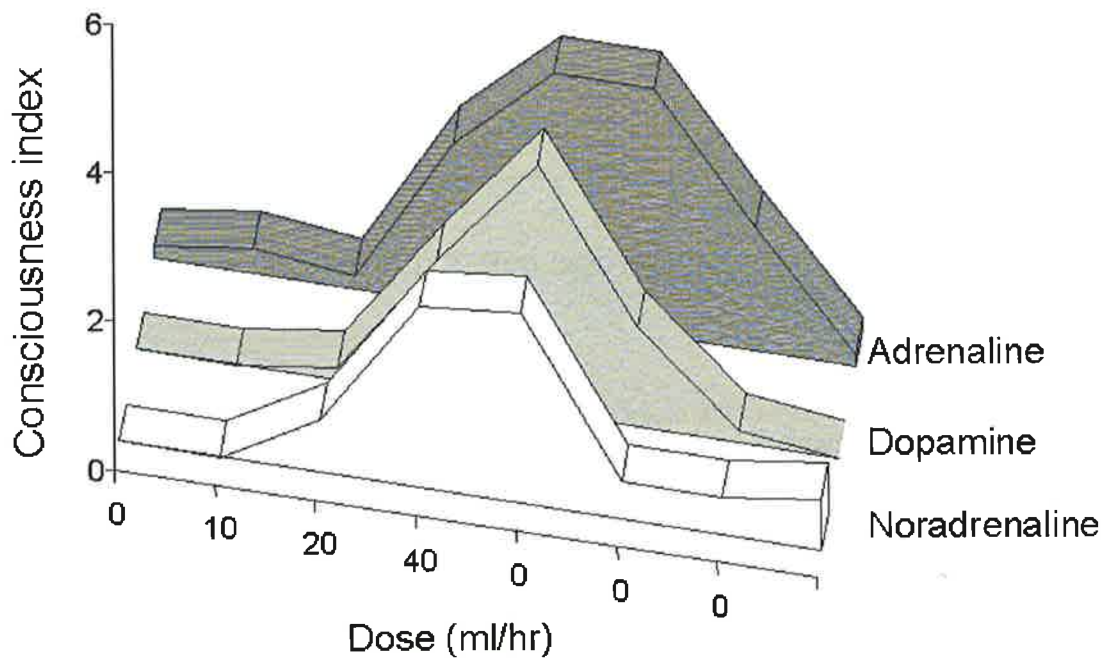


Figure 8.5
Schematic diagram demonstrating levels of consciousness during infusions of adrenaline, noradrenaline and dopamine during continuous propofol anaesthesia (15 mg/min). The scoring system is described in the text: the minimum value is 0, and is consistent with deep anaesthesia and no spontaneous movement; maximum value is 6 and consistent with an awake animal. The x-axis represents the dose of drug in 5 minute intervals in mL/hr.

The lowest blood concentrations were associated with emergence from anaesthesia, measured with the observational score (Figure 8.5). The pattern of emergence from anaesthesia was similar for all three catecholamines, beginning during the mid-range of the infusions (20mL/hr) and reaching a maximum in the 5 minute period after cessation of infusion. Anaesthesia was re-established after returning to baseline levels within 20 minutes after cessation.

As the expected relationship between cardiac output and arterial propofol concentration is an inverse one (218), linear regression analysis between the two parameters was determined by using the reciprocal of arterial propofol concentration yielding a r^2 value of 0.74 (Figure 8.6).

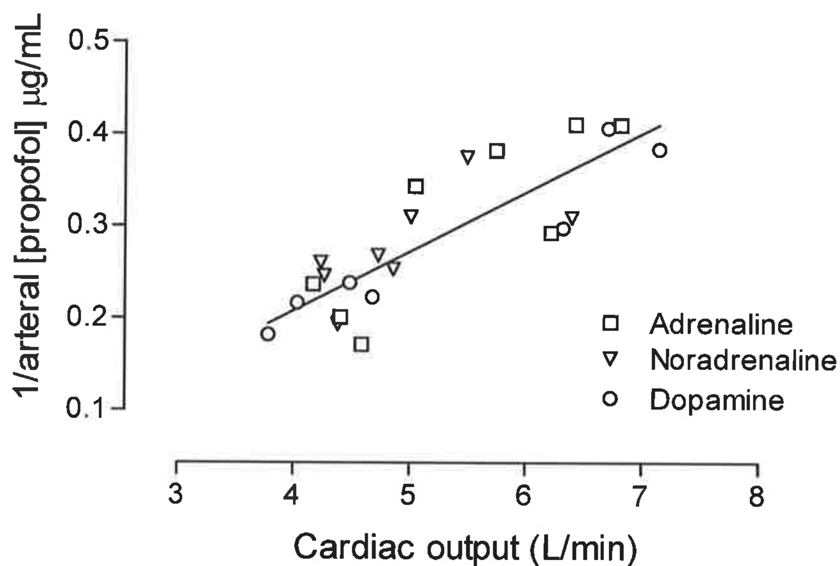


Figure 8.6

Linear regression analysis of the reciprocal of arterial propofol concentration (μg/mL) against the simultaneously measured cardiac output (L/min). Data points are the mean of 5 sheep, and represent measurements made during and after the catecholamine infusions.

The simulations using recirculatory pharmacokinetic model of propofol disposition are shown in Figure 8.7.

The predictions of the model where based on an induction dose of 250 mg propofol followed by a 15 mg/min infusion with a constant cardiac output of 4.4 L/min. Both the arterial and sagittal sinus concentrations rapidly reached steady-state.

Thereafter, the time-course of cardiac output changes induced by the catecholamines were entered into the model. The concentration changes observed were broadly consistent with the predictions of the model when cardiac output was changed. The predicted baseline concentrations were

slightly less than those observed, presumably due to some physiological difference between the present sheep and those used to define the model. However, the predicted arterial and sagittal sinus concentrations showed reductions during the catecholamine infusions that was within the 95% confidence intervals of the observed data.

It can be concluded that the observed concentration changes are consistent with an explanation based on kinetic changes secondary to the altered cardiac output produced by the catecholamines.

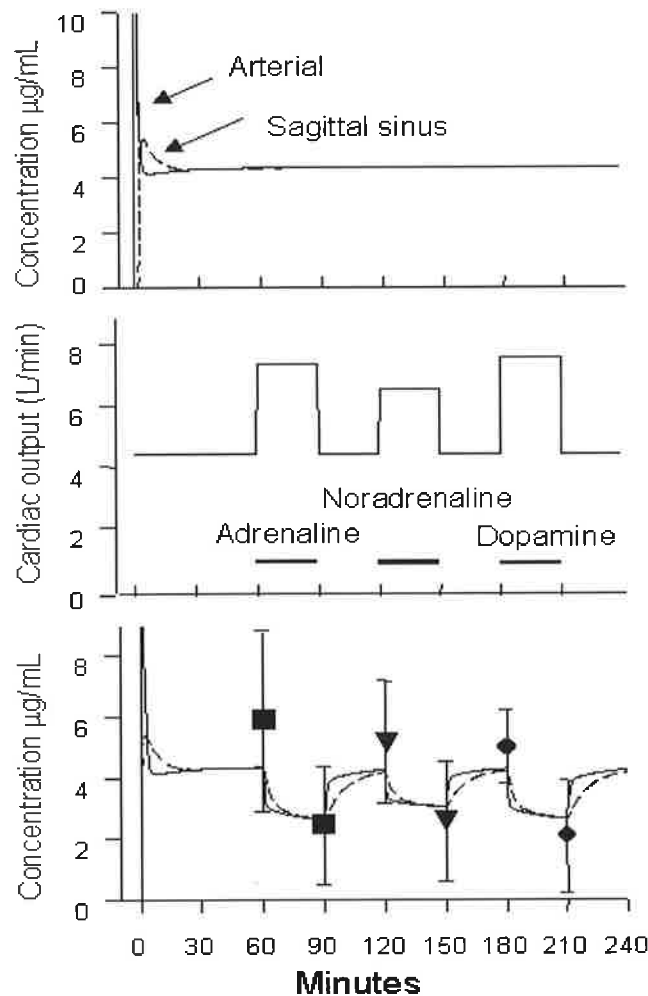


Figure 8.7
An investigation of mechanisms underlying the observed concentration changes using a recirculatory pharmacokinetic model of propofol disposition in sheep. Upper panel: The predictions of the model based on an induction dose of 250 mg propofol followed by a 15 mg/min infusion with a constant cardiac output of 4.4 L/min. Middle panel: The time-course of cardiac output changes induced by adrenaline, noradrenaline and dopamine which were entered into the model. Lower panel: The predicted time-course of the arterial (solid line) and sagittal sinus (dashed line) concentrations when cardiac output was altered. The observed baseline and trough values for each adrenaline (squares), noradrenaline (triangles) and dopamine (circles) are shown (expressed mean ± 95% confidence intervals).

8.2.7 Discussion

This study analysed the effects of exogenous catecholamine infusions on the arterial and brain effluent concentrations of propofol during continuous propofol anaesthesia.

Infusions of adrenaline, noradrenaline and dopamine produced equivalent, dose-dependent increases in cardiac output. The data supported the hypothesis that the increased cardiac output produced by catecholamines altered the pharmacokinetics of propofol. This phenomenon is not catecholamine-induced tachyphyllaxis, as the emergence from anaesthesia was associated with significantly lower blood concentrations of propofol in arterial blood and blood emerging from the brain. Although there have been few kinetic studies in which cardiac output has been changed experimentally, a study in which nitroglycerine was infused in rats has reported data generally consistent with the present data (282).

The mechanisms underlying this phenomenon are complex and require a reappraisal of the assumptions underlying traditional compartmental models of drug disposition (283). These two- or three-compartment models are generally defined in terms of abstract rate constants rather than anatomically identifiable blood flows or organ volumes. In particular, drug is added to a central distribution volume rather than a circulating flow of blood, which prevents such models accounting for cardiac output in a physiologically realistic manner. Consequently, a significant deficiency of these models, particularly with their application to critically ill patients, is their inability to predict the kinetic outcome of altered cardiac output and other circulatory changes.

It may be proposed that drug clearances in such models are proportional to cardiac output, in which case a change in cardiac output would require approximately five terminal half-lives before a new steady-state is achieved. However, this is not consistent with the rapid change in blood concentrations with altered cardiac output shown in Figures 8.2 and 8.3. By a similar argument, an effect of catecholamines on the binding or distribution volumes of propofol would not account for the observed rapid changes in propofol blood concentrations. Furthermore, similar changes have been observed when cardiac output is changed by other means (218).

These deficiencies are addressed in physiological models that have an underlying recirculatory structure. Such models, in their simplest form, divide the body into pulmonary and systemic sub-systems through which blood flows in a recirculatory manner at a rate given by the cardiac output. They often predict a marked dependence of pharmacokinetics on cardiac output (214,284-286). Two mechanisms are thought to be involved (218).

Firstly, there is a direct indicator dilution effect between the venous drug injection site and arterial blood (i.e. across the pulmonary sub-system). This is analogous to the indicator principle used in thermodilution cardiac output measurement - a fixed dose added to a higher cardiac output will result in less drug or indicator per unit blood volume, and therefore lower concentrations. The contribution of the first pass dilution effect (e.g. dose rate over cardiac output) to the total steady-state arterial drug concentration (e.g. dose rate over clearance) will simply be the drug clearance over the cardiac output. This effect is therefore most significant for drugs with a high clearance (283). For propofol, if the cardiac output is 5 L/min, and the clearance is 2 L/min, the contribution of the first pass concentrations to the total steady-state arterial concentration is approximately 20%.

Secondly, higher cardiac outputs imply higher blood flows to the organs of drug elimination and distribution in the systemic sub-system, which for some drugs increases the rate of their clearance and distribution resulting in lower recirculated concentrations. Taken together, both mechanisms imply higher cardiac outputs are associated with lower arterial concentrations after both intravenous bolus and infusion administration regimens. Both mechanisms are inherent in the model used for simulations in the present study.

Upton *et al* described an inverse relationship between cardiac output and the peak arterial concentrations of propofol after two-minute intravenous infusions in this sheep preparation (218). This was attributed principally to the first mechanism, and was considered of most importance after intravenous bolus injection of propofol, as used for the induction of anaesthesia. The present data and the model simulations imply that changes in cardiac output could alter the concentrations of propofol during a constant rate infusion by both the first and second mechanisms acting in concert. The general agreement between the model and the data in Figure 8.6 suggests that the contribution of other mechanisms to the altered kinetics of propofol (e.g. catecholamine induced increases in hepatic and pulmonary extraction of propofol) are likely to be minor, although these were not examined in this study

An important issue is the extent to which this phenomenon could be expected to occur in man. A feature of propofol disposition in sheep is nonlinear metabolism in the lung (214). It is possible that this nonlinearity contributes to the observed reduction in arterial concentration, as lung clearance will increase further as the arterial concentrations become lower. To examine this issue, the model was used to simulate the expected steady-state arterial propofol concentrations for an infusion rate of 15 mg/min when the cardiac output was 4, 6 and 8 L/min respectively. In the presence of nonlinear lung metabolism of propofol, the concentrations were 4.7, 3.2 and 2.4 μg/mL, respectively. If no lung metabolism is assumed, these concentrations were

6.8, 5.5 and 4.9 μg/mL, respectively. The total reduction in concentration was therefore 50% with lung metabolism and 28% without - thus approximately half of the observed cardiac output dependence can be attributed to the known lung metabolism of propofol in sheep.

With respect to man, there is expanding information regarding linearity and extent of propofol clearance in the lung. It is known that propofol clearance apparently exceeds liver blood flow (211), and metabolism of propofol can occur in the absence of a liver (212). There is some evidence for nonlinearity as shown by nonlinear increases in blood concentration with higher infusion rates (213) and progressive decreases in clearance with higher doses (287). Dawidowicz has described the pulmonary elimination of propofol where the drug is transformed into 2,6-diisopropyl-1,4-quinol by oxidative metabolism (288).

8.3 CONCLUSIONS AND FUTURE DIRECTIONS

The studies in this chapter highlight two important pharmacokinetic and pharmacodynamic interactions.

Induced hyperdynamic circulatory states by exogenous infusions of adrenaline, noradrenaline and dopamine increased propofol requirements for anaesthesia.. This was associated with a dose-dependent "reversal" of anaesthesia. A previously developed pharmacokinetic modelling program identified that this phenomenon could be attributed to reductions of arterial and brain effluent propofol concentrations consistent with recirculatory kinetics. A similar mechanism in man is likely.

Secondly, increasing doses of propofol were associated with haemodynamic instability in sheep, primarily presenting as cardiac irritability and bradycardia. For this reason, these studies comparing the effects of ramped infusions of catecholamines under propofol and isoflurane anaesthesia used a lower maximal dose of 40 mL/hr.

These phenomena have important clinical implications. Propofol is increasingly being used as a sedative agent in critically ill patients, many of whom receive catecholamine infusions to defend systemic blood pressure. There are anecdotal reports of increasing tolerance to propofol in such patients, which has been attributed to propofol tachyphyllaxis. Whilst a degree of tachyphyllaxis may occur with prolonged administration, these studies suggest that catecholamine induced changes in propofol kinetics may be a significant contributing factor.

A series of case reports have highlighted sudden cardiac arrest and death in head-injured patients receiving prolonged infusions of moderate to high doses of propofol and catecholamines, specifically noradrenaline (232). This was attributed to progressive propofol induced myocardial depression and

catecholamine induced adrenergic receptor down regulation. This is a plausible explanation, but other pharmacokinetic factors such as those identified in this study may provide insights into this important clinical issue. This was highlighted in a letter in response to the cases described (289).

Given the increasing use of propofol in critically ill patients with pathological or catecholamine induced hyperdynamic circulations, this phenomenon requires investigation in man with some urgency.

Aspects of the studies in this chapter were published in the following articles:

1. Myburgh JA, Upton RN, Grant C, Martinez A. (2001) Epinephrine, norepinephrine and dopamine infusions increase propofol clearance during continuous propofol anaesthesia in an ovine model. Intensive Care Medicine 27: 276-282
2. Myburgh JA, Upton RN. (2001) Propofol use head injured patients. Lancet 357: 1708-1710.

Chapter 9. Systemic and cerebrovascular effects of catecholamines under anaesthesia

This chapter will describe the effects of ramped infusions of adrenaline, noradrenaline and dopamine on systemic and cerebral haemodynamic and metabolic function under steady-state propofol and isoflurane anaesthesia.

Essentially, the studies in this chapter are a repeat of the studies conducted in Chapter 6, which described the effects of the catecholamines on systemic and cerebrovascular function in awake sheep.

The effects under intravenous and volatile anaesthesia may be different due to the potential effects that these anaesthetic agents have on cerebral blood flow, metabolism and autoregulation. As outlined at the end of the previous chapter, potential catecholamine:anaesthetic interactions may have important clinical implications, especially with propofol.

9.1 Introduction

The direct effect of catecholamines on the cerebral circulation under physiological and pathophysiological conditions remains contentious, due to variability of experimental models and methods of measurement of cerebrovascular mechanics.

Within physiological autoregulatory limits, catecholamines do not cross the intact blood-brain barrier, thereby limiting their direct cerebrovascular effects. However, this effect may be altered by changes in blood-brain barrier permeability or by systemic physiological perturbations (e.g. systemic hypertension) (207).

In Chapter 7, the effects of propofol and isoflurane on cerebral blood flow and carbon dioxide reactivity in this animal preparation were described. Propofol anaesthesia was characterised by a substantial, statistically significant decrease in cerebral blood flow (55% of baseline, $p=0.001$); isoflurane did not change cerebral blood flow. Neither of these changes in cerebral blood flow occurred in the presence of changes in systemic blood pressure.

Isoflurane and propofol anaesthesia produced equivalent, significantly linear carbon dioxide reactivity lines that were significantly different to those in awake sheep. However, no significant difference in carbon dioxide reactivity between propofol and isoflurane was demonstrated. This suggests that, in part, metabolic autoregulation may be altered under anaesthesia, albeit to an equivalent extent for the two agents studied.

These data were used as a basis for an altered cerebrovascular physiology model. The degree of cerebrovascular perturbation would be reproducible in this preparation and allow comparative studies of the catecholamines under these conditions.

Propofol and/or isoflurane may affect the physiological function of the blood-brain barrier due to alterations of blood-brain barrier permeability (120,197). Other studies have demonstrated that these anaesthetic agents may alter metabolic and/or pressure (myogenic) autoregulation (122).

Consequently, it was postulated that the interaction of exogenous catecholamines with propofol or isoflurane anaesthesia may result in direct effects on the cerebral vasculature.

9.2 Aim

The aim of these studies was to determine the effects of adrenaline, noradrenaline and dopamine on cerebral blood flow, intracranial pressure, cerebrovascular resistance and cerebral oxygen consumption under continuous propofol and isoflurane anaesthesia.

The effects under the two anaesthetised states were compared with each other and with the data for awake sheep described in Chapter 6.

9.3 Method

The general methods used in this preparation are presented in detail in Chapter 4.

Studies were performed in two cohorts of instrumented adult Merino ewes: anaesthetised with steady-state propofol or isoflurane.

9.3.1 Animal preparation

One to two weeks prior to the study, the animals were instrumented under anaesthesia as described in Chapter 4.3.

The complete "head" (Doppler transducer, sagittal sinus catheter and intracranial pressure monitor) and vascular (arterial, venous and pulmonary artery catheters) preparations were used.

9.3.2 Interventions

On the day of measurement, the animal was moved to the study laboratory.

Intubation, ventilation and maintenance of anaesthesia proceeded as described in sections 4.3.1 and 8.4.2.

Anaesthesia was induced with 200mg propofol for the propofol studies and 1000mg thiopentone for the isoflurane studies.

For the isoflurane cohort, maintenance of anaesthesia was with isoflurane delivered via a vaporiser in the anaesthetic circuit to maintain an end tidal concentration of 2%.

For the propofol cohort, propofol was delivered via a syringe driver to maintain a constant infusion rate of 15mg/min.

After one hour of anaesthesia, a pseudo-steady-state was assumed to be established based on previous studies of mathematical modelling of propofol disposition (218,261). Similarly, stability of end tidal isoflurane concentrations at 2% assumed steady-state anaesthesia.

The intervention timelines for these studies are shown in Figure 8.1. In random order, each animal received three ramped intravenous infusions of noradrenaline, adrenaline or dopamine through the infusion lumen of the pulmonary artery catheter over a dose range of 0-40 mL/hr.

One hour was allowed to elapse between completion of each study to ensure clearance of each catecholamine and restoration of baseline values.

9.3.3 Measurements

The methods of measurement are outlined in detail in section 4.4.2.

Mean arterial pressure was measured continuously. Cardiac output and right atrial pressure were measured at 5 minute intervals. At the same time, blood samples were taken from the arterial and pulmonary artery catheter for measurements of arterial and mixed venous blood gases.

Cerebral blood flow and intracranial pressure were continuously measured. Intermittent blood samples were taken from the sagittal sinus catheter for measurement of cerebral venous blood gas analysis.

Systemic and cerebrovascular vascular resistances, systemic and cerebral oxygen consumption and delivery were calculated according to the calculations outlined in section 4.4.2.7.

9.3.4 Statistical analysis

Normal distribution of datapoints before parametric analyses was determined using the Kolmogorov-Smirnov test.

Comparison of the effects of catecholamines on all parameters with baseline values were determined using two-way analysis of variance and Bonferroni corrections for multiple time points. Significance was determined by 95% confidence intervals, assuming a t-distribution. A p-value of <0.05 was considered to be statistically significant.

9.4 Results

Studies were conducted in 6 animals for each catecholamine in the propofol and isoflurane cohorts. The data for the awake cohort (described in Chapter 6) are included here to facilitate comparisons so that a complete statistical analysis is possible.

9.4.1 Systemic haemodynamic effects

All three catecholamines significantly increased mean arterial pressure from baseline in a dose-dependent manner in the three cohorts ($p<0.001$).

The maximal effects on mean arterial pressure by the catecholamines were significantly greater than under isoflurane anaesthesia than in awake sheep (adrenaline $p<0.01$; noradrenaline $p<0.001$;dopamine $p<0.05$). There were no significant differences in this respect between sheep under propofol anaesthesia and awake sheep; and between sheep under propofol and isoflurane anaesthesia. (Figure 9.1).

The effects on cardiac output are shown in Figure 9.2.

All three catecholamines significantly increased mean arterial pressure from baseline in a dose-dependent manner in the three cohorts ($p<0.001$), with the exception of noradrenaline in the awake cohort.

Cardiac outputs were significantly higher in the awake cohort compared to propofol and isoflurane cohorts. The difference between awake sheep and those anaesthetised with propofol was statistically significant for adrenaline and dopamine ($p<0.05$). There was no difference between the maximal cardiac outputs produced under propofol and isoflurane anaesthesia.

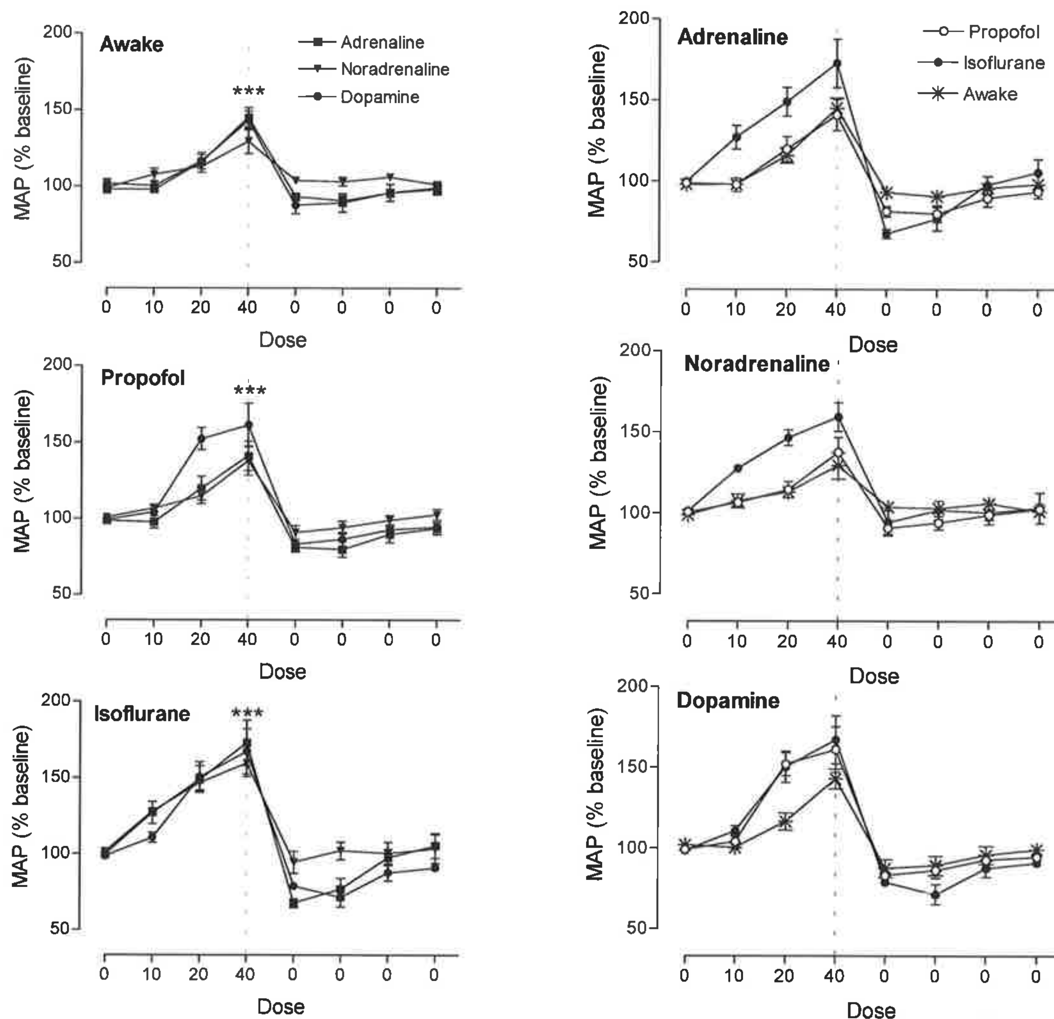


Figure 9.1

*The effects of adrenaline, noradrenaline and dopamine on mean arterial pressure (mmHg) in three cohorts: awake sheep, and those under steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort; right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr. ***=p<0.001. Data are expressed as mean±sem.*

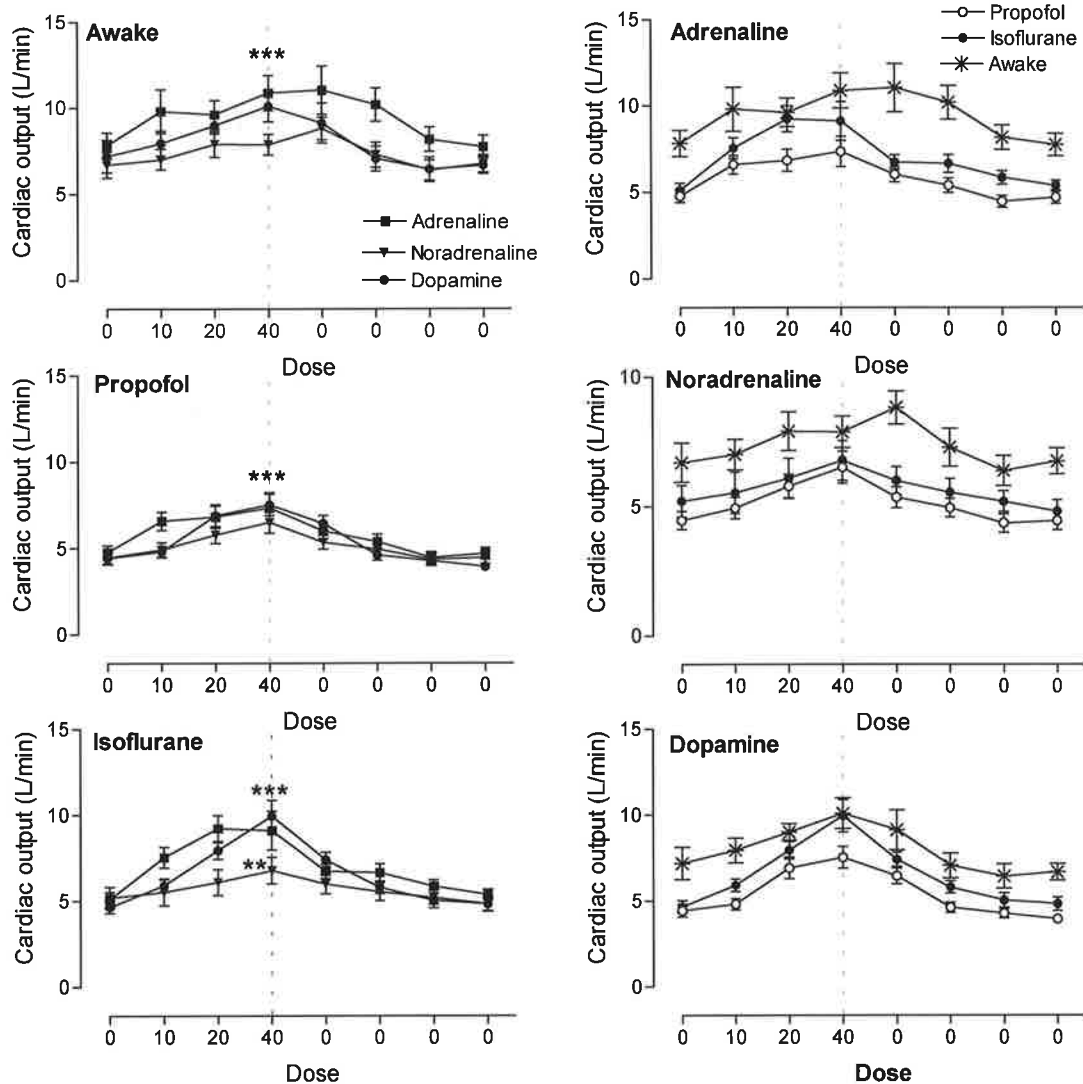


Figure 9.2
*The effects of adrenaline, noradrenaline and dopamine on cardiac output (L/min) in three cohorts: awake sheep, and those under steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort; right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr. **=p<0.01; ***=p<0.001. Data are expressed as mean±sem.*

No significant changes in calculated systemic vascular resistance from baseline were demonstrated with the three catecholamines in each cohort. Similarly, there were no significant differences in systemic vascular resistance changes between the awake, propofol and isoflurane cohorts. (Figure 9.3).

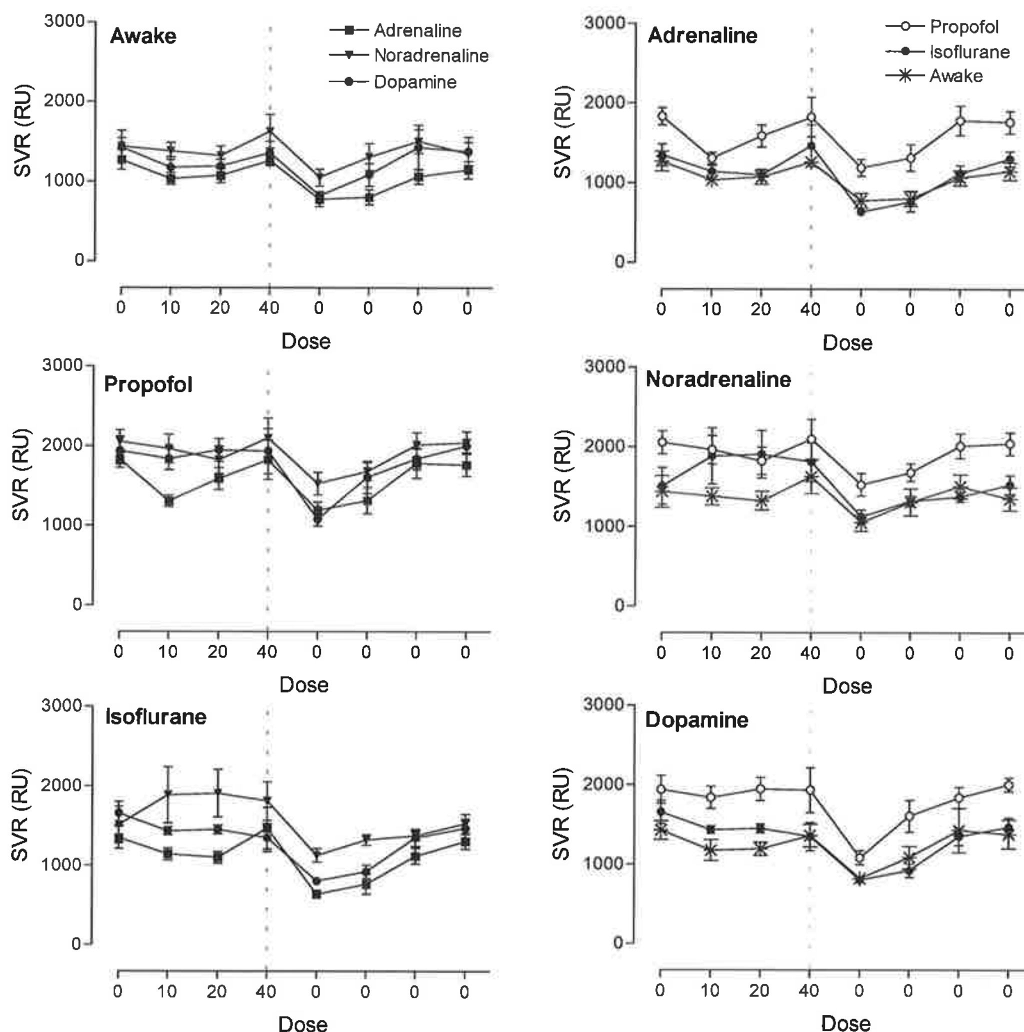


Figure 9.3
The effects of adrenaline, noradrenaline and dopamine on systemic vascular resistance (resistance units) in three cohorts: awake sheep, and those under steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort; right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr. Data are expressed as mean±sem.

9.4.2 Systemic metabolic effects

The effects on systemic oxygen consumption are shown in Figure 9.4. Under propofol and isoflurane anaesthesia, dopamine produced a significant increase in mean oxygen consumption at peak infusion rates($p<0.001$). In the awake sheep, only adrenaline produced a significant increase in oxygen consumption ($p<0.01$).

However, anaesthesia produced a significant reduction in systemic oxygen consumption compared to that in awake sheep. Propofol reduced baseline oxygen consumption compared to awake sheep before the commencement of the infusions ($p<0.05$) and this remained significantly lower than in awake sheep at the maximum infusion rate (adrenaline $p<0.001$, noradrenaline and

dopamine $p<0.05$). The findings were similar under Isoflurane anaesthesia for baseline and maximum infusion rates (adrenaline $p<0.001$, noradrenaline $p<0.01$, dopamine $p<0.05$).

There was no significant difference between baseline and catecholamine induced oxygen consumption between the propofol and isoflurane cohorts.

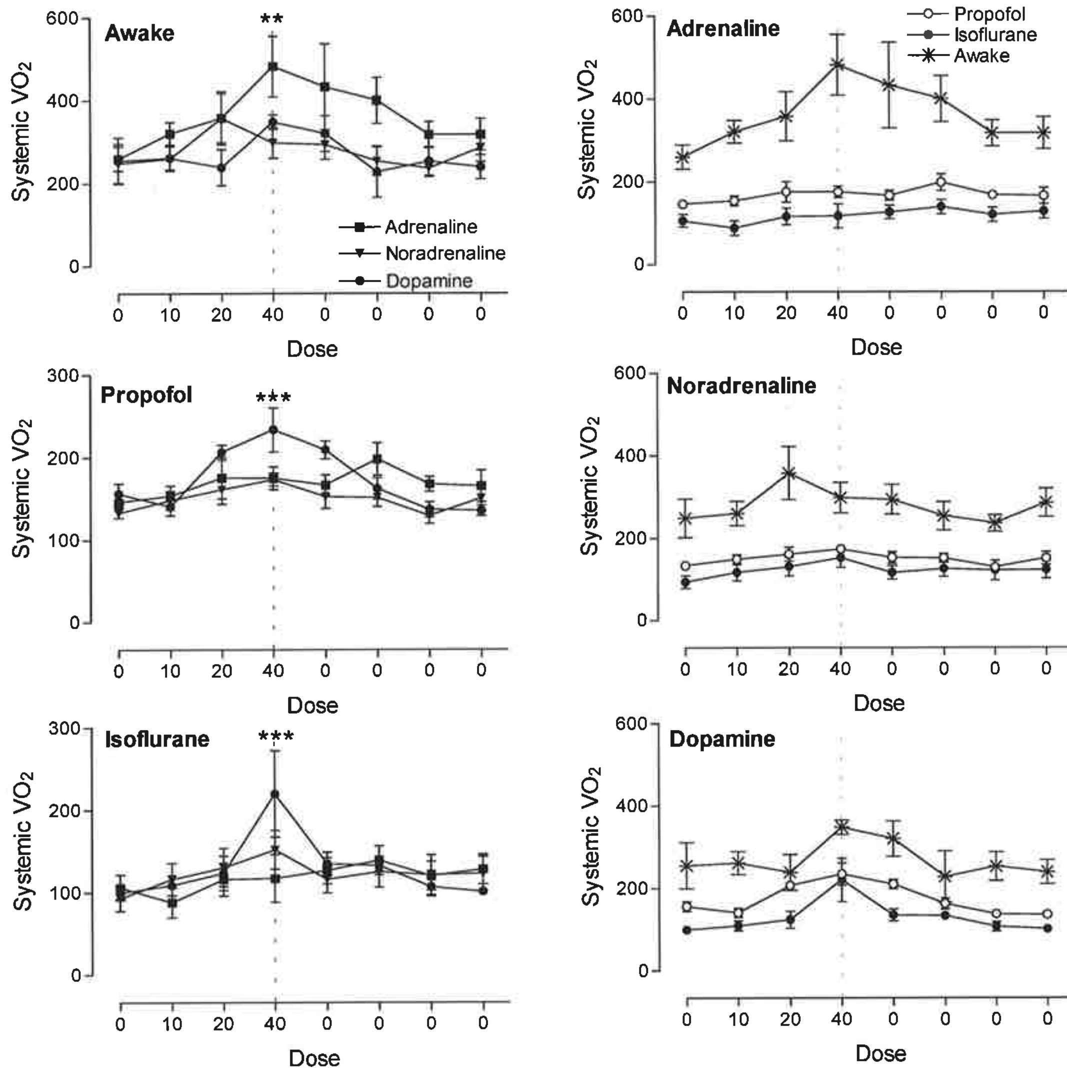


Figure 9.4
*The effects of adrenaline, noradrenaline and dopamine on systemic oxygen consumption in three cohorts: awake sheep and those under steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort (Note: different Y axis scale for anaesthetised cohorts); right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr. **=p<0.01;***=p>0.001. Data are expressed as mean ± sem.*

As described in Chapter 6.3.2, in awake sheep, dopamine produced a significant increase in arterial and venous $PaCO_2$ with an associated reduction in pH (Figure 6.7). The same phenomenon occurred using the

lower catecholamine infusion regimen. However, under anaesthesia where carbon dioxide was controlled with mechanical ventilation, pH did not significantly change from baseline with any of the catecholamines in the three cohorts.

Figure 9.5 shows the comparisons of the effects of the three catecholamines on arterial pH and $PaCO_2$ in awake sheep and under anaesthesia. There were no significant differences in arterial pH between the three cohorts, apart with dopamine in the awake group. Under controlled mechanical ventilation, the respiratory acidosis associated with dopamine was prevented.

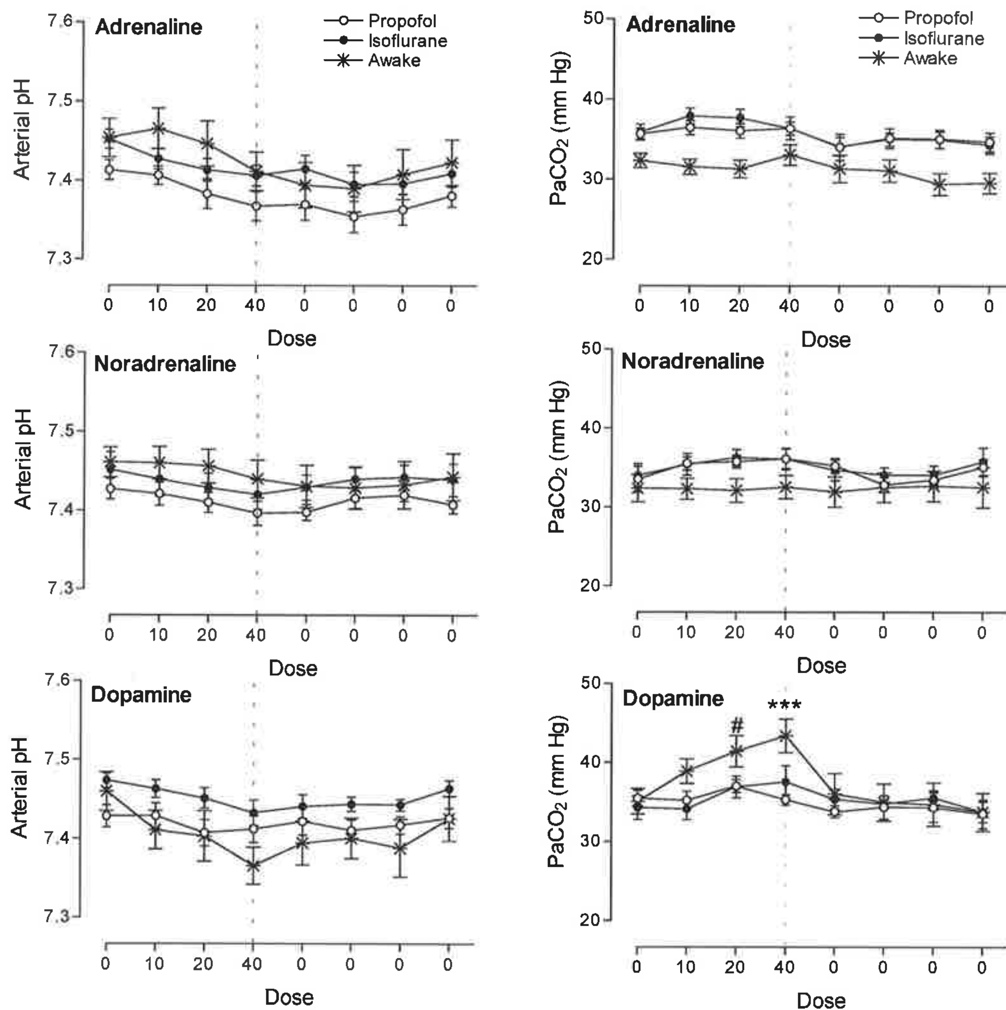


Figure 9.5

*The effects of adrenaline, noradrenaline and dopamine on arterial pH (left hand column) and $PaCO_2$ (right hand column) in awake sheep and those under steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Doses are expressed as mL/hr. #=p<0.05, ***=p>0.001 Data are expressed as mean ± sem.*

Similar effects were observed on mixed venous pH and carbon dioxide tensions, and are shown in Figure 9.6.

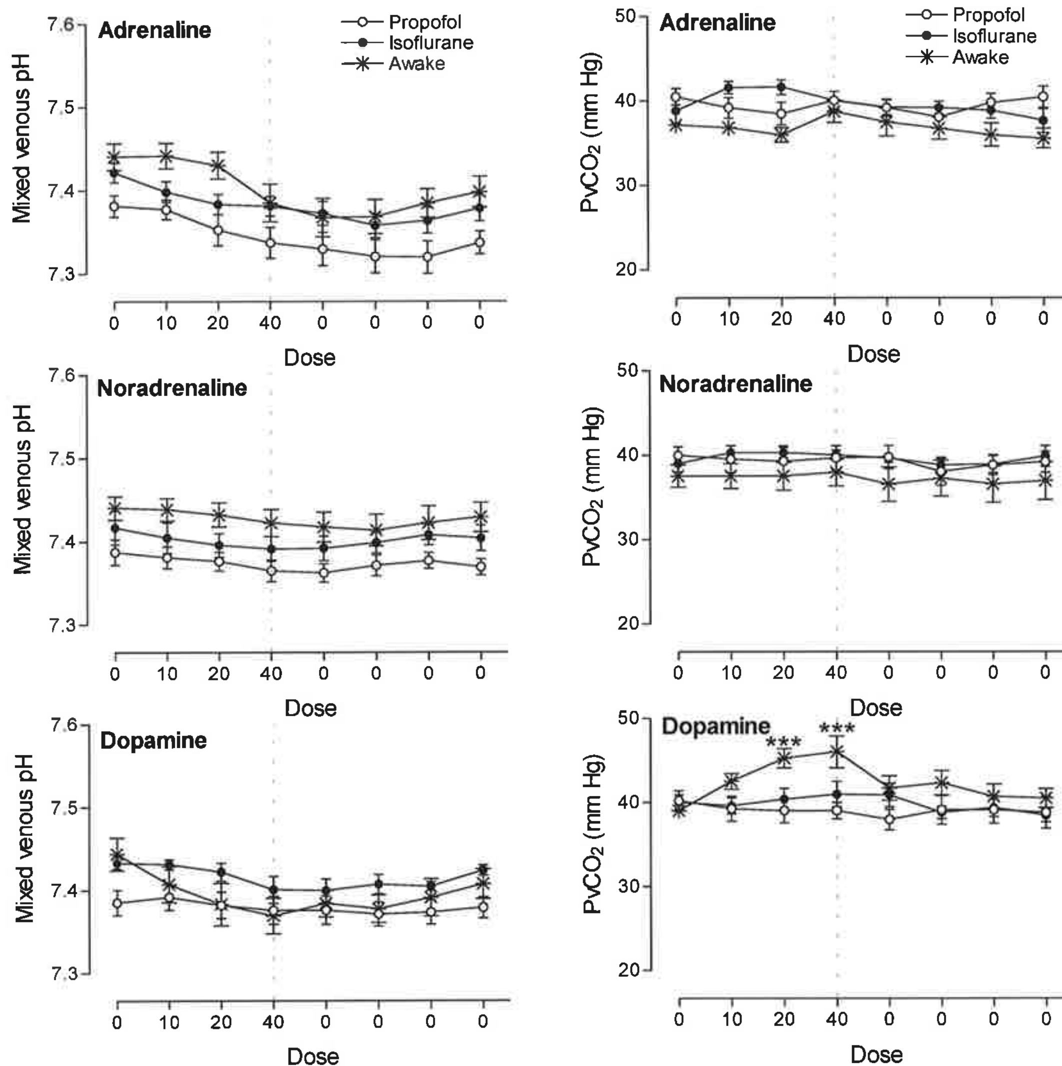


Figure 9.6
*The effects of adrenaline, noradrenaline and dopamine on mixed venous pH (left-hand column) and carbon dioxide tension ($PvCO_2$ mmHg) (right hand column) in awake sheep, and those steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Doses are expressed as mL/hr. #=p<0.05, ***=p>0.001 Data are expressed as mean ± sem*

9.4.3 Cerebrovascular haemodynamic effects

The effects of catecholamines on cerebral blood flow under anaesthesia were significantly different to those in awake sheep (Figure 9.7).

In the awake cohort, cerebral blood flow was significantly increased by dopamine ($p<0.01$), but not by adrenaline or noradrenaline ($p>0.05$).

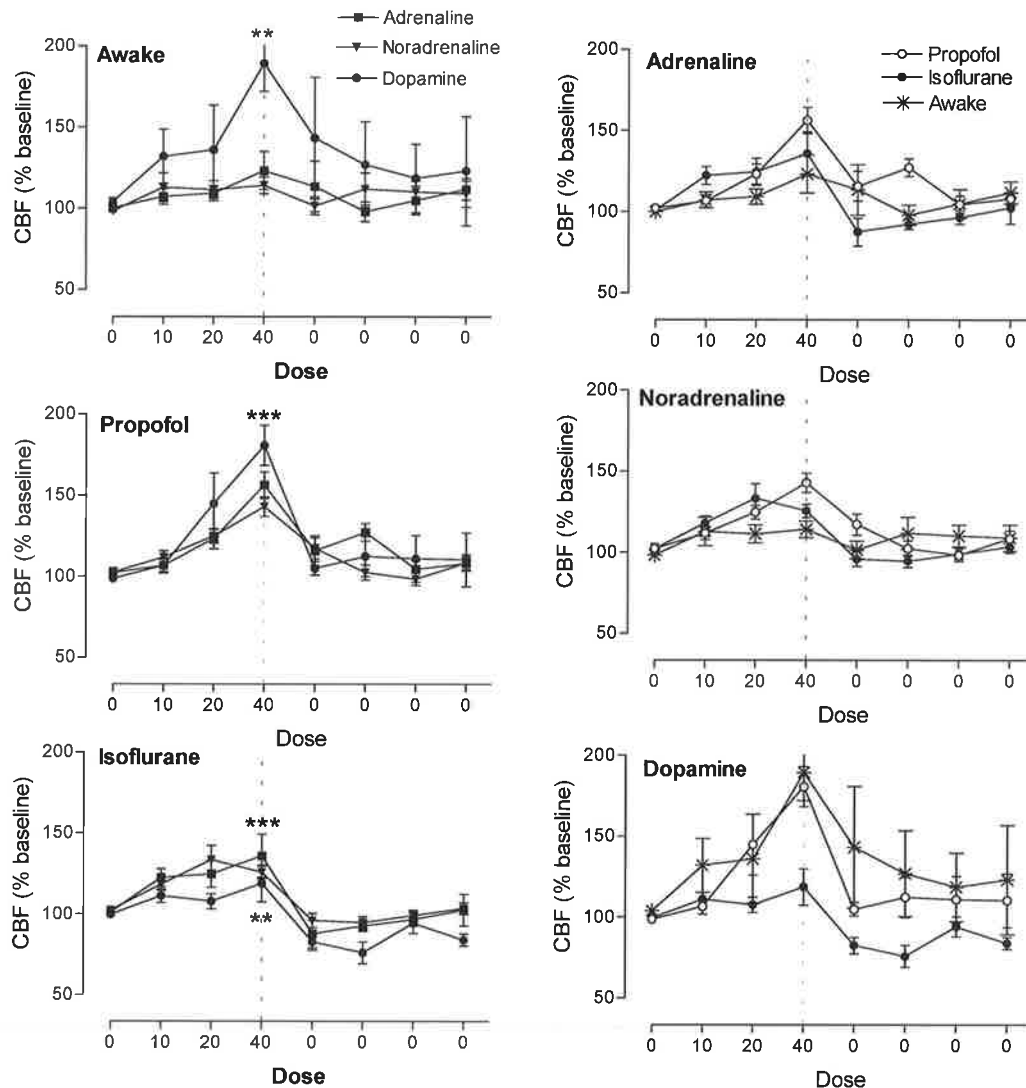


Figure 9.7
*The effects of adrenaline, noradrenaline and dopamine on cerebral blood flow (% baseline) in three cohorts: awake sheep, and those under, steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort; right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr **=p<0.01; ***=p<0.001. Data are expressed as mean±sem*

Under propofol anaesthesia, adrenaline, noradrenaline and dopamine produced statistically significant, equivalent increases in cerebral blood flow from baseline (p<0.001 for each drug).

Under isoflurane anaesthesia, adrenaline, noradrenaline (p<0.001) and dopamine (p<0.01) significantly increased cerebral blood flow from baseline. There was a significant difference between the peak effects of dopamine and noradrenaline (p<0.01).

For the inter-cohort comparisons, the effects of dopamine on cerebral blood flow were significantly greater under propofol anaesthesia and in awake sheep than under isoflurane anaesthesia (p<0.001).

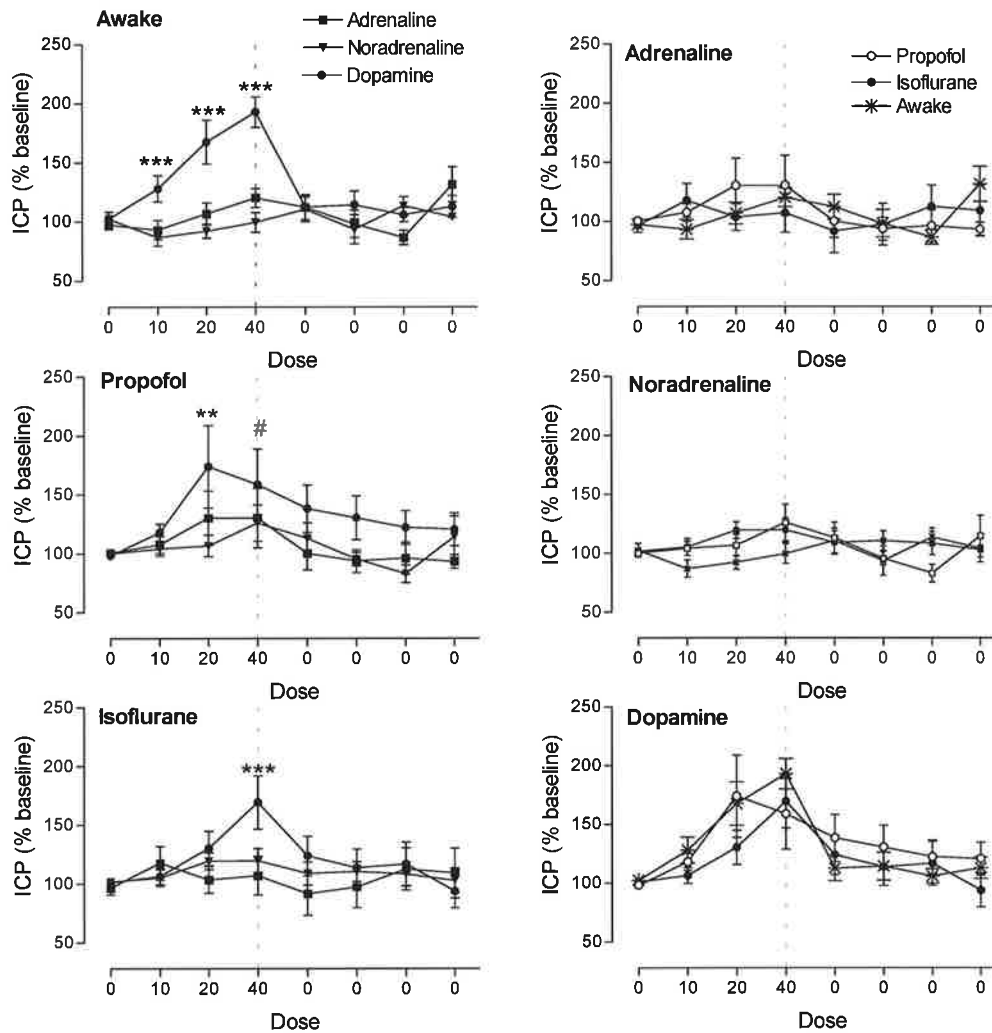


Figure 9.8
*The effects of adrenaline, noradrenaline and dopamine on intracranial pressure (% baseline (L/min) in three cohorts: awake sheep, and those under steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort; right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr #=p<0.05:**=p<0.01; ***=p<0.001. Data are expressed as mean±sem*

The effects on intracranial pressure are shown in Figures 9.8. In all cohorts, dopamine significantly increased intracranial pressure from baseline in a dose-dependent manner (in awake sheep p<0.001; under propofol p<0.05 and isoflurane anaesthesia p<0.001), whilst adrenaline and noradrenaline did not (p>0.05).

Dopamine increased intracranial pressure significantly more than adrenaline and noradrenaline in the awake cohort ($p<0.001$); as did noradrenaline in the propofol and isoflurane ($p<0.05$) cohorts.

Intracranial pressures were not significantly different at baseline between the awake and anaesthetised sheep or in response to the catecholamines in the inter-cohort comparisons.

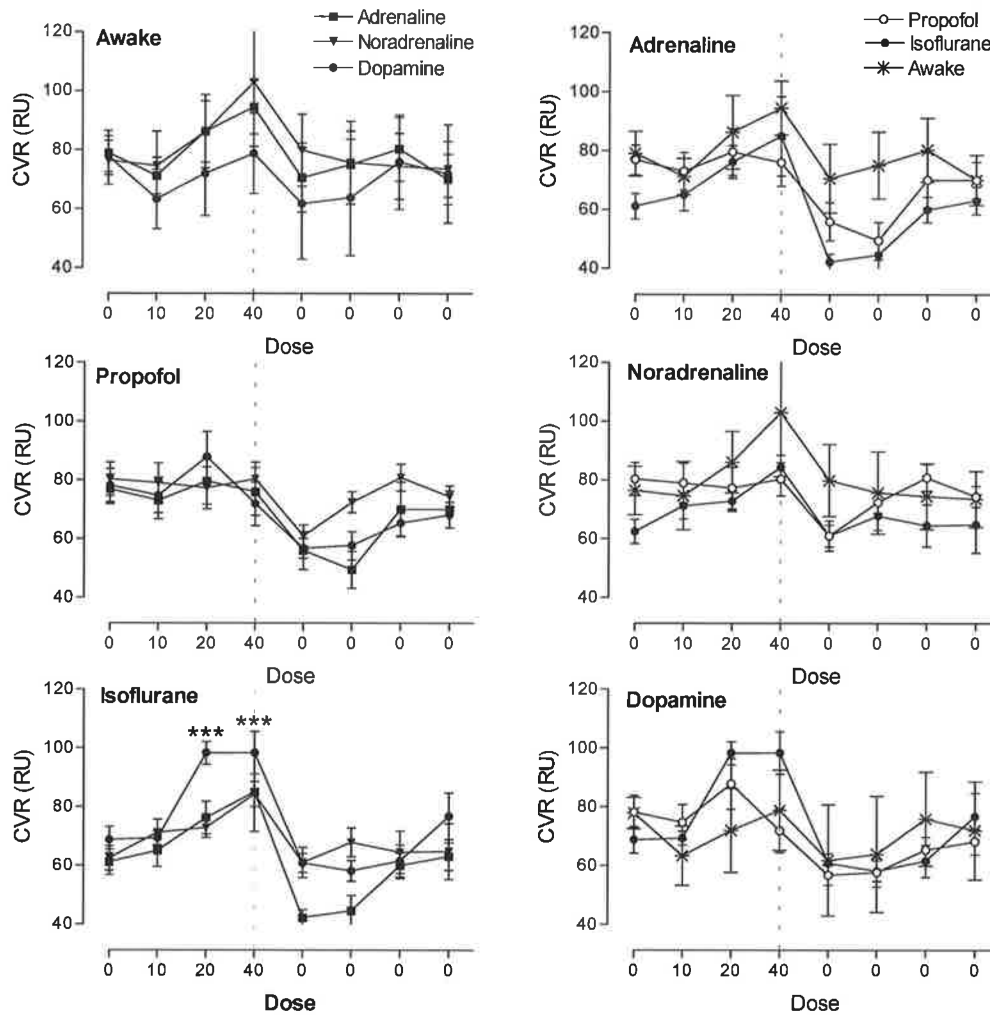


Figure 9.9

*The effects of adrenaline, noradrenaline and dopamine on cerebrovascular resistance (resistance units) in three cohorts: awake sheep, and during steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort; right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr. ***=$p<0.001$. Data are expressed as mean±sem*

No statistically significant changes in calculated cerebrovascular resistance from baseline were demonstrated during the infusions of adrenaline and noradrenaline in the awake and propofol cohorts ($p>0.05$). Dopamine

significantly increased cerebrovascular resistance from baseline under isoflurane anaesthesia ($p<0.001$), but not in the awake and propofol cohorts.

Prompt reductions in cerebrovascular resistance were demonstrated in all cohorts following cessation of catecholamine infusions. These reductions were only statistically significant following cessation of adrenaline in the awake ($p<0.05$) and isoflurane ($p<0.001$) cohorts, and dopamine in the propofol ($p<0.05$) and isoflurane ($p<0.001$) cohorts.

9.4.4 Cerebral metabolic effects

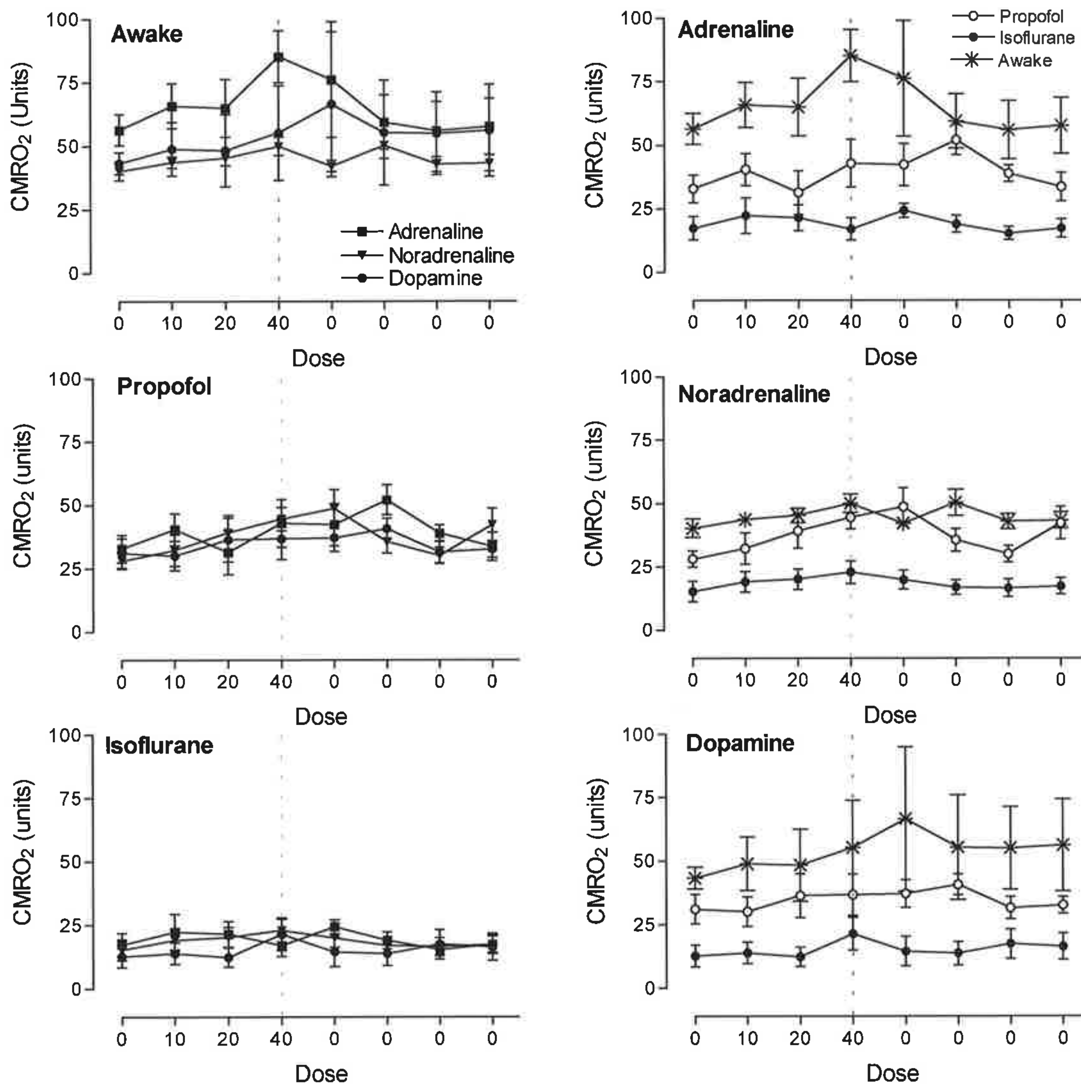


Figure 9.10
The effects of adrenaline, noradrenaline and dopamine on cerebral oxygen consumption ($CMRO_2$ Units) in three cohorts: awake sheep, and those during steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Left hand column: comparisons of the catecholamines for each cohort; right hand column: comparisons of the cohorts for each catecholamine. Doses are expressed as mL/hr. Data are expressed as mean±sem

Adrenaline, noradrenaline and dopamine did not significantly change cerebral oxygen consumption from baseline during the infusion and post infusion period in any of the cohorts ($p>0.05$) (Figure 9.10).

However, cerebral oxygen consumption was significantly lower under propofol and isoflurane anaesthesia than in awake sheep. During the adrenaline infusion, cerebral oxygen consumption was lowest under isoflurane anaesthesia - significantly lower than propofol anaesthesia. ($p<0.05$) and in the awake sheep ($p<0.001$).

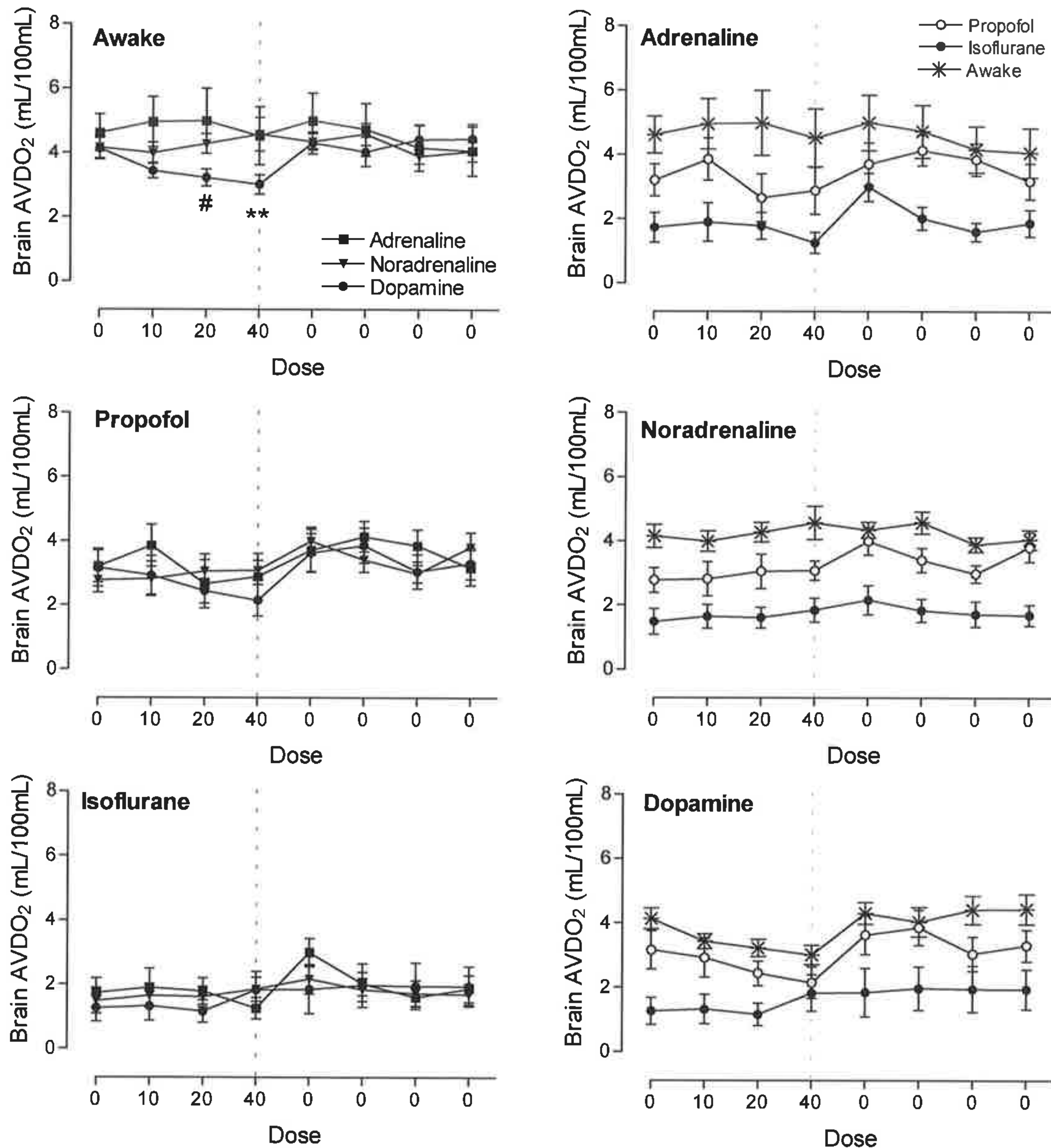


Figure 9.11

*The effects of adrenaline, noradrenaline and dopamine on brain arterio-sagittal sinus oxygen content difference ($AVDO_2$ g/100mL) in awake sheep, and during steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Doses are expressed as mL/hr. #=$p<0.05$, **=$p<0.01$. Data are expressed as mean ± sem*

The effects on arterio-sagittal sinus oxygen content differences are seen in Figure 9.11. As demonstrated in awake sheep (Figure 6.11), increased cerebral blood flow induced by dopamine was associated with a significant reduction in arterio-sagittal sinus oxygen content differences. Under propofol and isoflurane anaesthesia, there were no significant changes from baseline arterio-sagittal sinus content differences with any catecholamine, despite associated increases in cerebral blood flow (Figure 9.7).

Arterio-sagittal sinus oxygen content differences were significantly lower under anaesthesia than in awake sheep.

The lack of change in this parameter and calculated cerebral oxygen consumption suggests that cerebral blood flow:metabolism coupling was preserved under anaesthesia.

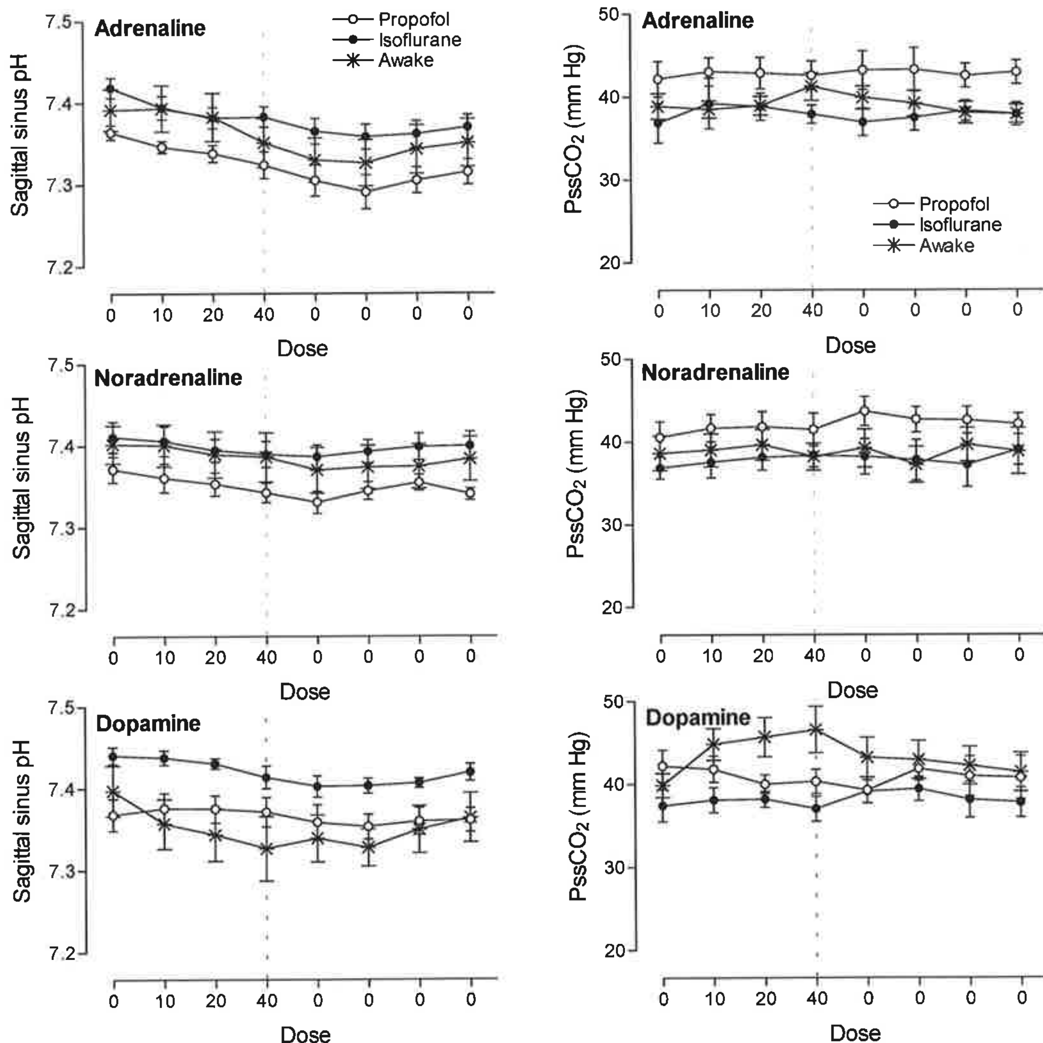


Figure 9.12
The effects of adrenaline, noradrenaline and dopamine on sagittal sinus pH (left hand column) and carbon dioxide tension (PssCO$_2$ mmHg) (right hand column) in awake sheep, and during steady-state propofol (15mg/min) and 2% isoflurane anaesthesia. Doses are expressed as mL/hr Data are expressed as mean ± sem

The effects on arterial and sagittal sinus pH and carbon dioxide tensions are shown in Figure 9.12. These mirrored trends in systemic pH and $PaCO_2$ (Figure 9.5). There were no significant differences in sagittal sinus pH between the three cohorts, apart from a trend for dopamine to reduce sagittal sinus pH in awake sheep. Under controlled mechanical ventilation, dopamine did not affect sagittal sinus pH.

9.5 Discussion

This study provided comparisons of the systemic and cerebrovascular haemodynamic and metabolic effects of adrenaline, noradrenaline and dopamine under propofol and isoflurane anaesthesia.

The data from awake sheep demonstrated that in the presence of equivalent systemic hypertension and increased cardiac output, dopamine significantly increased cerebral blood flow and intracranial pressure in a dose-dependent manner. The effects of dopamine on cerebral blood flow and intracranial pressure were significantly greater than those produced by adrenaline, which increased cerebral blood flow but to a lesser extent, and noradrenaline, which did not. Of the three catecholamines, noradrenaline had the least effect on cerebrovascular mechanics.

Compared to baseline measurements in awake sheep, anaesthesia was associated with reductions in cardiac output and systemic oxygen consumption. However, the response of the systemic circulation to infusions of catecholamines was similar to those in awake sheep. The three catecholamines produced equivalent systemic responses, particularly in augmentation of mean arterial pressure and cardiac output. The increase in $PaCO_2$ with dopamine observed in awake sheep was not present under anaesthesia with controlled mechanical ventilation.

As described in Chapter 7, propofol anaesthesia caused a 45% reduction in cerebral blood flow to those from values in awake sheep, whilst isoflurane did not significantly change cerebral blood flow. This occurred in the absence of changes in mean arterial pressure.

Under propofol and isoflurane anaesthesia, the cerebrovascular effects of catecholamines were significantly different to those in awake sheep. Cerebral blood flow was significantly increased by all three catecholamines under propofol and isoflurane anaesthesia compared to awake sheep, with dopamine demonstrating the most pronounced effects, particularly under propofol anaesthesia. Dopamine-induced hyperaemia was associated with other cerebrovascular changes. Dopamine consistently increased both intracranial pressure and calculated cerebrovascular resistance, whilst this was not a feature of noradrenaline and adrenaline, suggesting that a greater degree of hyperaemia was induced by dopamine infusions. In the presence of an equivalent effect on mean arterial pressure, these dopamine-specific

cerebrovascular effects appear to be centrally mediated, possibly by propofol or isoflurane mediated changes in blood-brain barrier permeability, thereby causing a direct influence on the cerebral vasculature.

Cerebral oxygen consumption was significantly lower under anaesthesia compared that in awake sheep. The lack of demonstrable effects on cerebral oxygen consumption induced by the catecholamines may be explained by the coupling of flow and oxygen consumption under anaesthesia.

There are few studies quantifying and comparing the effects of catecholamines on cerebrovascular mechanics. In an early study, King et al compared the effects of adrenaline (using doses of 6-22 μg/min) and noradrenaline (using doses of 19-73 μg/min) on cerebral blood flow, measured using the nitrous oxide method, in awake human volunteers (204). Induced hypertension with adrenaline was associated with increased cerebral blood flow that was attributed to increased cerebral oxygen consumption. Noradrenaline was associated with decreased cerebral blood flow that was attributed to increased cerebrovascular resistance. The differences between our results and those from King's study may be explained by the difference in doses used – a standardised infusion over a dose range compared to disparate doses in different individuals. Cerebrovascular resistance is a derived index that is prone to cumulative measurement errors. Caution should be used when attributing dynamic changes in complex systems to a single derived index.

The mechanisms responsible for the variable effects of catecholamines on the cerebral circulation in awake and anaesthetised sheep can only be speculated about.

In awake animals, Under physiological conditions, exogenous catecholamines do not cross the blood-brain barrier. The integrity of the endothelial cell lining of the cerebrovascular bed constitutes a morphological blood-brain barrier mechanism to catecholamines. The small percentage that may pass this membrane are deaminated within the endothelial cells and pericytes of brain microvessels and, in the case of large parenchymal and pial vessels, in the smooth muscle layers (12).

Selective transmission of dopamine may occur across the natural defects in the blood-brain barrier such as in the posterior pituitary or pineal gland which have specific dopaminergic receptors, or via non-adrenergic central neural mechanisms (193,194).

Brief or sustained hypertensive stimuli that exceed the upper cerebral autoregulatory threshold may transiently open the blood-brain barrier through an effect on endothelial cell linings. High circulating concentrations of catecholamines can also open the morphological barrier, but probably only indirectly by inducing an acute rise in systemic blood pressure (12,114).

Once the blood-brain barrier is open, systemically administered catecholamines may enter the brain parenchyma, where they may induce pronounced changes in cerebral blood flow and metabolism.

The effects of catecholamines such as adrenaline, noradrenaline and dopamine on blood-brain barrier permeability have been demonstrated in a number of experimental models including measurement of leakage of labelled albumin from blood to brain parenchyma (104), Evans blue (115) and horseradish peroxidase tracers (105). The effects of catecholamines on cerebral blood flow and metabolism were demonstrated using various methods of cerebral blood flow measurement including ^{14}C ethanol (290), quantitative autoradiography (114) and hydrogen clearance techniques (108). A consistent finding in these studies was that blood-brain barrier permeability was altered by induced hypertensive stimuli (114,291), particularly by dopamine and adrenaline (105). This phenomenon has been implicated in the pathogenesis of hypertensive encephalopathy (121).

Intravenous and inhalational anaesthetics have direct cerebrovascular effects that are complex. Propofol is regarded as an indirect cerebral vasoconstrictor, whilst isoflurane is thought to be a vasodilator. However, the mechanisms underlying the effects remain unclear as there are variable effects on blood-brain barrier permeability, flow-metabolism coupling and myogenic autoregulation with different agents (231,236,237,269).

Anaesthetic agents may affect blood-brain barrier permeability. Direct effects of isoflurane on blood-brain transfer coefficients and capillary permeability-surface area product have been demonstrated with 1 and 2% isoflurane (119). Propofol has been shown to independently increase the uptake of chemotherapeutic agents such as melphalan and etoposide phosphate across the blood-brain barrier following osmotic disruption (197). The effects of propofol/nitrous oxide anaesthesia on blood-brain barrier permeability have been shown to be qualitatively and quantitatively more pronounced than those of isoflurane/oxygen anaesthesia (120).

9.6 Conclusions and Future Directions

Propofol anaesthesia is associated with reductions in cerebral blood flow compared to flow under isoflurane anaesthesia and in awake sheep.

Under anaesthesia, cerebral blood flow was significantly increased by all three catecholamines compared to awake sheep, with dopamine demonstrating the most pronounced effects, particularly under propofol anaesthesia.

Potential mechanisms for these observations include alteration of blood-brain barrier permeability by propofol and isoflurane, potentially exacerbated by catecholamine induced hypertensive changes. These studies did not include

measurements of blood-brain barrier morphology or permeability, and therefore the mechanisms can only be the subject of speculation.

Propofol and isoflurane may induce changes in cerebral metabolic and myogenic autoregulation. The effects of propofol and isoflurane on cerebrovascular carbon dioxide reactivity are described in Chapter 7, demonstrating equivalent changes by the two anaesthetics from values in awake sheep. The effects of the catecholamines studied on the cerebral circulation under propofol and isoflurane anaesthesia may be due to alterations in upper myogenic autoregulatory thresholds. If this is the case, then catecholamine-induced hypertension may be associated with direct cerebrovascular effects.

Defining myogenic autoregulatory responses to adrenaline, noradrenaline and dopamine under propofol and isoflurane anaesthesia is the basis for the studies described in the next chapter.

Aspects of the studies in this chapter were published in the following article:

Myburgh JA, Upton RN, Grant C, Martinez A. (2002) Cerebrovascular effects of infusions of adrenaline, noradrenaline and dopamine under propofol and isoflurane anaesthesia. Anaesthesia and Intensive Care 30:725-733.

Chapter 10. Comparative studies of the effects of catecholamines on cerebral autoregulation

This chapter describes the effects of ramped infusions of adrenaline, noradrenaline and dopamine on cerebral myogenic autoregulation under steady-state propofol and isoflurane anaesthesia.

The effects of propofol and isoflurane anaesthesia on cerebral blood flow and carbon dioxide reactivity were described in Chapter 7, and the effects of catecholamine infusions on cerebrovascular function have been described in the previous chapter. These studies provide the platform from which to address the effects that the catecholamines may exert on cerebral myogenic autoregulation.

It was speculated that changes in cerebral myogenic autoregulation induced by propofol and isoflurane may potentiate direct cerebrovascular effects of exogenous catecholamines. Defining such changes may therefore provide further insight into potential mechanisms of the cerebrovascular effects of catecholamines under anaesthesia.

The analyses in this chapter are based on the data presented in Chapter 9; the effects of the ramped infusions of each catecholamine on cerebral blood flow/mean arterial pressure relationships in awake sheep and under propofol and isoflurane anaesthesia are examined.

10.1 Introduction.

Cerebral autoregulation is defined as the maintenance of cerebral blood flow at constant levels in the presence of changing systemic pressures. Also termed "myogenic" autoregulation, this phenomenon is attributed to changes in cerebrovascular resistance, although a number of metabolic systems have been implicated (14,194,292,293).

The capacity of this complex physiological system to maintain cerebral autoregulation may be influenced by abnormal physiological perturbations (e.g. systemic hypertension), drugs (e.g. volatile and intravenous anaesthetics, hypertonic agents) and pathological states (e.g. traumatic brain injury, subarachnoid haemorrhage and stroke).

Under these conditions, cerebral autoregulation may be altered so that cerebral blood flow becomes dependent on cerebral perfusion pressure. This is particularly relevant at lower autoregulatory thresholds where cerebral hypoperfusion may be potentiated by systemic hypotension. This has implications in traumatic brain injury. At the upper end of the autoregulatory

spectrum, exceeding higher thresholds by induced hypertension may result in cerebral hyperaemia and in extreme conditions, intracranial hypertension.

Infusions of adrenaline, noradrenaline and dopamine under propofol and / or isoflurane anaesthesia are commonplace in intensive care medicine and anaesthesia. However, the effects of interactions on upper autoregulatory thresholds have not been extensively studied.

10.2 Aim

The aim of the studies in this chapter was to determine the effects of adrenaline, noradrenaline and dopamine on cerebral autoregulation under steady-state propofol and isoflurane anaesthesia.

These effects were compared with each other and with those in awake sheep.

10.3 Methods

As stated above, the analyses in this chapter are based on the studies presented in Chapter 9: the animal preparation, interventions and measurements for these studies are described in section 9.3.

Ramped infusions of adrenaline, noradrenaline and dopamine (0-40 mL/hr) were administered in three cohorts: in awake sheep, and under steady-state propofol (15mg/min) or 2% isoflurane anaesthesia.

Following baseline measurements, continuous measurements of cerebral blood flow and mean arterial pressure were made during and after the infusions.

10.3.1 Data analysis

Normal distribution of datapoints before parametric analyses was determined using the using the Kolmogorov-Smirnov (KS) test.

Data for cerebral blood flow were normalised to an arterial carbon dioxide tension of 35mmHg to remove the influences of small differences in carbon dioxide tensions on cerebral blood flow. This was done by determining the correlation between cerebral blood flow and $PaCO_2$ during the catecholamine infusions. Cerebral blood flow measurements were then adjusted using the derived slope and intercept for a $PaCO_2$ of 35 mmHg.

As described in Chapter 7, propofol anaesthesia was characterised by a statistically significant 55% reduction in cerebral blood flow compared to values in awake sheep ($p=0.001$). Isoflurane anaesthesia was not associated with a significant change in cerebral blood flow (88.45% of baseline, $p=0.39$).

Accordingly, cerebral blood flows normalised for carbon dioxide were adjusted by the degree of change in cerebral blood flow induced by the respective anaesthetic agent, as expressed as percentage of baseline.

Cerebral autoregulation was defined as the change in cerebral blood flow for change in mean arterial pressure. This was determined using linear regression analysis.

Comparisons between regression lines were made using an analysis of covariance, using the pooled data for each dataset.

10.4 RESULTS

The effects of each catecholamine on normalised cerebral blood flow and mean arterial pressure in awake sheep and under propofol and isoflurane anaesthesia are shown in Figure 10.1.

All three catecholamines significantly increased mean arterial pressure from baseline in a dose-dependent manner ($p<0.001$) in both the awake and isoflurane cohorts

In the awake cohort, only adrenaline significantly increased cerebral blood flow from baseline ($p<0.01$).

During propofol anaesthesia, adrenaline significantly increased cerebral blood flow values in awake sheep ($p<0.001$); whilst noradrenaline and dopamine did not. There was no statistically significant difference between the effect of each catecholamine in any of the cohorts ($p>0.05$).

During isoflurane anaesthesia, none of the catecholamines significantly increased cerebral blood flow from baseline values in awake sheep ($p>0.05$).

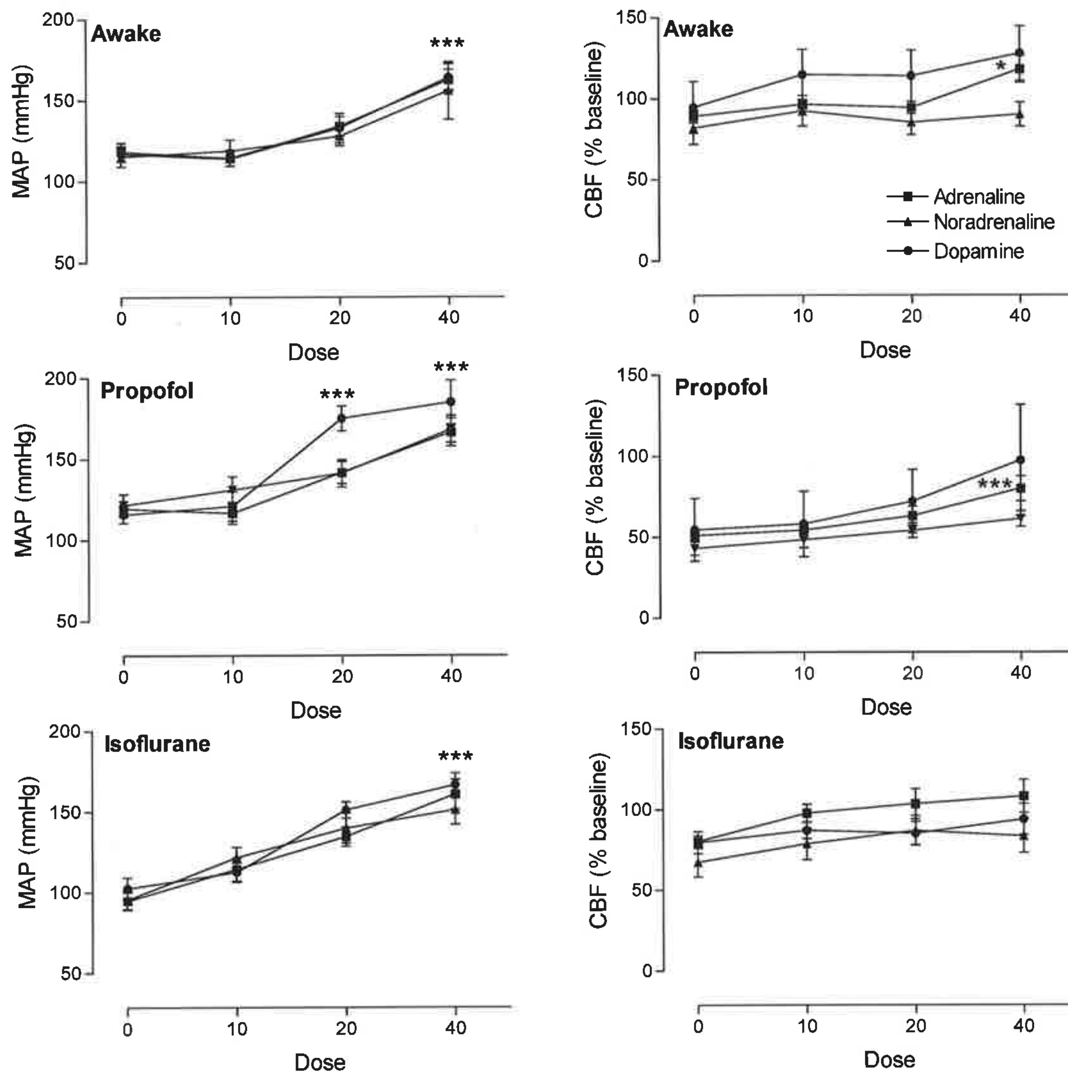


Figure 10.1
*Effects of infusions of adrenaline, noradrenaline and dopamine on mean arterial pressure (mmHg) (left hand column) and cerebral blood flow normalised for carbon dioxide (% baseline) (right hand column) in awake sheep (top row), and under steady-state propofol (15mg/min) (middle row) and 2% isoflurane anaesthesia (bottom row). Dose is expressed as mL/hr. *** = p<0.001. Data are expressed as mean±sem.*

Individual regression analyses between normalised cerebral blood flow and mean arterial pressure for adrenaline, noradrenaline and dopamine in awake sheep and under propofol and isoflurane anaesthesia are shown in Table 10.1, 10.1, 10.2 and 10.3.

	Awake		
	Adrenaline	**Noradrenaline**	**Dopamine**
r^2	0.81	0.16	0.59
Slope	0.53 ± 0.18	0.11 ± 0.16	0.45 ± 0.27
Intercept	29.4 ± 29.2	73.6 ± 21.8	52.2 ± 36.3

Table 10.1
Summary of regression analysis between normalised cerebral blood flow and mean arterial pressure for adrenaline, noradrenaline and dopamine in awake sheep. Slope is normalised cerebral blood flow (% baseline /mmHg), intercept normalised cerebral blood flow (% baseline).

	Propofol		
	Adrenaline	**Noradrenaline**	**Dopamine**
r^2	0.96	0.95	0.81
Slope	0.54 ± 0.07	0.38 ± 0.05	0.48 ± 0.16
Intercept	-12.6 ± 10	-1.6 ± 7.9	-2.1 ± 25.4

Table 10.2
Summary of regression analysis between normalised cerebral blood flow and mean arterial pressure for adrenaline, noradrenaline and dopamine under steady-state propofol anaesthesia (15mg/min). Slope is normalised cerebral blood flow (% baseline /mmHg), intercept normalised cerebral blood flow (% baseline).

	Isoflurane		
	Adrenaline	**Noradrenaline**	**Dopamine**
r^2	0.86	0.87	0.62
Slope	0.40 ± 0.11	0.33 ± 0.09	0.15 ± 0.08
Intercept	46.8 ± 14.8	37.5 ± 11.7	66.3 ± 11.4

Table 10.3
Summary of regression analysis between normalised cerebral blood flow and mean arterial pressure for adrenaline, noradrenaline and dopamine under steady-state 2% isoflurane anaesthesia. Slope is normalised cerebral blood flow (% baseline /mmHg), intercept normalised cerebral blood flow (% baseline).

The differences in the slopes and intercepts of the regression lines for individual catecholamines in awake sheep and under anaesthesia were compared using an analysis of covariance (Table 10.4).

No statistically significant differences were demonstrated between mean slopes for adrenaline, noradrenaline and dopamine within each cohort ($p>0.05$).

Significant differences in mean intercepts were demonstrated between dopamine and noradrenaline; and between noradrenaline and adrenaline in the awake cohort.

The differences between the mean intercepts of adrenaline and noradrenaline, and dopamine and noradrenaline were significant under propofol and isoflurane anaesthesia.

The differences between the mean intercepts of adrenaline and dopamine infusions were significantly different under isoflurane anaesthesia.

	Awake		Propofol		Isoflurane	
	Slope	**Intercept**	**Slope**	**Intercept**	**Slope**	**Intercept**
Adrenaline v noradrenaline	0.16	0.08	0.14	0.002*	0.65	0.002*
Adrenaline v dopamine	0.84	0.06	0.79	0.72	0.15	0.03*
Dopamine v noradrenaline	0.36	0.01*	0.68	0.02*	0.23	0.12

Table 10.4
Summary of the p values for the analysis of covariance comparing the differences in the slopes and intercepts of the regression lines for individual catecholamines in awake sheep, and under steady-state propofol (15mg/min) or 2% isoflurane anaesthesia. Data are expressed as the p values for individual regression comparisons. (*) indicates significant results where $p<0.05$.

The effects on the autoregulation curves for the three cohorts are shown in Figure 10.2. As no significant differences between the slopes for each catecholamine within each cohort were identified by analysis of covariance, the data for the autoregulation curves are presented using pooled data for the three catecholamines.

Intercohort comparisons between awake sheep and those under propofol anaesthesia, between awake sheep and those under isoflurane anaesthesia and between those under propofol and isoflurane anaesthesia were made by analysis of covariance of the data pooled for all catecholamines.

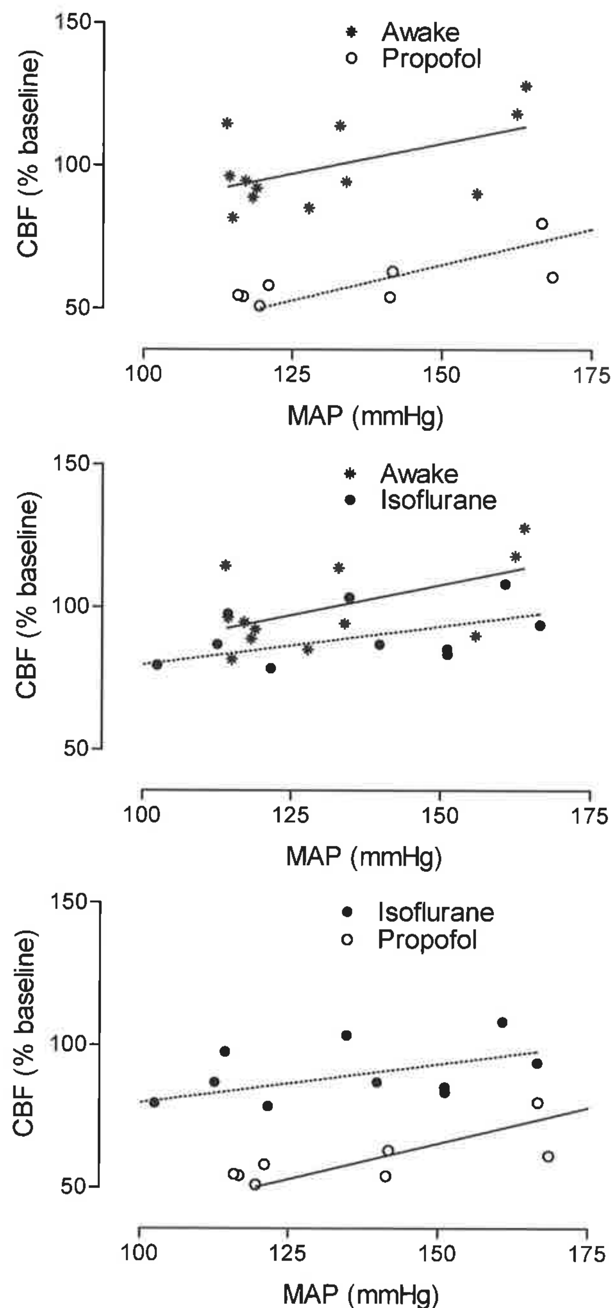


Figure 10.2
Autoregulation expressed as linear regression lines for normalised cerebral blood flow (CBF: % baseline) and mean arterial pressure (MAP: mmHg) in awake sheep (stars), and under steady-state propofol (15.mg/min) (open circles) and 2% isoflurane (solid circles) anaesthesia during infusions of adrenaline, noradrenaline and dopamine. Catecholamine data have been pooled for each cohort.

There was no significant difference between the slopes of the awake (pooled slope = 0.39) and propofol cohorts (pooled slope = 0.48) (p=0.69). However, there was a statistically significant difference between the intercepts (p<0.0001) of the two cohorts.

There was no significant difference between the slopes of the awake and isoflurane cohorts (pooled slope = 0.28) ($p=0.50$). However, there was a statistically significant difference between the intercepts ($p=0.02$) of the two cohorts.

There was no significant difference between the slopes of the propofol and isoflurane cohorts ($p=0.38$). However, there was a statistically significant difference between the intercepts ($p<0.001$) of the two cohorts.

10.5 Discussion

The studies in this chapter analysed and compared the effects of catecholamine induced hypertension on cerebral autoregulation in awake sheep and during steady-state propofol and isoflurane anaesthesia.

10.5.1 Defining autoregulation

In order to determine autoregulatory responses, the data in these studies were modified to define changes in cerebral blood flow for associated changes in mean arterial pressure under the different conditions.

Firstly, to define metabolic autoregulatory effects, carbon dioxide reactivity data were described in Chapter 7. These studies demonstrated that carbon dioxide reactivity under propofol and isoflurane anaesthesia was significantly different to that in awake sheep, but there was no significant difference between those under propofol and isoflurane anaesthesia. Studies under anaesthesia were conducted using mechanical ventilation, so that arterial carbon dioxide tensions and pH were maintained at normal levels.

Secondly, the direct effects of propofol and isoflurane on cerebral blood flow were quantified (Chapter 7). This identified that propofol anaesthesia was associated with a 55% reduction and isoflurane anaesthesia with a (non-significant) 11% reduction in cerebral blood flow compared to values in awake sheep.

Accordingly, cerebral blood flow data measured during the ramped infusions of adrenaline, noradrenaline and dopamine were normalised to an arterial carbon dioxide tension of 35 mmHg and adjusted by the degree of anaesthetic induced reduction in cerebral blood flow.

A comparison of the effects of the three catecholamines on cerebral myogenic autoregulation under reproducible conditions has not been previously published.

In the presence of induced hypertension, the effects of catecholamines on normalised cerebral blood flow in the three cohorts were different.

In awake sheep and under propofol anaesthesia, noradrenaline and dopamine did not significantly increase cerebral blood flow, whilst adrenaline

caused a modest increase in cerebral blood flow from baseline values. Under isoflurane anaesthesia, none of catecholamines increased cerebral blood flow.

There were no significant differences between the effects of catecholamines on cerebral blood flow within each cohort. This allowed comparisons to be made between the awake and isoflurane cohorts using pooled catecholamine data for the subsequent linear regression analyses.

An assessment of overall autoregulatory function was determined rather than a specific break point where cerebral blood flow became pressure-dependent. The data did not support this sort of analysis. As identical curves-of-best-fit were obtained using curvilinear regression analysis, regression analysis was used to conduct intra- and inter-cohort comparisons using analysis of covariance.

The autoregulatory lines describe these effects. The slope of the cerebral blood flow/mean arterial pressure regression line is representative of the degree of autoregulation. Maintenance of a horizontal line represents the ability to maintain cerebral blood flow at a constant rate over a range of increasing blood pressure. Elevation of the slope represents progressive exhaustion of autoregulatory capacity to a point where cerebral blood flow ultimately becomes dependent on mean arterial pressure.

Despite qualitative differences between the effects of the catecholamines on cerebral blood flow in awake sheep and under propofol and isoflurane anaesthesia, there were no differences between the slopes of the regression lines in the three cohorts.

The major differences were in the intercept of the regression lines. This represented the overall reduction in cerebral blood flow induced by propofol and to a lesser extent by isoflurane. This suggests that the net reduction in cerebral blood flow under propofol anaesthesia is associated with an autoregulatory relationship similar to that in awake sheep. A similar response was demonstrated under isoflurane anaesthesia, also suggesting preservation of autoregulation during catecholamine induced hypertension.

10.5.2 Comparative studies

Studies of cerebral autoregulatory function under physiological, pathological and anaesthetised states have been conducted using various methods of measurement of cerebral blood flow / mean arterial pressure relationships. Ideally, these measurements should be simultaneous and continuous, thereby assessing the response of the dependent variable (cerebral blood flow) over a range of cerebral perfusion pressures. Methods of cerebral blood flow measurement in these studies include transcranial Doppler (128,129),

intermittent techniques such as radiolabelled microspheres (125) and cerebral venous outflow measurements (101).

Autoregulatory relationships have also been determined by a number of methods. These include regression analysis between mean arterial pressure and cerebral blood flow (127) or cerebrovascular resistance (128); dynamic and static measurements of cerebral blood flow responses to changes in mean arterial pressure (102,122), and derived indices such as the autoregulatory index (129).

10.5.2.1 Propofol

In a study using a porcine preparation, Lagerkranser studied the effects of propofol on the cerebral circulation over a range of mean arterial pressures (101). Regional cerebral blood flow was measured using a cerebral venous outflow technique and flow/pressure autoregulation was tested with angiotensin infusions and gradual blocks of the caval vein for hyper- and hypotensive challenges. Changes induced under propofol were compared to a control group receiving low dose isoflurane / nitrous oxide anaesthesia, where autoregulation was considered to be well preserved. Propofol caused a significant reduction in cerebral blood flow and did not significantly alter the slope of the regression line of regional cerebrovascular resistance versus mean arterial pressure, although inter-individual differences were observed. In these studies, the effects of propofol anaesthesia were compared with another anaesthetised group rather than with awake subjects, which raises questions about the significance of the inter-cohort comparisons of autoregulatory function.

In a primate preparation, van Hemelrijck compared the effects of propofol in increasing doses on cerebral blood flow and autoregulation (231). Dose-dependent reductions in cerebral blood flow were demonstrated that were considered to be coupled to cerebral oxygen consumption. In this preparation, physiological responsiveness to alterations in mean arterial pressure were preserved. This study did not use a control group to determine changes in autoregulatory function from baseline.

10.5.2.2 Isoflurane

Isoflurane anaesthesia causes a relative increase in cerebral blood flow by uncoupling flow and metabolism (124,236,237). As this phenomenon is directly dose and time dependent (124,125), interpretation and comparisons of studies of autoregulatory function requires consideration of dose, duration of anaesthesia and methods of measurements. Consequently, there are diverse conclusions about the effects of isoflurane anaesthesia on cerebral autoregulation.

In a lapine preparation, Patel studied the effects of induced hypertension by angiotensin II, noradrenaline and phenylephrine on global and regional cerebral blood flows (measured by radioactive microspheres) during 1.0 MAC isoflurane anaesthesia (41). The slopes of pressure / flow curves produced by noradrenaline and phenylephrine were significantly steeper than that produced by angiotensin II in all cerebral blood flow regions. Patel concluded that noradrenaline and phenylephrine caused indirect cerebral vasodilation, whilst angiotensin II caused vasoconstriction during 1.0 MAC isoflurane. However, deductions about specific vascular effects of vasoactive agents from the slope of regression curves must be made with circumspection. Factors that determine autoregulatory relationships are more complex than solely attributing changes to vasoreactivity. The difference in the conclusion from our study may, in part, be explained by the difference in cerebral blood flow measurement – intermittent (microsphere) versus continuous (Doppler).

Engelhard compared the effects of 1.5 MAC isoflurane on dynamic cerebrovascular autoregulation values in awake subjects (128). These were assessed using transcranial Doppler ultrasonography and deriving an autoregulatory index from an induced change in systemic blood pressure. Autoregulatory responses were delayed under isoflurane anaesthesia compared to the awake state. The difference in our study may be explained by the effects that catecholamines may have on the cerebral vasculature under isoflurane anaesthesia. This has been attributed to isoflurane induced changes in cerebral blood flow and blood-brain barrier permeability (119,124,236) and by the catecholamines themselves if associated induced hypertension exceeds the upper autoregulatory threshold (105,207).

A study that provides the closest comparison to the studies in this thesis was conducted in humans. Strebel assessed alterations in dynamic autoregulation from the responses of middle cerebral artery blood flow velocities in patients anaesthetised with low (0.5 MAC) and high (1.5 MAC) dose isoflurane anaesthesia; and with low and high dose propofol anaesthesia (122). Transient step increases and decreases in mean arterial pressure were induced by infusions of phenylephrine and rapid inflation/deflation of thigh cuffs respectively. Patients receiving the four anaesthetic regimens were compared to those under "baseline" anaesthesia with fentanyl and nitrous oxide. This study demonstrated differences between the autoregulatory responses under propofol and isoflurane anaesthesia. Strebel concluded that neither static nor dynamic rates of regulation were altered by propofol. Low dose isoflurane delayed autoregulatory responses to changes in systemic blood pressure, whilst high dose isoflurane ablated autoregulation. These results need to be interpreted recognising the lack of an intact autoregulatory baseline, the use of intermittent indirect measurements of cerebral blood flow and derived variables for the assessment of autoregulation. However, it does emphasise the importance of

anaesthetic dose and autoregulatory function. In our study, steady-state concentrations of isoflurane were used for standard periods of time, which demonstrated baseline stability and allowed inter-cohort comparisons.

The studies in this thesis therefore appear to be consistent with other published studies in concluding that cerebral autoregulation is essentially preserved during propofol anaesthesia. This has been attributed to maintenance of cerebral blood flow:metabolism coupling and preservation of carbon dioxide reactivity (24). Whist this may be applicable to steady-state anaesthetic conditions, the concomitant administration of catecholamines may independently alter cerebral autoregulation. This has been attributed to propofol induced changes in cerebral blood flow and blood-brain barrier permeability (120,122,197) and by the catecholamines themselves if associated induced hypertension exceeds the upper autoregulatory threshold (105,207).

10.6 Conclusions and Future Directions

The results of the studies in this chapter need to be interpreted in the light of the following considerations.

In the earlier chapters, the qualitative effects of adrenaline, noradrenaline and dopamine on cerebral blood flow using a higher dosing regimen were described. In awake sheep (Chapter 6), the catecholamines were infused to peak doses of 60 mL/hr. At these doses, dopamine significantly increased cerebral blood flow from baseline. In these autoregulatory studies, doses were limited to a maximum of 40 mL/hr so that linearity between cerebral blood flow and mean arterial pressure was maintained. At higher doses, it is probable that the relationship is non-linear.

Secondly, regression lines were determined using pooled data for each study rather than individual data. As described in the studies of carbon dioxide reactivity (Chapter 7), this was done to minimise the effects of inter-animal variability.

Thirdly, mean arterial pressure was used as an index of cerebral perfusion pressure. As this was a physiological study conducted in the absence of antecedent raised intracranial pressure and at normocapnia, this should be an acceptable assumption. However, direct effects of catecholamines on intracranial pressure, once upper autoregulatory thresholds have been exhausted, may be a confounding variable that was not quantified.

In conclusion, over the dose range used, catecholamine induced hypertension caused increased cerebral blood flow during steady-state propofol anaesthesia. However, this was offset by an associated reduction in cerebral blood flow caused by propofol.

Under steady-state 2% isoflurane anaesthesia, induced systemic hypertension by adrenaline, noradrenaline and dopamine did not significantly alter cerebral blood flow.

The concomitant administration of catecholamines under propofol or isoflurane anaesthesia was not associated with altered autoregulatory function compared to that in awake sheep.

There may be clinical implications from these animal studies. Patients with altered cerebral blood flow and autoregulatory capacity are frequently anaesthetised with isoflurane or sedated/anaesthetised with propofol, e.g. following traumatic brain injury and aneurysmal subarachnoid haemorrhage. In these patients, particularly following traumatic brain injury, cerebral blood flow may be significantly reduced by the pathological process (36,37).

Potentially, propofol may further reduce cerebral blood flow, either directly or by reduction in systemic blood pressure. These studies suggest that absolute reductions in cerebral blood flow may be offset by concomitant administration of adrenaline, noradrenaline of dopamine without alteration in autoregulatory capacity.

Despite recognised cerebral vasodilatory effects of isoflurane, and potential vascular effects of the catecholamines under isoflurane anaesthesia, there did not appear to be alterations in autoregulatory capacity in this preparation.

However, extrapolations of the results from our study to these pathophysiological states in humans are speculative and further studies are warranted before conclusions can be drawn.

Aspects of the studies in this chapter will be published in the following articles:

1. Myburgh JA, Upton RN, Grant C, Martinez A. The effects of adrenaline, noradrenaline and dopamine infusions on cerebral autoregulation during propofol anaesthesia in an ovine model. Intensive Care Medicine (Accepted for publication January 2003).
2. Myburgh JA, Upton RN, Grant C, Martinez A. The effects of infusions of adrenaline, noradrenaline and dopamine on cerebral autoregulation during isoflurane anaesthesia in an ovine model. Anaesthesia and Intensive Care (Accepted for publication January 2003).

Chapter 11. Summary and conclusions

The use of the catecholamines adrenaline, noradrenaline and dopamine to augment the failing circulation under a variety of conditions is an established part of clinical practice. These drugs have been used for over fifty years.

Despite intensive research into the neurophysiology and molecular basis of the adrenergic system, definitive studies about the consequences of manipulating the effector arm of this system by infusing exogenous catecholamines, remain elusive.

Specifically, the potential and real interactions of these drugs and anaesthetic agents such as propofol and isoflurane have not been conclusively evaluated in representative physiological and pathological models or in humans.

The results of the studies done in our animal preparation provide some insights into the pharmacodynamic effects of adrenaline, noradrenaline and dopamine on systemic and cerebrovascular haemodynamic and metabolic function. The studies were conducted under in awake sheep and during steady-state intravenous and inhalational anaesthesia.

In concluding this thesis, each aim of this thesis (outlined in Chapter 3) will be addressed.

11.1 Systemic effects of catecholamines in awake sheep

In Chapter 6, the systemic haemodynamic and metabolic effects of infusions of adrenaline, noradrenaline and dopamine were described.

Despite many years of clinical experience, opinion amongst clinicians varies considerably about the effects that these drugs have on standard parameters such as blood pressure, cardiac output and systemic vascular resistance. The purported effects of the catecholamines are based on simplistic conclusions from limited studies in humans. These effects are then applied to a simplified circulatory theory based on Ohm's Law that defines the relationship between pressure, flow and resistance. Consequently, there is great disparity in the reporting of how these drugs affect the circulation, in both standard physiological textbooks and clinical studies. The theoretical limitations of current clinical based thinking is discussed in Chapter 1 and a hypothesis is proposed that, on teleological grounds, adrenaline, noradrenaline and dopamine should have equivalent systemic effects in equivalent doses. The responses are dependent on dose, receptor affinity, and responsiveness that may vary greatly from organ to organ under pathophysiological conditions.

In the studies in Chapter 6, adrenaline, noradrenaline and dopamine significantly and equivalently increased mean arterial pressure and cardiac output from baseline in a dose-dependent manner. These changes occurred in the absence of significant changes in calculated systemic vascular resistance.

Oxygen consumption was also measured. This is a widely used method of assessing systemic metabolism but is limited by the indirect nature of measurement and potentially confounded by mathematical coupling. The catecholamines produced equivalent, non-specific changes in oxygen consumption.

11.2 Cerebrovascular effects of catecholamines in awake sheep

The effects of adrenaline, noradrenaline and dopamine on cerebrovascular haemodynamic function and oxygen consumption were analysed in Chapter 6.

These studies were based on the use of a validated, continuous measurement of cerebral blood flow that was discussed in Chapter 2. The Doppler flow meter model developed in the University of Adelaide sheep laboratory provides a continuous, accurate measurement of approximately 75% of cerebral blood flow with minimal contamination from other vascular beds. Validation studies confirmed that the dorsal sagittal sinus is a vessel containing representative cerebral venous effluent blood that is not subject to potential confounding variables that may effect Doppler based measurements. Of these, stability of vessel calibre, linearity between sagittal sinus flow and Doppler velocities and stable cerebral blood flow time course measurements were demonstrated.

The studies of the effects of adrenaline, noradrenaline and dopamine on cerebrovascular function were done in the context of simultaneous systemic perturbations. In the presence of equivalent and significant catecholamine induced hypertension, variable effects on the cerebral circulation were observed. Dopamine produced cerebral hyperaemia that was associated with increased intracranial pressure. These changes occurred in the absence of demonstrable changes in calculated cerebrovascular resistance.

The associated effects on cerebral oxygen consumption, pH and arterio-sagittal sinus oxygen content differences were measured. No significant changes in cerebral oxygen consumption were observed. However, systemic and cerebral arterial and venous hypercapnia and acidosis were observed with dopamine. Furthermore, significant reduction in arterio-sagittal sinus oxygen content differences suggested that dopamine induced hyperaemia was associated with increased cerebral oxygen consumption.

Mechanisms of specific cerebrovascular effects of dopamine were suggested and included a direct central dopaminergic effect, selective transmission of dopamine across naturally occurring defects in the blood-brain barrier, exhaustion of the upper autoregulatory threshold by dopamine induced hypertension.

It was concluded that, in this preparation, dopamine exerted a specific, dose-dependent cerebral hyperaemic and associated increases in cerebral oxygen consumption in the presence of induced systemic hypertension.

11.3 SYSTEMIC AND CEREBROVASCULAR EFFECTS OF ANAESTHESIA

The observations in the studies of Chapter 6 provided the platform for determining similar responses under non-physiological conditions. Intravenous anaesthesia using propofol and inhalational anaesthesia with isoflurane were selected as reproducible perturbations.

The studies conducted in Chapter 7 addressed the direct effects of propofol and isoflurane anaesthesia on cerebral blood flow. Dose ranges for propofol and isoflurane were selected in accordance with previously published pharmacokinetic studies in sheep. Propofol anaesthesia was associated with a 45% reduction in cerebral blood flow compared to values in awake sheep. Isoflurane anaesthesia was not associated with a significant reduction in cerebral blood flow. Neither agent caused a significant reduction in mean arterial pressure.

As part of defining the cerebrovascular effects of the catecholamines under anaesthesia, carbon dioxide reactivity in awake sheep and under steady-state propofol (15mg/min) and 2% isoflurane anaesthesia were determined. These studies demonstrated that, over a representative range of arterial carbon dioxide tensions, cerebrovascular reactivity under propofol and isoflurane were significantly different to that in awake sheep, but not different from each other. Carbon dioxide reactivity studies in the awake animals were subject to inter-individual variability.

The effects of infusions of catecholamines on systemic and cerebrovascular haemodynamic and oxygen consumption were determined. During pilot studies, higher doses of catecholamines (>40mL/hr) were not tolerated by the animals. Accordingly, the same studies conducted in awake sheep (Chapter 6) were repeated under propofol and isoflurane anaesthesia, but using a lower dose regimen (Chapter 9).

The systemic effects of catecholamines were similar to those in awake sheep. However, anaesthesia was associated with reductions in cardiac output and systemic oxygen consumption.

The cerebrovascular effects of adrenaline, noradrenaline and dopamine were significantly different to those in awake sheep. Under propofol and isoflurane

anaesthesia, cerebral blood flow was significantly increased by all three catecholamines, with dopamine demonstrating the most pronounced effects, particularly under propofol anaesthesia. These changes were not associated with significant intracranial hypertension with adrenaline and noradrenaline. However, dopamine produced dose-dependent increases in intracranial pressure.

Intravenous and inhalational anaesthesia significantly reduced cerebral oxygen consumption. There were no changes in arterio-sagittal sinus pH with any of the catecholamines, suggesting that catecholamine induced increases in cerebral blood flow were coupled to cerebral metabolism. As carbon dioxide was controlled by mechanical ventilation under anaesthesia, no associated changes in carbon dioxide tensions were observed.

It was concluded that, under anaesthesia, catecholamines exert direct effects on the cerebral vasculature. However, it appears that the changes are coupled to metabolism. However, this relationship cannot be established by these studies.

Potential mechanisms for these observations include alteration of blood-brain barrier permeability by propofol and isoflurane, potentially exacerbated by catecholamine induced hypertensive changes. As these studies did not include measurements of blood-brain barrier morphology or permeability these mechanisms remain speculative.

Other potential mechanisms include changes in cerebral metabolic and myogenic autoregulation. The latter were addressed in Chapter 10.

Another important catecholamine:anaesthetic interaction was observed during infusions of propofol. As the dose of catecholamines were increased, reversal of anaesthesia by propofol became apparent. This occurred despite establishing steady-state anaesthetic conditions for 1.5 hours beforehand.

A study to determine the effect of catecholamine induced increases in cardiac output on propofol concentrations was conducted (Chapter 8). Adrenaline, noradrenaline and dopamine significantly and equivalently increased cardiac output in a dose-dependent manner. At the same time, blood samples taken for propofol assays demonstrated significant reductions in arterial and sagittal sinus propofol concentrations. These changes in propofol concentration correlated with “reversal” of anaesthesia.

A previously developed recirculatory pharmacokinetic model was used to predict changes in propofol concentrations with changes in cardiac output. These correlated with the measured changes in propofol concentrations.

It was concluded that induced hyperdynamic circulatory states by exogenous infusions of adrenaline, noradrenaline and dopamine increased propofol requirements for anaesthesia.

11.4 Effects of catecholamines and anaesthesia on autoregulation

The final aim of the studies described in this thesis was to determine the effects of catecholamine induced changes on mean arterial pressure and cerebral blood flow on cerebral myogenic autoregulation (Chapter 10).

Measurements of cerebral blood flow obtained in the studies described in Chapter 9 were adjusted to account for propofol and isoflurane induced changes in cerebral blood flow. Similarly, in order to minimise any effects of carbon dioxide, the cerebral blood flow values were normalised to a $PaCO_2$ of 35mmHg.

Autoregulation was determined by cerebral blood flow responses to systematic increases in mean arterial pressure and expressed as the linear regression relationship between the two variables. Regression analyses were determined between cerebral blood flow (%baseline) and mean arterial pressure (mmHg) in the three cohorts: awake sheep and those under propofol and isoflurane anaesthesia.

Despite qualitative differences between the effects of the catecholamines on cerebral blood flow in awake sheep and under propofol and isoflurane anaesthesia, there were no differences between the slopes of the regression lines in the three cohorts. The slope of the cerebral blood flow/mean arterial pressure regression line is representative of the degree of autoregulation. The major difference was demonstrated in the intercept of the regression lines consistent with the overall reduction in cerebral blood flow induced by propofol anaesthesia and, to a lesser extent, by isoflurane anaesthesia. The concomitant administration catecholamines, propofol or isoflurane was not associated with altered autoregulatory function.

Over the dose range studied, catecholamine induced hypertension caused increased cerebral blood flow during steady-state propofol anaesthesia. However, this was offset by an associated reduction in cerebral blood flow caused by propofol. Under steady-state isoflurane anaesthesia, induced systemic hypertension by adrenaline, noradrenaline and dopamine did not significantly alter cerebral blood flow. The concomitant administration of under propofol or isoflurane anaesthesia was not associated with altered autoregulatory function compared to values obtained in awake sheep.

11.5 Clinical implications

A secondary aim of this thesis was to provide information from a validated animal model that may have implications for clinical practice.

The pharmacodynamic effects of the catecholamines described in the studies in this thesis suggest that the endogenous catecholamines are equieffective in augmenting systemic blood pressure and cardiac output in a dose-

dependent fashion. Direct measurements of these two variables provide the most accurate information about their systemic circulatory effects.

On the basis of these results, a prospective randomised, double blind trial on the comparative effects of adrenaline and noradrenaline in critically ill patients receiving these catecholamines is planned as a post-doctoral study.

The cerebrovascular effects of the catecholamines demonstrated in these studies provide some insights that may be of relevance to the clinical use of these drugs. It appears that dopamine has specific dose-dependent cerebrovascular effects. These effects may be more pronounced under conditions of altered cerebral autoregulation or blood-brain barrier permeability, although it may be hypothesised that all catecholamines would exert similar effects if blood-brain barrier and homeostatic mechanisms are disrupted.

It does not appear that cerebral myogenic or metabolic autoregulation is significantly impaired under propofol or isoflurane anaesthesia in this preparation. The use of catecholamines may be beneficial to compensate for reductions in cerebral blood flow produced by propofol particularly in patients with pathological reductions in cerebral blood flow.

An observation which may well be clinically relevant is the effect of catecholamine induced changes in cardiac output on propofol clearance. This may be the principle mechanism underlining propofol "tachyphyllaxis" observed in patients receiving moderate to high doses of propofol together with catecholamine infusions.

As outlined in Chapter 9, a series of case reports highlighted sudden cardiac arrest and death in such patients. This was attributed to progressive propofol induced myocardial depression and catecholamine induced adrenergic receptor down regulation. This is a plausible explanation, but the pharmacokinetic factors identified in this study may be the basis for these clinical observations. Of potential concern is the situation in which a patient is receiving high doses of both propofol and a catecholamine, followed by temporary withdrawal of the catecholamine. This could be associated with a sudden increase in propofol concentrations that would compound any cardiac depression. Given the increasing use of propofol in critically ill patients with pathological or catecholamine induced hyperdynamic circulatory states, this phenomenon requires investigation in man with some urgency and an awareness of this possibility in those directly caring for such patients.

Fin

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