# The Cerebral Pharmacokinetics and Pharmacodynamics of Propofol in Sheep

Guy Lawrence Ludbrook

Department of Anaesthesia and Intensive Care
Faculty of Medicine
Division of Health Sciences
University of Adelaide
Adelaide, S.A. 5005
Australia

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## ABSTRACT

Propofol is a popular drug for the induction and maintenance of anaesthesia, but currently there is a poor understanding of its pharmacokinetics when administered rapidly. Previous studies have suggested that regional pharmacokinetic techniques can provide insight into drug distribution to target organs during rapid administration, and can be used to develop optimal dosing strategies. This thesis therefore examined the systemic and cerebral pharmacokinetics and pharmacodynamics of propofol following rapid administration, using regional pharmacokinetic techniques in a sheep preparation. New methods for measurement of cerebral blood flow, cerebral metabolic rate and depth of anaesthesia were developed and validated. Studies of rapid injection of propofol revealed that brain, but not arterial, concentrations of propofol correlated closely with cerebral effects, demonstrating the inadequacies of conventional compartmental pharmacokinetics based on blood concentrations. This was confirmed by compartmental pharmacokinetic analysis of the measured propofol concentrations from these studies, which poorly described the time-course of arterial and brain concentrations of propofol during rapid administration, and was therefore inadequate for the determination of accurate dose regimens. Later studies revealed that brain concentrations of propofol were relatively independent of administration rate and that the claimed "dose-sparing" with slow administration is an illusion. Very rapid administration may only increase the risk of hypotension, without affecting depth of anaesthesia. The consistent relationship between brain concentrations and depth of anaesthesia over time also shown in these studies failed to support the previously proposed concept of "acute tolerance" during induction of anaesthesia. The final studies showed that distribution of propofol to the brain was dependent on cardiac output, suggesting that dose regimens may be based on estimates of cardiac output rather than conventional factors such as body mass, and that the phenomenon of dose-sparing with "co-induction" is likely to have a pharmacokinetic basis. The analysis of the cerebral pharmacokinetics and pharmacodynamics of propofol in this thesis therefore provided new insights into its distribution and subsequent cerebral effects during the induction phase of anaesthesia. These data pave the way for the development of new dose strategies for the use of this agent in the clinical setting in man.

## DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, 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 consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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# ABBREVIATIONS

CBF = cerebral blood flow

CMR = cerebral metabolic rate

EEG = electroencephalography

CPP = cerebral perfusion pressure

CSF = cerebrospinal fluid

MAP = mean arterial pressure

$CO_2$ = carbon dioxide

$O_2$ = oxygen

$PaO_2$ = partial pressure of oxygen in arterial blood

$PaCO_2$ = partial pressure of carbon dioxide in arterial blood

$PetCO_2$ = end tidal partial pressure of carbon dioxide

# CHAPTER 1. A LITERATURE REVIEW OF STUDIES OF THE CEREBRAL PHARMACOKINETICS AND PHARMACODYNAMICS OF PROPOFOL

## 1.1 INTRODUCTION

General anaesthesia in adults is today most commonly induced with the rapid intravenous administration of a sedative-hypnotic agent, with anaesthesia then being maintained with inhalation of volatile anaesthetic agents or, less commonly, by the slower, continuous administration of intravenously administered agents. The aim of intravenous administration at induction is to rapidly achieve a sufficient depth of anaesthesia to avoid recall or sympathetic responses to external stimuli such as surgery or placement of an endotracheal tube, while avoiding an excessive dose which can be associated with such side effects as hypotension, dysrhythmias, myocardial ischaemia and excessive respiratory depression. As these agents generally have narrow therapeutic indices, carefully judged dose regimens are required to deliver the appropriate amount of drug to the brain to achieve an acceptable depth of anaesthesia whilst avoiding the consequences of under- or over-dosing. Examination of clinical practice suggests that these aims are not always achieved. Underdosing at induction has led to a documented incidence of associated adverse events including recall of events at induction (McKenna and Wilton, 1973) and sympathetic stimulation producing tachycardia, hypertension and myocardial ischaemia (Ludbrook et al., 1997). Overdose has also been associated with a significant incidence of hypotension and potential mortality (Ludbrook et al., 1993).

For the last 50 years, sodium thiopentone has been the most popular agent used for routine intravenous induction. This popularity stems from the rapid onset of anaesthesia following its bolus administration and its relatively safe record in clinical practice in experienced hands. It does, however, have a narrow therapeutic index and there is a significant incidence of hypotension if excessive doses are used (Reilly, 1994). This was particularly evident during the early stages of its introduction into clinical anaesthetic practice in the 1930's and 1940's. Reported data from this era suggest a high incidence of hypotension associated with administration of thiopentone according to published dosing formulae based on body weight, particularly when administered without a clear understanding of factors, such as hypovolaemia, which might drastically reduce dose requirements (Bennetts, 1995). These types of situations remain, even today, a trap for the inexperienced anaesthetist .

Propofol is a hindered phenol that was introduced as an intravenous induction agent in the late 1970's, and now challenges the popularity of thiopentone. When

first introduced, it superficially appeared little different from thiopentone. Early reports suggested similar pharmacokinetic properties, although the recovery from anaesthesia appeared more rapid, with similar effects on the central nervous and cardiovascular systems (Jones, 1982; Glen, 1980).

Further experience has revealed some significant pharmacokinetic and pharmacodynamic differences between these agents and it has become apparent from clinical studies that administration of propofol at induction of anaesthesia requires careful choice of dose regimen. For example, there is an increased incidence of hypotension at induction with propofol rather than with more established induction agents (Canessa et al., 1991). The frequency of this problem is well documented. An analysis of 25,000 cases of propofol use in anaesthesia found that hypotension developed in over 15% of cases. In over three quarters of these cases, hypotension developed within 10 minutes of the commencement of induction (Hug, Jr. et al., 1993). Fatal cardiovascular collapse following rapid administration of propofol at induction is also well described (Warden and Pickford, 1995; Mackay, 1996). While it has been suggested that altered dose regimens such as slower administration may reduce this incidence (Dundee et al., 1986), the rationale behind this is not explicitly stated.

It is possible that an incomplete understanding of the pharmacokinetics of this drug at induction of anaesthesia underlies this problem, as appeared to be the case with the introduction of thiopentone. Although there has been extensive analysis of the pharmacokinetics of propofol, the majority of these studies have focussed upon the maintenance phase of anaesthesia, and the use of pharmacokinetic modelling to devise optimal dose regimens for this period of anaesthesia (Smith et al., 1994). However, the incidence of adverse events at induction suggests that the pharmacokinetics during this phase of administration may be at least as important. In fact, studies of the pharmacokinetics of propofol at induction are quite limited. The most common approach is to use conventional compartmental pharmacokinetic theory to describe this period of rapid injection. Close examination of the available data reveals many flaws and inconsistencies with this approach, and these deficiencies are particularly apparent when dealing with observed clinical phenomena such as dose sparing with either slower administration rates (Peacock et al., 1992; Peacock et al., 1990; Stokes and Hutton, 1991) or co-administration of other agents ("co-induction") (Short and Chui, 1991; Johansen et al., 1995). There has been considerable debate on the processes involved in these phenomena, such as a change in drug distribution or a change in drug-receptor interactions, but this debate remains speculative because of the paucity of data on drug distribution to the brain during this period.

Further advances in optimising dose regimens of propofol require an understanding of the process of drug distribution from the point of injection to the site of action. Simultaneously, a systematic examination of the effects of propofol on factors such as cerebral blood flow (important for both drug delivery to the brain and cerebral functioning), blood pressure, and depth of anaesthesia is required. Only with these data interrelating drug delivery, distribution and effects can the process of induction with propofol be fully understood, and guidelines for optimal dose regimens be developed.

In the following sections of this chapter, the published data on the pharmacokinetics and pharmacodynamics of propofol will be discussed, with an emphasis on data relevant to periods of rapid administration, such as at induction of anaesthesia. The methods used to collect these data will also be reviewed, with a view to the development of techniques to allow the simultaneous study of the time-course of both the cerebral concentrations and effects of propofol following rapid intravenous administration.

## 1.2 PHARMACOKINETICS

### 1.2.1 GENERAL PHARMACOKINETIC APPROACHES

Drugs are administered via a range of routes and in different dose regimens, usually with the aim of achieving a desired effect by delivery of an appropriate quantity of drug to a specific organ or effector site (sometimes termed the "biophase"). Critical to the determination of the dose regimen to achieve this aim is a knowledge of drug disposition in the body and, in particular, the determinants of uptake and elution of the relevant drug into and out of the target site. To this end, a variety of pharmacokinetic methods have been developed that can be broadly divided into "systemic" and "regional" pharmacokinetic methods. The use of systemic pharmacokinetics seeks to infer the organ drug concentrations from analysis of the pattern of drug concentrations in systemic blood. Regional pharmacokinetics attempts to address this problem by measurements of drug concentrations at the target organ, thus moving the site of drug measurement closer to the biophase and ultimately leaving only drug-organ parenchyma-receptor interactions to explain any discrepancies between the time-courses of drug concentrations and pharmacological effects. In the following section a range of pharmacokinetic approaches will be reviewed, particularly in relation to the study of cerebral pharmacology.

#### 1.2.1.1 Pharmacokinetic modelling

In general, pharmacokinetic models are of 3 types: compartmental; physiological; and empirical (Tucker, 1994).

1.2.1.2 Compartmental modelling

This approach is usually based on analysis of the time-course of concentrations of the drug in question in the systemic blood, most commonly an analysis of the decay of concentrations following completion of administration. A compartmental model is generated from these concentrations, with the number of compartments, and the rate and extent of distribution between these compartments, determined only by the fit of mathematical functions to the data. There is usually no attempt to match these compartments to anatomical structures, and the concentrations in a compartment can be heterogeneous. Definitions of a compartment generally only require that there are proportional changes in drug concentration within the compartment (Cutler, 1978). In practice, most models involve a central compartment into which a drug instantly mixes (and which is sometimes mistakenly assumed to represent mixing in the blood and well perfused tissues), and from where the drug is both distributed and eliminated.

While these models are easily formulated and applied because of the relatively simple mathematical analyses required, and can be used to describe and predict concentrations in blood under many circumstances, the fact that they have no physiological or anatomical basis imposes a number of limitations.

The first is the assumption that instantaneous mixing occurs in a central compartment. It is intuitively obvious that the drug concentration in a central compartment at time zero, the time administration is commenced, is zero. In practice, the first blood sample is taken after the drug appears in the systemic circulation, and the concentration at time zero is determined by back extrapolation from the concentration-time curve. The highest theoretically predicted drug concentration is therefore at time zero, when in practice mixing cannot have occurred. This is obviously at odds with reality. Furthermore, the value of parameters, such as volumes of distribution and rate constants, may be substantially influenced by the timing and location of the initial blood sample (Chiou, 1979; Tucker, 1981; Hull, 1994). Although in many cases this lack of consideration of initial mixing does not induce significant inaccuracies in predicting concentrations over prolonged periods, it may lead to major inaccuracies in predicting early drug concentrations when drugs are administered rapidly. For example, there are heterogeneous concentrations at different vascular sites during this initial mixing process. Thus, the site of sampling will also influence the parameters which are later calculated from the measured drug concentrations. As arterial concentrations will be higher than those in peripheral veins soon after commencement of intravenous administration of a drug, the initial volume of distribution determined using arterial samples may be much lower than if venous sampling is used (Schuttler et al., 1986). In addition, distribution from a central compartment occurs

during this mixing process, further limiting the application of a compartmental approach.

The second limitation arises because the compartments seldom have an anatomical identity, which means this approach has limited ability to predict concentrations at physical sites (Westlake, 1971). For example, the time-course of concentrations of hexobarbitone predicted in a peripheral compartment of a compartment model were greatly different than those measured in fat after rapid intravenous injection of hexobarbitone in mice (Noordhoek, 1971). That model, therefore, could not account for what is a distinct anatomical location. Despite this, some drugs do distribute according to known anatomy. Inulin distributes to a volume consistent with the known volume of the extracellular space, and indeed can be used for estimations of that volume (Belknap et al., 1987).

The third limitation is that compartmental models have limited ability to account for physiological and pharmacological changes. For example, distribution between compartments is often considered to be linear, and described by a rate constant (k). In reality, this process is often non-linear. Indeed, some drugs can alter their own uptake and elution into and out of organs by, for example, altering organ blood flow (Bjorkman et al., 1992). This will also be a dose dependent phenomenon and obviously cannot be accurately accounted for by a rate constant. These phenomena need not be drug induced. There can be significant alterations in CBF and responses due to changes such as age, $CO_2$ tension and trauma (Ludbrook et al., 1992; Bouma and Muizelaar, 1992), all of which may affect the process of drug distribution. Thus, while a compartmental model may allow a close fit of a single measured data set of blood concentrations, it may poorly predict drug concentrations under pathophysiological conditions.

*1.2.1.2.1 Effect compartment modelling*

The effect compartment approach to pharmacokinetics stemmed from simultaneous analysis of systemic pharmacokinetics and pharmacodynamics of neuromuscular blocking agents (Sheiner et al., 1979; Hull et al., 1978). Initially, this was restricted to an extension of a systemic compartmental model, with the effect compartment linked to the central compartment by a rate constant $k_{eo}$. Subsequently, a semi parametric approach which links only the plasma concentration and effect with $k_{eo}$ has also been used (Fuseau and Sheiner, 1984).

The addition of an effect compartment to a systemic compartment model to a degree overcomes the problem of lack of correlation between compartments and anatomical sites, particularly when the site of action is not central, and hysteresis between blood concentrations and effect is evident. The relationship between systemic concentrations and effect therefore allows predictions of the desired

response to be made without the need for knowledge of the time-course of regional concentrations of a drug. This technique has been successfully applied to the brain. Using EEG to measure cerebral effect, a differential hysteresis between blood concentrations and cerebral effect was observed for fentanyl and alfentanil after rapid intravenous administration (Scott et al., 1985). The values of $k_{eo}$ (expressed as half-lives) were estimated as approximately 6 and 2 minutes for the two opioids respectively, and consistent with the observed clinical duration of action of these opioids. A similar approach has been used with benzodiazepines, revealing different rates of central nervous system penetration of midazolam and diazepam (Buhrer et al., 1990), and a number of other sedative/hypnotic agents, including propofol (Schuttler et al., 1987; Schwilden et al., 1985; Schuttler et al., 1986).

Although this approach, by apparently moving closer to the biophase, can to a degree account for the disequilibrium between blood concentrations and effect, it still has some limitations. Firstly, it assumes that the effect measured is appropriate. Although this requirement is satisfied for neuromuscular blockade mentioned above, there is generally a poor correlation between EEG and clinical indicators of depth of anaesthesia (see 1.4.2.3.2), and so the time-course of "anaesthesia" may not be what is being measured. Secondly, like compartmental pharmacokinetics, rate constants are commonly used to describe the distribution to the effector site and cannot account for factors such as non-linear kinetics. The influence of CBF on brain uptake and elution of ketamine could not, for example, be accounted for using this method (Bjorkman et al., 1992). Thirdly, this approach assumes that there is a constant relationship between a drug concentration at the effector site and drug effect, and so circumstances where drug-receptor interactions may be complex may lead to inaccuracies. This is evident in the complex relationship between receptor binding and analgesic effect described for opioids such has lofentanil (Leysen et al. 1980), and has also been raised with the anaesthetic agents in the form of "acute tolerance". This term refers to the suggestion that an acute change in the effector site-response relationship occurs during the induction process with intravenous anaesthetic agents (Toner et al., 1980), and arose from the fact that systemic drug concentrations were lower during recovery from rapid drug administration than during induction. Effect compartment modelling cannot identify whether changes in drug-receptor interaction or a regional drug distribution underlie these observations.

One example of these limitations is provided by the use of a compartmental model, which includes an effect compartment, to examine the effects of inhaled anaesthetic agents and body weight on atracurium concentrations and effect (Parker and Hunter, 1993; Parker et al., 1993). Firstly, no significant correlation was found

between body mass and calculated central or steady state volume of distribution, suggesting there were unknown factors affecting concentrations which this model could not account for. Secondly, it was found that age and gender influenced $k_{eo}$. As no basis for these observations could be offered by the model, the appropriateness of the model for predicting dose regimens under a range of physiological or pathophysiological conditions is clearly limited.

1.2.1.3 Physiological modelling

Rather than fitting data to an abstract model, the physiological approach attempts to devise a model based on known anatomy, physiological responses and physico-chemical processes, and then uses these models to predict the time-course and magnitude of drug concentrations or effects (Jarvis, 1994). For example, flow models can be extremely complex and attempt to mimic as many steps as possible in the chain of absorption, distribution and elimination by taking into account factors such as perfusion, tissue structure and drug diffusion for each tissue bed. Interestingly, it was in the study of anaesthetic agents that significant early development of physiological modelling occurred (Bischoff and Dedrick, 1968; Mapleson, 1963); this has continued with increasingly complex models (Fukui and Smith, 1981; Chen and Andrade, 1976; Ebling et al., 1994).

While attempting to account for as many variables as possible, physiological modelling is not itself without problems, such as mathematical complexity and incomplete data on which to base the model. For example, in the absence of specific data it is commonly assumed that the tissue distribution of many lipophilic substances can be described by a flow limited model, where each tissue is represented as a well stirred compartment (Jarvis, 1994; Davis and Mapleson, 1993). This was assumed when data on tissue distribution of opioids in rats following rapid administration were used in a physiological model of opioids applied to man (Bjorkman et al., 1990). Data on tissue distribution in man were not available. However, a later study in the rat which specifically analysed the time-course of tissue concentrations of fentanyl and alfentanil, two lipophilic synthetic opioids, revealed diffusion barriers for most tissues, demonstrating that the assumption of flow limitation was incorrect (Bjorkman et al., 1993). The disequilibrium between myocardial venous concentrations and calculated myocardial concentrations demonstrates a similar phenomenon for lignocaine (Huang et al., 1993). This highlights both the problems of obtaining data on which to base a physiological model, as well as the problems that can result when assumptions need to be made.

### 1.2.2 PHARMACOKINETICS OF PROPOFOL

Much has been published about the disposition of propofol, but, to date, has been limited to a compartmental analysis based on concentrations in the systemic blood, with some examination of the relationship between these concentrations and effects. The limitations of this type of approach have been frequently revealed, particularly at induction, but, despite this awareness, more complex relevant pharmacokinetic analysis has not yet been undertaken. The current knowledge of the pharmacokinetics of propofol along with some of the limitations of this approach will be reviewed below.

#### 1.2.2.1 Systemic pharmacokinetics of propofol

The pharmacokinetics of propofol has most commonly 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. A large number of studies and analyses of this type have been performed, and have been the subject of a number of reviews (Smith et al., 1994; Kanto and Gepts, 1989; Cockshott, 1985). The findings have been quite consistent, even across species, with most studies finding the time-course of blood concentrations to fit a 3 compartment model.

Propofol is usually assumed to instantaneously mix in a central compartment ($V_1$), with a calculated mean volume of distribution in man of approximately 0.2 L/kg, but with a large variation (ranging between 0.1 and 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 and 4 minutes. Following this there is a slower decrease, thought to represent metabolic clearance from the blood, with a half-life of between 25 and 60 minutes. The third phase of prolonged slow decrease is believed to represent exchange between blood and fat, and has a half-life usually between 150 and 800 minutes. This third phase was not identified in some early modelling of propofol, probably because of inadequate duration of sampling after administration (Adam et al., 1983).

These systemic compartmental pharmacokinetic models have been used to predict the time-course of propofol concentrations in the blood during anaesthesia in a number of studies (White and Kenny, 1990; Tackley et al., 1989; Schuttler et al., 1988), and have generally done so reasonably well, although with considerable scatter. In a recent study in man, the performances of five models, each with different parameters values, were analysed by comparing the concentrations predicted by each model with those achieved following administration of propofol using a computer and one of the models to deliver a dose regimen predicted to produce stepwise increases in blood concentrations (Vuyk et al., 1995). Four of the models performed similarly, and one consistently underestimated blood

concentrations, but the accuracy of all models decreased at higher concentrations of propofol. These types of studies, however, involve slow administration and generally ignore the period of rapid distribution of propofol immediately after administration, a time during which they may not perform well. This was not revealed in the cited study because of delayed sampling after administration was commenced.

#### 1.2.2.2 Cerebral pharmacokinetics

There are relatively few data on the distribution of propofol to the brain, particularly after rapid administration. An autoradiographic study of propofol distribution in rats during relatively slow administration demonstrated rapid distribution of propofol into the brain, with final brain-blood partition coefficients of approximately 3:1 (Shyr et al., 1995). In this study, measurement of propofol concentrations in multiple sites in the brain and spinal cord revealed even distribution throughout the central nervous system, data consistent with the homogeneous effect of propofol on cerebral metabolic rate, which will be discussed later in this chapter (Cavazzuti et al., 1991). No data on the relative time-courses of propofol in the blood and brain were presented, however, and so no estimation of the degree or duration of blood-brain disequilibrium during the period of administration was possible. Furthermore, the absence of measurements of cerebral effects did not allow brain concentration-effect relationships to be determined.

There was evidence of disequilibrium between blood and brain in the period immediately after rapid administration of propofol in another radiographic study in rats (Simons et al., 1991a). While there was a consistent brain-blood concentration ratio of approximately 2.5 for approximately 30 minutes after bolus injection of propofol, the value at 4 minutes after administration was significantly higher (3.4), suggesting a large difference in the time-course of drug concentrations in the two regions. Disequilibrium prior to this could not be assessed as samples were not taken until 2 minutes after administration.

The relationship between blood and brain concentrations has been indirectly examined in man by simultaneous sampling of arterial and jugular venous (effluent from the brain) blood after administration of propofol at rates of 6 and 12 mg/kg/hr (Peacock et al., 1995). Following both administration rates there were large and prolonged concentration gradients between arterial and cerebral venous blood, which were maximal between 2 and 4 minutes following commencement of administration. While mass balance principles could not be used to accurately estimate the time-course of brain concentrations in the absence of measurements of cerebral blood flow, it is apparent from these data that there is prolonged disequilibrium between propofol concentrations in the arterial blood and the brain, even at the relatively slow rates of administration used in this study. It is interesting

to note that the times of onset of anaesthesia in this study (approximately 5 minutes and 2.5 minutes after the 6 and 12 mg/kg/hr dose rates, respectively) coincided with the largest concentration gradients, suggesting that large degrees of uptake were still occurring at that time and that further deepening of anaesthesia would be expected after cessation of administration.

### 1.2.2.3 Concentration-effect relationships

Because of the paucity of data on the time-course of cerebral concentrations of propofol, the time-course of effect on some cerebral parameter such as depth of anaesthesia or an electrophysiological parameter has frequently been substituted.

Studies examining this relationship during prolonged administration, when pseudo-steady-state between blood and brain is expected, have generally demonstrated a consistent relationship. The recovery phase of anaesthesia has often been used to take measurements, as the rates of change of concentrations and effects are relatively slow. For example, concentrations in man of approximately 1 μg/ml were associated with a high rate of response to verbal stimulus, while concentrations above approximately 2.5 μg/ml had a low rate of response (Wessen et al., 1993; Kay et al., 1986; Adam et al., 1983; Shafer et al., 1988). Concentrations required for surgical anaesthesia in man have generally been found to be relatively consistent, but with some variation with different levels of surgical stimulus (Kanto and Gepts, 1989). There is evidence of some variation in this concentration-effect relationship between species, but consistent relationships have usually been found within species (Adam et al., 1980). These types of studies, often conducted using probit analysis, can be effective at determining blood levels at pseudo-equilibrium at which onset of anaesthesia occurs, but the 'all or nothing' nature of the response means that the time-course of effect cannot be followed. In man, a consistent relationship between propofol concentrations in blood and both EEG changes and probability of response to verbal stimuli in man has been demonstrated when measurements were made 30 minutes after commencement of propofol (Forrest et al., 1994). The infusion regimen in this study had previously been shown to produce stable blood levels of propofol within 5 minutes of commencement of administration.

Despite these findings, there is evidence that this approach of relating blood concentrations to effect is insufficient to completely account for the pharmacokinetics of propofol. During a period of relatively rapid changes in drug concentrations, such as following rapid administration at induction of anaesthesia, it might be expected that these disequilibria would be more pronounced. This in fact can be identified. For example, it is interesting to note the 30 minute interval between onset of administration of propofol and measurements of depth of anaesthesia in the study cited in the previous paragraph (Forrest et al., 1994). This

interval was used because the same group had found evidence of a rightward shift of the blood concentration-cerebral response relationship earlier in the induction process. Using the same dose regimen, the dose found to be effective in 50% of cases was found to be higher at 10 minutes compared to that recorded between 20 and 120 minutes (Dunnet et al., 1994). This would suggest either a persistence of blood-brain disequilibrium at 10 minutes or a change in drug-receptor interaction, but these possibilities were not considered further.

Two studies examining blood concentrations of propofol and effects on electroencephalography and tail flick in rats have concluded that there is a good relationship between blood concentrations and cerebral effects after both rapid bolus injection and after slow infusion at 60 mg/kg/hr (Tan et al., 1993; Shyr et al., 1993). While this is apparent in the displayed EEG trace following infusion administration, examination of changes in various EEG parameters over time following the bolus administration reveals that the peak changes occurred approximately 2-3 minutes after administration. It is likely that peak blood concentrations were reached at some time prior to the first arterial blood sample taken at 30-60 seconds, as propofol concentrations were decreasing at the time of the first sample. There is therefore evidence of hysteresis between blood concentration and cerebral effect. Interestingly, in the same study, simultaneous measurement of tail flick response was reported to show that effective antinociception occurred only during the period of 60-90 seconds post dose. As peak EEG effects occurred later than that, this is a demonstration of the lack of correlation between electrical and antinociceptive measurements of cerebral effect of some drugs.

The problems this hysteresis might produce have previously been recognised. While a good correlation between concentrations in blood and response to verbal stimulus during recovery from anaesthesia with propofol has been demonstrated in man (Adam et al., 1983), the same study recognised a mixing phase very early after administration which was not accounted for by the compartmental model fitted to the data after that time. The authors concluded that blood concentrations would therefore be of little value in predicting hypnotic effect during this phase.

This hysteresis has been confirmed. Simultaneous measurement of EEG and blood concentrations of propofol in man has found that higher blood concentrations were associated with fixed endpoints of effect, such as burst suppression, during induction rather than maintenance of anaesthesia (Hazeaux et al., 1987), suggesting a period of disequilibrium. In a similar study, blood levels at onset of anaesthesia were approximately double those which produced satisfactory anaesthesia during maintenance (Herregods et al., 1989).

### 1.2.2.4 Effect compartment modelling of propofol

Despite the recognition of disequilibrium between blood concentrations and cerebral effects, only one group appears to have formally analysed this phenomenon using effect compartment modelling (Schuttler et al., 1986). The EEG effects of propofol after very rapid intravenous administration were related to blood concentrations, with the findings of a prolonged disequilibrium with a half-life of 2.9 minutes. While this provides valuable information on the relative time-course of blood concentrations and effect, there are limitations to this approach (see section 1.2.1.2.1).

### 1.2.2.5 Specific pharmacokinetic problems at induction

It would appear that the general limitations of a compartmental approach to pharmacokinetics discussed previously (see section 1.2.1.2) are particularly relevant in the early phase following rapid distribution of propofol. As propofol is usually initially administered in this manner and as it is a drug with a relatively low therapeutic index, these limitations might be critical when using compartmental models to generate dose regimens. Indeed, the complexity of the pharmacokinetics of propofol, the limitations of the current approach, and the relationship to cerebral effects during this stage of anaesthesia were all acknowledged in a large review of the pharmacokinetics of propofol (Kanto and Gepts, 1989). A solution, however, was not offered.

Examination of the published pharmacokinetic literature on propofol reveals some specific inconsistencies which may relate to these limitations of compartmental modelling; these will be discussed separately.

#### 1.2.2.5.1 Blood sampling

The site of blood sampling is critical to modelling when using blood concentrations of a drug to determine the model parameters. The time-course of concentrations in arterial blood reflects the concentration of drug delivered to the organs, while the time-course of venous concentrations reflects factors such as the delay in passage of drug through an organ bed, blood flow through that organ, and the balance between rates of drug uptake and elution in that organ. It is therefore not surprising that large differences have been shown in arterial and venous concentrations of propofol for 1-2 minutes after rapid administration (Major et al., 1983).

Model parameters calculated from the time-course of concentrations taken simultaneously from arterial and venous sites have been shown to be markedly different. In one study of rapid propofol administration, the initial volume of distribution calculated from arterial concentrations was approximately half that calculated simultaneously from venous concentrations (Schuttler et al., 1986). Discrepancies between parameters that are not related to the initial mixing phase in

the circulation, such as steady state volume of distribution, were much smaller. A comparison of two independently determined models of propofol pharmacokinetics, one calculated using arterial and the other using venous concentrations, shows similar discrepancies (Sear and Glen, 1995).

Venous concentrations may themselves differ depending on the site of sampling. For example, in one study cerebral venous propofol concentrations were shown to be much lower than those recorded in more peripheral venous sites in previous studies using similar administration rates, reflecting different rates of regional distribution (Peacock et al., 1995). This must be considered when comparing different compartmental pharmacokinetic models. In recognition of this, an interesting approach to the problem of using "arterial" blood for sampling has been to attempt to minimise the differences between arterial and venous concentrations by a process termed arterialisation of venous blood. Techniques such as limb warming have been used to induce high limb blood flow rates which greatly exceed tissue metabolic needs, therefore in essence generating a functional shunt. While this has succeeded in minimising the concentration differences between the two sampling sites (Okell et al., 1991; Johnston et al., 1996), it still does not overcome the problem of disequilibrium between the blood and the brain.

1.2.2.5.2 Rate of administration

The rate of administration of propofol may influence calculated pharmacokinetic parameters. In a study of the pharmacokinetics of propofol in which the first blood sample was taken 5 minutes after cessation of administration, the calculated initial volume of distribution increased with decreasing administration rates (Adam et al., 1983). Conventional pharmacokinetics, in which initial distribution is assumed to be instantaneous, does not provide a mechanism to account for this observation, which perhaps relates to the early mixing phase of propofol in the blood. In fact, the authors of this study commented on a very rapid redistribution phase identified in a few subjects which could not be accurately characterised. This may reflect the initial mixing phase in the blood which was poorly identified because of the delay in commencement of blood sampling. If pharmacokinetic models vary with the rate of administration, they should not be used to predict blood concentrations for dose regimens involving different administration rates.

Rate of administration has also been shown to affect the relationship between blood concentrations and cerebral effects. In man, reducing the rate of administration of propofol was associated with a trend towards greater blood concentrations at the point of onset of loss of consciousness, although this did not prove to be statistically significant due to inter-individual variation (Stokes and Hutton, 1991). Furthermore, slower rates of administration were associated with a statistically significant decrease in administered dose. Similar findings have been shown in rats where

both high and low administration rates of propofol were associated with increased dose requirements to produce onset of anaesthesia (Larsson and Wahlstrom, 1994). Speculation about these findings in both papers centred around potential disequilibrium with the "biophase", assumed to be the brain, and the factors which therefore might determine the rate of uptake of propofol into the brain.

1.2.2.5.3 Extra-hepatic metabolic clearance

Calculated clearance values for propofol are high, in the order of 25-30 ml/kg/min (Shafer et al., 1988; Kay et al., 1985), and generally exceed liver blood flow which is of the order of 15 ml/kg/min; these findings have usually been interpreted as representing extra-hepatic metabolism.

There is evidence of significant extraction of drugs during the initial passage of drugs from injection site to the systemic circulation. Up to 75% of injected opioids may fail to reach the systemic circulation after i.v. injection, with evidence of uptake and elution of these drugs occurring rapidly during the initial pass through the lung (Roerig et al., 1987). A similar phenomenon has been described with other drugs such as verapamil and diazepam, and the degree of this distribution has been attributed to physicochemical properties of these drugs such as lipid solubility (Roerig et al., 1987). Furthermore, the degree of extraction may be variable, as co-administration of propranolol and fentanyl, both lipophilic amines, has been shown to reduce lung uptake of fentanyl (Roerig et al., 1989). Differentiation between simple distribution through lung tissue and active metabolism in lung tissue is rarely performed, as this requires either demonstration of efflux of all administered drug from the lung using mass balance principles (by prolonged sampling of systemic and pulmonary arterial blood), or direct measurement of drug, or drug metabolites, in lung tissue.

Significant lung extraction of propofol has been proposed, although there is a lack of data on this area in man. Temporary large gradients between the central venous and arterial concentrations of propofol have previously been demonstrated immediately after rapid injection of propofol (Major et al., 1983), although such studies do not differentiate between lung uptake and processes such as vascular mixing during passage along the vascular tree. In another study, a comparison of the time-course of central venous and systemic arterial concentrations of propofol failed to find evidence of significant extraction (Gray et al., 1992).

There is evidence of extraction across the lung in other species. Extraction of 60% of the administered dose of propofol during first pass through the lung has been reported in cats using a double indicator technique and indocyanine green (Matot et al., 1993). The accuracy of these results could be questioned, however, as the double indicator technique used in this study may only measure delayed passage

through the lung. Also, this technique does not differentiate between uptake and metabolism, although evidence of diffusion back into the circulation in the study cited above suggested that uptake was at least partially involved. In the same study, the rate of extraction was reduced by co-administration of halothane and fentanyl, but not positive pressure ventilation. This is consistent with metabolism of propofol in the lung, as halothane and other volatile agents are known to reduce lung extraction of bioamines via inhibition of monoamine oxidase (Cook and Brandom, 1982), but it could also be an effect on active transport and simply relate to distribution rather than metabolism. Metabolism of propofol in the lung certainly appears to occur in sheep. Large concentration gradients across the lung in sheep have been measured during prolonged administration (Mather et al., 1989), and the lack of detectable quantities of propofol in lung tissues on subsequent tissue analysis supports the hypothesis of active metabolism. This study also demonstrated decreasing extraction with increasing dose rate, suggestion a saturable metabolic process, unlike the dose-rate independent extraction which simultaneously occurred in the liver. The finding in rats of a high blood-lung ratio of propofol metabolites within a few minutes of bolus injection of propofol lends further support for rapidly acting metabolism in the lungs (Simons et al., 1991b).

Propofol is a phenol, and metabolism is predominantly via conversion to glucuronide and sulphate conjugates, which is usually thought to occur predominantly in the liver. Enzymes for conjugation of phenols, however, also exist in the lung (Aitio, 1976; Cassidy and Houston, 1984) and high extraction (eg 60%) due to metabolism, with evidence of saturation at higher dose rates, has been demonstrated during first pass in rats (Cassidy and Houston, 1980a; Cassidy and Houston, 1984; Cassidy and Houston, 1980b).

1.2.2.5.4 Variability in calculated parameters

While the average values for specific parameters were outlined above, there is large inter-individual variation. Although some of this can be explained by factors such as changing clearance with changing hepatic function, there is no obvious reason for this difference in the early phase after rapid administration.

Changes in the initial distribution of propofol with age provide an example of such inter-individual variation. Higher relative induction dose requirements of children are well recognised, a fact "explained" in conventional compartmental pharmacokinetic terms by the higher calculated volume of distribution of the central compartment (for body weight) reported for children (Kataria et al., 1994; Saint-Maurice et al., 1989). On the other hand, advanced age has been shown to be associated with a reduction in dose requirements at induction (Dyck et al., 1991; Dundee et al., 1986), again usually "explained" by a reduction in initial distribution volume. As these compartments are not defined physiologically, it is difficult to

determine the factors responsible for this variation. Changes in fluid volumes, cardiac output and relative perfusion of different organ beds have been proposed, but not clearly identified. There are other examples where it is even more difficult to "account" for observed variability with conventional compartmental pharmacokinetic approaches. A large difference in calculated initial volume of distribution between volunteers and surgical patients (56 L vs 37 L, respectively) has been reported for propofol (Schuttler et al., 1985), despite good weight matching between groups. As age differences (24 vs 40 years) should have had little effect on fluid volumes, other factors affecting initial mixing in the blood, such as differences in cardiac output, need to be considered.

1.2.2.5.5 Co-administration at induction

A further example of the problems of relating blood concentrations of propofol to cerebral effect relates to the phenomenon of co-administration of drugs with propofol at induction of anaesthesia. Under conditions of pseudo steady-state, such as during the maintenance phase of anaesthesia, an additive effect of co-administration of anaesthetic agents can be demonstrated. This has clearly been shown with minimum alveolar concentration (MAC) equivalents for inhaled anaesthetic agents. MAC is the alveolar concentration at which 50% of patients do not respond to a standard surgical skin incision. One MAC can be achieved by the administration of two different agents with a combined concentration equivalent to 1 MAC (Torri et al., 1974; Saidman and Eger II, 1964), suggesting that the effects of volatile agents are simply additive. Similarly, it has been shown that the blood concentration of propofol required to achieve a set endpoint of anaesthesia can be reduced with co-administration of drugs such as opioids (Wessen et al., 1993).

This relationship appears more complex, however, during a period of rapid drug administration. In unpremedicated man, administration of a small dose of midazolam, a sedative-hypnotic agent, immediately prior to induction of anaesthesia with propofol has been shown to produce a large reduction in the dose required to achieve loss of response to either a noxious or a verbal stimulus (Short and Chui, 1991). This "dose sparing" was shown to exceed the simple additive effects of the individual agents. This was considered to be due to a synergistic effect at a receptor level (both agents act at least partly through the GABA receptor (Salonen and Maze, 1993)) and not a pharmacokinetic phenomenon, and was thought to have been supported by a subsequent study which failed to demonstrate a change in blood concentrations of propofol with co-administration of these two drugs (Teh et al., 1994).

A pharmacokinetic mechanism for these observations, however, may deserve greater consideration. Firstly, it is a feature of these studies that subjects are, of necessity, unpremedicated and therefore likely to be anxious. Furthermore, they

are usually young and systemically fit. As anxiety can be associated with a high cardiac output state, the potential for redistribution of propofol away from the brain may exist (Gaffney et al., 1988). Co-induction could therefore potentially act via anxiolysis and reversal of this redistribution, rather than any receptor synergistic effect. Secondly, a similar synergism has also been demonstrated following co-induction with midazolam and fentanyl (Ben Shlomo et al., 1990). As the cerebral sites of action of these drugs are quite different, it is difficult to propose a mechanism of receptor based synergism. Fentanyl may, however, have a significant anxiolytic effect and therefore alter drug distribution. Thirdly, studies which have examined the pharmacokinetic effects of co-induction with propofol have often not been well designed to do so. The study cited above which found no change in blood concentrations following co-administration of midazolam and propofol (Teh et al., 1994) was conducted during the maintenance phase of anaesthesia, when disequilibrium and effects on drug redistribution would be expected to be minimal. In another study, which found no effect of co-administration of propofol and fentanyl on the pharmacokinetic profiles of these drugs, initial sampling of blood was delayed, thereby reducing the chance of detecting early changes in concentrations (Gill et al., 1990). In fact, one of the early studies of the pharmacokinetics of propofol following pre-administration of fentanyl did find a large increase in initial propofol concentrations in blood with co-induction, resulting in a 50% decrease in the calculated initial distribution volume (Cockshott et al., 1987). As suggested above, this is consistent with anxiolysis and drug redistribution .

Separation of pharmacokinetic and receptor effects is possible with measurement of effect site concentrations (in this case the brain), but techniques for this are difficult, as will be discussed later in this chapter. It is also possible to separate these two effects using co-induction with drugs which alter pharmacokinetics but are not active at brain receptor sites. The use of esmolol (a beta receptor antagonist) as a co-induction agent has provided some evidence to support a pharmacokinetic explanation of synergism in man. Administration of esmolol immediately prior to administration of propofol provided a large "dose-sparing" effect at induction of anaesthesia, a finding which could not be explained by the authors (Johansen et al., 1995). As esmolol has no effect on conscious state or at the possible sites of action of propofol in the brain or on cerebral blood flow, the most likely explanation was an esmolol-induced reduction in cardiac output, resulting in a relative increase in distribution of propofol to the central nervous system.

#### 1.2.2.6 Summary

From the above review of the pharmacokinetics of propofol, it is evident that the compartmental approach undertaken to date does not adequately characterise the

time-course of the effects of propofol on the brain during the period of induction, or provide insight into the mechanisms underlying some of the observed phenomena associated with propofol at induction. This restricts the development of effective dose strategies to optimise the delivery of propofol at induction and thereby minimise the incidence of adverse events associated with propofol administration. Many questions about dose regimens have been raised by the studies performed to date, but only detailed examination of the time-course of distribution of propofol to the brain, the predominant effector site for anaesthesia, can provide answers.

## 1.3 PHARMACODYNAMICS

While a drug can have effects on almost any organ system, administration of anaesthetic agents usually targets the central nervous system, and this will be the primary focus in this review. The general approach to modelling the relationship between drug effects and drug concentrations will be addressed first.

### 1.3.1 PHARMACODYNAMIC MODELLING

Pharmacodynamic modelling relates the observed drug effects with drug concentrations under steady state conditions, but does not relate the time-courses of concentrations and effect. Various approaches have been reviewed (Holford and Sheiner, 1981; Schwinghammer and Kroboth, 1988) and are briefly discussed below.

#### 1.3.1.1 Linear and log linear models

In linear models there is a straight line relationship between concentration and effect, and parameters of this type of model are easily determined using linear regression of the time-course of effects. This approach can be mathematically simple, and can account for systems where there is no effect when drug concentration is zero. It is inadequate, however, for the description of many biological systems, particularly when effects have baseline values or when the effects have a ceiling of values. For example, the maximal changes in CBF recorded following administration of thiopentone (Michenfelder, 1974) and propofol (Artru et al., 1992) require that non-linear functions are used to describe the relationship between drug concentrations and effect on CBF. Log transformation of drug concentrations can be performed, but these models are then not able to describe drug effect when drug concentrations are zero.

#### 1.3.1.2 $E_{max}$ models

These models are popular because the mathematical relationship between drug concentration and effect accomodates both no drug effect at a zero drug concentration and a maximum drug effect (Holford and Sheiner, 1981; Schnider et al., 1994). They also allow standard measures of drug potency to be determined,

such as the concentration ($EC_{50}$) or dose ($ED_{50}$) which produces 50% of the effect. This type of model more closely represents the relationship between drug concentration and effect on CBF in the papers cited above and, for example, provided the best fit of data relating midazolam concentrations to changes in EEG amplitude in rats (Mandema and Danhof, 1992).

#### 1.3.1.3 Sigmoid $E_{max}$ models

This model produces an 'S' shaped relationship between concentration and changes in effect, and is calculated using the Hill Equation which was originally derived to describe the haemoglobin-oxygen dissociation curve (Hill, 1910). This has been used to provide a close fit of the relationship between thiopentone concentrations in blood and depression of spectral edge analysis of EEG signals (Hudson et al., 1983). A good fit has also been found with this type of model and data relating ketamine (Schuttler et al., 1987) and benzodiazepine concentrations to EEG effect (Mandema et al., 1992).

### 1.3.2 CEREBRAL PHARMACODYNAMICS OF PROPOFOL

In reviewing the pharmacokinetics of propofol, three specific effects of propofol on the brain which are important during the stage of induction of anaesthesia (depth of anaesthesia, CBF and blood pressure) were identified. Depth of anaesthesia, a complex and poorly defined parameter, is of obvious importance, being the primary objective of propofol administration. Cerebral blood flow changes are also potentially important for a number of reasons. Maintenance of adequate flow is essential for delivery of metabolic substrates for cerebral metabolism, itself altered by propofol. In addition, minimising CBF without risking cerebral ischaemia may be important in the treatment of some pathophysiological states, such as raised intracranial pressure. This will be discussed later in this chapter. Furthermore, as was mentioned previously, CBF changes may themselves affect regional pharmacokinetics by altering the distribution of propofol to the brain. Blood pressure changes at induction have already been discussed as a clinically significant problem, and blood pressure is one of the determinants of CBF. There are data suggesting that the risk of hypotension may be altered with different dose regimens, and therefore is potentially related to drug distribution.

In addition to these three effects, the influence of propofol on cerebral metabolism, while not specifically addressed in the pharmacokinetic review, is also an important consideration at induction. This relates both to a link between cerebral metabolism and depth of anaesthesia, and the need for "coupling" of flow and metabolism to optimise substrate delivery mentioned above.

The available data on these pharmacodynamic effects of propofol will be therefore be reviewed below.

### 1.3.2.1 Effect of propofol on neuronal function

As with many of the sedative-hypnotic drugs used in anaesthesia, there is a limited understanding of the mechanisms underlying propofol's actions, and this relates in part to the incomplete understanding of cerebral physiology and pharmacology.

It appears likely that propofol, like the benzodiazepines, has an action at the $GABA_A$ receptor. This is composed of glycoprotein subunits which form a functional membrane channel (Tenelian et al., 1993), and binding of an agonist leads to opening of that channel and influx of chloride ions. The resulting depolarisation can be either inhibitory or excitatory depending on whether sufficient depolarisation is achieved to activate voltage dependent conduction, but, in general, administration of GABA analogues induces sedation. Specific effects, however, depend on which neuronal pathways are affected.

The interaction of propofol with the GABA receptor is as yet incompletely understood. In the rat there are data to suggest that propofol enhances GABA binding to the $GABA_A$ receptor (Peduto et al., 1991), and propofol in clinically relevant concentrations has been shown to potentiate GABA mediated pre- and post-synaptic inhibition (Collins, 1988). Propofol has also been shown to enhance GABA binding in a dose dependent fashion, in a similar manner but perhaps through different mechanisms or receptor sites to barbiturates and steroid anaesthetics (Concas et al., 1991). The concept of separate sites of action for these drugs is supported by the observation that the benzodiazepine antagonist flumazenil may partially reverse the anaesthetic action of thiopentone but does not affect the duration of effect of propofol (Fassoulaki et al., 1993).

There are other possible explanations for the action of propofol on the brain. Volatile anaesthetic agents may inhibit sodium channel opening, necessary for neuronal depolarisation in the central nervous system (Wann, 1993), and propofol may act similarly. Propofol has been shown to depress the opening of isolated human brain sodium channels *in vitro*, with the concentration to produce half maximal depression being approximately 20 μmol/L (Frenkel et al., 1993). This is comparable to the free plasma concentrations commonly achieved after induction of anaesthesia with conventional dose regimens (35 μmol/L or 6 μg/ml), suggesting that this effect may occur at therapeutic doses.

The regions in the brain where propofol has its major effects are also incompletely understood. A study of the effect of propofol on cerebral metabolism found widespread homogeneous effects throughout the brain (Cavazzuti et al., 1991), data consistent with the widespread distribution of $GABA_A$ receptors in the brain. Measurements of the effects of propofol using electrophysiological monitoring have identified some specific sites which relate to anaesthetic effect. Propofol appears to have an action in the brainstem at the thalamic level (Angel, 1993), and so disrupts

ascending sensory information, but there is evidence that there is also a direct widespread cortical effect producing a reduced response to sensory input (Angel, 1993; Angel and LeBeau, 1992).

Thus, although the exact site of effect of propofol in the brain is unclear, it is apparent that its sites of action are widespread throughout the brain. As it appears that propofol distributes relatively homogeneously throughout the brain, measurements of global brain concentrations of propofol will probably suffice as organ concentrations relevant to cerebral effects.

#### 1.3.2.2 Cerebral metabolism

As approximately 50-60% of cerebral metabolic rate (CMR) is a result of synaptic transmission, propofol might be expected, from the cerebral mechanisms of propofol described above, to have a depressant effect on CMR. This has been supported by a number of studies in different species, including man. One of the first studies of propofol described a 36% decrease in CMR for oxygen ($CMRO_2$) and accompanying EEG evidence of neuronal depression in man after bolus administration of propofol in a dose sufficient to induce anaesthesia (Stephan et al., 1988). Similar methods were used in a second study that year using a three stage infusion regimen, with EEG evidence of neuronal depression and a 28% decrease in CMR (Vandesteene et al., 1988). Both studies examined CMR at only one level of anaesthesia. These results have been supported in studies in other species which have looked at the dose-response relationship. The CMR and EEG power have been reported to gradually decrease during increasing doses of propofol administered to rabbits, and one study estimated that EEG isoelectricity, the point of maximum drug induced depression of neuronal activity, was associated with a 47% decrease in CMR (Ramani et al., 1992). This figure is consistent with the propofol induced inhibition of synaptic transmission mentioned above, and is almost identical to the maximal CMR depression associated with isoelectricity found for thiopentone, a barbiturate anaesthetic agent (Michenfelder, 1974). It is also consistent with the maximal degree of depression of glucose utilisation recorded after propofol administration to rats (Cavazzuti et al., 1991).

Other studies have found smaller degrees of CMR depression with propofol. A study in dogs also found a dose-dependent effect of propofol on CMR and EEG, although there was only a 30% reduction in CMR at doses of propofol which appeared to have induced near maximal EEG depression (Artru et al., 1992). In baboons, dose dependent effects on both CMR for oxygen and CMR for glucose were recorded, with maximal recorded decreases (not statistically significant from baseline) of 22% and 36% respectively (Van Hemelrijck et al., 1990). As neuronal electrical activity was not measured, it cannot be determined whether this represented maximal neuronal depression, but the administered doses were not

large. Furthermore, the results appear to have been skewed by one animal with a low starting metabolic rate and little observed change after propofol.

While the effects of propofol on global CMR are well described, it is possible that there are significant regional differences in propofol effects because of the heterogeneous nature of the brain. This has been examined in rats using a labelled deoxy-glucose technique to measure metabolism in 35 regions of the brain and spinal cord following a single dose of propofol (Cavazzuti et al., 1991). There was minimal regional variation in the decrease in cerebral metabolism induced by propofol, with a mean decrease of approximately 60%. This uniform effect is similar to that found following administration of barbiturates (Crosby et al., 1978), but other general anaesthetic agents such as the volatile agents can induce more regional variation, a fact which may be attributable to different, but as yet poorly understood, mechanisms of action (Shapiro et al., 1978).

#### 1.3.2.3 Cerebral blood flow

Anaesthetic agents have a variable effect on cerebral blood flow (CBF). Volatile agents generally induce cerebral vasodilatation, particularly at higher doses, producing gradual relative increases in CBF as CMR decreases (Reinstrup and Uski, 1994). In contrast, many of the commonly used intravenous anaesthetic agents such as benzodiazepines, barbiturates and etomidate produce parallel dose-dependent decreases in both CBF and CMR (Michenfelder, 1990). Coupling of flow and metabolism are considered to be preserved with these agents, with the observed CBF decreases thought to be secondary to drug induced decreases in CMR.

There is some evidence that the effects of propofol on CBF are similar to those of other intravenous anaesthetic agents. At single administration rates, decreases in CBF of 51% and 27% were recorded in man in some of the first studies of CBF and propofol in 1988 (Stephan et al., 1988; Vandesteene et al., 1988). In both cases, however, these CBF decreases exceeded the concomitant decreases in CMR (36% and 18% respectively), a pattern also demonstrated in a study in baboons (Van Hemelrijck et al., 1990). Propofol-induced CBF changes were shown to be dose-dependent in both rabbits and dogs, with maximal decreases of approximately 60% in both species (Ramani et al., 1992; Artru et al., 1992). Although CBF and CMR appeared to be matched in rabbits, maximal reductions in CBF were nearly double those of CMR in dogs.

Despite a general picture with propofol administration that decreases in both CMR and CBF occur, most studies have recorded relatively greater CBF changes. It has been proposed that inadvertent hypotension or concomitant use of other anaesthetic agents in the experimental preparations may partially underlie these

observations. It is certainly true that in some of these studies propofol administration was associated with significant hypotension (a 10-25% decrease in mean arterial pressure) which may have lowered CBF by altering perfusion pressure, but this may only be a partial explanation. In some studies discrepancies in CMR and CBF changes have been recorded despite no significant change in blood pressure. Furthermore, there is now evidence that cerebral autoregulation, the process by which CBF is maintained despite large changes in cerebral perfusion pressure, is well preserved during propofol administration (Strebel et al., 1995; Matta et al., 1995), and some of the recorded CBF-CMR discrepancies occurred with perfusion pressures within the auto-regulatory range. In contrast, parallel changes in both parameters have been demonstrated in rabbits when blood pressure was maintained with angiotensin (Ramani et al., 1992).

The influence of concomitant anaesthetic agents is not clear. A range of drugs such as opioids, volatile anaesthetics and nitrous oxide ($N_2O$), each with different and sometimes incompletely understood effects on CBF and CMR, have been used in these studies and may have contributed to the observed results. It is perhaps significant that a decrease in CBF relative to CMR has been recorded in man despite the use of no other anaesthetic agent (Stephan et al., 1988).

#### 1.3.2.4 Blood pressure

It was recognised nearly 10 years ago that administration of propofol, particularly via rapid bolus at induction, was frequently associated with decreases in mean arterial pressure (MAP) (Stephan et al., 1986; Monk et al., 1987); this effect has been implicated in documented examples of mortality and morbidity (Warden and Pickford, 1995). The subsequent decrease in cerebral perfusion pressure (CPP), the gradient between mean arterial pressure and intracranial pressure, can be detrimental to the brain in two ways. Initial small decreases in blood pressure can induce reflex cerebral vasodilatation, potentially increasing intracranial volume and pressure. This has the potential to reduce CPP and induce cerebral ischaemia if cerebral elastance is high. Marked reductions in blood pressure may however, critically reduce CBF simply by reducing CPP directly (Zauner and Muizelaar, 1996).

There have been numerous studies into the haemodynamic effects of this drug in order to define the exact mechanisms involved in hypotension, but the picture is still incomplete. It is clear that propofol has an effect on the peripheral vasculature, with studies generally finding decreases in both total peripheral resistance and pre-load (Pagel and Warltier, 1993; Stephan et al., 1986; Monk et al., 1987). This has been demonstrated to produce hypotension *in vivo* in man even when cardiac performance has been totally preserved by the use of implanted artificial hearts (Rouby et al., 1991). The mechanism behind this is not completely clear, and both

endothelium dependent and calcium channel effects have been proposed (Petros et al., 1993; Chang and Davis, 1993).

Propofol also has an effect on myocardial contractility. *In vitro* studies on isolated myocardial muscle have shown a dose dependent negative inotrope effect through a mechanism which may be different to that seen following administration of volatile anaesthetic agents (Azuma et al., 1993; Azari and Cork, 1993; Puttick and Terrar, 1993). Propofol administered by infusion to both pigs and dogs was found to induce systemic hypotension and dose dependent depression in contractility, even when blood concentrations were as low as 1 μg/ml (Coetzee et al., 1989; Pagel and Warltier, 1993). However, it would appear that for equivalent anaesthetic concentrations, the negative inotropic effects of propofol are less than with thiopentone (Cork et al., 1991). These results are largely supported by *in vivo* findings.

Although this general pattern of cardiovascular effects has been found in man, the relative contributions of myocardial depression and systemic vasodilatation to hypotension varies between studies (Aun et al., 1993; Grounds et al., 1985; Gauss et al., 1991; Lippmann, 1991). For example, in one study patients administered a large dose of propofol rapidly (3 mg/kg of propofol over 20 seconds) developed a 20% decrease in mean arterial pressure, due to a combination of a 10% decrease in cardiac index and a decrease in systemic vascular resistance (Vohra et al., 1991). In this study, however, the lack of invasive monitoring did not allow discrimination between direct myocardial depressant effect or pre-load reduction as a cause of the cardiac index changes. When more direct assessment of myocardial performance has been possible, for example using ventriculography and invasive cardiovascular monitoring, it has been concluded that cardiac performance is relatively preserved during propofol administration, perhaps by indirect reflex sympathetic activity, despite the known direct negative inotropic effects of this agent (Lepage et al., 1991).

A further contributing factor to hypotension may be direct CNS depression. A reduction in renal sympathetic activity during propofol administration has been recorded (Tiurmina et al., 1993) and proposed as a mechanism contributing to hypotension following propofol administration. Furthermore, propofol induced depression of the arterial baroreceptor reflex has been reported (Cullen et al., 1987; Ebert and Muzi, 1994) and may limit the normal homeostatic responses to hypotension.

In reality, it is likely that the cardiovascular effects following administration of this drug are a result of a complex interaction of direct drug effects on the myocardium and systemic vessels as well as cardiovascular homeostatic responses such as those mediated through the baroreceptor reflexes. The rate and pattern of propofol

administration may therefore be important with respect to the extent and nature of cardiovascular effects. This is supported by findings of a reduction in the degree of hypotension when slower rates of propofol administration have been used (Stokes and Hutton, 1991; Dundee et al., 1986).

## 1.4 METHODS AVAILABLE FOR STUDIES OF CEREBRAL PHARMACOKINETICS AND PHARMACODYNAMICS

The data reviewed above suggest that a knowledge of the time-course of propofol concentrations in the brain as well as the blood are important if the process of induction is to be understood. In addition, simultaneous measurement of certain effects on the brain would allow clarification of the relationship between the pharmacokinetics and pharmacodynamics of propofol. The available methods for undertaking such studies were therefore reviewed.

### 1.4.1 CEREBRAL PHARMACOKINETIC METHODS

Measurement of regional drug concentrations has been performed for a number of regions and organs, and the available methods will be reviewed, with particular emphasis on the relevance to studies of cerebral concentrations during rapid drug administration.

#### 1.4.1.1 Tissue biopsies

Direct tissue sampling for later drug analysis can be utilised to determine regional drug concentrations. Either serial samples can be taken in the same subject, or post mortem samples can be taken at different time points after drug administration in large numbers of animals. Serial biopsies in the same subject rely on ready access to the target organ, sufficient organ size to avoid organ dysfunction, and the ability to take samples with sufficient frequency to accurately delineate the profile of changing drug concentrations. These conditions can be met for such examples as the distribution of thiopentone into subcutaneous fat (Price et al., 1960) or myocardial concentrations of local anaesthetic agents in sheep (Nancarrow et al., 1987), but the brain presents more of a problem because of the anatomy of the skull, limitations on the number and frequency of biopsies possible, potential problems of changes in drug disposition due to cerebral damage and resultant changes in CBF or tissue diffusion, and, usually, the need for concomitant anaesthesia. For example, while serial brain biopsies over 5 hours have been used to determine the cerebral pharmacokinetics of misonidazole in dogs, the use of barbiturate anaesthesia to obtain biopsies in some animals induced increases in plasma concentrations of misonidazole (White et al., 1979). The anaesthesia no doubt altered drug disposition. In addition, this technique only allowed infrequent sampling to be performed. Similar techniques have been used to examine the tissue:blood partition coefficients for local anaesthetics in the brain of sheep

(Nancarrow et al., 1987). This required an anaesthetised preparation and biopsies of 50-75 mg were taken only at steady-state. Determination of the time-course of brain drug concentrations during rapid drug administration is limited by the number of biopsies possible in a sheep brain weighing approximately 90-100g.

Alternatively, biopsies can be taken post mortem using animals killed at different time points following drug administration in order to delineate the time-course of drug distribution in different tissues, including the brain. This approach has been used in rats to characterise the tissue pharmacokinetics of fentanyl and alfentanil, two synthetic opioids with markedly different physicochemical properties; animals were killed as frequently as every minute (Bjorkman et al., 1993). These data were then used to determine that a one compartment tissue model could account for the brain uptake of alfentanil, but not fentanyl, for which significant diffusion limitation existed. The organ disposition of thiopentone was studied in the rat using similar techniques, in the development of a complex physiological model to describe thiopentone pharmacokinetics (Ebling et al., 1994). While these methods allow measurement of drug concentrations at specific tissue sites, they require large numbers of animals (Ebling et al., for example, used 73 rats) and rely on the assumption that the process of killing the animals does not affect drug disposition.

#### 1.4.1.2 Autoradiographic studies

A similar approach is taken in autoradiographic studies, with distribution of radioactivity being used to determine the distribution of a radio-labelled drug. For example, differences in the rate of uptake and elution in the central nervous system of thiopentone and phenobarbitone have been identified in a number of species, data which correlates with the known duration of central nervous system effect of these two barbiturates (Cassano et al., 1967). While this method has been used for measurement of propofol distribution in the brain (Rhodes and Longshaw, 1977), this study provided no information on the time-course of distribution. As with tissue biopsies, these studies would require the sacrifice of large numbers of animals at different time points to allow accurate measurement of distribution after rapid intravenous injection.

#### 1.4.1.3 Microdialysis

Microdialysis is a technique in which intermittent samples of organ interstitial fluid are taken for drug analysis to define the pharmacokinetics in particular regions, including the brain. Recently, pharmacokinetic studies have been reported in awake animals. For example, systemic and brain pharmacokinetic parameters of SDZ ICM 567, a serotonin antagonist, have been reported in the awake rat (Alonso et al., 1995). In a study of cerebral pharmacokinetic and pharmacodynamics in rats, this technique was used to measure blood and brain concentrations of

morphine and its 6-glucuronide metabolite, whilst drug induced analgesia was measured using the tail flick response (Stain et al., 1995). Although similar rates of elimination of morphine from both brain and blood were found, and it was determined that morphine 6-glucuronide was a more potent analgesic agent than morphine, they did not examine kinetics and dynamics whilst concentrations were changing rapidly. In summary, microdialysis does allow the accurate measurement of regional drug concentrations and it can be used in awake animals.

#### 1.4.1.4 Isolated organs

Artificial isolation of a specific organ can simplify the vascular supply and allow ready access to afferent and efferent blood for both flow measurements and blood sampling for drug analysis. This can be achieved *in vitro*, with an isolated artificially perfused organ or by auto-transplantation. For example, an artificially perfused isolated heart preparation and mass balance principles have been used to measure the uptake of propafenone into the rabbit heart and to relate this to the myocardial effects of the drug (Gillis and Kates, 1988). Auto-transplantation *in vivo* is practical for some organs and has been used for pharmacokinetic studies of the liver in the dog (Jacqz et al., 1986). Although these methods allow accurate characterisation of the time-course of drugs in specific organs, induced changes in the blood or perfusate composition and alterations in organ function and blood flow control may prevent extrapolation of the results to the physiological setting. Complex anatomy and blood flow control preclude such studies in the brain.

#### 1.4.1.5 Mass balance principles

Mass balance principles for use in regional pharmacokinetics apply the Law of Conservation of Matter. Organ blood flow and the time-course of concentrations of a drug in arterial and organ venous blood are simultaneously measured, and it is assumed that any drug which enters, but is not measured to leave, remains in the organ. Knowledge of the volume or mass of that organ then allows the concentration to be determined. While simple in principle, there are a number of conditions which must be met in order for accurate estimations of the time-course of drug concentrations to be made (Upton et al., 1988), which will be elaborated upon below.

##### 1.4.1.5.1 Vascular anatomy

Vascular anatomy and physiology should be well described, and appropriate sampling and flow measurement techniques used. Although it can generally be assumed that drug concentrations in the arterial "tree" will accurately reflect input into an organ, sites of venous sampling must be carefully chosen to ensure that venous blood is representative of that organ, and in particular to avoid contamination from other vascular beds. For example, ligation of the hemiazygous

vein is necessary in the sheep to avoid contamination of myocardial effluent blood in the coronary sinus blood with systemic venous blood (Huang, 1991). The cerebral venous anatomy is complex and careful catheter positioning in man (Peacock et al., 1995) and ligation of some vessels in dogs (Michenfelder et al., 1968) has been proven necessary to ensure "pure" cerebral venous blood is sampled. The relevant cerebral venous anatomy for the sheep will be discussed later in this thesis in chapter 4. Changes in the distribution of blood flow should be also minimal during the period of measurement, to avoid a potential change in the area of an organ from which blood is sampled. For example, redistribution of CBF has been recorded following the use of isoflurane as an anaesthetic agent, while halothane has a more homogeneous effect (Hansen et al., 1988).

1.4.1.5.2 Non-vascular drug loss

As only blood draining from the organ is usually sampled, it is important that other sites of drug loss are excluded if these mass balance principles are to remain valid. For example, direct diffusion of some drugs through skin layers has been detected (Singh and Roberts, 1993) and indeed this skin diffusion is now increasingly used therapeutically as a route of administration of drugs. Lymphatic drainage has also been identified as a site of drug loss from individual organs. For example, even after a rapid intravenous injection of L-dopa, it appeared in the lymph within a few minutes of administration (Sudo et al., 1995); different sites of sampling of lymph demonstrated different rates of transfer from different regions. However, the unique anatomy of the brain means that these routes of drug loss are not a significant problem. The brain does, however, have a separate compartment containing cerebrospinal fluid (CSF), a possible site of drug loss. Relatively rapid penetration of the CSF by barbiturates has been reported with drug concentrations comparable with those in the plasma (Frey et al., 1979). For other sedative/hypnotic drugs, however, the concentrations achieved are relatively low (Moffat et al., 1995). Drug metabolism in an organ is another potential source of error and can lead to over-estimation of drug concentrations. For example, sulphation of drugs in the brain has been reported (Baranczyk Kuzma et al., 1993) and enzyme systems which can metabolise phenols have been identified in the human brain (Hwang et al., 1995; Hurd et al., 1993).

1.4.1.5.3 Transit times

Transit time of blood should be small in proportion to the time delay between equal concentrations being reached in arterial and venous concentrations, otherwise transit time can contribute to calculations of drug concentrations. For example, at the instant a drug is first detected in arterial blood it is evident that the venous concentration will be zero, and so mass balance calculations will produce a positive flux into the organ (and a positive drug concentration in the organ) prior to the drug

actually reaching the organ. In organs such as the heart and kidney maximum transit times have proven to be sufficiently small (Coulam et al., 1966) in comparison to the time to reach equal arterial and venous concentrations for most drugs, thus contributing a negligible error to mass balance calculations. Transit times in the brain have been reported in man with techniques for measurement of CBF (Tamaki et al., 1984; Nighoghossian et al., 1996) and in pigs (Bjorkman et al., 1992); the similar small values to those reported for the heart and kidney suggest the error in cerebral mass balance calculations due to transit time should be minimal.

1.4.1.5.4 Blood sampling and assay

To accurately characterise the concentration versus time curves for both arterial and organ venous blood, especially when concentrations are changing rapidly, it is important that methods used for blood sampling should be adequate and that samples are taken at sufficiently frequent intervals (Huang et al., 1991; Upton et al., 1988). Analysis of mass balance principles has now allowed sampling intervals to be easily determined in individual studies to provide sufficient accuracy without the need to collect excessive numbers of samples (Upton, 1994).

1.4.1.5.5 Organ blood flow

Cerebral blood flow must be frequently measured for accurate calculation of organ drug concentrations, as the drugs, such as anaesthetic agents, may themselves alter CBF. For example, ketamine and midazolam have both been shown to reduce CBF and thus their own rate of uptake into the brain (Bjorkman et al., 1992), and so an assumption of constant flow would overestimate brain concentrations.

### 1.4.2 CEREBRAL PHARMACODYNAMIC METHODS

As was previously mentioned, induction agents are usually administered at the induction of anaesthesia with the primary intention of altering specific aspects of cerebral function. The methods available for measurement of these will be reviewed.

#### 1.4.2.1 Cerebral blood flow

Accurate measurement of cerebral blood flow (CBF) has proven difficult both experimentally and clinically, due in part to the relative inaccessibility of the brain and the complexities of the cerebrovascular anatomy. A diverse range of methods to measure CBF have been devised, each with particular advantages and limitations; some of the most popular methods will be discussed below.

1.4.2.1.1 Indicator methods

Indicator methods are those in which the rate of delivery or removal of a substance to or from the brain by the blood stream is measured, and can be broadly grouped

into those which use indicators that readily diffuse in and out of the brain, and those that remain intravascular. All these methods measure CBF intermittently, with the measurement interval dependent on the rate of uptake and elution of the indicator, and require a constant flow rate during the measurement period. This limits their application to studies requiring very frequent measurements of CBF such as those described in this thesis. Nevertheless, commonly used methods using specific indicators will be briefly reviewed.

*Diffusible indicators*

These methods are based on the Fick principle which states that "if the quantity of a tracer increases or decreases during 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" (Fick, 1870). Alternatively, the rate of change of the indicator can be measured directly in the brain (Kety, 1957). The indicator should be neither metabolised nor pharmacologically active, should have a short half-life, and should equilibrate rapidly between blood and the tissues in the area of interest.

Nitrous oxide was one of the first indicators used (Kety and Schmidt, 1945) and remains in use today, but, faces problems such as incomplete pseudo equilibrium with the brain after 10 minutes (Reid et al., 1992) and nitrous oxide induced changes in CBF (Reinstrup et al., 1994). Hydrogen clearance is an established method of CBF measurement (Doyle et al., 1975; Fein et al., 1975) and is popular because hydrogen concentrations can be readily measured using implanted platinum electrodes and polarography and also because local measurements are possible (von Kummer, 1984). Problems with this method include the need for simultaneous measurements from multiple sites to determine global flow and potential changes in CBF behaviour in the region being measured due to traumatic damage to the brain during electrode insertion (Young, 1984; Tuor and Farrar, 1984). Xenon (Xe) is an inert freely diffusible gas, and can be detected in the brain using radioactive xenon ($^{133}$Xe) and scintillation counters (Risberg et al., 1975) or non-radioactive Xe and computer tomography (Gur et al., 1989). These techniques provide repeatable intermittent measurements of CBF, but the equipment required for scintillography limits its application in awake unrestrained animals and there are suggestions that Xe can affect CBF (Junck et al., 1985).

*Non diffusible indicators*

Using similar principles to those involved in measurement of cardiac output with dye dilution or thermodilution, CBF can be measured by injecting a known quantity of an indicator that remains intravascular and measuring the time-course of its appearance in cerebral venous blood. While the principles involved are relatively

simple, measurements can still only be made intermittently and, as with cardiac output measurements (Runciman et al., 1981), errors due to incomplete indicator mixing, variable rate of injection and recirculation may decrease accuracy. Alternately, microspheres may be used. Plastic microspheres of appropriate diameter are trapped in capillary beds, and labelling with radioactive tracers allows those trapped in a region to be counted. A comparison with the number counted in another body organ with known blood flow indicates flow to that region (Heymann et al., 1977; Buckberg et al., 1971). These techniques were first used to measure CBF in 1970 (Roth et al., 1970) and have subsequently been used and validated extensively. Problems with this method include the need for appropriate selection of microsphere size, which is critical for both microsphere distribution and trapping (Marcus et al., 1976), expense, potential inaccuracy under pathophysiological states (Heiss and Traupe, 1981; Marcus et al., 1981), and the fact that the number of measurements is limited by the number of available isotopes.

1.4.2.1.2 Flowmeter methods

*Cerebral vasculature*

Although electromagnetic and ultrasonic Doppler flow meters implanted on arteries or veins can be used to accurately and continuously measure flow to specific organs, the vascular anatomy of the brain limits the effectiveness of these techniques in the measurement of CBF. The arterial supply to the brain is variable across species, but is frequently via multiple vessels. Furthermore, the proportion of total flow in any single vessel may not be constant. For example, the arterial supply of the brain in man is via two carotid and two vertebral vessels yet, in the absence of arterial disease, occlusion of a single vessel will not induce cerebral ischaemia because of the collateral supply from the Circle of Willis. Furthermore, flow redistribution between major cerebral vessels can be detected (Abboud, 1981), therefore accurate measurement of global CBF cannot be assured unless flow in all vessels is simultaneously measured. Measurement of arterial flow is further limited in a number of species such as goats, sheep and cattle where carotid arterial blood passes through a rete mirabile, a vascular bed for heat exchange, before entering the brain (Garcia Villalon et al., 1989; Draehmpaehl, 1988; Lluch et al., 1985). Indeed such a rete has even been reported in isolated cases in man (Fuwa, 1994; Itoyama et al., 1993). This restricts access to vessels carrying blood solely to the brain.

Alternatively, flow in cerebral veins or sinuses may be used to measure CBF. Post capillary blood passes through cerebral veins and then to sinuses, which are structures with walls devoid of smooth muscle and composed of only endothelium surrounded by dura mater (Capra and Kapp, 1987). This anatomical feature of sinuses removes one of the problems associated with the use of flow meters on

cerebral arteries. Some flow meters measure blood velocity, which is only proportional to flow when the vessel cross-sectional area and the pattern of flow remain constant. The lack of smooth muscle in cerebral sinuses, and their rigid walls, results in a constant vessel diameter over a wide range of intraluminal pressures and in the presence of drugs or physiological changes which alter smooth muscle tone. Blood velocity therefore remains proportional to total flow, if the flow profile remains constant.

Probe placement is also critical because of the distribution of cerebral blood and the risk of measurement of extra-cerebral blood flow, especially as significant species variations in cerebral venous anatomy exist. In man, cerebral venous blood drains via the cerebral sinuses to reach the jugular veins and is joined by significant quantities of extra-cerebral venous blood from the facial veins. Sampling from the proximal jugular vein, however, results in blood with less than 3-5% contamination with extra-cerebral blood (Zauner and Muizelaar, 1996) and this site has been used for the continuous measurement of CBF in man, although this method predominantly measures ipsilateral flow (Touho et al., 1991).

In animals, access to veins or sinuses containing relatively pure cerebral blood is more difficult because of influx of extra-cerebral blood from the subcutaneous tissues (Hegedus and Shackelford, 1965). In some species, such as the dog and rat, it has been necessary to ligate the veins containing extra-cerebral blood (Michenfelder et al., 1968; Nilsson and Siesjo, 1983). This has allowed continuous accurate measurements of CBF to be made successfully (Stange et al., 1989; Michenfelder and Milde, 1988).

The relevant cerebral venous anatomy of the sheep has been described. It has been demonstrated using dissection and microsphere flow measurement techniques that blood in the dorsal sagittal sinus represents approximately 75% of cerebral venous blood, that this blood drains from both hemispheres and that it is almost completely free of extra-cerebral blood as long as blood sampling from the transverse sinuses is avoided (Hales, 1973; Hales, 1972). This evidence has been used to sample cerebral effluent blood for the purposes of measurement of cerebral extraction of oxygen and drugs (Szeto et al., 1980; Rosenberg, 1988), but measurement of flow as an indicator of CBF has not been attempted despite the relatively easy access to the dorsal sagittal sinus through a craniotomy or burr hole.

*Implanted ultrasonic Doppler flow meters*

The velocity of blood in specific vessels can be accurately calculated from the Doppler frequency shift following reflection of sound waves off the moving blood components if the angle between the ultrasonic beam and blood flow is known. This can be translated into a measurement of blood flow if the nature of the flow

profile and the cross sectional area of the vessel are also known (Hartley and Cole, 1974). Technological advances mean that Doppler transducers can be chronically implanted onto even very small vessels, including intracranial arteries and sinuses, allowing continuous measurements in the unrestrained animal (van Bel et al., 1994). As discussed previously, vessel selection is critical in measurement of CBF as local flow may not be representative of other areas of the brain. Although velocity measurements are highly accurate using this type of method, because the relationship between velocity and flow depends partly on the cross-sectional area, calculations of flow measurements assume no change in vessel diameter. This is obviously not the case for cerebral arteries where vessel diameter is under the control of smooth muscle tone which can change with numerous factors including nitric oxide, adenosine production, autonomic discharge, pH and drugs (Armstead and Leffler, 1992; Faraci and Heistad, 1990).

Studies which have examined the relationship between cerebral arterial blood velocity recorded using Doppler technology and CBF flow have frequently detected significant discrepancies between the two variables, especially when techniques known to alter cerebrovascular resistance were used to change CBF (Sorteberg et al., 1989; Sonesson and Herin, 1988). For example, in piglets there was a non-linear relationship between most indices of velocity and flow during carbon dioxide induced changes in CBF, with velocity increases always less than increases in CBF (Hansen et al., 1983). This is consistent with velocity measurements not accounting for increases in vessel cross-sectional area and the authors concluded that this method could not be used as a quantitative measure of CBF. In another study in rabbits, basilar artery blood velocity and cerebral blood flow using hydrogen clearance were simultaneously measured during changes in arterial $PaCO_2$ (Nelson et al., 1990). There were major discrepancies between recorded changes in blood flow and velocity. For example, an increase in $PaCO_2$ induced a 133% increase in CBF but only a 70% increase in blood velocity; data consistent with velocity measurements underestimating flow changes because of increases in cerebral vessel cross sectional area.

*Trans-cranial Doppler flow meters*

This method allows signal penetration through high density bone to a distance of up to 10 cm from the probe by the use of low frequency ultrasound, and thus the velocity of blood in segments of the Circle of Willis can be selectively measured continuously. Since its inception, this method has been further refined and is now a widely used monitor of CBF. It does share the potential problems of the relationship between blood velocity and flow with other arterial Doppler methods and has the added problem of inconsistencies in the angle of the ultrasound beam with blood flow, a factor which can add errors of up to 15% (Seiler et al., 1986).

There is evidence that velocity and flow are not linearly related with this method. There was a non-linear relationship between Doppler signal and an electromagnetic flowmeter in a study in man, although the magnitude of the discrepancies in changes in the two variables was relatively small (Lindegaard et al., 1987). This may be related to the cross sectional area of the vessels examined. These problems were reviewed by Kontos, 1989 who, in reference to trans-cranial Doppler, concluded that "Under conditions of rapidly changing blood flows and blood pressures, the results of such techniques are of doubtful value and may be misleading" (Kontos, 1989). Although various indices such as pulsatility index can be derived from the Doppler signal in an attempt to assess the state of cerebrovascular resistance and to improve the accuracy of the recorded signal (Murkin and Lee, 1991), without a frequent assessment of vessel diameter the relationship between flow and velocity will always be subject to error. The extremes of this situation are well demonstrated by trans-cranial Doppler measurements during pathological cerebral vasospasm when, because of marked decreases in vessel diameter, cerebral velocity increases can occur concurrently with decreases in flow (Steiger et al., 1994). Ultrasonic Doppler technology would therefore appear useful as a semi-quantitative measure of changes in CBF, especially as it allows rapid changes in velocity to be continuously measured, but accurate knowledge of any changes in vessel diameter are essential if accurate quantitative flow measurements are to be made. This was highlighted in a study in cats and dogs when a very close linear correlation (r=0.98) between cerebral blood velocity using a Doppler transducer and CBF using microspheres could be achieved when observed changes in vessel diameter following changes in $PaCO_2$ were accounted for (Busija et al., 1981).

An alternate solution is to use vessels with little or no vascular smooth muscle such as cerebral sinuses or cerebral veins. Recently, a technique of continuous blood velocity measurement in the jugular bulb in man using Doppler technology was described. Cerebral blood flow measurements with this technique were compared with CBF measurements using a Kety-Schmidt method (Ohsumi et al., 1994). A close linear relationship was shown, presumably because of the relatively fixed diameter of this vessel. This suggests that this technique may have a future role in the continuous measurement of CBF in man.

*Laser Doppler*

Laser Doppler is a relatively new technique for continuous measurement of flow, and there are now numerous reports of its use to measure organ blood flow, including cerebral blood flow (Fukuda et al., 1995). While, like ultrasonic Doppler, it relies on a frequency shift for the detection of velocity changes, in laser Doppler the micro-circulation is examined. Coherent light at wave lengths of 600-800 nm is

delivered to the tissues via fibre optic cable, with the magnitude and frequency shifts of the reflected light related to the number and velocity of tissue red blood cells. This method measures frequency shifts due to red blood cell movement only in a small area of cerebral tissue, perhaps as small as 1 $mm^2$, as the light beam is narrow and probably penetrates less than 1 mm (Kiel et al., 1985; Fukuda et al., 1995). Therefore, although measurement is continuous, as with hydrogen clearance there is an assumption that regional flow is representative of the rest of the brain. However, unlike hydrogen clearance, this technique is relatively non-invasive and measurements should not themselves alter the measured flow.

Evaluation of this method in a variety of tissue types has generally found good correlation with flow measured using techniques such as hydrogen and radioactive tracer clearance. For example a close linear correlation (r=0.96) between $H_2$ clearance and laser Doppler has been reported in measurements of the regional cerebral circulation in rabbits (Fukuda et al., 1995) and in cats (Haberl et al., 1989b). However, studies comparing laser Doppler signals and observed pial vessel diameters in rabbits revealed some of the problems of regional measurements (Haberl et al., 1989b; Haberl et al., 1989a). While both variables changed in parallel following systemic stimuli such as changes in $PaCO_2$, discrepancies were revealed following topical application of vasoactive substances. It was suggested that this reflects differential penetration of the adenosine and bradykinin and that different vessels were being measured by each technique. Although a relatively new technique for the measurement of CBF, data to date suggests it has a place for the continuous measurement of regional flow, but as only local flow is measured it was not considered suitable for the studies in this thesis.

#### 1.4.2.2 Cerebral metabolism

Measurement of the rate of metabolism of biological systems is most commonly measured by the rate of consumption of metabolic substrates or the rate of production of metabolic products. Examples range from the measurement of oxygen consumption in the whole body or individual organs (indirect calorimetry) to measurement of body heat production (direct calorimetry) (Guyton, 1981). Indirect calorimetry methods are generally more easily performed, and 'metabolic carts' can be used to provide a reasonable assessment of metabolic activity in the clinical setting (Sykes et al. 1991). It must be realised, however, that the metabolic pathways and therefore the pattern of substrate consumption must remain constant for accurate measurements to be made. For example, oxygen consumption is commonly used as an index of metabolic rate, yet it may underestimate metabolic activity if anaerobic metabolism develops or if substrate consumption changes.

The metabolic rate of individual organs can also be measured using similar principles to those for the whole body, although anatomical restrictions usually restrict these to indirect calorimetry methods. These techniques have been applied to the brain.

Cerebral metabolic rate is most commonly measured indirectly by measuring metabolic substrate extraction across the brain, using arterio-venous concentration differences, and CBF. The difficulties of accurate CBF measurement have been discussed above and will not be reviewed further, but errors in measurement of this parameter will obviously impact on the accuracy of CMR measurements. The problems of sampling pure cerebral effluent blood were also discussed in the previous section, and it is essential that the blood collected for measuring extraction across the brain is not contaminated with blood draining from extra-cerebral tissues.

These techniques have been applied to CMR measurement in man and other species. In man, sampling of relatively pure cerebral venous blood for CMR estimation has been possible using the jugular bulb (Stephan et al., 1988; Vandesteene et al., 1988; Pinaud et al., 1990; Matta and Lam, 1995). Earlier studies which used sampling of blood from the jugular vein (Thomsen et al., 1989) probably provided erroneous results because of contamination with facial blood. In dogs, as with CBF measurements discussed above, the dorsal sagittal sinus is a suitable site following ligation of vessels containing venous blood from the extra-cerebral tissue of the head (Artru et al., 1992). In sheep, the minimal contamination of dorsal sagittal sinus blood has allowed this site to be used to measure cerebral oxygen consumption (Pappenheimer and Setchell, 1973). Consumption of both glucose and oxygen have commonly been used in these studies as indices of CMR and, unlike other organs, the problems of altered metabolic pathways do not contribute significantly to measurement errors because of the obligate aerobic metabolism of the brain.

An alternative approach to measurement of substrate consumption is to directly measure their concentrations in the brain . Autoradiographic measurements of the cerebral uptake of labelled glucose has been used as an index of CMR (Sokoloff, 1976), and these techniques have been applied to the effects of propofol (Cavazzuti et al., 1991) and other anaesthetic agents (Crosby et al., 1978; Shapiro et al., 1978) on cerebral metabolism. While these techniques provide an accurate picture of the rate of substrate utilisation and permit measurement of regional metabolism, autoradiographic counting has required euthanasia and sectioning of the brain.

To circumvent this, a technique using similar principles has been developed. Labelled deoxy-glucose is trapped in the brain during metabolism, and then detected using positron emission tomography (PET) for autoradiography (Alkire et al., 1995). This allows real-time measurement of glucose consumption without

anatomical dissection, but the frequency of measurements is limited by the speed at which tomography can be performed. The capital cost of PET technology is another obstacle to its use as a research tool. This technique has, however, been successfully used in man to measure the effects of propofol on CMR, but only during prolonged administration. It does not allow the very frequent measurements which would be necessary to examine the process of induction of anaesthesia.

### 1.4.2.3 Depth of anaesthesia

There is a need for a method which allows accurate reproducible measurement of the cerebral effects of sedative and anaesthetic drugs for use in clinical anaesthetic practice and in experimental pharmacological investigations of the pharmacokinetics and pharmacodynamics of these drugs. Although a number of methods have been developed which examine drug effects on the central nervous system either directly, by observing changes in cerebral function, or indirectly, by observing the response to an external stimulus, most of these have significant limitations (Newton, 1993). The field has been summarised as follows: "endeavours to find a single observation that truly represents depth of anaesthesia have been compared to those of the alchemists in their search for the philosopher's stone" (Evans and Davies, 1984).

#### 1.4.2.3.1 Clinical signs

The first formal description of the effect of anaesthetic agents was published nearly 140 years ago (Snow, 1858) and related to the inhalational agent chloroform. This classification was subsequently applied to ether anaesthesia and refined by Guedel (Guedel, 1937). The most commonly used classification included 4 stages of anaesthesia. This ranged from analgesia (without obvious change in level of consciousness), through surgical anaesthesia (a state of loss of response to surgical stimuli), to respiratory paralysis (onset of apnoea secondary to central nervous system depression). Attention was obviously focussed on the stage of surgical anaesthesia, the depth of anaesthesia at which inhaled anaesthetic agents were clinically useful. The degree of surgical stimulus which did not elicit a motor response (and also appeared to include no conscious awareness of the event) was known approximately for each of the 4 sub stages (or "planes"), allowing the anaesthetist to titrate drug administration to induce the minimum depth of anaesthesia necessary for a particular operation. For example, tooth extraction could be performed at Plane 1 of surgical anaesthesia, whilst surgery on the upper abdomen involving an increased level of sensory stimulus required deeper anaesthesia such as Plane 3 (MacIntosh and Bannister, 1943). Although this form of classification is of some practical value in clinical anaesthesia, it is qualitative and thus of limited value for scientific research. Studies are therefore usually restricted

to probit analysis of likelihood of response to a single stimulus of standard intensity, such as verbal command or noxious stimulus.

1.4.2.3.2 Electrical activity of the brain

*The Electroencephalogram (EEG)*

The EEG records cerebral post-synaptic potentials and, as there is a general relationship between EEG potentials and both cerebral blood flow and cerebral metabolism (Prior, 1987), EEG may be useful in the measurement of depth of anaesthesia during drug-induced cerebral depression. In the unprocessed EEG there is a general pattern as anaesthesia deepens; an increase in EEG amplitude, followed by an increase in slower frequencies, and then intermittent silent periods (burst suppression) until electrical silence is achieved at deep planes of anaesthesia (Michenfelder, 1974). Although this may provide a guide to the depth of anaesthesia induced by a particular drug, there is considerable variability in the response to different anaesthetic agents, especially when more than one are used in combination. For example, there is a large variation in the incidence of burst suppression observed when equipotent doses of different volatile anaesthetic agents are used as anaesthetic agents (Schwilden and Stoeckel, 1987). This, in combination with the difficulties in interpretation of the complex pattern of the unprocessed EEG, limits the usefulness of the technique as an anaesthetic monitor. These problems may be further compounded in the unrestrained animal or uncooperative subject by the addition to the low voltage signal (typically 10-100 μV) of artefact from skeletal muscle potentials (Harmel et al., 1978) and electrical power line noise. Although electronic filtering systems may minimise this interference, this may significantly alter the recorded signal from the brain (Donegan and Rampil, 1990).

The EEG output may be processed in an attempt to simplify interpretation of the signal and to minimise artefact. These techniques fall into in two broad categories. The first, time-domain, involves analysis of the EEG voltage of the raw signal with time and is used in such techniques as aperiodic analysis and burst suppression ratio (Rampil et al., 1988; Gregory and Pettus, 1986). The second, frequency domain, involves breakdown of the raw signal to measure the relative voltage (power) of the different EEG frequencies, examples of which include the compressed spectral array and density spectral array (Levy et al., 1980; Fleming and Smith, 1979). The usefulness of these measurements has been examined in a large number of studies relating conventional clinical signs of anaesthesia with recorded EEG signals during different phases of anaesthesia. While a relationship has generally been found (Thomsen et al., 1989; Rampil and Matteo, 1987; Dwyer et al., 1994; Rampil and Laster, 1992; Sidi et al., 1990; Drummond et al., 1991; Hollingsworth and Rampil, 1993; Greenwald et al. 1994), the sensitivities and

specificities are insufficient to allow processed EEG alone to be considered a reliable measure of depth of anaesthesia. For example, correlation coefficients of only 0.46-0.71 between various processed EEG parameters and conventional indicators of depth of anaesthesia such as response to voice and response to surgical incision have been reported (Greenwald et al. 1994). In evaluating EEG as a clinical tool, it was evident in one study that despite predictable changes in a number of EEG parameters with increasing concentrations of isoflurane, recorded EEG variables were not significantly different in the group which moved in response to a surgical incision compared to those that did not (Dwyer et al., 1994). Despite these difficulties, electroencephalographic techniques allowing continuous quantitative measurements of cerebral activity have been successfully used experimentally to examine the pharmacokinetic-pharmacodynamic relationships of a number of anaesthetic agents (Van Steveninck et al., 1993; Danhof and Mandema, 1992; Schuttler et al., 1987; Schuttler et al., 1985; Schwilden et al., 1985; Mandema et al., 1992; Hovinga et al., 1992; Breimer et al., 1991). However, it is apparent that care must be taken in selection of the EEG parameter recorded, depending on the drug and drug effect to be studied (Mandema and Danhof, 1992).

The place of the EEG as a measurement of depth of anaesthesia in man, a species in which detailed verbal feedback on experiences is readily attained, has been summarised by an eminent neuroanaesthetist: "as presently derived, these EEG descriptors at best provide trend information to be used in concert with other clinical signs of depth of anesthesia" (Drummond et al., 1991).

*Evoked potentials*

Peripheral stimuli produce afferent electrical signals which ascend through the brainstem, and the resulting changes in the pattern of cerebral neuronal activity can be recorded (Kaada et al., 1967). Drug induced cerebral depression will alter these electrical responses to stimulation ("evoked potentials") in response to specific visual, auditory or tactile somatosensory stimuli, and this has been used as a means of measuring depth of anaesthesia. Recordings are most frequently made from the brainstem as these responses may persist even under deep anaesthesia when cortical responses have been lost, thus allowing measurement of a wide range of depths of anaesthesia. There are technical difficulties associated with this technique, predominantly because of the comparatively small magnitude of the potentials (in the order of less then 5 μV compared with 10-100 μV for EEG), and so between 50 and 2000 potentials are usually averaged over some minutes, restricting the frequency at which measurements can be made (Hanning and Aitkenhead, 1994). As with EEG, interference due to skeletal muscle movement is a potential problem in the awake subject.

Following a stimulus, both the latency and amplitude of subsequent electrical waveforms are recorded and changes in both may have relationships with drug induced cerebral depression. For example, changes in both peak latency and amplitude have been demonstrated in some auditory evoked potentials following increasing doses of propofol (Savoia et al., 1988; Thornton et al., 1989). These studies also showed that propofol selectively altered middle latency potentials and not brainstem auditory evoked potentials, emphasising that the correct recording sites must be used and that drug effects on evoked potentials are "drug specific". While a similar dose dependent pattern similar to that seen following propofol has been detected after administration of althesin and etomidate (Thornton et al., 1986; Thornton et al., 1985), volatile agents affect evoked potentials differently. There is a dose dependent depression of both brainstem and cortical potentials, with various agents differentially affecting latencies, but not amplitudes, of the recorded cortical waves (Newton et al., 1992; Heneghan et al., 1987). There is also some evidence that changes in evoked potentials relate to conventional signs of anaesthesia, such as responses to commands following inhalation of increasing doses of isoflurane, with generally reasonable correlation between indices of anaesthesia and evoked potentials (Estrin et al., 1988; Newton et al., 1992; Schwender et al., 1994; Schwender et al., 1995). There is, however, evidence of inter-subject variability. For example, after administration of isoflurane, individual subjects developed loss of response to commands despite no change in evoked potentials, and in others the evoked potential pattern did not clearly change in a dose dependent manner (Newton et al., 1992).

Of the electro-physiological techniques available, the field of evoked potentials probably shows the greatest promise as a monitor of depth of anaesthesia, but inter-subject variability and the problem of movement artefact has limited its application to date.

1.4.2.3.3 Surface Electromyography (EMG)

Skeletal muscle receives CNS input even in the resting state, and so recording of muscle electrical activity has been considered as a monitor of changing brain activity during anaesthesia. These recordings can be performed simply, and do not require the multiple lead system usually required for EEG. While there is a demonstrated relationship between onset of anaesthesia and recorded EMG (Hameroff and Grantham, 1990; Edmonds, Jr. et al., 1986; Yli Hankala et al., 1994), the recorded signal may be differentially affected by various drugs and cannot be considered a reliable or sensitive monitor of depth of anaesthesia.

1.4.2.3.4 Oesophageal manometry

It has been proposed that CNS activity can be deduced from measurement of oesophageal motility. The oesophageal musculature exhibits three types of activity, reflecting both functional peristaltic contractions and basal activity, which are controlled at least to some degree by input from the brainstem (Christensen, 1978). This activity may therefore be influenced by drug induced brainstem depression. Such a relationship has been demonstrated following administration of anaesthetic agents (Sehhati et al., 1980; Evans et al., 1987), although poor correlation with measured drug blood levels has been reported following propofol administration (Thornton et al., 1989). As with many other methods for measurement of depth of anaesthesia, inter-patient variability reduces the effectiveness of this type of monitoring. In one study there were examples of patients inadequately anaesthetised despite no recordable oesophageal activity and others with persistent activity when apparently adequately anaesthetised (Cox and White, 1986). In addition, administration of other drugs such as anticholinergic agents may independently influence oesophageal motility and thus interfere with the relationship between anaesthesia and motility (Sehhati et al., 1978).

1.5.2.3.5 Algesimetry

An alternative method for measuring the depressant effects of anaesthetic drugs is the observation of behavioural responses to artificial applied external noxious stimuli; a concept similar to that used for evoked potentials but with a motor rather than an electrical endpoint. This technique has most frequently been applied to the examination of analgesic drugs, a field known as algesimetry. This relies upon delayed or absent responses to noxious stimuli as a result of pharmacological blockade of specific pain pathways. The techniques used can be classified according to the types of stimuli and responses utilised.

*Stimuli*

Constant intensity stimulus has been used most commonly, relying on the latency between stimulus and response to determine the degree of analgesia (Vyklicky, 1979). Alternatively, stimuli of increasing intensity may be used, with the degree of analgesia being related to the stimulus intensity required to induce an observed response (Waterman et al., 1988; Nolan et al., 1985; Nolan et al., 1987). Numerous forms of stimulus have been used, each with advantages and disadvantages. None has all the properties of an "ideal stimulus". These properties were originally proposed in 1957 and are still relevant (Beecher, 1957). Ideally, a chosen stimulus would activate solely pain receptors and pathways, thus avoiding the risk of recording a response to a non-noxious sensation. For example, application of sufficient heat will activate heat receptors prior to pain receptors, and so withdrawal

behaviour may occur once a temperature rise rather than pain is perceived. This is the problem of learned behaviour when repeated stimuli are applied.

Thermal stimulus of the skin is widely used in experimental studies, with heat applied by techniques including hot plates, heated wires, heated liquids, radiant heat sources and lasers (Nolan et al., 1987; Tarkka et al., 1992; Sotgiu et al., 1995; Necker and Hellon, 1978). The advantages of this form of stimulus include the ability to infer nociception by comparing the applied temperature to achieve a response with the temperature which induces pain in man, as it is relatively constant across species (Watkins, 1989), and the fact that only specific sensory receptors are stimulated. Its disadvantages include a risk of thermal injury to the skin, difficulties in precise control of the stimulus and a limit to the frequency with which measurements can be made because of the slow rate of tissue cooling.

Mechanical stimuli involve application of pressure to the skin or visceral organs to stimulate mechanoreceptors until a response indicative of nociception is observed (Gobel and Westphal, 1989; Vyklicky, 1979). While this form of stimulus is easy to apply and the forces are easy to measure and can be equated to those inducing pain in man, frequent application of these forces risks tissue injury.

Chemical stimuli are usually irritant substances injected subcutaneously or intra-peritoneally and induce relatively slow onset and certainly slow offset noxious stimuli. Thus, frequency of measurements are limited.

Electrical stimuli have the advantages of extremely rapid onset and offset allowing frequent measurements without risk of tissue damage, if maximum currents are limited, and have been used in algesimetry studies in a number of species including man (Vyklicky, 1979; Chapman et al., 1985; Ayhan et al., 1983; Delitto et al., 1992; Willer, 1977). There are potential problems with this form of stimulus, including stimulation of a range of modalities of sensation, changes in response threshold during first exposure to the stimulus (Vyklicky, 1979; Watkins, 1989) and pain relief induced by cutaneous electrical stimulation (Willer et al., 1982; Roche et al., 1984; Guieu et al., 1990). Cutaneous electrical stimulation activates more than one receptor type, thereby potentially cuing the subject and eliciting a response before pain is experienced. This is demonstrated through verbal responses in man where different sensations associated with increasing current levels are clearly documented (Russell, 1983). This can be partially overcome by the use of electrical tooth pulp stimulation; a stimulus thought to induce only pain, although pre-pain sensations have now been demonstrated and mechanoreceptors and heat sensitive fibres have been found to exist in teeth (Byers, 1984; Chatrian et al., 1975; Matthews et al., 1976).

*Response*

The merits of standard responses have been reviewed (Chapman et al., 1985); responses are broadly grouped into reflex and voluntary behaviour . An assumption in recording behaviour in response to an intended noxious stimulus is that nociception has triggered the response. Human studies allow complex feedback on perceived pain such as analogue pain score, but responses in other species must be interpreted. For example, while the writhing response to intra-peritoneal injection of irritant substances undoubtedly reflects nociception, it may not be equivalent to writhing in man (Siegmund et al., 1957), and limb withdrawal in response to mechanical pressure may be a learned response to avoid nociception rather than nociception itself (Ley et al., 1990).

While the techniques described above are usually related to measurement of drug induced analgesia, similar principles have been applied to the study of sedative/hypnotic drugs which act globally to depress the CNS rather than on specific pain pathways. The advantage of this form of measurement of depth of anaesthesia is that it allows a functional assessment of the effects of the drug, as ablation of a response to noxious stimuli is what is intended by administration of these drugs. A potential limitation, however, is that measurements can only be made up to light planes of anaesthesia because these techniques rely on observation of a motor response.

### 1.4.2.4 Minimum alveolar concentration (MAC)

Minimum alveolar concentration (MAC) is a concept which was introduced in an attempt to quantitate and compare the potencies of volatile anaesthetic agents and to allow correlation between the administered dose of an inhaled anaesthetic agent and its expected anaesthetic effects. MAC is defined as the concentration in the alveoli (at atmospheric pressure and steady state) that prevents a muscular response to a standard surgical skin incision in 50% of the population. A further extension of this concept is $MAC_{bar}$, the concentration at which any autonomic response to such a stimulus is prevented in 50% of the population (Roizen et al., 1981). MAC has proven valuable for comparison of the relative potencies of anaesthetic agents, especially as additivity has been demonstrated when inhaled agents are mixed (Torri et al., 1974), but it has limitations. A surgical skin incision is a difficult sensory stimulus to standardise, and as a result alternative noxious stimuli such as application of a constant electrical current to the skin have been introduced (Hornbein et al., 1982; Saidman and Eger II, 1964; Schultz et al., 1987). Subsequently these techniques have been successfully applied to measurement of the anaesthetic effects of intravenous anaesthetic agents like propofol and benzodiazepines (Short and Chui, 1991).

One limitation of these methods is the use of a constant stimulus, usually restricting measurement to an "all or none" phenomenon and probit analysis, a problem overcome in algesimetry studies by the use of a variable intensity stimulus. A method of algesimetry in sheep has been described which uses the responses to increasing intensities of both thermal and mechanical stimuli (Nolan et al., 1987). The intensity of heat required to initiate an ear flick and the intensity of mechanical pressure required to initiate limb withdrawal have been recorded following administration of different analgesic agents in a large number of studies by the same group of workers (Nolan et al., 1985; Waterman et al., 1988; Nolan et al., 1987; Livingston et al., 1991; Kyles et al., 1995; Ley et al., 1989; Kyles et al., 1993; Ley et al., 1992; Ley et al., 1991; Ley et al., 1990). These studies have demonstrated that this technique allows reproducible responses to analgesic drugs to be recorded. In particular, mechanical pressure to the limb in an awake unrestrained sheep has been demonstrated to elicit a clear leg lift at levels considered to be noxious, although this response may be masked by dysphoria and agitation following intravenous administration of opioids (Livingston et al., 1991). Of interest is the effect of sedative/hypnotic drugs on the recorded stimulus intensities. An initial study using this method reported no effect of acepromazine, a sedative non-analgesic drug (Nolan et al., 1987), on the stimulus intensity to elicit a response and concluded that this method measures analgesic but not sedative effects of drugs. A subsequent study found a change in stimulus intensity following administration of midazolam, again a sedative drug, and concluded that midazolam has an analgesic effect in sheep (Kyles et al., 1995). Although there have been claims that midazolam has an analgesic action, particularly in the spinal cord (Niv et al., 1988; Serrao et al., 1991), it seems possible that changes in conscious state induced by midazolam may have altered the response, as in MAC testing described previously. This suggests that differentiation between analgesic and sedative/hypnotic effects may not in fact be possible, and also that these techniques in sheep potentially could be used for measurement of changes in conscious state, or depth of anaesthesia.

## 1.5 SUMMARY AND AIMS OF RESEARCH

This chapter has identified that problems exist with the current administration of propofol at induction of anaesthesia, and that it would appear likely that this stems from the pharmacokinetical approaches used to date. It is apparent that the conventional systemic compartmental pharmacokinetic approach used almost exclusively with this drug to date cannot be accurately applied to rapid drug administration, such as occurs at induction of anaesthesia. Dose regimens based on this approach are therefore likely to poorly predict the time-course and magnitude of the desired drug effects on the brain. In addition, this systemic

approach cannot provide insight into phenomena such as dose-sparing with "co-induction", dose sparing and a reduced risk of hypotension with slower administration rates, or "acute tolerance". This review has also revealed that the data on specific effects of propofol on the brain are incomplete, with current data on the relative effects of propofol on CBF and CMR casting doubt on the preservation CBF-CMR "coupling".

These issues can be addressed with an integrated approach which simultaneously examines the time-course of propofol concentrations in the systemic blood and the brain as well as the time-course of effects on the brain and systemic blood pressure. The available methods for this approach were reviewed, but most have significant limitations or involve the use of assumptions. For the measurement of cerebral pharmacokinetics, the application of mass balance principles appeared suitable, but development and validation of new methods were necessary for this to be applied to the brain. To measure CBF and CMR continuously also required the development of new methods.

It was decided to use an integrated pharmacokinetic-pharmacodynamic approach to study the cerebral pharmacokinetics and pharmacodynamics of propofol during rapid intravenous administration, and to adapt an existing sheep preparation for use in these studies. The specific aims of this thesis were:

1. To develop and validate methods for application of mass balance principles to measurement of cerebral drug concentrations, and for measurement of the effects of propofol on the brain, in sheep.

2. To use these methods to examine the relationship between concentrations of propofol in the blood and brain and its cerebral effects in unrestrained sheep.

3. To examine the effects of changes in cardiac output and propofol administration rate on propofol uptake into the brain.

# CHAPTER 2. GENERAL METHODS AND MATERIALS FOR STUDIES IN SHEEP

## 2.1 ETHICAL APPROVAL

All studies conducted were approved by The Animal Ethics Committee of The University of Adelaide. Additional approval was received from The Ethics Committee of the Royal Adelaide Hospital for studies conducted in the Royal Adelaide Hospital.

## 2.2 ANIMAL SELECTION AND HANDLING

Sheep were used in these studies because of ready availability and tolerance to repeated handling and chronic instrumentation. In addition, their size means that many physiological parameters are quantitatively similar to those found in man, and their blood volume means that repeated blood sampling can be performed over time without risk of anaemia or hypovolaemia. A chronically instrumented sheep preparation (Runciman et al., 1984) was chosen to be adapted for the studies in this thesis. This preparation has been used in an extensive range of integrated pharmacokinetic and pharmacodynamic studies (Upton et al., 1991a; Upton et al., 1988; Upton et al., 1991b; Huang et al., 1993a; Huang et al., 1993b). These studies found sheep to readily adapt to housing 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 very rapid return of normal behaviour patterns.

Female Merino sheep (Ovis Aries) were purchased from Mortlock Farm of the University of Adelaide. They had been purpose bred for experimentation and, because they were selected from a single flock, were well matched for age and weight. Only sheep with haemoglobin type A, determined using electrophoresis, were used in these studies. There is some variability in the properties of different haemoglobin types in sheep, but type A haemoglobin in sheep and human adult haemoglobin have similar affinity for oxygen (Chamley and Holland, 1969; Langston et al., 1993), thereby resulting in values of haemoglobin saturation and oxygen content similar to man.

Sheep were housed in the animal house of the Faculty of Medicine at the University of Adelaide, and cared for according to the guidelines for animal care of the National Health and Medical Research Council of Australia. Within the animal house sheep were housed in a room isolated from animals used in other projects and lived in mobile metabolic crates. In these crates they had free access to lucerne chaff and water at all times, except during studies. Faeces and urine were separated by a wire grill and collected in containers beneath the crate, and food consumption and fluid balance were measured daily. Sheep were inspected at

least once per day. 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.

## 2.3 PREPARATION AND INSTRUMENTATION

### 2.3.1 ANAESTHESIA

Sheep underwent general anaesthesia for all procedures involving implantation of catheters and flow probes. Anaesthesia was induced with rapid injection of sodium thiopentone (Abbott Australasia, Kernell, NSW, Australia), 15-20 mg/kg, into the left internal jugular vein, followed by intubation of the trachea using a cuffed endotracheal tube with the aid of direct laryngoscopy. Anaesthesia was maintained with halothane (Zeneca, Cheshire, England, UK), 2% in oxygen, delivered via a vaporiser and a circle system. Artificial ventilation to normocapnia was maintained using a gas powered ventilator (Bird Mk 8, Bird Corporation, Palm Springs, CA, USA) and end expired carbon dioxide tension was monitored using an infra-red carbon dioxide analyser (Model OIR 7101, Nihon Kohden Corporation, Tokyo, Japan) attached to the endotracheal tube.

### 2.3.2 INSTRUMENTATION

Animals were initially prepared under general anaesthesia with catheters and a flow probe using aseptic techniques according to the general method of Runciman et al. (1984). In brief, after an intramuscular administration of procaine penicillin (1.25 g) and streptomycin (1.25 g) (Penstrep™, Troy Laboratories, Smithfield, NSW, Australia) the neck was shorn and the right carotid artery and jugular vein were exposed through a right sided longitudinal skin incision. All catheters (Multi-purpose A1 catheter, Cordis Corporation, Miami, FL, USA) were inserted using a Seldinger technique and correct positioning confirmed with fluoroscopy and injection through the catheters of radio-opaque dye (Angiografin™, Schering AG, Germany). For sampling of arterial blood and for measurement of arterial pressure, two 7F catheters were placed in the carotid artery and positioned with their tips at the origin of the brachiocephalic trunk. One 7F catheter, for drug administration, was placed in the jugular vein and positioned with its tip in the right atrium. Catheters were fastened to the strap muscles of the neck using a small plastic plate and exteriorised.

A flow probe for measurement of cerebral blood flow and a sagittal sinus catheter for sampling of cerebral venous blood were placed through a 19 mm trephine hole in the skull. These methods are described in detail in chapter 4. The catheter and Doppler probe wire were exteriorised and the bone plug replaced.

Sheep were then recovered from anaesthesia and transferred to their metabolic crates (figure 2-1). The catheters were connected through three-way stopcocks

(Abbott Laboratories, North Chicago, Ill, USA), extension lines (Abbott Ireland Ltd, Slago, Republic of Ireland) and continuous flushing devices (Intraflo, Abbott Laboratories, North Chicago, USA) to a gas powered flushing system which allowed each catheter to be flushed with heparinised saline (0.9% saline to which 5 I.U./ml of sodium heparin (David Bull Laboratories, Melbourne, Australia) had been added) at a rate of 3 ml/hr.

Post-operatively, sheep were able to stand and were eating within 3-4 hours of surgery. Analgesia was provided with methadone 10 mg i.m. (Parnell Laboratories) or xylazine 2.5-5 mg i.m. (Troy Laboratories, Smithfield, NSW, Australia) as necessary.

## 2.4 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 0.9% saline 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 $^{o}$C, antibiotic therapy was immediately commenced using i.v. gentamycin (Delta 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.

## 2.5 DRUG STUDIES

On experimental days, chronically instrumented sheep were transported into an experimental room in their mobile metabolic crates. Sheep were then placed in a canvas sling that allowed them to partially weightbear on their hind limbs (figure 2-1) and a strap was placed horizontally under the mandible. This technique prevented excessive movements prior to, and during, onset of anaesthesia, thus minimising interference with measurements of haemodynamic parameters and depth of anaesthesia. Transportation and placement in the sling did not appear to distress the animals.

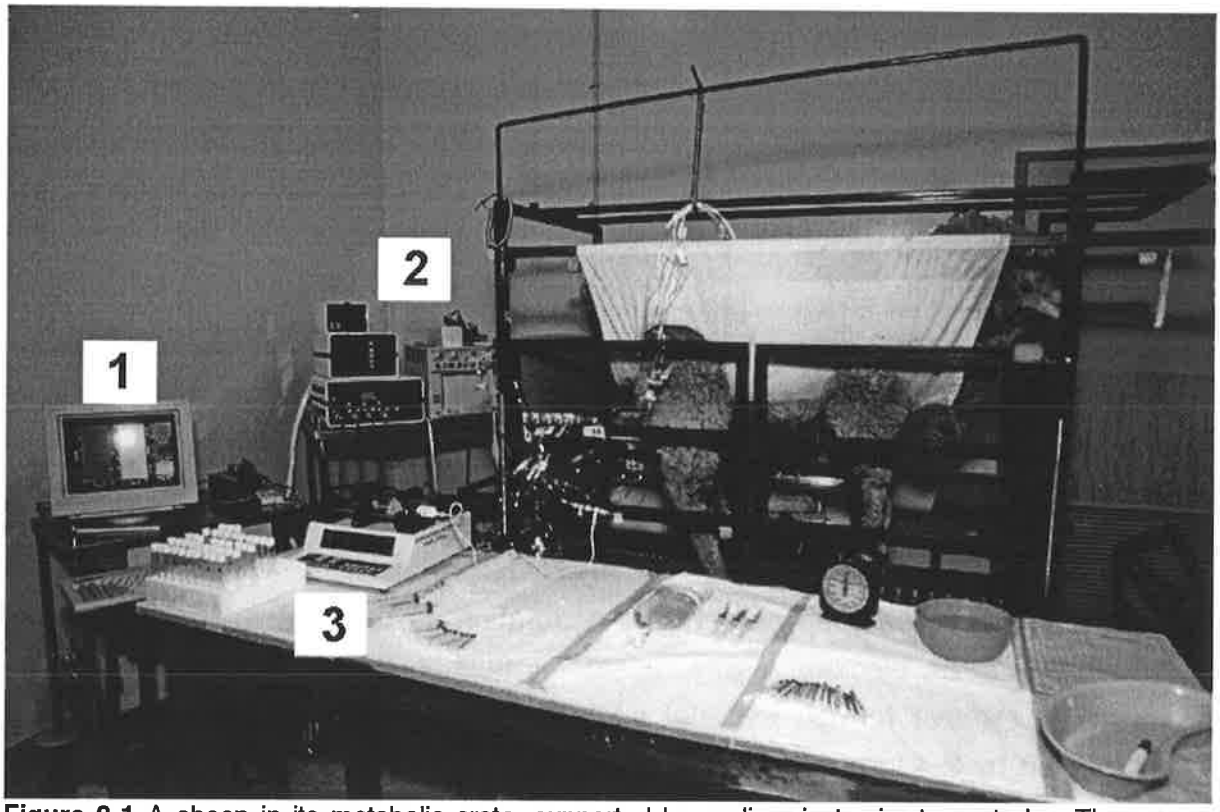


**Figure 2-1** A sheep in its metabolic crate, supported by a sling, just prior to a study. The analogue-digital system (1) and monitoring equipment (2) are visible in the background to the left. On the table in the foreground are syringes for sample collection and the syringe pump (3) with a syringe of propofol attached.

For blood pressure measurement, one of the arterial catheters was connected to a transducer (Abbott Ireland Ltd, Slago, Republic of Ireland) and the waveform continuously displayed on a monitor (Hewlett-Packard model 78345A, Boeblingen, Germany). The Doppler transducer on the sagittal sinus was connected to a Doppler flowmeter (Bioengineering, The University of Iowa, 56 M.R.F. Iowa City, Iowa, USA) operated at 20 kHz. The outputs from the blood pressure monitor and Doppler flowmeter were recorded at 1 Hz using an analogue to digital (A-D) card (Metrabyte DAS 16-G2) and a personal computer (Microbits 486-based IBM compatible) and recorded digitally on computer disc.

Drugs were administered in a 50 ml syringe (Becton Dickinson, Dublin, Ireland) attached to the right atrial catheter by a 75 cm extension line (Tuta Laboratories, Lance 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 following initial assembly of the system, and was filled with the drug solution prior to commencement of each study. All drugs were administered by constant rate infusion, with the syringe driven by a syringe pump (Model 33, Harvard Apparatus Ltd, Kent, England). The amount of drug administered was confirmed by measurement of the volume of drug in the syringe at the commencement and completion of infusion.

## 2.6 BLOOD SAMPLE HANDLING

Blood samples from the sagittal sinus and arterial brachiocephalic trunk 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 5 ml syringe attached to the stopcock distal to the sheep to account for the average catheter-stopcock dead space of approximately 1.8 ml. Next, 1 ml of blood was withdrawn into a separate 1 ml syringe attached to the proximal stopcock and transferred into 1.5 ml Eppendorf microtubes (Micro Test-tubes 3810, Eppendorf, Hamburg, Germany) which contained 25 µl of heparin (1000 I.U./ml) for later drug assay. At times when blood gas analysis or oxygen content measurement was required, a further 0.5 ml was withdrawn and placed in a 1 ml syringe to which 25 µL of heparin (1000 I.U./ml) had been added. The air was then expelled from the syringe, the tip was sealed, and the syringe was stored at 0 $^{o}$C for up to 30 minutes until gas analysis was performed.

After blood samples were taken, the stopcocks and catheter were flushed with 5 ml of heparinised saline and sealed until the next sample was taken. At the end of each study, samples were stored at -5 $^{o}$C along with 10 ml of "blank" blood (drug free blood taken prior to commencement of the study) until thawed for assay.

## 2.7 PROPOFOL ASSAYS

Propofol concentrations in whole blood were assayed using a modification of a previously described high-performance liquid chromatography (HPLC) and fluorescence detection technique (Mather et al., 1989). Samples were frozen to -5 $^{o}$C within 45 minutes of collection, and were stored for an average of 1 month and then thawed at room temperature for assay. For solvent extraction, 25 µL of thymol (0.1% solution in methanol and water, in a ratio of 1:4) to act as an internal standard, 200 µL of 1 molar $KH_2PO_4$ and 200 µL of n-hexane were added to the 1 ml sample which was then vortex-mixed (Thermolyne Maximix, Thermolyne Corp., IO, 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 $^{o}$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), and 10 µL samples 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 an HPLC column (Activon Goldpak ODS E18, Sydney, Australia) and a fluorescence detector (Perkin-Elmer LS40) using an excitation wave length of 210 nm and emission wavelength of 320 nm.

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 an $R^2$ value of 0.995 or greater. The limit of sensitivity was approximately 0.02 μg/ml.

### 2.7.1 STORAGE STABILITY

While stability of propofol in blood has previously been reported (Adam et al., 1981), more recent studies have suggested that significant loss of drug (nearly 1% per day) from frozen sheep blood occurs early in the storage phase (Weaver et al., 1995). To examine this phenomenon, the stability of propofol in sheep blood was examined over a 2 week period using the conditions of collection and storage used in all subsequent studies in this thesis.

#### 2.7.1.1 Methods

Venous blood (100 ml) was collected from the internal jugular vein of a sheep and stored as 1 ml samples which contained 25 μl of heparin (1000 I.U./ml). Known quantities of propofol were added to 3 of the samples and they were frozen to -5 $^{o}$C within 30 minutes of propofol being added. The remaining 1 ml samples were also frozen. At Day 7, 3 samples were thawed, had propofol added as described above, and were refrozen. At Day 14, a further 3 samples were thawed and had propofol added. All samples were then thawed. This method therefore provided samples of propofol in sheep blood which had been stored at -5 $^{o}$C for 0, 7 and 14 days. All samples were then assayed on Day 14 for propofol using the techniques described above. The results were then graphed, and regression analysis performed to look for concentration changes over time.

#### 2.7.1.2 Results

The changes in measured concentrations over time are displayed in figure 2-2A, and regression analysis in figure 2-2B. It was evident that, after 14 days, measured concentrations remained within the 95% confidence limits of the data.

#### 2.7.1.3 Discussion

It is evident from these data that loss of propofol from samples stored at -5 $^{o}$C over 2 weeks was significantly less than that reported by Weaver et al. This finding, in combination with the fact that relative changes over time, rather than absolute values, are critical to the findings in the studies in this thesis, suggests that storage of samples is likely to minimally influence the findings of the studies in this thesis.

## 2.8 BLOOD GAS AND OXYGEN CONTENT ANALYSIS

Arterial gas analysis was performed on blood stored at 0 $^{o}$C within 30 minutes of collection using a gas analyser (CIBA Corning 278, MA, USA). Oxygen content analysis was performed using an oximeter (IL482, Instrumentation Laboratory,

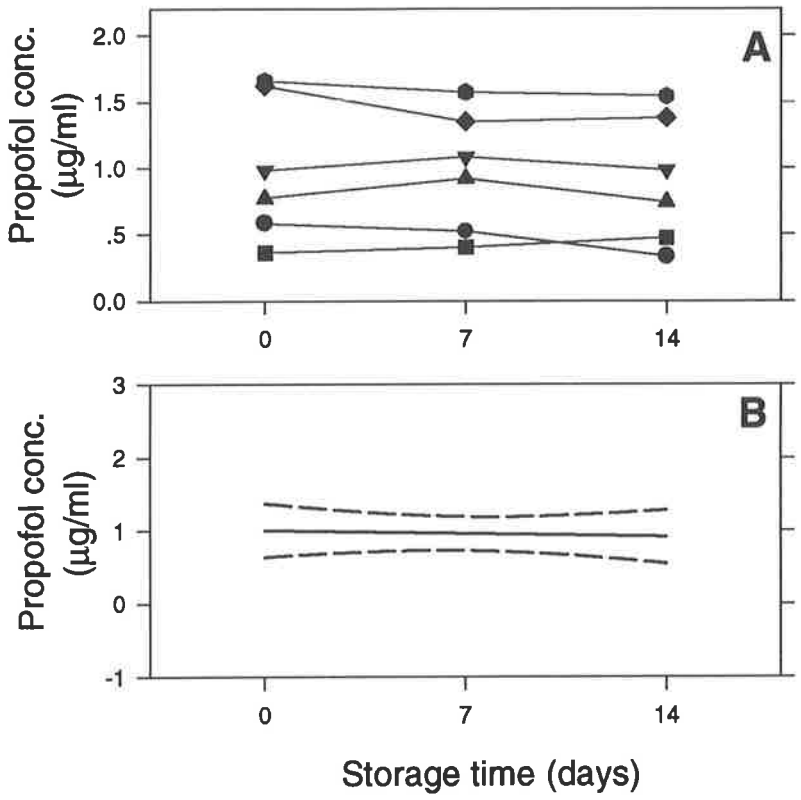


**Figure 2-2** The measured concentrations of propofol following increasing periods of storage at -5 °C. (A) shows the separate concentrations, and (B) shows regression analysis of pooled data.

Lexington, MA, USA) which had been calibrated for sheep blood (Langston et al., 1993).

## 2.9 DATA HANDLING

Output from the Doppler flowmeter and the Hewlett-Packard pressure monitor was recorded digitally at a frequency of 1 Hz and transferred to a commercial spreadsheet program (Excel™, Version 4.0, Microsoft Corporation, Redmont, WA, USA) run on an IBM compatible personal computer (Microbits 486-based IBM compatible). Drug concentration data were manually entered on the same spreadsheet. This spreadsheet was used to manipulate and calculate all data. Statistical analysis was made using a commercial statistics package (Systat, SPSS, Chicago, IL, USA) run on the same personal computer. Data for graphing were transferred to a commercial graphing package (SigmaPlot for Windows™, Jandel Scientific, San Rafael, CA, USA) and printed on a laser printer (Brother Laser Printer HL-600, Irvine, CA, USA).

# CHAPTER 3. A METHOD FOR THE FREQUENT MEASUREMENT OF THE ANTINOCICEPTIVE EFFECTS OF DRUGS

## 3.1 INTRODUCTION

While knowledge of the time-course of systemic and regional concentrations of a drug is important for understanding the pharmacokinetics and in designing dose regimens, the application of this information is limited unless the relationship between the concentrations and the desired effect is established. In the case of propofol, the primary desired effect is usually an altered state of consciousness, or depth of anaesthesia.

The available methods for measurement of depth of anaesthesia have been reviewed in chapter 1. For the proposed studies for this thesis, a method which allowed frequent, reproducible measurements to be made in awake sheep was required. Techniques which measured changes in cerebral function such as observed clinical signs related to changes in cerebral function, EEG, oesophageal manometry and electromyography did not appear practical, either because of the technical problems associated with measurement of very low voltage signals in an awake unrestrained animal, or their general poor correlation with accepted standards of depth of anaesthesia. Techniques in which an altered response to an external stimulus is used to reflect changes in cerebral function were therefore considered. Measurement of evoked potentials relies on detection of changes in measured cerebral electrical activity due to drug induced depression of the sensory pathways in the brain, but suffers similar problems of low voltage measurement to those which occur with EEG. Therefore, techniques used for algesimetry studies were chosen. In algesimetry, a noxious stimulus is applied and a response believed to represent the perception of pain, such as withdrawal behaviour, is observed. As with evoked potentials, drug induced depression of sensory pathways will alter the relationship between stimulus and response.

This concept essentially differs from evoked potentials only in that a mechanical rather than an electrical response is measured. This was considered a valid approach, particularly as a motor response to a noxious stimulus has been used as an indicator of depth of anaesthesia for many years; this is reflected in the scale of clinical signs of depth of anaesthesia and the indicator of volatile anaesthetic potency MAC, both outlined in chapter 1. Indeed, in clinical practice, movement following surgical stimulus, a not uncommon event, remains one of the "gold standard" indicators of inadequate depth of anaesthesia.

### 3.1.1 IDEAL CHARACTERISTICS OF THE PROPOSED TECHNIQUE

For the proposed studies, a technique for the measurement of the antinociceptive effects of drugs required the following characteristics.

#### 3.1.1.1 Stimulus:

Rapid onset and offset

To accurately define the time-course of changes of drug effects following rapid drug administration, measurements needed to be made as frequently as every 30 seconds. Therefore, there should be almost immediate loss of perceived sensation once the stimulus was ceased.

Accurate delivery

In order to allow accurate repeated measurements, the time-course and magnitude of the applied stimulus should be consistently reproducible. If a ramped stimulus were to be used, the magnitude and rate of change should be accurately controlled and the magnitude of the stimulus should be able to be accurately measured in real time.

Tissue effects

Frequent application of a stimulus should not damage body tissues, both for ethical reasons and because of a risk of changes in perceived sensation for a given level of stimulus over time.

#### 3.1.1.2 Response:

Reliable endpoint

The observed motor response should be consistent, with a clear point of onset. This response should be present over a range of conscious states.

Stable over time

There should be no change in the magnitude of stimulus required to induce the response for a period of baseline measurement equivalent to the duration of the proposed experimental period.

Voluntary action

The response should be a voluntary action and not a reflex response, thereby involving cerebral, rather than only spinal, pathways.

It appeared that no method existed which had all these characteristics. Thermal, mechanical and chemical techniques are all commonly used noxious stimuli in algesimetry paradigms, but were considered impractical as they do not have rapid onset and offset and may convey the risk of tissue damage if applied at frequent intervals. Electrical current, however, has been frequently used to induce

nociception (Vyklicky, 1979; Chapman et al., 1985; Ayhan et al., 1983) and satisfied most of the criteria outlined above. It can produce a noxious stimulus of a controlled magnitude which rapidly dissipates once the flow of current is ceased. However, application of electrical current has been associated with the development of changes in perception of noxious stimuli, for example in TENS therapy for longstanding pain (Tasker, 1988), and it was considered important that this be specifically examined.

A range of behaviours associated with nociception including tail flick, vocalisation, jaw opening, jumping, paw licking and writhing have been used as response endpoints, depending on the type and location of the applied stimulus (Schultz et al., 1987; Vyklicky, 1979). Limb withdrawal in sheep has been shown to be a consistent easily recorded response to mechanical and nociceptive stimuli (Nolan et al., 1985) and hence appeared suitable for the proposed studies.
It was therefore decided to develop and validate a method for measuring the effects of analgesic and sedative drugs in sheep at frequent intervals using a ramped electrical current applied to a limb as a noxious stimulus, with the limb lift described by Nolan et al. (1987) as the response.

### 3.1.2 DEVELOPMENT OF AN ALGESIMETRY DEVICE

#### 3.1.2.1 Current Generator

A 9V battery powered electronic peripheral nerve stimulator (Digistim 3, Neuro Technology, Houston, Texas, USA) was chosen to be adapted for this purpose because it was readily available and designed as a constant current generator (i.e. to deliver a constant but manually adjustable DC current relatively independently of electrical resistance). In addition, it had a continuous display of the delivered current on a liquid crystal screen display (LCD).

#### 3.1.2.2 Pilot study in man

The stimulator was initially used on a human volunteer (the author) in order to assess the type of sensation induced by a ramped cutaneous electrical stimulus, to assess baseline stability over time, to confirm the current range likely to produce the sensation of pain, and to determine the effect of changes in current frequency. Studies on electrical safety had suggested that the sensory threshold for a 50 Hz current in man was approximately 0.3 mA, with the pain threshold approximately 1 mA (Russell, 1983).

##### 3.1.2.2.1 Methods

The peripheral nerve stimulator was connected via insulated electrical wires to two skin surface electrodes placed 1 cm apart over to the subject's anterior lower limb. This site was chosen because of its similarity with the hind limb of the sheep,

particularly in relationship to the relative lack of muscle bulk which might be directly stimulated by the electrical current. A separate operator controlled current delivery.

Current frequencies available on this unmodified stimulator were 1 Hz, 50 Hz and 100 Hz, and initially 100 Hz was chosen. The output was manually increased by the operator and the currents at which sensation was first felt, pain was first perceived, and maximum stimulation could be tolerated were recorded.

To study baseline stability, measurements were made every minute for 20 minutes. To study the effect of frequency on perceived sensations, the current frequency was altered serially and sensations were recorded. To study the effect of rate of change in current, the threshold current to induce pain was recorded at manually controlled slow and fast rates of change.

3.1.2.2.2 Results

The results are displayed in figure 3-1. The sensory threshold was approximately 1 mA, with pain perceived at approximately 2.5-4 mA. These values were relatively stable over the 20 minute period. Pain was originally felt at approximately 5 mA, but this increased slowly to reach a stable level of approximately 7 mA after 5-7 minutes.

Quite different sensations were noted at the different frequencies, with a much sharper pricking pain felt at the lowest frequency. At the highest frequency the pain was dull, and more easily tolerated. Alterations in the rate of change in applied current also altered the perceived sensation, with a slower rate producing less perceived pain for a given current.

### 3.1.2.3 Pilot study in sheep

This was conducted in one sheep to determine whether the limb withdrawal previously described (Nolan et al., 1987) was consistently produced by an electrical stimulus, and to observe whether a change in the threshold current to induce limb withdrawal occurred after administration of an antinociceptive drug.

3.1.2.3.1 Method

The stimulator was connected to the lower hind limb of an unrestrained sheep in a metabolic crate using skin surface electrodes in a similar manner to that described above. Measurements of threshold current were made prior to a single intramuscular injection of 10 mg of methadone (Parnell Laboratories), a dose expected to produce mild-moderate analgesia in an adult sheep, and then at intervals afterwards for approximately 1 hour. At each time point, 3 measurements of threshold current were made at 15 second intervals and averaged and recorded as mean $\pm$ sem.

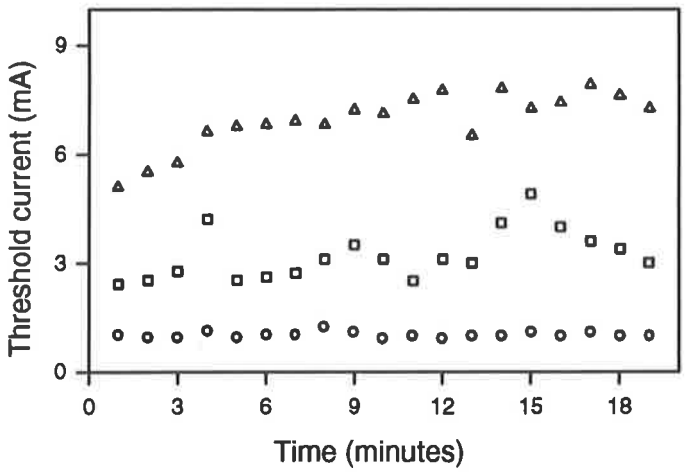


**Figure 3-1** Threshold current to produce sensation (circles), pain (squares) and the maximum tolerable current (triangles) following cutaneous application in a human subject.

3.1.2.3.2 Results

The results are shown in figure 3-2. Application of a ramped electrical stimulus at 100 Hz produced consistent avoidance behaviour. There was brisk limb withdrawal, with a clear point of onset, following each measurement. The baseline threshold current was 5.28 mA $\pm$ 0.12. This decreased to 4.36 mA in the interval of approximately 20 minutes before the next measurement, during which time methadone was administered. The mean threshold current then increased transiently, following a time-course consistent with the expected duration of analgesic action of methadone in sheep. The small variation in threshold current between the three measurements at each time point confirmed that limb withdrawal occurred at a consistent level of electrical stimulus in sheep.

### 3.1.2.4 Electronic modifications

These pilot studies suggested that the proposed technique might be a useful method for the measurement of effects of antinociceptive drugs. They also suggested that choice of current frequency, rate of change in current delivery, and measurement interval might be important to achieve consistent results. It was decided to study the effects of different stimulus patterns and measurement intervals on the observed response to determine the optimal methodology. For these studies, a device was needed which would allow consistent delivery and recording of a wide range of patterns of electrical stimuli. No suitable device was commercially available and so an electronic engineer was commissioned to electronically adapt the commercially available electronic peripheral nerve stimulator used in the pilot studies. Modifications and additional electronics were

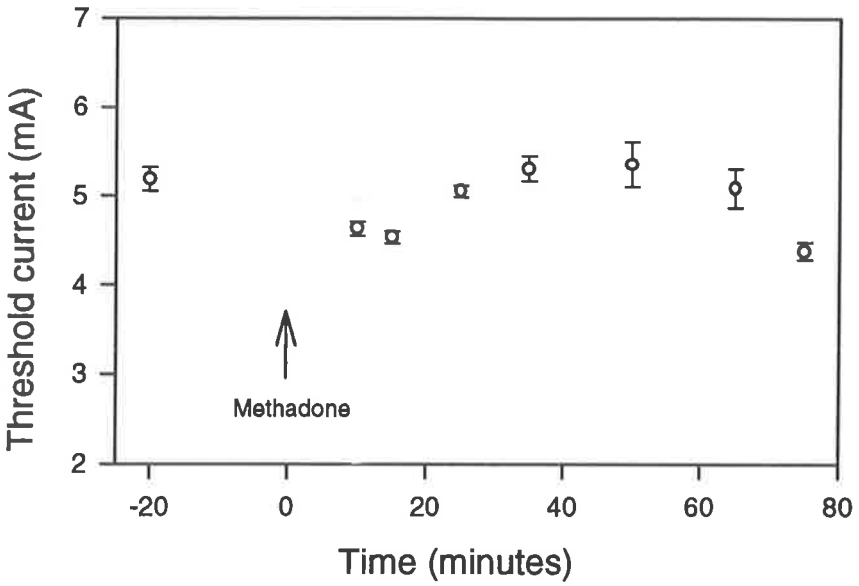


**Figure 3-2** Threshold current to induce limb withdrawal in a sheep over time following administration of methadone, 1 ml, intramuscularly at time 0. Data are presented as mean & sem.

designed and incorporated to allow flexibility of current output, rate of current increase and current frequency. The functions of these modifications is outlined below.

- Frequency control: Continuously variable adjustment of the pulse repetition rate of the DC current output between 10 and 60 Hz.

- Stepped adjustment of current amplitude: Division of the current range into 20 equal steps; the magnitude and duration of each step being determined by the set maximum output and the ramp duration respectively.

- Ramp duration control: Adjustment of the time for current increase from minimum to maximum output.

- Remote cutout switch: A push-button hand-held "deadman's handle" type cutout button was fitted to be controlled by the operator, to allow rapid cessation of current delivery following observation of the withdrawal response and thus prevent inadvertent or excessive current delivery to the sheep.

- Liquid crystal display (LCD): Addition of a "sample and hold" characteristic so that delivered current was continuously displayed on the LCD and the highest delivered current at the time of release of the remote cutout switch was held on the screen until current delivery was recommenced.

- Current delivery time: An electronic stopwatch (Radio Spares, Australia) was interfaced with the stimulator. Timing was commenced by initialisation of current delivery and stopped by release of the remote cutout switch, thus providing a readout of the duration of current delivery before the withdrawal response was observed.

- Output control: The maximum deliverable output current could be continuously adjusted, to a maximum of approximately 50 mA (depending on electrical resistance in the circuit). This value was chosen to prevent tissue damage.

The modified stimulator is displayed in figure 3-3 and will subsequently be referred to as the algesimetry device.

*Nomenclature*

A schematic representation of the ramped output, including the nomenclature to be used with the algesimetry device, is detailed in figure 3-4.

Having examined the feasibility of the use of an algesimetry paradigm to measure the antinociceptive effects of drugs, and having developed a device which would allow current generation, the device was formally tested in sheep. The specific aims of the following studies were to:

1. Determine the variability in threshold current over time under baseline conditions.

2. Examine the effects of current frequency, ramp duration and measurement interval on the threshold current to induce a withdrawal response.

3. Use these data to determine the optimal pattern of applied stimulus.

4. Examine the effects of drug administration on threshold current.

## 3.2 METHODS

### 3.2.1 ANIMAL PREPARATION

Animals were selected and housed according to the general methods described in chapter 2.

### 3.2.2 STUDY DESIGN

#### 3.2.2.1 Measurement protocol

3.2.2.1.1 Current delivery

The consistent withdrawal response demonstrated in the pilot studies following application of electrical current to the lower hind limb of sheep confirmed this as a satisfactory site for current delivery. In these studies current was initially delivered via 2 insulated electrical wires connected to gel coated conductive pads. However, to reduce the current delivered and to overcome potential inconsistent excessive

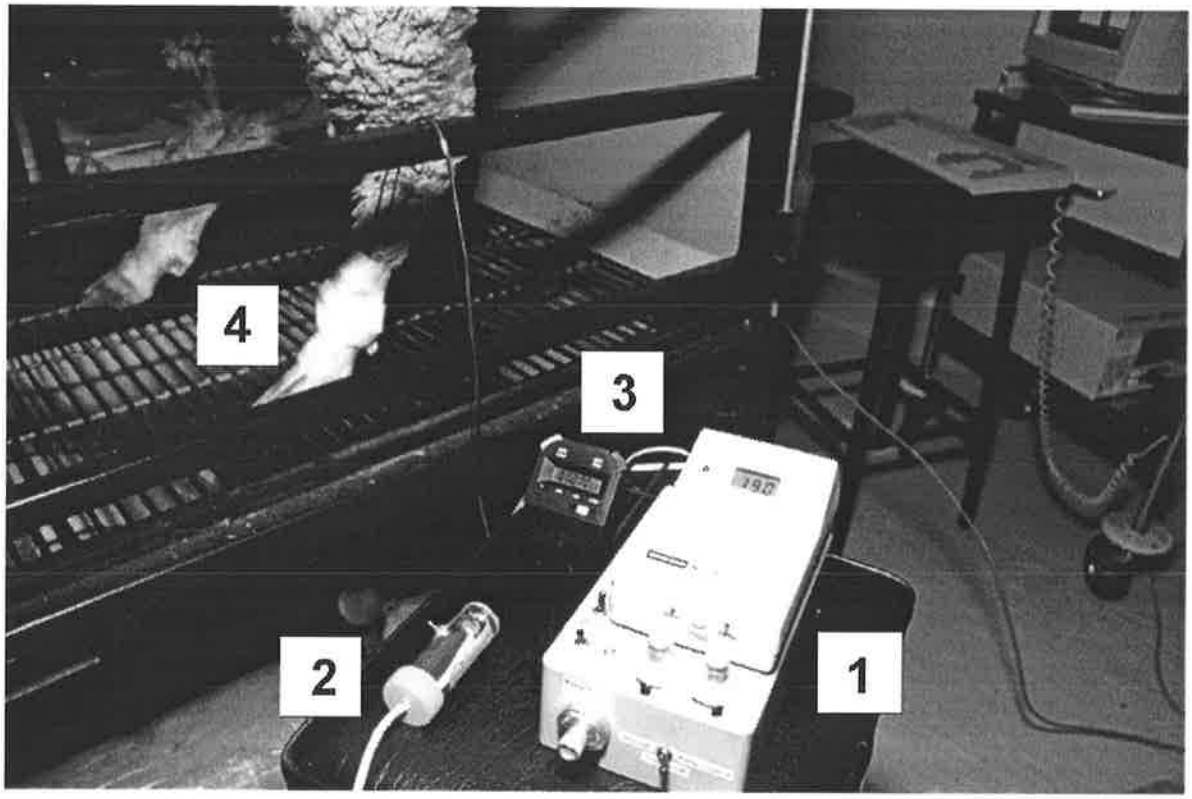


**Figure 3-3** The algesimetry device attached to the hind limb of a sheep. Displayed are (1) the control box, (2) the "dead man's handle", (3) the stop watch and (4) the wires connected to the sheep limb.

skin impedance, particularly with regrowth of wool over time, current was subsequently delivered through 26 g needles placed subcutaneously 1 cm apart and taped in place. The current ramp rate was initially set at approximately 1 mA per second. The ramp rate was then adjusted so that the threshold current to achieve limb withdrawal was reached in approximately 5 seconds, in 5-6 steps. This ramp duration would allow measurements at frequent intervals while minimising "overshoot" from excessively rapid current rise.

3.2.2.1.2 Response

The limb withdrawal response was a discrete movement. When applied current reached an apparently noxious level, the sheep responded with a rapid but controlled flexion of the limb, and the limb remained elevated until the stimulus was ceased. The rate of withdrawal was variable, for example it decreased with administration of antinociceptive drugs, and so the point at which the hoof lost contact with the floor of the crate was chosen as the endpoint to be recorded. To improve consistency in response, sheep were placed in a sling, which preserved partial weight bearing on the hind limbs, to minimise spontaneous movements.

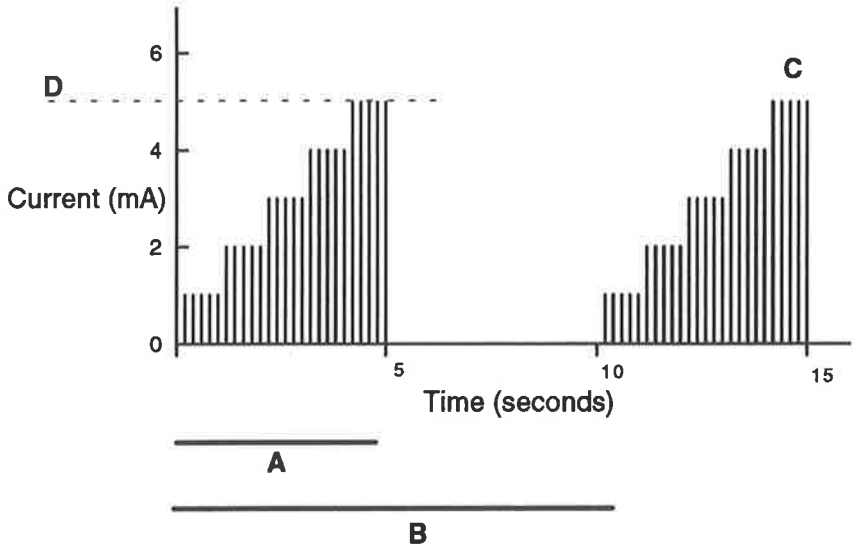


**Figure 3-4** Schematic representation of the stimulus output, including nomenclature
A. Ramp duration - the time over which current was applied
B. Measurement interval - the time between commencement of consecutive measurements
C. Current frequency - the number of current pulses per second
D. Threshold current - the delivered current at the point of limb withdrawal
This example shows a current frequency of 5 Hz, a ramp duration of 5 seconds, a measurement interval of 10 seconds, and a threshold current of approximately 5 mA.

3.2.2.1.3. Operator

The operator was seated to the side of the animal, where limb movement was easily visible and operator movements were removed from the direct field of view of the sheep. This, and the silent operation of the machine, also minimised visual or auditory cues for the animal. After pre-setting of the current ramp rate, current delivery was initiated first by depression of the push button on the "deadman's handle" and then operation of a simple "on-off" switch. Current delivery was then automated, leaving the operator to concentrate on sheep behaviour. Once the end point was observed, the push button on the "deadman's handle" was released, instantly ceasing current flow. The operator then recorded the maximum delivered current and the duration of delivery from the LCD screen and stopwatch. Before the next measurement, the current ramp rate could then be manually adjusted, if necessary, so that the duration of delivery remained constant.

### 3.2.2.2 Determination of optimal current delivery regimen

Each of the following studies of current delivery were performed on 5 sheep using the general method described above. Intermittent measurements were made for a period of approximately 5 minutes prior to commencement of all studies in order for the animal to become adjusted to the procedure.

3.2.2.2.1 Current frequency

Using a ramp duration of 5 seconds and a measurement interval of 30 seconds, the frequency of the delivered current was altered serially at 15 minute intervals from 20 to 60 to 15 to 20 Hz in 5 animals. At each time point the threshold current from all animals were pooled and expressed as mean ± sem. The data were examined for variation over time using repeated measures analysis of variance; a significance level of p=0.05 was chosen (Ludbrook, 1994; Ludbrook, 1991).

3.2.2.2.2 Ramp duration

A current frequency of 20 Hz was used with a measurement interval of 30 seconds. Threshold current was measured while alternating every 5 readings between a short ramp duration (approximately 5 seconds) and a long ramp duration (a rate of change of approximately 0.1 mA per second). The threshold current from each of the 2 groups were pooled and expressed as mean ± sem, and a paired Student's t-test used to compare the threshold currents for the groups; a significance level of p=0.05 was chosen.

3.2.2.2.3 Measurement interval

Using a current frequency of 20 Hz and a ramp duration of 5 seconds, threshold current was measured at 30 second intervals for 15 minutes, then at 60 second intervals for 15 minutes and then 10 second intervals for 15 minutes. The threshold currents at each ramp time were pooled and expressed as mean ± sem. The data were examined for variation over time using repeated measures analysis of variance; a significance level of p=0.05 was chosen.

3.2.2.2.4 Baseline stability

Using the stimulus pattern considered to be optimal (20 Hz, 5 second ramp duration, 30 second measurement interval), variation in measured threshold current over 60 minutes was examined in 5 sheep. The threshold currents from each ramp time were pooled and expressed as mean ± sem. The data were examined for variation over time using repeated measures analysis of variance; a significance level of p=0.05 was chosen.

### 3.2.2.3 Effects of analgesic and sedative/anaesthetic agents on threshold current

Using the optimal stimulus pattern and measurement interval determined above, a number of drugs with antinociceptive properties were administered via different routes and in different dose regimens to individual sheep while the threshold current was measured. A range of analgesic and sedative/hypnotic drugs with different mechanisms of action was deliberately chosen for these studies to assess the

scope of applications of this technique. Doses chosen were those expected to produce sedation as well as anaesthesia, or moderate analgesia (Flecknell, 1987).

3.2.2.3.1. Propofol

The sedative/hypnotic agent propofol (ICI Pharmaceuticals, Cheshire, England) was administered intravenously over 2 minutes in doses of 50, 100 and 200 mg. Propofol was also administered intravenously as a 100 mg bolus over 20 seconds followed by a continuous infusion of 10 mg/minute for 25 minutes.

3.2.2.3.2 Sodium thiopentone

The sedative/hypnotic agent thiopentone (Abbott Australasia, Sydney, Australia) was administered intravenously over 2 minutes in doses of 250 or 750 mg.

3.2.2.3.3 Xylazine

Xylazine (Troy Laboratories, Smithfield, NSW, Australia), an alpha-2 adrenoreceptor agonist, was administered intramuscularly in doses of 2.5, 5 or 10 mg, and a dose of 2.2 mg of xylazine was also administered via rapid intravenous bolus.

3.2.2.3.4 Opioids

The opioids pethidine (David Bull Laboratories, Mulgrave, Victoria, Australia) in doses of 100 or 300 mg, and alfentanil (Janssen Pharmaceutica, Beerse, Belgium) in doses of 1 and 2 mg were administered intravenously over 2 minutes.

## 3.3 RESULTS

### 3.3.1 DETERMINATION OF OPTIMAL CURRENT DELIVERY REGIMEN

#### 3.3.1.1 Current frequency

The threshold currents following changes in frequency are displayed in figure 3-5. There was no significant change over time ($p=0.99$).

#### 3.3.1.2 Ramp duration

The mean ramp duration to reach the threshold current was $5.38 \pm 1.41$ seconds and $54.7 \pm 12.6$ seconds in the short and long ramp duration groups respectively.

The mean threshold current was $4.98 \pm 1.46$ mA and $5.44 \pm 1.25$ mA in the short and long ramp duration groups respectively. These were not significantly different ($p=0.23$).

#### 3.3.1.3 Measurement interval

The threshold current following changes in measurement interval are displayed in figure 3-6. There was no significant change over time for the duration of the study ($p=0.28$). However, a measurement interval of 10 seconds (5 seconds ramp

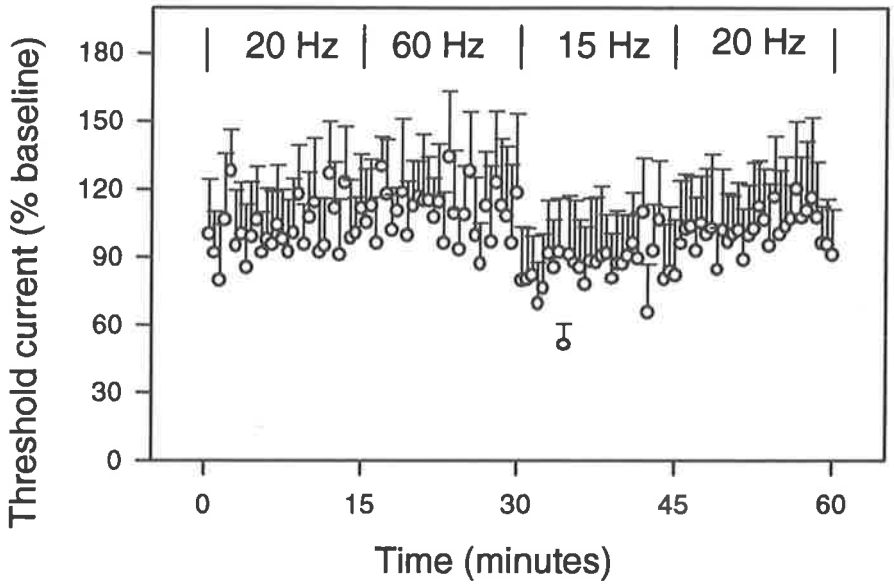


**Figure 3-5** Threshold current over time in 5 sheep following changes in current frequency. The data are presented as mean & sem.

duration and 5 seconds rest) produced mild agitation, as evidenced by some spontaneous leg and head movements, within the first 5 minutes of measuring at this interval. This was accompanied by increased variability in measurements during this time period, although recorded values did not change significantly over time.

#### 3.3.1.4 Baseline stability

The threshold current over a 60 minute period of measurements is displayed in figure 3-7. There was no significant change over time (p=0.99).

### 3.3.2 EFFECTS OF ANALGESIC AND SEDATIVE/ANAESTHETIC AGENTS ON THRESHOLD CURRENT

#### 3.3.2.1 Sedative/anaesthetic agents

Both propofol and sodium thiopentone produced temporary onset of sedation in the sheep, as evidenced by lowering of the head, a reduction in spontaneous movements and depression of the eyelash reflex. Simultaneously, there were dose dependant increases in the threshold current to produce limb withdrawal which slowly returned to baseline over approximately the same time period as the traditional visible indices of sedation or anaesthesia. Typical data are displayed in figures 3-8 and 3-9. At high doses of both agents deep levels of anaesthesia were achieved, with complete loss of the lash reflex, and there were periods during which no withdrawal response could be detected with the highest currents that could be

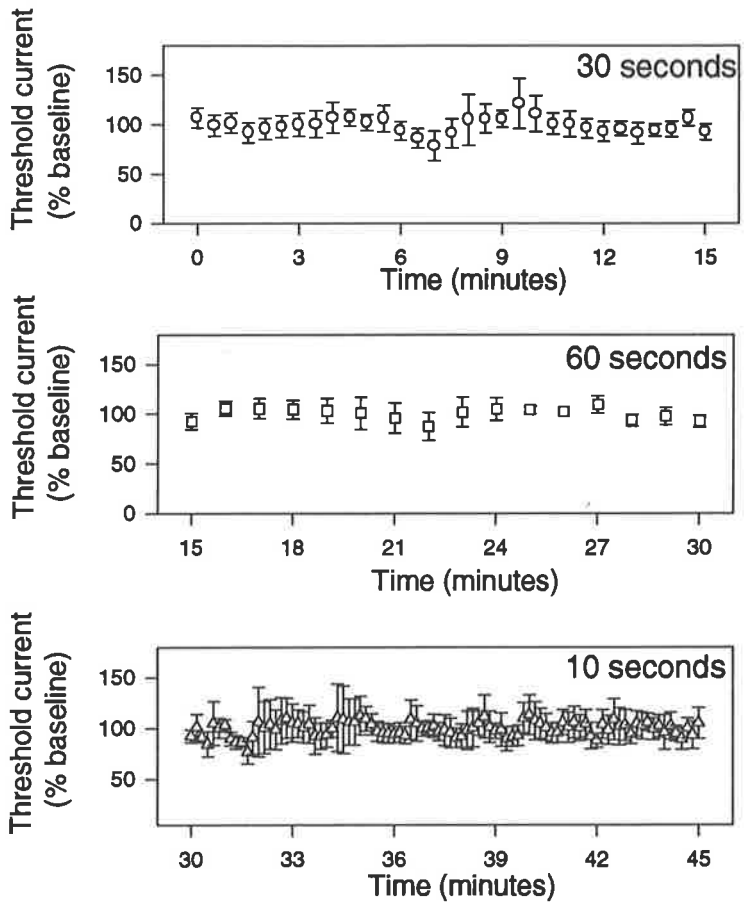


**Figure 3-6** Threshold current, as % baseline, following measurements in 5 sheep at intervals of 30 seconds, 1 minute and 10 seconds. Data are presented as mean & sem.

safely delivered (approximately 50 mA). No measurements were therefore recorded at these times. Administration of a rapid bolus of propofol followed by a continuous infusion produced a rapid rise in threshold current followed by a slower prolonged increase, which fell rapidly once the infusion was ceased. Typical data are displayed in figure 3-10.

### 3.3.2.2 Effects of analgesic agents

Xylazine produced dose dependant increases in threshold current when administered intramuscularly (figure 3-11A). Signs of sedation or anaesthesia such as changes in head posture, reduced spontaneous movements and loss of eyelash reflex were not evident following the lowest 2 doses, but were present following the 10 mg dose. Intravenous administration of xylazine (2.2 mg) produced a change in magnitude and time-course of threshold current similar to that seen after 5 mg intramuscularly, without signs of sedation (figure 3-11B).

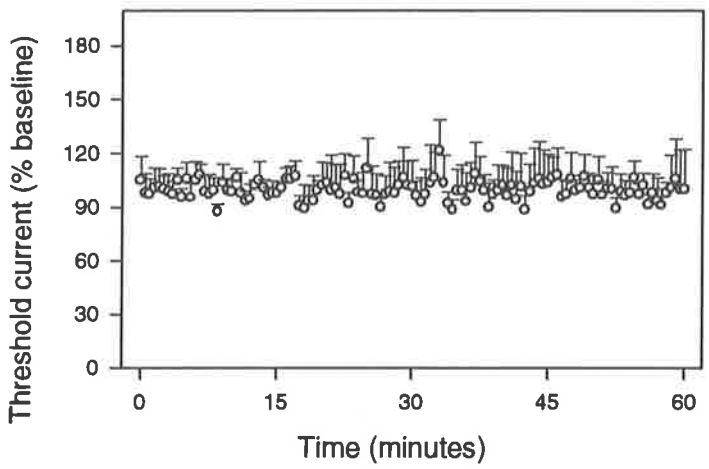


**Figure 3-7** Threshold current following measurements in 5 sheep at 30 second intervals over 60 minutes. Data are displayed as mean & sem.

When administered intravenously, the opioids alfentanil and pethidine produced significant agitation, as manifest by irregular movements of the head and limbs, nystagmus and chewing, in all sheep. This made observation of the point of limb withdrawal unreliable.

## 3.4 DISCUSSION

As discussed in chapter 1, numerous methods have been used in an attempt to measure depth of anaesthesia; the number of methods available and their lack of generalised use in anaesthetic practice perhaps bearing testimony to their limitations. Algesimetry paradigms, however, appear to be the most accurate and reliable. The simplest of these is the clinical sign of movement in response to surgical stimulation (in the absence of muscle relaxants), and this remains the standard against which trials of alternative measurement methods are usually judged. Of the newer techniques, evoked potentials (measurement of the brain's electrical response to a peripheral stimulus) appears to hold most promise; perhaps not surprisingly considering the parallels which can be drawn with algesimetry. Recording of movement responses, evoked potentials, algesimetry techniques and the method described here are all examples of some cerebral response to peripheral sensory stimulation ranging from surgical incision to electrical current.

In the original validation studies of the algesimetry technique in sheep on which the current technique was based (Nolan et al., 1987), a change from baseline was observed with opioids, but not acepromazine, an agent believed to induce sedation but not analgesia, suggesting that this technique could differentiate between analgesia and sedation. To a degree, this must be dose dependent, as it was obvious from the current studies with propofol and thiopentone that, in sufficient doses, sedative/hypnotic agents will alter the noxious threshold to induce

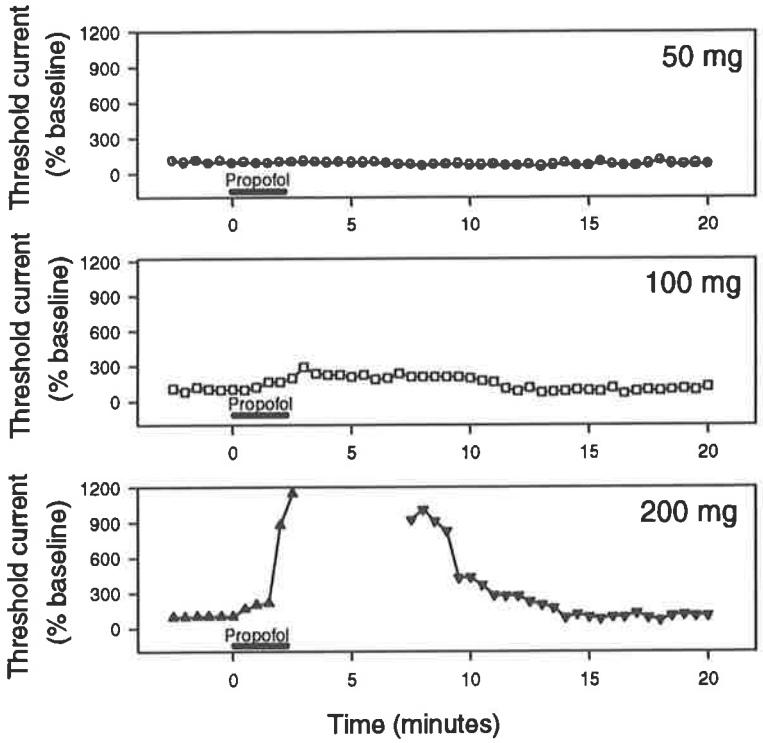


**Figure 3-8** Changes in threshold current following administration of propofol i.v. over 2 minutes in doses of 50 mg, 100 mg, and 200 mg. Between 2.5 and 7.5 minutes after the 200 mg dose there was no response to maximal stimulus, and so no value could be recorded.

withdrawal. The conclusions from a second study from that group that midazolam has analgesic properties, because administration was followed by an increase in both thermal and mechanical thresholds, must therefore be questioned (Kyles et al., 1995).

Differentiation between the drug induced changes in perceived sensation can only be made when complex feedback on sensory perception is available; essentially requiring a human model. Such studies have, for example, been performed in examination of the effect of low doses of sedative/hypnotic drugs such as propofol and thiopentone on algesia (Anker-Moller et al., 1991; Wilder-Smith et al., 1995). While these and other studies (Ewen et al., 1995; Goto et al., 1994) have recorded an hyperalgesic effect of both propofol and thiopentone, the magnitude of change is small compared to the changes recorded following the doses of these drugs used in the current study, and therefore unlikely to significantly affect the results of the proposed studies in this thesis.

Different stimulus types may produce different drug responses in algesimetry studies, and may explain some of the differences seen with this method compared to the thermal and pressure stimuli of Nolan et al (Nolan et al., 1987). Thermal

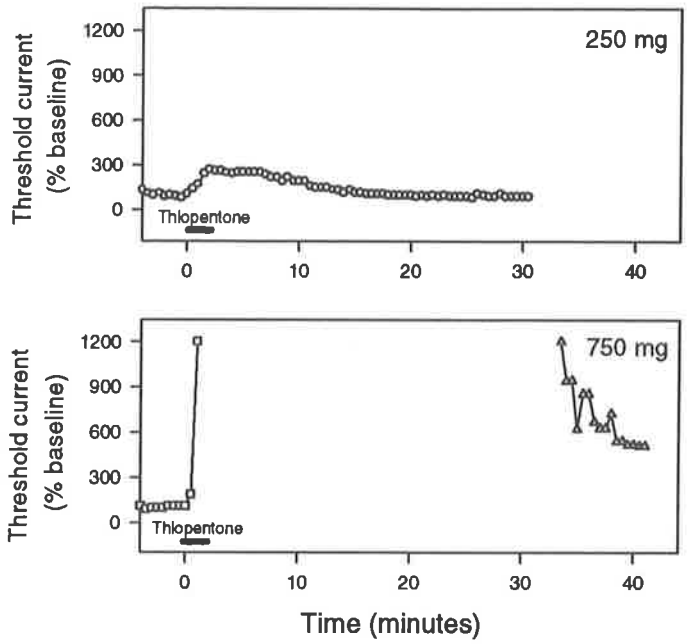


**Figure 3-9** Changes in threshold current following administration of sodium thiopentone i.v. over 2 minutes in doses of 250 mg and 750 mg. Between 0.5 and 34 minutes after the 750 mg dose there was no response to maximal stimulus, and so no value could be recorded.

stimuli activate predominantly Aγ innervated nociceptors, while pressure additionally activates touch and pressure receptors (Anker-Moller et al., 1991). An electrical stimulus activates a broad range of sensory receptors. Differential responses have in fact been recorded between thermal and pressure measured analgesia in sheep (Nolan et al., 1987).

In order to use this method to measure of the cerebral effects of antinociceptive drugs, it is assumed that the withdrawal response reflects cerebral activity, and not simply a reflex response at a spinal cord level, and that the stimulus is indeed noxious. This issue has previously been discussed in relation to indicators of voluntary performance in algesimetry methodology (Chapman et al., 1985). Although complex feedback from human studies would be necessary to prove this assumption, there is some supportive evidence.

Firstly, the current densities required to achieve limb withdrawal with our method are of a similar magnitude to those which appear to produce nociception in man and a number of other species (Vierck et al. 1983). Secondly, associated behaviour of the sheep suggests nociception is experienced. At low and presumably non-noxious currents, sheep were mildly aroused in a similar manner to when they perceive a non noxious stimulus such as noise or movement in the room. As the current increased sheep became agitated, showing behaviour such as head and

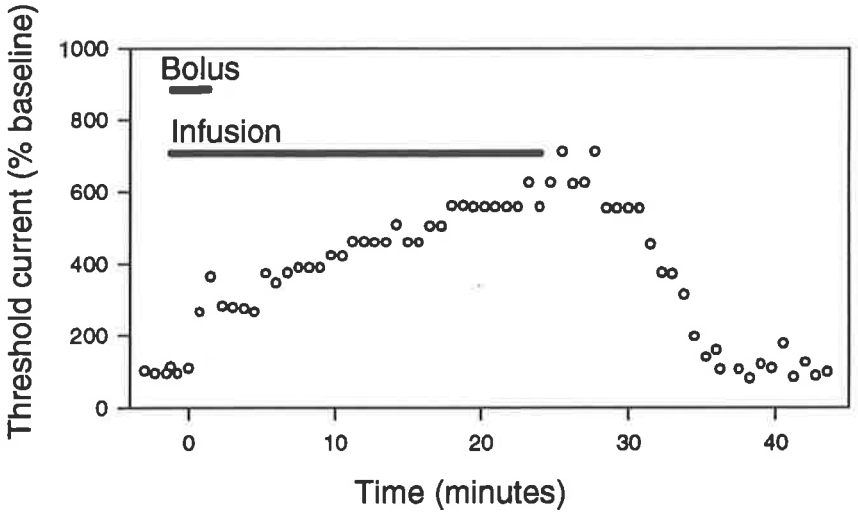


**Figure 3-10** Changes in threshold current following propofol 100mg i.v. over 20 seconds at time 0 followed by a continuous infusion of 10 mg/min for 25 minutes.

ear movements usually associated with noxious stimuli until eventually they withdraw from the stimulus. Thirdly, the observed limb withdrawal was a slow controlled movement rather than a flinch or flick movement usually associated with reflex responses. Fourthly, sheep demonstrated evidence of learning behaviour. When measurements were first made on sheep not previously studied, they became agitated when high currents were delivered but failed to withdraw the stimulated limb. Only after a few minutes of measurements did they appear to learn that limb withdrawal relieved the stimulus. While this could imply that sheep learn to withdraw to a sensation rather than a noxious stimulus, the fact that even after sheep had been used in a large number of experiments they would still only withdraw when the delivered current was sufficiently high to produce some agitation suggests that a noxious stimulus was required each time to induce withdrawal.

### 3.4.1 BASELINE STABILITY

Animals usually initially required 2-5 minutes of measurements before stable threshold currents were achieved; perhaps evidence of learning behaviour as discussed above. After this period there was little variation in baseline values over a 60 minute period. This variation was certainly very small compared to the changes recorded after administration of effective doses of anti-nociceptive drugs and therefore unlikely to significantly influence the results in these studies.

Alterations in sensory thresholds over time have previously been described after application of electrical currents; this being the basis for TENS treatment (trans cutaneous nerve stimulation). This is based on the Melzack-Wall gate theory of pain which states that stimulation of large sensory fibres would suppress

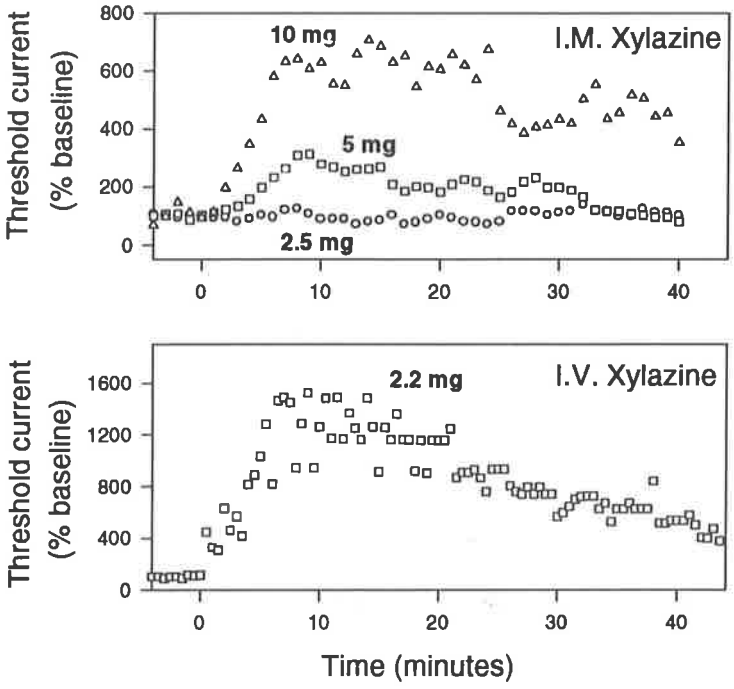


**Figure 3-11** Changes in threshold current following xylazine intramuscularly in doses of 2.5 mg (circles), 5 mg (squares) and 10 mg (triangles) at time 0, and changes in threshold current following xylazine i.v. in a dose of 2.2 mg at time 0.

transmission of sensation through small fibres (Melzack and Wall, 1965). Patterns of stimulus used in TENS vary, but include application of a monophasic rectangular pulsed current with continuous stimulation at 1-150 Hz or intermittent application of high frequency current (100-150 Hz) (Tasker, 1988). Despite the similarity of these patterns of stimulation with those used in the current study, no such phenomenon was recorded. This may relate to the relative insensitivity of the technique of pain detection in animals studies, or possibly to the poly-modal sensory input generated by the algesimetry device.

### 3.4.2 OPTIMAL STIMULUS PATTERN

The different sensations perceived with different patterns of stimulus in the human pilot study suggested that the pattern of stimulus, in particular the current frequency and ramp rate, might alter the current threshold in sheep. The lack of effect of changing these parameters demonstrated in the sheep study probably reflects the lack of sensitivity in nociception detection in animal studies compared to that allowed by the complex verbal feedback on perceived sensation available in human studies. Measurement interval, however, may be more important in production of consistent measurements. While reducing the interval between measurements to approximately 5 seconds did not produce a statistically significant increase in

baseline values (figure 3-6), variability in response appeared greater, particularly in the first minutes of measurements at that rate, and was associated with agitative behaviour in the sheep. Although not specifically examined, infrequent measurements may also reduce the consistency of response as the 'learning' behaviour exhibited when measurements are commenced may be reproduced if an interval of some minutes is left between measurements. The initial pilot study of the response to intramuscular methadone may be an example of this (figure 3-2). The mean threshold current recorded approximately 20 minutes prior to administration of the methadone was greater than the value at 2 minutes post dose, and in fact was no different to the peak value achieved after methadone. Measurement interval should therefore be kept constant. In subsequent studies which examined the antinociceptive effects of different drugs, measurements were made every 30 seconds; this allowed a satisfactory delineation of the time-course of drug effects after rapid i.v. injection while still maintaining consistency in response.

### 3.4.3 DRUG ADMINISTRATION

As mentioned in chapter 1, there is no "gold standard" against which to measure the effectiveness of this technique. In assessment of this technique, it was therefore decided to examine specific aspects of animal behaviour following drug administration. For effective measurements of drug effects following administration of the doses used in these studies, it was essential that consistent stable measurements were achieved prior to drug administration. As discussed above, this was achieved following determination of the apparent optimal pattern of stimulation and measurement frequency.

It was also necessary that the response was dose dependent over the range of doses considered to be effective in these animals. This was determined both by looking at the magnitude of response and whether the time-course of response was consistent with the known systemic pharmacokinetics of these drugs in sheep, depending on the route and mode of drug administration. Lastly, it was necessary to look for correlation with known indicators of drug effects, such as changes in the degree of spontaneous movements following onset of sedation.

#### 3.4.3.1 Response to propofol and thiopentone

Both these agents are sedative/anaesthetic drugs which act on the central nervous system and, although their exact mechanisms of action are incompletely defined, both may at least partially act at the GABA receptor producing a similar cellular effect on chloride conduction (Salonen and Maze, 1993). Administration of both propofol and thiopentone produced measurable increases in threshold current which were clearly greater than the variability seen with pre-administration measurements (figures 3-8 and 3-9). There was also evidence of a dose

dependent effect with both drugs, with increasing doses producing a greater maximal increase in threshold current as well as a longer duration of deviation from baseline measurements.

It was, however, apparent that only a restricted range of effects could be measured. For example, a small dose of propofol (50 mg i.v. over 2 minutes) produced no measurable change in threshold current, suggesting that this method is insufficiently sensitive to measure any sedative effect at this comparatively low dose. The largest dose of propofol (200 mg i.v. over 2 minutes) produced a period between 2 and 7 minutes during which no response was obtained to the highest delivered current considered ethical with respect to causing tissue damage (approximately 50 mA), and so again no measurement of drug effect could be made. The 100 mg dose produced a measurable increase in threshold current and allowed measurements to be made for the duration of the experimental period. This is therefore the dose range which allows simultaneous measurements of drug pharmacokinetics and pharmacodynamics to be made with this preparation.

The relationship between the pattern of changes in threshold current and sheep behaviour with different dose regimens is further supportive evidence that this technique is effective at measuring the effects of sedative/hypnotic drugs. Bolus administration of 50 mg of propofol produced no evidence of a change in animal behaviour and no change in threshold current. Following the 100 mg dose, sheep displayed evidence of moderate sedation or light anaesthesia (reduced spontaneous movements, eye closure, lowering of head) in the first minute after onset of administration and this correlated with measurable increases in threshold current. At the point of maximal increase in threshold current sheep appeared fully anaesthetised. They were slumped in the sling and had lost swallowing reflexes, producing a flow of saliva from the mouth. After the drug was ceased the current threshold and animal behaviour again changed in parallel. A similar relationship was found with thiopentone.

These studies also demonstrated how insensitive observations of animal behaviour are at detecting sedative drug effects. At the point of maximal effect of the 100 mg dose of propofol the sheep appeared to be anaesthetised, yet were still able to withdraw from a noxious stimulus. Furthermore, increasing the dose to 200 mg produced no further change in observed animal behaviour but a further increase in the recorded threshold current. This technique, therefore, allows differentiation between levels of sedation/anaesthesia not possible by simple observation of the animals.

This was also demonstrated when propofol was administered by bolus then slow infusion over 30 minutes (figure 3-10). The initial bolus of drug produced loss of all observable endpoints such as eye closure, loss of weight bearing and loss of

swallowing reflexes (apparent anaesthesia) and the recorded increase in threshold current of approximately 200% was consistent with that seen after the 100 mg dose over 2 minutes. Continued measurement of threshold current, however, demonstrated that the animal had preserved ability to withdraw to a noxious stimulus and increasing drug effects could be recorded for the next 30 minutes.

#### 3.4.3.2 Response to xylazine

Xylazine is an alpha-2 receptor agonist which is used as an analgesic agent in animals including sheep (Eisenach et al., 1994). In fact, the sedative and anaesthetic properties of alpha-2-agonists are increasingly recognised (Maze and Tranquilli, 1991) and, at higher doses, these agents will produce antinociception due to both sedation and analgesia. As with propofol and thiopentone, a dose-dependent effect on threshold current was recorded after increasing doses of xylazine administered intravenously (figure 3-11A). Intramuscular administration of 5 mg produced a similar time-course and magnitude of effect to the intravenously administered 5 mg dose, suggesting rapid absorption and a high bioavailability.

As mentioned earlier, it is not possible to differentiate between changes in threshold current induced by analgesic or sedative/anaesthetic effects of administered drugs using this device. The increases in threshold current recorded after the lower doses of xylazine therefore could be due to antinociception through either, or both mechanisms. Although behaviour suggestive of sedation was observed only after the highest dose of xylazine, the studies with propofol had demonstrated the insensitivity of this as an indicator of drug activity, and minor degrees of sedation may have been present with all doses.

#### 3.4.3.3 Response to opioids

The marked agitation exhibited by all sheep following all doses of opioids precluded any reliable measurements of antinociceptive effects. In particular, leg movements made it impossible to reliably identify limb withdrawal in response to the noxious stimulus. This response in sheep is well described (Davis, 1983). In the early descriptions of a thermal and mechanical algesimetry device in sheep, the responses of sheep to i.v. injection of opioids such as fentanyl and pethidine, in doses similar to those used in the current study, were described. No pooled data were displayed, only typical data showing large increases in mechanical threshold to induce limb withdrawal (Nolan et al., 1985; Nolan et al., 1987). Behavioural problems which interfered with measurements were not discussed despite the limb lift endpoint being the same as that used in the current study. In later work, the same group described agitation and locomotor activity following administration of similar doses of opioids and its amelioration following administration of droperidol, a dopamine antagonist (Livingston et al., 1991). It is also possible that differences in

measurement frequency (approximately 5 minutely in the above work) underlies the inability to detect the withdrawal endpoint reliably in the current study. As described previously, measurements more frequently than every 30 seconds produced agitation in sheep and it is possible that the effect of stimulus induced agitation and opioid induced locomotor activity may have combined to make measurements every 30 seconds impractical. Less frequent measurements could be attempted in future studies, but as pharmacokinetic-pharmacodynamic studies following rapid injection of drugs require very frequent measurements of effect, this has not been pursued in the current series of experiments.

In summary, the studies presented in this chapter demonstrate the efficacy of an algesimetry paradigm in the frequent measurement of the central nervous system effects of sedative/anaesthetic and analgesic drugs. Application of an appropriate pattern of ramped electrical stimulus induces a reproducible readily identified limb withdrawal response. The threshold current to induce withdrawal changes in a dose dependent manner following administration of a range of centrally acting drugs, allowing frequent measurement of the time-course of drug effect with improved sensitivity over simple observation of observed spontaneous behaviour. This would therefore appear to be a suitable method to use in studies comparing the time-course of central nervous system pharmacokinetic and pharmacodynamics of anaesthetic agents following rapid administration.

# CHAPTER 4. DEVELOPMENT OF A CEREBRAL BLOOD FLOW METHOD FOR STUDIES OF CEREBRAL PHARMACOKINETICS AND PHARMACODYNAMICS IN UNRESTRAINED SHEEP.

In chapter 1 it was shown that measurements of blood concentrations of drugs often fail to provide adequate information on the distribution and effects of intravenous anaesthetic agents administered rapidly during induction of anaesthesia. In contrast, studies of organ drug concentrations have provided clear insight into drug distribution and effect following similar rapid administration which cannot be derived from blood concentrations alone.

The various methods of measuring cerebral drug concentrations were reviewed, and it was shown that the direct application of mass balance principles provided a method that allowed frequent accurate measurement of the time-course of organ drug concentrations in conscious animals. This has previously been applied successfully to sheep for measurement of regional concentrations in the heart, liver, lung and hindquarters (Huang et al., 1993a; Upton et al., 1991c; Runciman et al., 1984). In this chapter, the requirements for the successful application of this method to the brain in sheep are reviewed, and the methods developed and validated.

Accurate calculation of organ drug concentrations at frequent intervals requires methods for:

1. frequent collection of arterial blood (input into the organ)

2. continuous accurate organ blood flow measurement

3. frequent collection of pure organ venous blood (output from the organ).

Previous studies in the sheep have demonstrated that arterial and organ venous blood can be frequently sampled in the awake sheep through chronically implanted catheters (Huang et al., 1994; Runciman et al., 1984), and that pulsed Doppler flow probe technology allows long-term measurement of flow through blood vessels (Huang et al., 1992) in awake unrestrained animals. For these techniques to be applied to the brain, a method was needed for continuous CBF measurement and rapid sampling of "pure" cerebral venous blood.

Previously described methods for CBF measurement did not appear suitable for simultaneous pharmacokinetic-pharmacodynamic studies in awake unrestrained animals. Placement of Doppler crystals on cerebral arteries was considered, but rejected because multiple arteries supply blood to the brain in most species and major flow redistribution can occur between these vessels (Abboud, 1981). Measurement at only one arterial site could therefore lead to inaccuracies in flow

estimation. Furthermore, changes in vessel diameter which may occur under physiological or pathophysiological circumstances, can alter vessel diameter and hence the relationship between flow and blood velocity measured with a Doppler technique (Hansen et al., 1983).

There are descriptions of successful CBF measurement using cerebral outflow vessels in species other than sheep (Michenfelder et al., 1968; Stange et al., 1989; Mattle et al., 1990). As a site for "pure" cerebral venous blood sampling would need to be identified for pharmacokinetic calculations regardless of how CBF was measured, it was decided to attempt to combine the techniques by measuring flow and collecting blood at a common venous site. 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. These will be considered separately.

## 4.1 IDENTIFICATION OF A SUITABLE ANATOMICAL SITE

### 4.1.1 INTRODUCTION

The anatomy of blood outflow from the brain has been described in detail in a number of species, and in general follows a similar pattern (Hegedus and Shackelford, 1965). Intracranially, capillary blood drains through cerebral veins and into cerebral sinuses that have walls composed of fibrous dura mater and lined with endothelium (Capra and Kapp, 1987). 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. 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. For example, in man this has been the rationale for retrograde insertion of catheters into the jugular bulb, one of the exit points of cerebral sinuses from the skull, for measurement of cerebral venous oxygen content (Sheinberg et al., 1992; Lewis et al., 1995) in the management of neurotrauma. More recently, Doppler probes have been placed at this location and used for the measurement of CBF (Ohsumi et al., 1994). A similar site was not considered suitable in sheep because this collects blood predominantly from only one hemisphere, 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. The cerebral venous drainage of the sheep was therefore examined to attempt to identify an intracranial site which would provide ready access to pure cerebral venous blood. Previous reports (Pappenheimer and Setchell, 1973) suggested the dorsal sagittal sinus may be a suitable site. This was therefore confirmed using the following methods.

### 4.1.2 METHODS

#### 4.1.2.1 In vivo angiography studies

The vascular anatomy of the brain was examined by following the passage of a bolus of radio opaque dye through the brain following arterial injection. Two Merino sheep from the same flock used in all subsequent studies were anaesthetised as described in chapter 2, with normocarbia maintained using hand ventilation and capnography. A 7-French catheter was placed percutaneously in each carotid artery and the sheep transferred to the digital angiography suite of the Royal Adelaide Hospital. They were placed supine, and a rapid bolus of contrast medium (Optiray 320, Mallinkrodt Medical) injected at separate times into each carotid artery catheter, and digital subtraction angiographs of the head were taken at 0.5 second intervals in the vertical and horizontal planes during the passage of contrast through the head. These radiographs were then viewed in "real time" by a radiologist to determine the pattern of blood flow.

#### 4.1.2.2 Retrograde dye studies

The area drained by the sagittal sinus was examined post-mortem by retrograde injection of India ink. Following systemic anticoagulation with 10,000 I.U. of heparin (David Bull Laboratories, Melbourne, Australia) intravenously, two Merino sheep from the same flock were killed with an overdose of pentobarbitone. The dorsal sagittal sinus was then exposed through a 20 mm trephine hole and cannulated with a 20 g cannula at a point corresponding to the caudal junction of the parietal and frontal bones. The sagittal sinus was ligated immediately caudal to the cannula ("downstream") and 20 ml of India ink infused slowly through the cannula so it passed retrograde to normal blood flow. The extra-cranial distribution of dye was then examined by extensive dissection of the soft tissues of the head. Following this, the brain was removed through a large craniotomy, sectioned in the coronal plane at 0.5 cm intervals, and also examined for dye distribution.

### 4.1.3 RESULTS

#### 4.1.3.1 Digital subtraction angiography

The high resolution obtained by digital subtraction of background images allowed clear demonstration of the passage of contrast through the arterial tree, brain parenchyma, and venous/sinus system. Static films displayed the anatomy of the vessels, but "real-time" viewing of serial films also allowed estimations of proportional flows. The arterial phase of passage of contrast revealed multiple branches of the carotid artery and the relatively small blood flow to the brain (figure 4-1). 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 and then to the

two transverse sinus which passed laterally and inferiorly (figure 4-2). Blood from a relatively small area of the brain on the inferior aspects of the hemispheres, cerebellum and the brainstem drained posteriorly via the straight sinus. Extra-cerebral blood was seen to join the transverse sinus ("downstream" from the sagittal sinus) and a small quantity of blood entered the sagittal sinus from two vessels adjacent to the nose (v. lateralis nasi). This was estimated to represent less than 5% of the blood flow at this point.

#### 4.1.3.2 Retrograde dye studies

Examination of the extra-cranial soft tissues revealed no dye except for a small amount adjacent to the nose in the v. lateralis nasi. Examination of the brain found dye extensively distributed throughout the brain parenchyma, with the exception of the inferior aspect of the cerebral hemispheres, the cerebellum and the brainstem (figure 4-3). This intra- and extra-cerebral distribution was consistent with the findings on angiography described above.

### 4.1.4 DISCUSSION

It is critical for the proposed studies that only the flow of cerebral blood is measured. Mass balance principles can only be applied to an organ if both "effluent" blood from which samples are taken, as well as flow measurements, are representative of that organ (Upton, 1994). Figure 4-1 clearly shows that measurement of flow in extra-cerebral arteries supplying the brain is impractical in sheep. The rete mirabile (a vascular network which acts as a cooling mechanism to prevent hyperthermia of cerebral blood) interposed between the carotid artery and the brain parenchyma makes an approach to the brain from the carotid artery impossible, and the very small vessels leading from the rete are not surgically accessible. The studies described here show that 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. A more anterior approach would access a lesser proportion of the total cerebral blood flow and a more distal approach would risk sampling blood from the transverse sinus contaminated with extra-cerebral blood. These findings are consistent with those previously described in sheep, but reassuring given that venous anatomy can be variable between species and possibly breeds. Anatomical studies have suggested that extra-cerebral contamination from the face is minimal at this point of the sagittal sinus (Hegedus and Shackelford, 1965) and that the dorsal sagittal sinus represents blood from approximately 70-80% of the brain. Other work has specifically sought the presence of extra-cerebral blood in the cerebral sinuses. Injected microspheres, which enter the extra-cerebral venous blood from the head through arteriovenous anastomoses in the sheep nose, have been detected in significant numbers in blood collected from the transverse sinus, but not to any

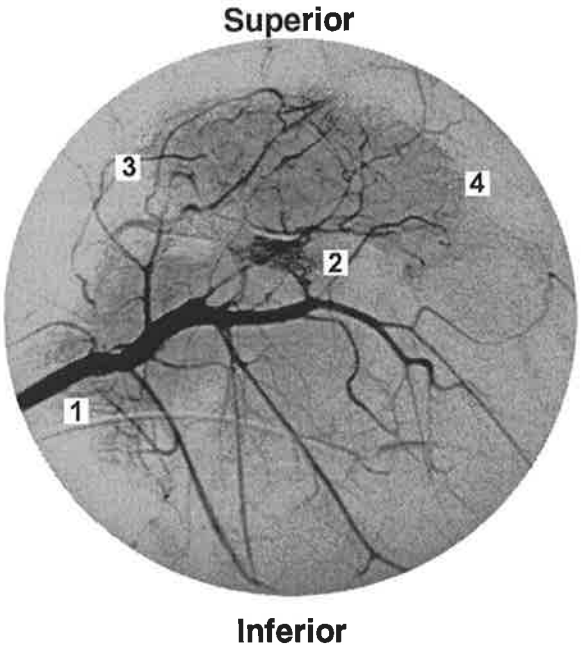


**Figure 4-1** A digital subtraction angiograph of the sheep head (right lateral view) showing dye passing through the arteries. Displayed are (1) the carotid artery, (2) the rete mirabile, (3) the occipital lobe and (4) the frontal lobe.

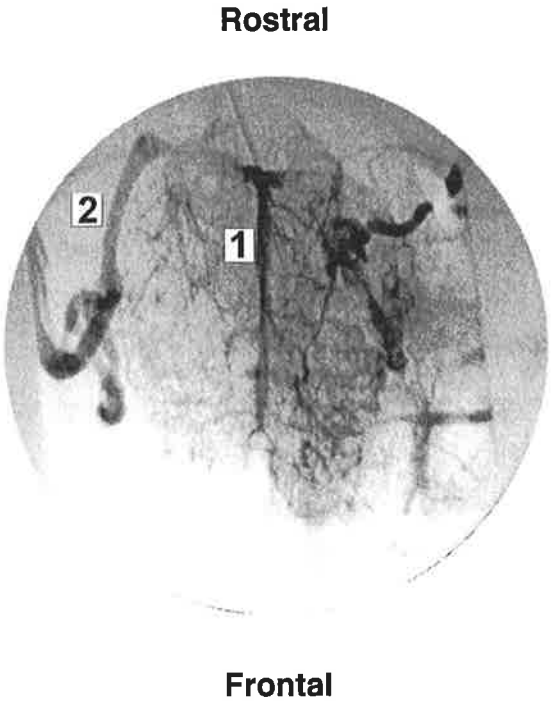


**Figure 4-2** A digital subtraction angiograph of the sheep brain in the coronal plane (superior view) showing dye passing from cerebral veins rostrally (upwards in this picture) into the dorsal sagittal sinus (1) and then into the straight sinus (2).

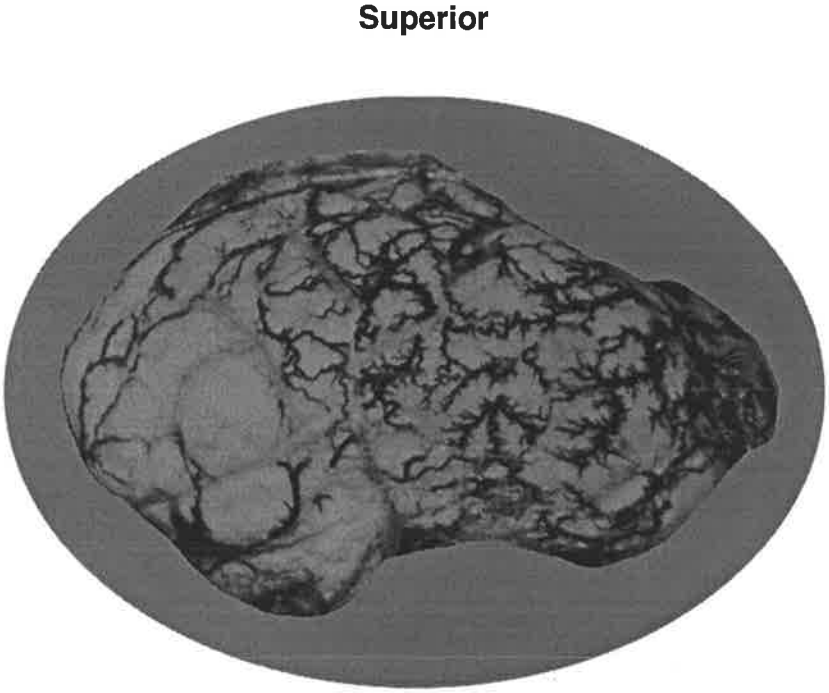


**Figure 4-3** A right lateral view of a sheep brain showing India Ink staining the brain following retrograde injection down the sagittal sinus. The dye has spread throughout the brain, except for a portion of the infero-posterior aspect (bottom left in this picture).

extent in blood from the sagittal sinus (Hales, 1972). In addition, a temperature change following injection of cold saline into the para nasal veins of a sheep was detected by thermocouples implanted in the transverse sinus but not the dorsal sagittal sinus (Hales, 1972). This differs significantly from other species, like the dog, where contamination from facial blood is much more significant and ligation of facial veins is necessary before accurate measurements of CBF can be made using sagittal sinus blood (Michenfelder et al., 1968).

While angiography demonstrated that a proportion of the venous outflow from the brain is not measured at this point on the sagittal sinus, it is evident that blood in the sagittal sinus drains from a wide range of anatomical locations in the brain. Furthermore, while there are known to be regional differences in the metabolic requirements that determine regional CBF (Miller and Bell, 1987), data on the regional effects of propofol on cerebral metabolism suggest that its effects are relatively homogeneous throughout the brain (Cavazzuti et al., 1991). In studies on propofol it would therefore appear likely that changes in both flow and venous blood content at this site would be representative of the whole brain.

There is, therefore, good evidence that the majority of cerebral blood in the sheep passes uncontaminated through the sagittal sinus and that it would appear suitable

for the purposes of sampling cerebral venous blood and measurement of CBF. Indeed, the sagittal sinus of the sheep has been similarly used previously to sample cerebral venous blood (Hales, 1973a; Pappenheimer and Setchell, 1973; Szeto et al., 1980).

## 4.2 VALIDATION OF THE CBF METHOD

### 4.2.1 INTRODUCTION

After identification of a suitable site to access cerebral blood, the validity of the flow method was examined in a second group of sheep. A pulsed Doppler flow probe, which measures the Doppler shift of ultrasonic pulses when reflected from moving particles (Franklin et al., 1959), was selected for placement on the sinus. As such, it is more accurately termed a velocity probe. 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. 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. As the suitability of the site had already been determined, it was decided that it was now necessary to demonstrate:

- that the sagittal sinus diameter and flow profile remains constant over a range of flows

- that the relationship between flow and velocity is linear

- that flow responses to physiological and pharmacological perturbations are consistent with previously described work.

### 4.2.2 METHODS

#### 4.2.2.1 Equipment

A 545C-4 directional pulsed Doppler flowmeter (Bioengineering, University of Iowa, Iowa, USA, (Haywood et al., 1981) and "suture down" style 1 mm diameter 20 MHz piezoelectric transducers mounted on a cloth patch (Figure F, Tritonics Medical Instruments, Iowa City, Iowa, USA) was selected. The Doppler flowmeter and the probe send 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$^{o}$ to the vessel wall. The resulting echoes from the vessels walls and blood cells are received by the same transducer and amplified by the Doppler flowmeter. The emitted ultrasound is pulsed so that the returning echoes are separated in time according to the distances travelled. Functionally, "time-gating" of the returning ultrasound echoes can be used to determine the distance into the vessel that the flowmeter is measuring velocity. This can be controlled by the "range" of the Doppler flowmeter, which in general use is adjusted until the velocity signal from the

flowmeter is maximal, which 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 was used to determine the influence of CBF on vessel diameter. This is described below.

The output from the flowmeter was 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 1 Hz.

#### 4.2.2.2 Surgical placement of the flow probe

Adult female Merino sheep aged between one and two years were used for the study. Anaesthesia was induced using intravenous thiopentone (20 mg/kg), a cuffed endotracheal tube was inserted into the trachea and the lungs ventilated with a mixture of 2% halothane in oxygen. Each animal was placed in the "sphinx" position and the head shaved and soaked in a povidone-iodine antiseptic solution. All surgical procedures were performed under aseptic conditions. A midline longitudinal incision of approximately 8 cm in length was made in the scalp, and the periosteum reflected. The caudal junction of the frontal and parietal bones was located and a hole made on the longitudinal median suture using a 19 mm trephine. The bone plug was removed to expose the dorsal sagittal sinus and dura. 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 Doppler transducer was fed from the trephine hole, between the skull and the dura, and the wire pulled through 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 at the position identified in the angiography and dye studies previously reported. The transducer was moved laterally until maximum output from the probe was recorded (figure 4-4).

The bone plug from the trephine hole 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, and the periosteum and scalp sutured closed. The animals were allowed to recover and, after a week to allow the probe and bone plug to become embedded in scar tissue, used for experimental studies.

#### 4.2.2.3 Validation and calibration of the flow measurement

##### 4.2.2.3.1 Vessel diameter over a range of CBF values

The aim of this study was to confirm a constant vessel size, which would suggest a constant relationship between Doppler output and flow, over a range of CBF values.

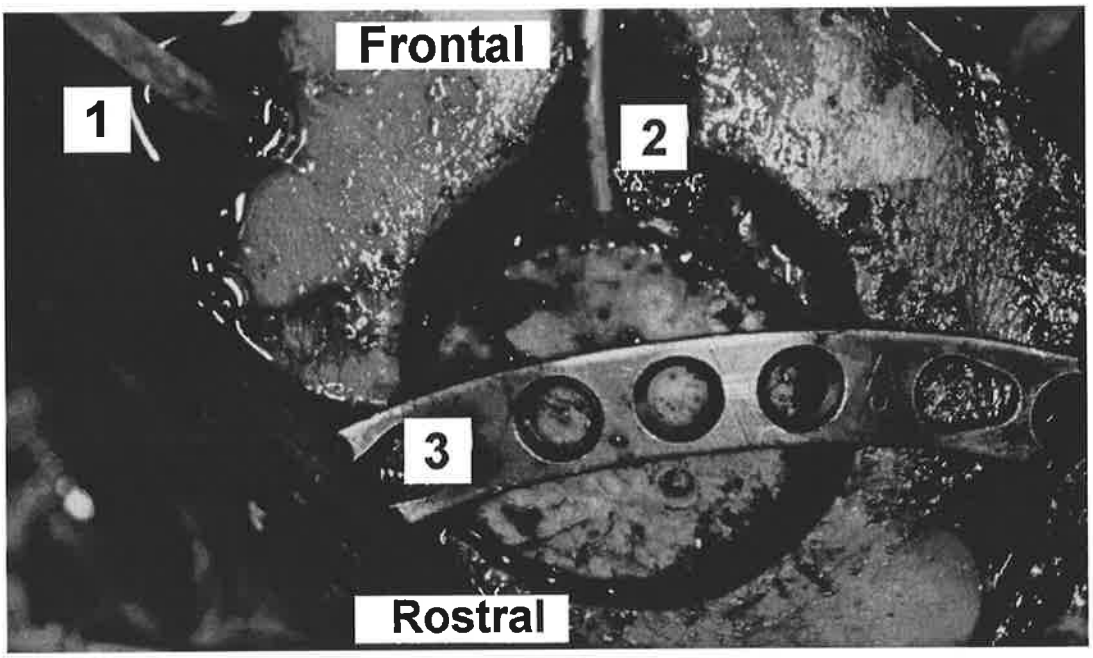


**Figure 4-4** A picture of the dorsal aspect of the sheep head during surgery. Displayed are (1) the wire from the flow probe exiting the skull, (2) the sagittal sinus catheter and (3) the plate to fix the bone plug and flow probe in position.

Six sheep previously prepared with Doppler probes were anaesthetised with 2% halothane as outlined in chapter 2. The ventilation rate of the sheep was initially increased to achieve hypocarbia ($P_{et}CO_2$ approximately 25-30 mmHg). When the Doppler velocity had reached its minimum, the "range" was altered to define the velocity profile in the vessel and its diameter as described previously. The ventilation rate was then decreased to achieve hypercarbia ($P_{et}CO_2$ approximately 65-70 mmHg). Once the maximum Doppler velocity was reached, the measurements were repeated.

4.2.2.3.2 The relationship between Doppler velocity and sagittal sinus blood flow

The aim of this study was to ensure a constant relationship between Doppler output and actual flow over a range of CBF values. Following the examination of diameter changes, a direct venous outflow method was used to measure the true sagittal sinus flow. An incision was made in the scalp of the anaesthetised sheep rostral to the original site and the periosteum reflected. A trephine hole was then made 2 cm caudal to, or "downstream" of, the site of the transducer. Stainless steel screws were used to fix a plastic spout around the hole for the subsequent collection of blood from the sinus. The sheep were systemically heparinised with the administration of 10,000 I.U. of heparin intravenously and placed in the lateral position. The sagittal sinus was located and a non-cutting tapered needle was used to place a ligature around the vessel, this was tightened and the vessel occluded.

An incision was made rostral to the tie allowing blood from the sinus which had just passed under the Doppler probe to be collected from the spout. The ligature prevented any backflow of blood. While the ventilation rate was changed as described previously to give a range of sagittal sinus flow rates, 30 second collections of the volume of blood leaving the sinus were made consecutively using a stop-watch and measuring cylinder. The flow rate was expressed as millilitres per minute and was later correlated with the corresponding mean sagittal sinus Doppler velocity recorded over each 30 second period of blood collection to produce a calibration curve. The sheep were then killed with a barbiturate overdose.

**4.2.2.3.3 The time-course of CBF and its response to perturbations**

The aim of these studies was to examine the behaviour of CBF in response to changes in carbon dioxide tension (both awake and under halothane anaesthesia) and changes in blood pressure, to confirm that CBF responses were consistent with previously published reports.

4.2.2.3.4 Control measurements

The aim of this study was to define the normal range of CBF for sheep using this method, and to define the intra-sheep variability in CBF on different days. In six sheep, measurements of sagittal sinus blood velocity (as Doppler shift in kHz) were made for a 40 minute period to define the natural time-course of CBF. The sheep remained in their metabolic crates and were supported in a comfortable sling that prevented them from lying down. Their heads were free to move, but a strap was placed across their crates such that they could not lower their head below the horizontal. These measures minimised posture related systemic haemodynamic changes. Access to food and water were denied during the measurements.

Measurements of CBF were also made over a 3 minute period at approximately the same time on 3 different days in six awake sheep that were subsequently calibrated as described above to give CBF in ml/min.

4.2.2.3.5 $CO_2$ reactivity

*Halothane anaesthesia*

Six sheep were anaesthetised and ventilated as described previously. The end-tidal carbon dioxide concentration in expired breath ($P_{et}CO_2$) was measured using a capnograph (Model OIR 7101, Nihon Kohden Corporation, Tokyo, Japan). While $P_{et}CO_2$ and the Doppler blood velocity in the sagittal sinus were measured continuously as described previously, the ventilation rate of the sheep was increased to achieve hypocarbia ($P_{et}CO_2$ approximately 25-30 mmHg), and then decreased to achieve hypercarbia ($P_{et}CO_2$ approximately 65-70 mmHg). Cerebral

blood flow reactivity curves were determined from the relationship between $P_{et}CO_2$ and sagittal sinus Doppler output.

*Awake*

Five sheep were prepared and placed in a comfortable sling, as described above. A soft plastic mask connected to a non-rebreathing circuit was placed over the sheep's mouth and nose and a total flow of fresh gas through the circuit of 20 L/min was maintained at all times. Initially, 100% oxygen was administered during a period of baseline measurement of CBF and mean arterial pressure. Carbon dioxide was then introduced into the circuit in increments, while oxygen flow rates were reduced to maintain total flow at 20 L/min. Cerebral blood flow and mean arterial pressure were continuously measured, and an arterial sample was taken for gas analysis when CBF had reached a plateau following each increase in inspired carbon dioxide concentration. To allow a simple comparison of awake carbon dioxide response with values recorded under anaesthesia, the inspired concentration of carbon dioxide was restricted to keep arterial carbon dioxide tension within the range of 35-60 mmHg. This is the range in which the relationship between carbon dioxide tension and CBF can be reasonably described by a linear function.

4.2.2.3.6 The response to hyper- and hypotension

Five sheep were prepared with the Doppler probes as described above. One week following surgery, the response to administration of both metaraminol (Merck Sharp and Dohme, Granville, NSW, Australia) and sodium nitroprusside (David Bull Laboratories, Melbourne, Victoria, Australia) was measured on separate occasions. On each occasion the sheep were placed in a comfortable sling as described previously and, following a period of baseline measurement of CBF and mean arterial pressure, were administered either metaraminol (0.5 mg/min for 5 minutes) or sodium nitroprusside (0.4 mg/min for 10 minutes). Pilot studies had revealed that these doses were sufficient to induce a slow increase and decrease in mean arterial pressure, respectively. Changes from baseline were determined using repeated measures analysis of variance.

## 4.2.3 RESULTS

### 4.2.3.1 Validation and calibration of the flow measurement

4.2.3.1.1 The influence of CBF on vessel diameter

Examples of the velocity profile across the sinus at high and low flows are shown in figure 4-5. It is apparent from this figure that it is difficult to accurately determine the exact location of the far edge of the sinus, and that the location of the centre of the vessel is difficult to determine with an accuracy of less than 15%. To determine

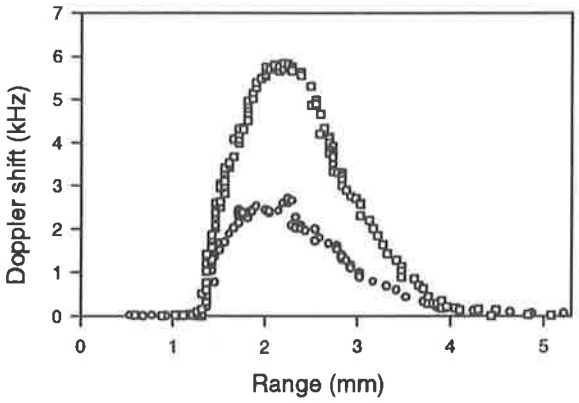


**Figure 4-5** The flow profile in the sagittal sinus during hypocarbia (circles) and hypercarbia (squares).

if the dimensions of the vessel changed with flow against this inherent variability in measurement, an extra 2 sheep were studied, and the distance from the probe to the centre of the vessel (the radius) was determined from the velocity profiles at high and low flows. For a total of 8 sheep, the average increase in velocity from low to high flows was 135%. Concurrently, the radius decreased on average by 5.5 ± 4.4% at high flows. More importantly, a paired Students t-test found no statistical difference between the radius at low and high flows ($p = 0.24$), showing that there were no significant changes in vessel dimensions with changes in flow.

4.2.3.1.2 Relationship between Doppler velocity and sagittal sinus flow

Regression analysis of calibration data from the 6 animals showed a linear relationship between the measured Doppler blood velocity (kHz of Doppler shift) and actual flow (ml/min) determined using the direct venous outflow method. For all animals, the $r^2$ value ranged between 0.91 and 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. The slopes ranged from 0.010 to 0.027 and the intercept from 0.028 to 0.53. This implies that every animal must be individually calibrated if actual flow values are required. An example of calibration data is shown in figure 4-6.

### 4.2.3.2 The time-course of CBF and its response to perturbations

4.2.3.2.1 Control measurements

The time-courses of the CBF measured in six sheep were generally constant over a 40 minute period (an example is shown in figure 4-7). However, it was found that the measured velocity was dependent on the level of arousal of the animal, and for consistent measurements it was necessary to acclimatise the sheep to the

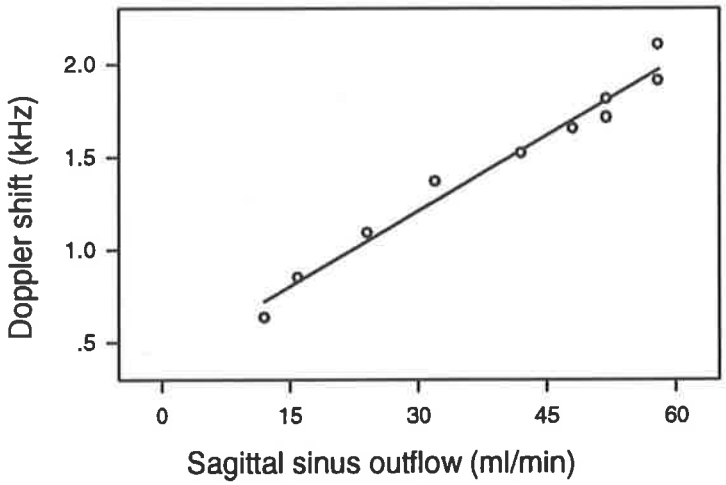


**Figure 4-6** An example of the relationship between sagittal sinus outflow and recorded Doppler signal during flow probe calibration under anaesthesia in one animal. Regression analysis produced an $r^2$ value of 0.97.

experimental conditions, and to ensure that they were not presented with distractions (such as someone walking into the room) during a study. There was little obvious relationship between CBF and head movement.

The frequency response of the University of Iowa Doppler flowmeter is known to be sufficient to characterise the beat by beat blood velocity profile in arteries, thus it can be assumed that the trace shown in figure 4-7 represents the true time-course of sagittal sinus blood velocity. Typically, there was a slow cycling of velocity with a period of 1-3 min, superimposed with rapid transient spikes of increased velocity which appeared to be related to the level of arousal of the animal.

For the six sheep in which calibrated measurements were made on 3 different days, it was not possible to make a measurement on the third day for 2 sheep. For the six sheep, the coefficient of variation of the control CBF for the 3 min recording periods for was between 5 and 15%, with the exception of one measurement for which the value was 44%. The control CBF varied between sheep, with a highest value being a mean of 53 ml/min for the three measurements in one sheep, and the lowest value being a mean of 31 ml/min in another.

For two of these sheep, the weight of their brains were determined to be 79 and 90 g at autopsy. Assuming the mass of brain drained by the caudal dorsal sagittal sinus under the Doppler probe was 75% of these values, as discussed previously, this gave CBF values of 34 and 30 ml/min per 100 g of tissue. For greater accuracy, the brain could be stained with a retrograde injection of Indian ink and dissected as described previously to determine the actual mass of the region

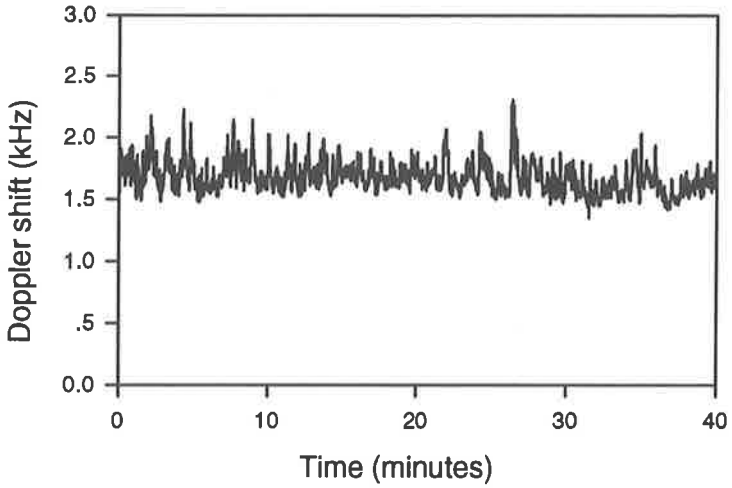


**Figure 4-7** The signal from the Doppler probe placed on the sagittal sinus recorded at 1 Hz over 40 minutes in an awake unrestrained animal.

drained by the sinus beneath the probe, although this information is not necessary for the drug studies for which the method was developed.

4.2.3.2.2 $CO_2$ reactivity

*Halothane anaesthesia*

The combined results from 6 animals are shown in figure 4-8. As expected, these data showed a sigmoid relationship between $P_{et}CO_2$ and CBF. The data were curve-fitted to a sigmoid equation using the least squares package MINSQ II (Micromath Scientific Software, Salt Lake City, Utah, USA). The line of best fit ($R^2$ = 0.99) showed that the maximum and minimum CBF occurred at a $P_{et}CO_2$ of approximately 65 mmHg and 30 mmHg respectively, and that the relationship between the two parameters was approximately linear over the $P_{et}CO_2$ range 40-60 mmHg.

*Awake*

The combined results from 5 animals are shown in figure 4-9. In this carbon dioxide range, regression analysis demonstrated a linear relationship between carbon dioxide tension and CBF in all animals, with $r^2$ values ranging from 0.75-0.99. During each baseline period, however, there were variations in the carbon dioxide tension in each animal which contributed to the scattering of data shown in figure 4-10 A. This scatter was minimised by normalising carbon dioxide values, and the pooled data are displayed in figure 4-9 B. Regression analysis of these data revealed a linear relationship between carbon dioxide tension and CBF ($r^2$=0.76). This normalisation also allowed direct comparison between the carbon dioxide

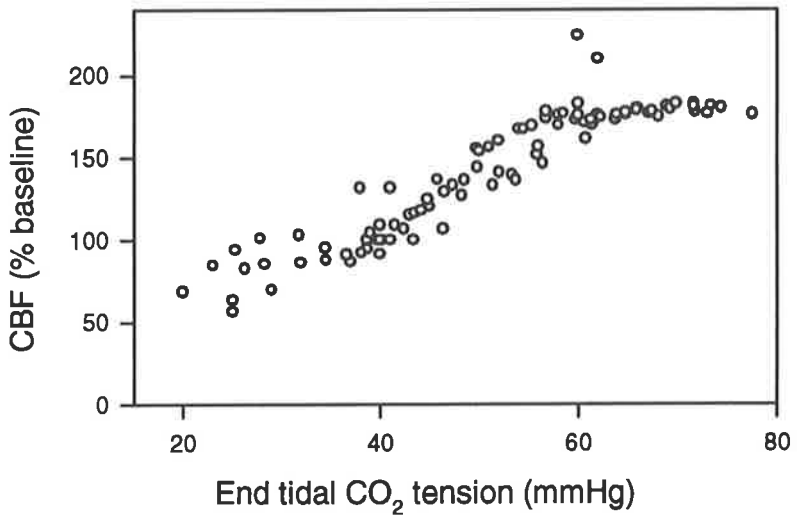


**Figure 4-8** The relationship between end tidal $CO_2$ tension and CBF in 6 animals anaesthetised with 2% halothane.

response awake and under halothane anaesthesia (figure 4-11). The slopes of the regression lines for awake and anaesthetised animals were 1.88 ± 0.19 and 1.59 ± 0.10 respectively, which were not statistically significantly different when compared with a Student's t-test (p=0.13).

The response to hyper- and hypotension

Administration of metaraminol produced a gradual increase in mean arterial pressure to reach a maximum increase of 30 mmHg at 5 minutes (p<0.0001) but CBF did not change significantly (p=0.17) (figure 4-11). Administration of sodium nitroprusside produced a gradual decrease in mean arterial pressure from approximately 100 mmHg to 55 mmHg (p<0.0001). During this period CBF decreased by a maximum of 20% (p<0.0001) despite a decrease in mean arterial pressure from 100 to 50 mmHg (figure 4-12).

### 4.4.4 DISCUSSION

While many methods of CBF measurement exist, continuous measurement of global flow in awake animals presents particular problems. Flow probes have generally been necessary for the continuous measure of CBF, and have been applied to cerebral arteries (Sonesson and Herin, 1988) as well as the sagittal sinus (Michenfelder and Milde, 1988; Stange et al., 1989) Most reports are in anaesthetised animals. 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 (Haywood et al., 1981; van Bel et al., 1994; O'Brien et al., 1991). Problems remain, however, with the flow-velocity

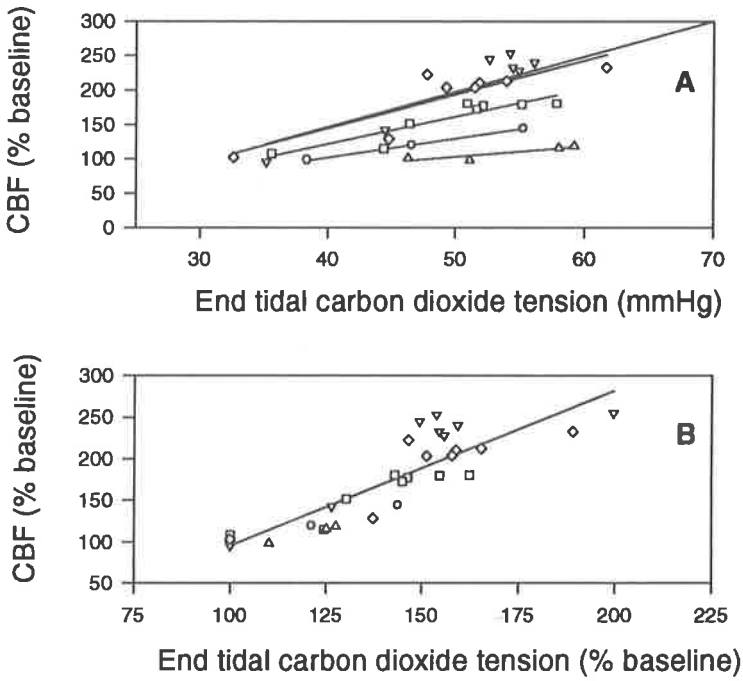


**Figure 4-9** The relationship between end tidal $CO_2$ tension and CBF in 6 awake unrestrained animals anaesthetised with 2% halothane. Data are displayed with end tidal $CO_2$ tension in mmHg (A) and normalised as % baseline (B).

relationship when vessel diameter can alter. In one study, for example, increases in the velocity of blood in cerebral arteries recorded using Doppler shift following hypercarbia were only half the increases recorded simultaneously using hydrogen clearance (Nelson et al., 1990), suggesting marked increases in vessel diameter. While calibrated Doppler flow probes are now available (Anonymous, 1995), they rely on placement of a fixed diameter probe around a vessel; not feasible in the sagittal sinus of a sheep.

The sagittal sinus was deliberately selected because of its structure. The walls of cerebral sinuses are comprised of dura and contain no smooth muscle, so active change in diameter does not occur (Capra and Kapp, 1987). Furthermore, the thick fibrous nature of the dura is likely to be resistant to dilatation from changes in intraluminal pressure (Lee and Hoff, 1996). The data presented here support the choice of the sagittal sinus and a velocity probe. Vessel diameter did not change across a four-fold change in flow (figure 4-5) and the flow-velocity relationship remained constant (figure 4-6). Despite these findings, it is important to recognise that this validation is limited to the normal brain. The hypercarbia induced in these studies was unlikely to induce major changes in intracranial pressure (ICP) because changes occurred relatively slowly and normal homeostatic mechanisms such as shunting of cerebrospinal fluid would be expected to be intact. Because of the rigid

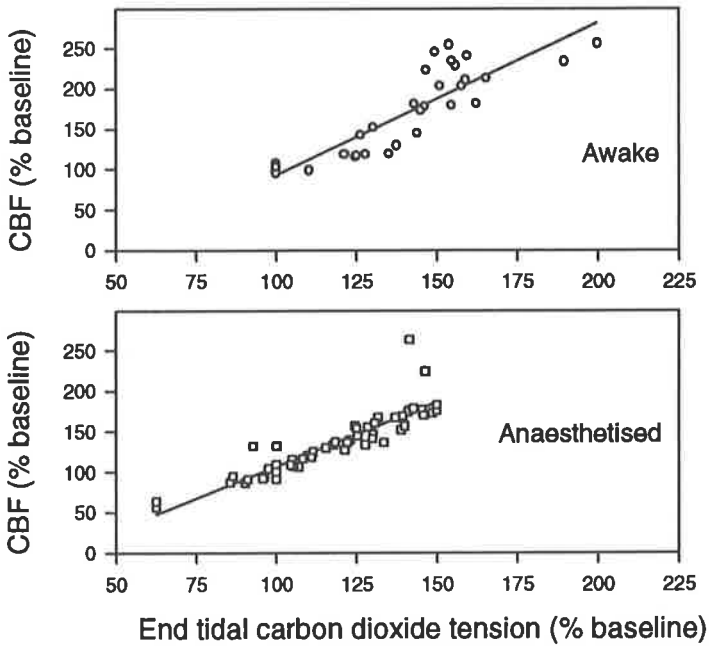


**Figure 4-10** The relationship between end tidal $CO_2$ tension and CBF in 5 awake unrestrained animals and 6 animals anaesthetised with 2% halothane. Data are displayed with end tidal $CO_2$ tension normalised as % baseline.

structure of the skull, large increases in ICP might compress the sinus and alter the flow-velocity relationship, leading to inaccuracies in flow measurement. Confirmation of a constant vessel diameter using the range studies described above should therefore be sought in studies where these conditions may occur.

It is of interest to compare the measured values and time-course of CBF with the values cited in the literature . With the Doppler method, the global CBF in sheep was 30-34 ml/min/100g. This is approximately half the 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 (Hales, 1973b; Hales, 1973a). These figures are significantly higher than the values of approximately 50 ml/min/100g commonly found across other species (Michenfelder, 1990; Madsen et al., 1993). It is not possible to determine the reasons for these differences, but, as noted above, any arousal or stress can transiently increase CBF and may influence the control values of CBF measured with a given technique.

The recorded flow responses to changes in $CO_2$ tension and blood pressure provide further support to this method. The $CO_2$ response curve for CBF is well described in the literature (Michenfelder, 1990; Anonymous, 1972). There is a sigmoid relationship over the full range of $CO_2$ tensions, with a pseudo-linear increase in CBF over the range of $CO_2$ of approximately 35-60 mmHg. At extremes

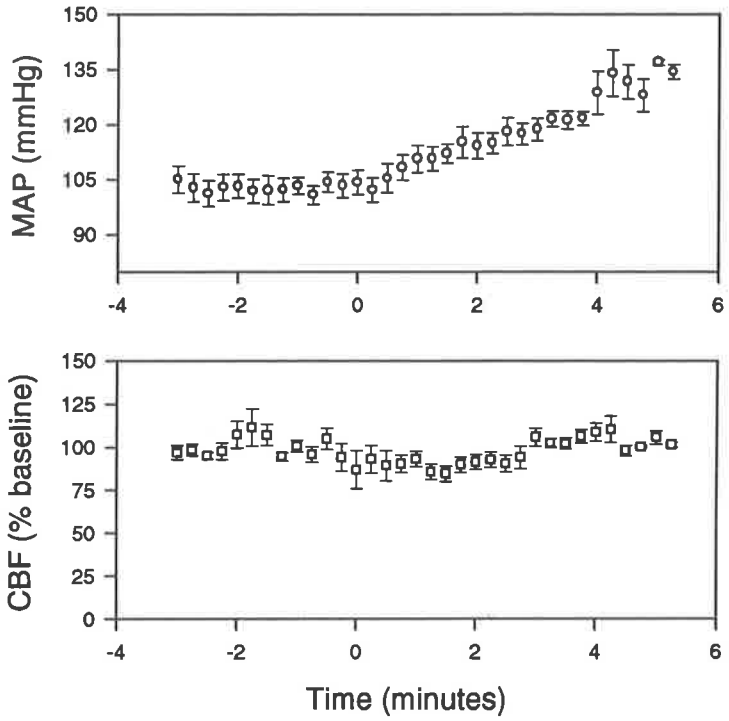


**Figure 4-11** The effects of administration of metaraminol (0.5 mg/min for 5 minutes from time=0) on mean arterial pressure (circles) and CBF (squares). Data are displayed as mean & sem.

of hypercarbia and hypocarbia, CBF increases and decreases over a fourfold range. In the current study, the response of CBF to changes in $P_{et}CO_2$ under halothane anaesthesia reproduced this classical sigmoid shaped curve (figure 4-8) with inflection points that closely matched literature values. In addition, the slope of the CBF response in the range of $CO_2$ between 35 and 60 mmHg was similar to that reported in previous studies (Michenfelder, 1990). When the CBF responses to $CO_2$ were examined in awake animals, there was more inter individual variation in the data than that recorded under anaesthesia (figure 4-9). While this is likely to partially reflect the variability in CBF that was detected in awake animals during normocarbia, it may also relate to the variation in baseline $CO_2$ tension between spontaneously breathing animals evident from the lowest (baseline) $CO_2$ values in each animal in figure 4-10. Accounting for this by normalising $CO_2$ tension revealed a more consistent response to $CO_2$ increases (figure 4-9). The similarity in the CBF response to $CO_2$ in awake animals and those anaesthetised with 2% halothane revealed in the current study (figure 4-10) is consistent with previous reports suggesting 1 MAC of halothane does not affect $CO_2$ responses (Madsen et al., 1987; Leon and Bissonnette, 1991).

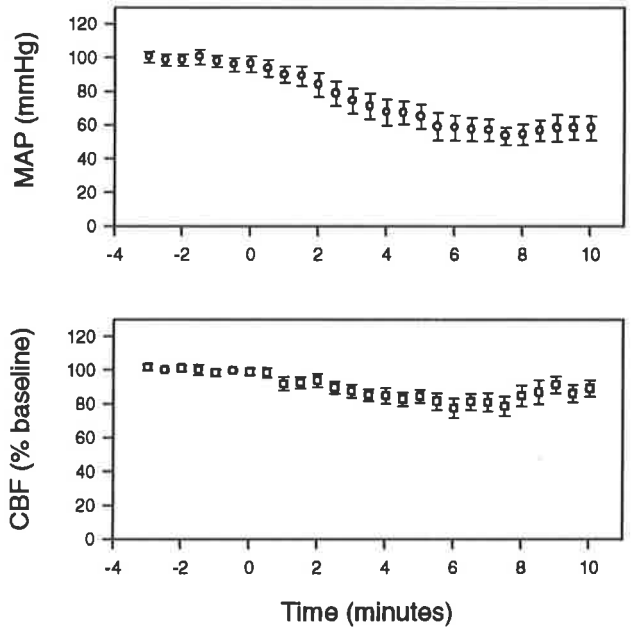


**Figure 4-12** The effects of administration of sodium nitroprusside (0.4 mg/min for 10 minutes from time=0) on mean arterial pressure (circles) and CBF (squares). Data are displayed as mean & sem.

The CBF responses to drug induced changes in blood pressure recorded in this study are also consistent with reported behaviour of the cerebral circulation. The effect of hypertension on CBF has been extensively studied, with the majority of studies using pharmacological agents to increase blood pressure. Hypertension induced with metaraminol has previously been shown to have no effect on CBF, despite significant increases in mean arterial pressure, as long as the systemic pressure is kept below approximately 150 mmHg (Eyre et al., 1988; Burke et al., 1987). Similar results have been found with other vasopressors such as phenylephrine and noradrenaline (Ong et al., 1986; Drummond and Shapiro, 1990). As none of these agents is thought to have a significant direct effect on the cerebrovascular smooth muscle, these findings are believed to reflect the normal autoregulatory mechanisms of the cerebral circulation. The lack of change in CBF following an increase in mean arterial pressure of approximately 30 mmHg demonstrated in the current study is therefore consistent with this phenomenon.

Hypotension is also associated with only minor changes in CBF until blood pressure decreases below approximately 50-60 mmHg (Drummond and Shapiro, 1990). Ideally these studies are performed using non-pharmacological methods such as haemorrhage or local compression to reduce perfusion pressure (Kontos et al., 1978; Florence and Seylaz, 1992), but control of blood pressure using these methods can be difficult and there are many published studies which have examined the CBF responses to drug induced hypotension. The response to

sodium nitroprusside was used in the current study because it was easy to administer and allowed rapid and easily reversible control of blood pressure, thus minimising the risk of inducing distress when studying awake animals. Furthermore, the responses of the cerebral circulation to sodium nitroprusside induced hypotension are well described, allowing ready comparison with the findings reported here. A decrease in mean arterial pressure to approximately 50-60 mmHg has been shown to cause minimal change in CBF in species such as man (Thomsen et al., 1989; Bunemann et al., 1987), monkeys (Sivarajan et al., 1985) and sheep (Ong et al., 1986). In the last of these studies, there was no difference between the CBF responses following hypotension induced with sodium nitroprusside or haemorrhage, suggesting that sodium nitroprusside has no direct effect on the cerebral vasculature in sheep. The minor changes in CBF recorded in the current study despite a large decrease in blood pressure are therefore consistent with the published data on the cerebral circulation and, from the work in the previous study, may demonstrate relative preservation of autoregulation.

In conclusion, the findings presented in the studies in this chapter demonstrate that chronic implantation of a Doppler flow probe on the sagittal sinus of the sheep allows continuous accurate measurement of approximately 75% of CBF in awake animals, with minimal contamination from other vascular beds. In addition, these studies have shown that placement of a catheter in the sinus would allow sampling of almost pure cerebral venous blood. The requirements for application of mass balance principles to calculations of the time-course of drugs in the brain are therefore met. In addition, they can be used to simultaneously measure the effects of drugs on CBF and cerebral metabolic rate (from oxygen extraction across the brain) in awake animals. This preparation would therefore appear to be a useful tool for the studies proposed in this thesis as well as potentially having broader applications for neurophysiological research.

# CHAPTER 5. THE RELATIONSHIP BETWEEN BRAIN AND BLOOD CONCENTRATIONS OF PROPOFOL, AND CEREBRAL EFFECTS AFTER RAPID INTRAVENOUS INJECTION IN SHEEP

## 5.1 INTRODUCTION

The systemic pharmacokinetics of propofol have been extensively investigated and the resultant data used to develop dose regimens in an attempt to provide optimal profiles of depth of anaesthesia during surgery (Short et al., 1994; Taylor et al., 1993; Marsh et al., 1991; Coetzee et al., 1995; Marsh et al., 1990; White and Kenny, 1990). These analyses have almost exclusively used conventional compartmental pharmacokinetic methods as a basis for these dose regimens. While this can be very successful for the prediction of the time-course blood concentrations and effect of propofol during relatively slow administration of this agent (Vuyk et al., 1995; Viviand et al., 1991), the review of compartmental pharmacokinetics in chapter 1 revealed major flaws in this approach during rapid administration. It was evident from this review that the hysteresis between drug concentrations in the blood and the brain during rapid administration means that analysis of the systemic concentrations of propofol cannot be used to accurately predict cerebral effects. While disequilibria between propofol concentrations in blood and anaesthetic effects at induction of anaesthesia are sometimes acknowledged in the literature, their causes remain partly speculative in the absence of data on the time-course and determinants of distribution of propofol to the brain (Stokes and Hutton, 1991; Larsson and Wahlstrom, 1994). In addition, insights into the scientific basis behind attempts to reduce the well documented incidence of adverse effects of rapid administration of propofol using techniques such as altered rate of administration and co-administration of other agents cannot be provided by a systemic pharmacokinetic approach, leaving researchers having to rely on empirical observations.

These issues are particularly relevant to the clinical practice of induction of anaesthesia, a situation where propofol is usually administered via rapid intravenous "bolus" over a period of 20-50 seconds, with rates of administration commonly reaching 200-300 mg/min. (Ooi, 1995; Aun et al., 1993; Kirvela et al., 1992). During this period of induction, the effects of propofol on a number of parameters are important. Changes in depth of anaesthesia, the primary reason for drug administration, are obviously important. The time-course of changes in cerebral blood flow (CBF) and cerebral metabolic rate for oxygen (CMR) are also important considerations.

The anaesthetic effects of propofol probably reflect drug induced suppression of synaptic transmission and electrical activity (Salonen and Maze, 1993) and it might therefore be expected that changes in depth of anaesthesia and brain drug concentrations would be accompanied by parallel changes in CMR. Studies in human and animal subjects have usually found CBF to also decrease along with CMR (Stephan et al., 1988; Vandesteene et al., 1988; Pinaud et al., 1990; Artru et al., 1992). It is usually therefore assumed that CBF changes are not direct effects of propofol, but simply secondary to reduced oxygen requirements of the brain (ie preservation of 'coupling' of CBF and CMR). Many of these studies, however, have been complicated by concomitant use of general anaesthetic agents, known to themselves alter CBF and CMR, or concurrent hypotension which may alter CBF, leaving the evidence for maintenance of CMR-CBF coupling incomplete. As both high and low CBF states produced by uncoupling of flow and metabolism can produce adverse cerebral effects, it is important that these effects of propofol be fully understood.

To examine these pharmacokinetic and pharmacodynamic questions in relation to propofol, it was decided to simultaneously measure the time-course of propofol concentrations in blood and the brain, and the effects on aspects of cerebral function during administration of propofol at rates comparable to those used at induction of anaesthesia. The data from the studies in the previous chapters suggested that a chronically instrumented sheep preparation would allow simultaneous measurement of both systemic and brain drug concentrations and relevant cerebral effects. The specific aims of this study were therefore to use this preparation to:

- examine the relationship between concentrations of propofol in arterial blood and the brain

- examine the relationship between these concentrations and depth of anaesthesia

- examine the relationship between CBF and CMR

following rapid intravenous administration of propofol.

## 5.2 METHODS

### 5.2.1 ANIMAL PREPARATION

Animals were prepared with catheters and a flow probe according to the general methods described in chapters 2 and 4.

### 5.2.2 STUDY DESIGN

Studies were commenced at least 1 week after surgery to allow wound healing. For each study sheep breathing room air remained in their metabolic crates with their weight partially supported by a sling.

#### 5.2.2.1 Parameter measurement

Cerebral blood flow was measured using a Doppler flow probe implanted on the dorsal sagittal sinus, as described in chapter 4. An index of depth of anaesthesia was measured using the threshold of electrical current used to induce limb withdrawal, as described in chapter 3. Mean arterial pressure was measured using an arterial catheter and pressure transducer as described in chapter 2. Haemoglobin saturation and carbon dioxide tension were measured in arterial blood using a gas analyser (CIBA Corning 278, MA, USA). Blood oxygen content was measured in arterial blood using an oximeter (IL482, Instrumentation Laboratory, Lexington, MA, USA) calibrated for sheep blood (Langston et al., 1993).

#### 5.2.2.2 Blood sampling

Blood samples were taken from the catheters at the following times: arterial 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 10, 12.5, 15, 17.5, 20, 25, 30, 35, 40 and sagittal sinus 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12.5, 15, 17.5, 20, 25, 30, 35, 40 minutes after the commencement of administration of propofol. One millilitre of blood from samples taken at each time point was placed in Eppendorf tubes to which heparin (25 I.U.) had been added and then frozen and stored below -5 $^{o}$C for later drug assay using the method described previously in chapter 2. One millilitre samples from both catheters at the time points of 2, 4, 10 & 20 minutes were sealed in gas-tight heparinised syringes for later gas tension and oxygen content analysis.

#### 5.2.2.3 General design

In each study, after the baseline variables were recorded for 3 minutes and baseline arterial blood samples taken, either 50 mg, 100 mg or 200 mg of propofol was administered intravenously at a constant rate over 2 minutes using a syringe pump (Model 33, Harvard Apparatus Ltd, Kent, England). All variables were recorded for 40 minutes and blood samples taken as described above.

Each dose was administered to 5 different sheep but, because of probe or catheter failure, a total of 8 sheep were studied. Two sheep received all doses in random order, 3 sheep received 2 doses and 3 sheep received only 1 dose. In sheep receiving more than 1 dose, at least 48 hours was allowed between studies.

### 5.2.3 DATA HANDLING AND STATISTICAL ANALYSIS

For analysis of drug induced changes in mean arterial pressure, values in each animal were averaged at 30 second intervals. For CBF measurement, calibration of all Doppler probes was not possible due to probe failure in some animals before calibration was possible. The Doppler output was therefore normalised to a baseline of 40 ml/min based on the data in chapter 4. For analysis of drug induced changes in CBF, Doppler output was averaged at 30 second intervals.

For calculation of oxygen extraction, the difference between arterial and sagittal sinus oxygen contents at each time point blood samples were taken was calculated in each animal. Cerebral metabolic rate for oxygen was calculated from the product of cerebral oxygen extraction and CBF. For measurement of depth of anaesthesia, because of variations in baseline values discussed in chapter 3, current threshold values at each time point in individual animals were expressed as the percent increase above the average baseline values. To allow comparison of the relative changes in CBF and CMR, these were also expressed as percent of baseline.

Mass balance calculations for the calculation of brain concentrations were performed as follows. For each animal the net flux of propofol into the brain was calculated from the arterio-sagittal sinus concentration difference and the CBF. As the Doppler probe could not be calibrated in all animals and because sagittal sinus blood velocity is closely related to blood flow under a wide range of values of CBF, CBF values were normalised to a baseline of 40 ml/min (from chapter 4). The total amount of propofol in the brain was calculated from the integral of the net propofol flux over time, and the brain concentration calculated assuming a brain mass in the area drained by the sagittal sinus of 75g (from chapter 4). To simplify mass balance calculations, sagittal sinus propofol values were calculated at the time points 0.25, 0.75. 1.25 and 1.75 minutes by linear interpolation.

All data at each time point for all animals were then pooled and expressed as mean and standard error of the mean (sem). Changes in all variables over time were examined using repeated measures analysis of variance.

#### 5.2.3.1 Pharmacokinetic analysis

To examine for dose dependent effects on arterial concentrations (drug input to the brain), the arterial concentrations were normalised for dose by scaling all values following the 50 mg and 200 mg doses by a factor of 2 and 0.5 respectively. To examine for concentration dependent kinetics in the brain, the effect of different arterial concentrations (input to the brain) after each dose was removed by normalisation. The curves of sagittal sinus and brain concentrations were scaled by the ratio of actual peak arterial concentration and the peak mean arterial concentration after the 100 mg dose.

The relationship between propofol concentrations and cerebral effects was examined by plotting blood and brain concentrations against both current threshold increase and CBF following the 100 mg dose, the only dose which produced a significant change in depth of anaesthesia which could be measured for the entire duration of the study. The drug concentration-effect curves were examined for the presence of hysteresis using the following method. For individual animals, each plot was divided into 2 sections at the point of peak propofol concentration so that

one section represented concentration-effect relationships when concentrations were increasing, and the other when they were decreasing. The area under the curve (AUC) in each section was calculated using the trapezoidal method and the values for all animals were pooled. Hysteresis was considered present when the pooled areas for the sections representing ascending and descending concentrations were statistically different when compared using a paired t-test (Huang et al., 1993).

## 5.3 RESULTS

### 5.3.1 PHARMACODYNAMICS

Cerebral blood flow decreased significantly following 50 mg, 100 mg and 200 mg of propofol ($p=0.002$, $p<0.0001$, $p<0.0001$ for each dose, respectively). The 200 mg dose prolonged the duration of the CBF decrease but did not alter the magnitude of maximum decrease (figure 5-1). These CBF changes were accompanied by small decreases in MAP, with maximal decreases of 9 mmHg, 11 mmHg and 7 mmHg after the 50 mg, 100 mg and 200 mg doses respectively, but the decrease was statistically significant only after the 200mg dose ($p=0.43$, $p=0.47$, $p=0.002$ for each dose, respectively). All doses of propofol produced significant respiratory depression. There was a transient increase in $PaCO_2$ ($p=0.001$, $p<0.0001$, $p<0.0001$ for each dose, respectively) with maximum increases of 7.0 mmHg, 7.4 mmHg and 10.6 mmHg, respectively (figure 5-2). There was, however, no significant change in arterial oxygen content ($p=0.06$, $p=0.28$ and $p=0.07$ for each dose, respectively).

There was no significant change in sagittal sinus oxygen content ($p=0.23$, $p=0.76$ and $p=0.28$ for each dose, respectively) nor in cerebral oxygen extraction ($p=0.84$, $p=0.65$ and $p=0.51$ for each dose, respectively)

Cerebral metabolic rate decreased following 50 mg, 100 mg and 200 mg of propofol ($p=0.007$, $p=0.059$ and $p=0.023$ for each dose, respectively) but the decrease after the 100 mg dose failed to reach statistical significance at the 0.05 level. Changes in CBF and CMR followed a similar time-course after all doses (figure 5-3) with no statistically significant change in the ratio of CBF-CMR over time ($p=0.81$, $p=0.66$ and $p=0.58$ after each dose, respectively).

Threshold current changed significantly after all doses of propofol ($p=0.045$, $p<0.0001$ and $p<0.0001$ after each dose, respectively) with a time-course of change similar to those of CBF and CMR. Increases in threshold current were closely and consistently accompanied by subjective signs of sedation and anaesthesia such as reduced spontaneous movements and eye closure. The current increase was maximal (173%) at 5 minutes after 100 mg, but the maximum increase after 200 mg

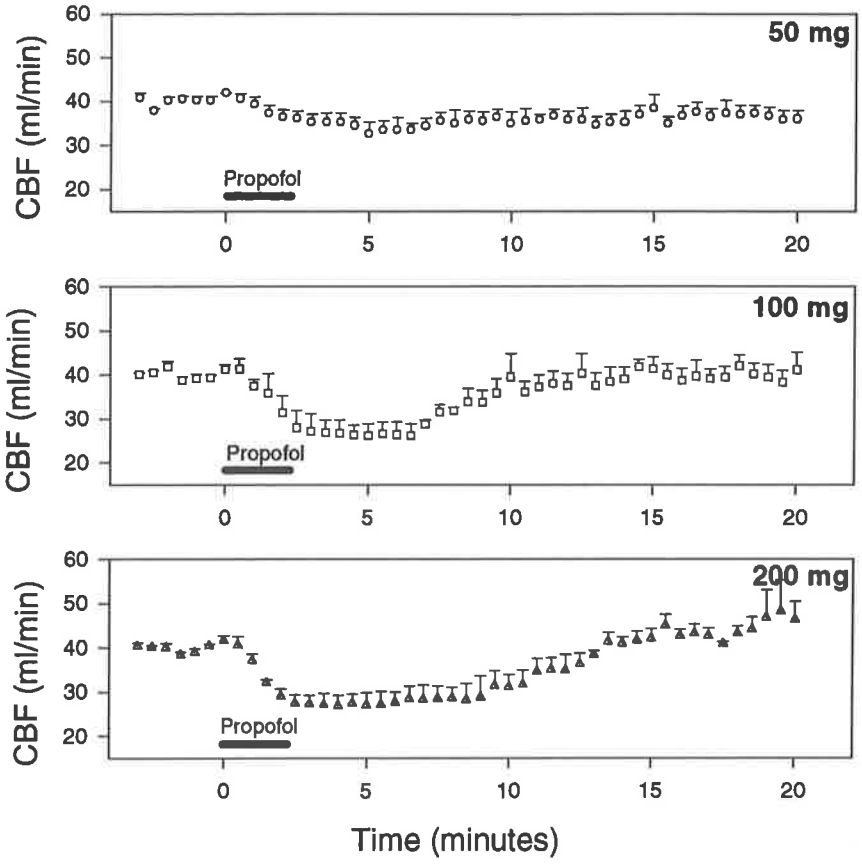


**Figure 5-1** Changes in CBF following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100 mg (squares) and 200 mg (triangles). Data presented as mean & sem.

could not be quantified because no response occurred at the highest current levels deliverable (figure 5-4).

### 5.3.2 PHARMACOKINETICS

The time-courses of the propofol concentrations for each dose were highly reproducible between animals and are shown in figure 5-5. Peak arterial concentrations of 1.5, 9.3 and 17.1 µg/ml for the 50, 100 and 200 mg doses, respectively were reached at 2 minutes following all doses of propofol, and decreased very rapidly after drug administration was ceased. There was no propofol detectable at the limit of detection in arterial blood 2 minutes after administration of any dose was ceased. After values were normalised for dose, peak arterial concentrations were similar after the 100 mg and 200 mg dose, but lower after the 50 mg doses, suggesting non-linear lung kinetics and a dose dependent effect on drug input to the brain (figure 5-6). The time-course of arterial concentrations, however, was similar when values were normalised for peak concentration (figure 5-7).

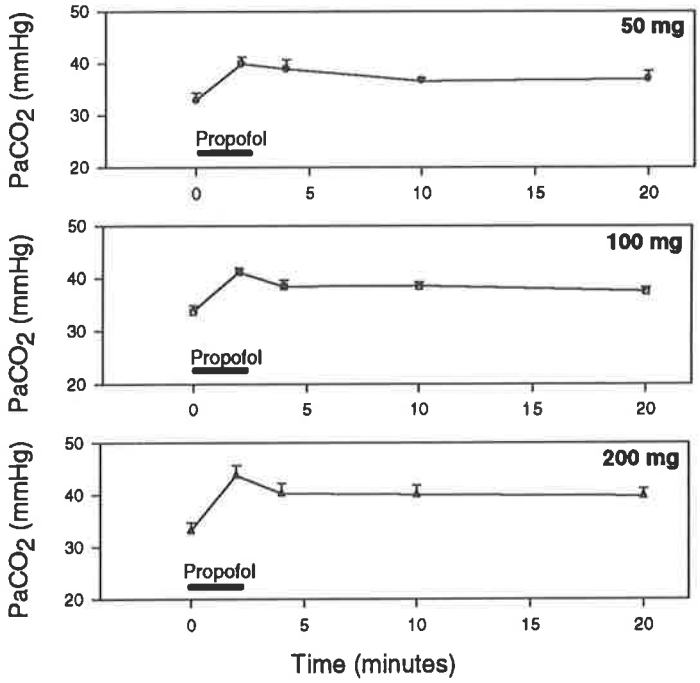


**Figure 5-2** Changes in $PaCO_2$ following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100mg (squares) and 200mg (triangles). Data presented as mean & sem.

Sagittal sinus concentrations increased and decreased more slowly than arterial values, reaching maxima of 0.4, 2.3 and 4.5 µg/ml after each dose, respectively, all at 2.5 minutes (figure 5-5). Scaling for peak arterial concentrations demonstrated that the relatively small peak sagittal sinus concentration after the 50 mg doses was secondary to relatively low arterial concentrations (figure 5-7), and therefore that brain uptake of propofol was proportional to arterial concentrations.

The time-course of brain concentrations was similar to that in the sagittal sinus, with concentrations peaking at 0.97, 4.9 and 9.2 µg/ml at 2.5 minutes, but with a slower decrease after administration was ceased (figure 5-8). Scaling for peak arterial concentrations (figure 5-7) revealed similar peak brain concentrations for all doses but more rapid elution after the 50mg doses.

### 5.3.3 CONCENTRATION-EFFECT RELATIONSHIPS

#### 5.3.3.1 Depth of anaesthesia

Significant changes in threshold current were recorded for the entire experimental period only after the 100 mg dose, as threshold current increased minimally after the 50 mg dose and after the 200 mg dose there was a period during which no withdrawal response was induced by the maximum current which could be safely delivered. Therefore, only data from the 100 mg dose was used for comparison with the simultaneously measured propofol concentrations.

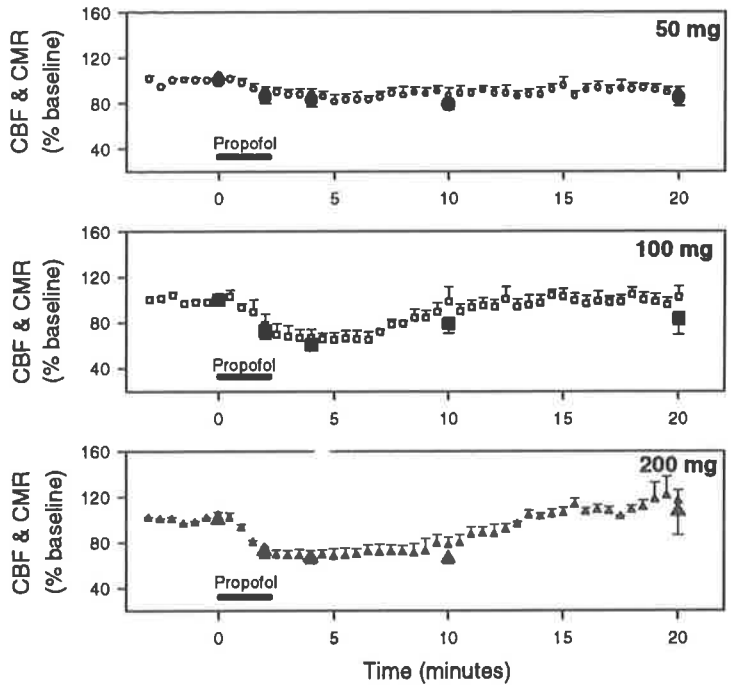


**Figure 5-3** Changes in CBF (open symbols) and CMR (closed symbols) expressed as percent of baseline following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100mg (squares) and 200mg (triangles). Data presented as mean & sem.

Following the 100 mg dose there was significant anti-clockwise hysteresis in the relationship between both arterial and sagittal sinus concentrations and depth of anaesthesia (p=0.033 and p=0.012 respectively), but no significant hysteresis between brain concentration and depth of anaesthesia (p=0.37; figure 5-9). Depth of anaesthesia returned to baseline levels while there were still significant brain concentrations of propofol.

#### 5.3.3.2 Cerebral blood flow

Changes in CBF were poorly related to arterial propofol concentrations with significant anti-clockwise hysteresis (p=0.04), but closely related to sagittal sinus and brain concentrations with no significant hysteresis (p=0.94, p=0.66 respectively; figure 5-10).

## 5.4 DISCUSSION

### 5.4.1 PHARMACOKINETICS

Although examination of the systemic pharmacokinetics of propofol can readily be performed in man, the invasive techniques involved in measuring specific organ pharmacokinetics, particularly in the case of the brain, generally limit these studies to animal preparations. Chronic instrumentation of the sheep used in this study

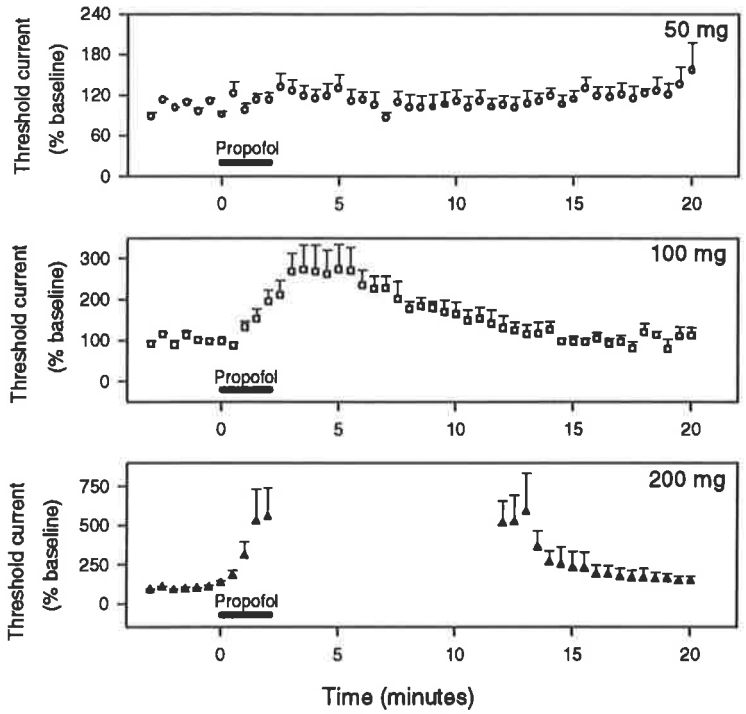


**Figure 5-4** Changes in threshold current (% baseline) following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100mg (squares) and 200mg (triangles). Data presented as mean & sem.

allowed calculation of the time-course of drug concentrations in the brain with simultaneous measurement of drug effects following rapid administration, but without the influence of concomitant anaesthetic agents on both cerebral drug kinetics and dynamics. This has not previously been possible for anaesthetic drugs.

#### 5.4.1.1 Arterial kinetics

If the propofol concentrations emerging from the lungs were linear with dose, the concentrations normalised for dose shown in figure 5-6 would be superimposed, which was clearly not the case for the 50 mg dose. It is possible that lung extraction of propofol may account for the relatively small quantity of drug emerging from the lung after the 50 mg dose. If that were the case, the similar normalised arterial curves following the 100 mg and 200 mg doses would suggest that this process is non-linear and probably saturable at clinically significant doses. Large and saturable lung uptake of drugs is well described, but exact mechanisms have not been identified (Roerig et al., 1989; Roerig et al., 1987; Taeger et al., 1988). Substantial removal of propofol from the circulation by the lung (approximately 60% on first pass) has also been previously described in sheep (Matot et al., 1994) and in cats (Matot et al., 1993), although a dose dependent effect has not been

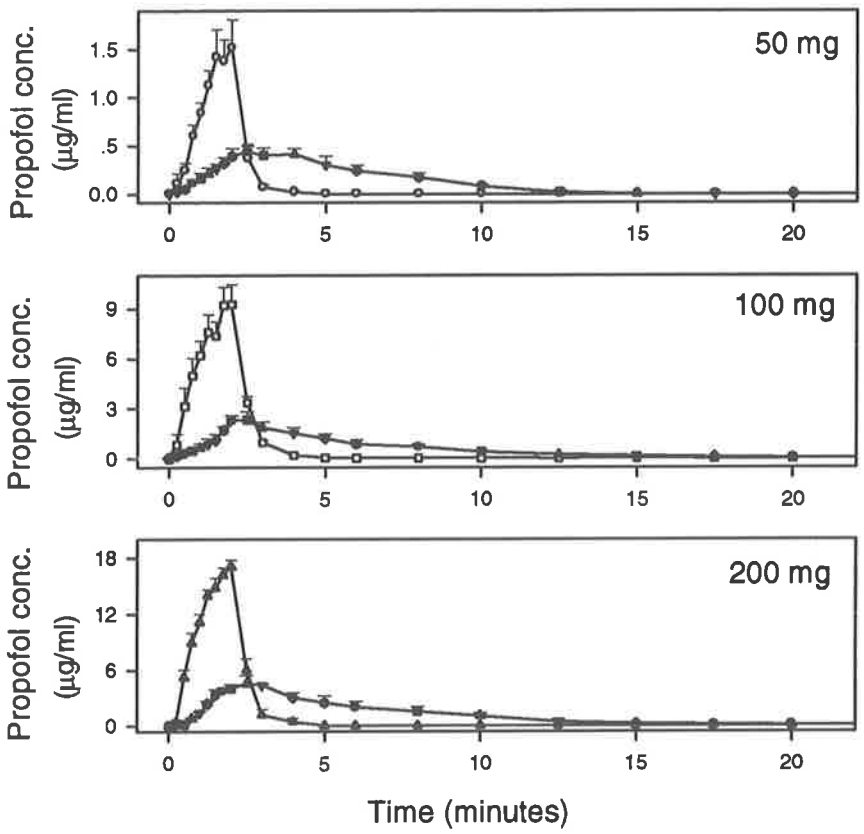


**Figure 5-5** Changes in arterial (open symbols) and sagittal sinus (closed symbols) concentrations of propofol following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100 mg (squares) and 200 mg (triangles). Data presented as mean ± sem.

specifically examined. This type of extraction may also occur in man, although there are currently few available studies. Because of the high lipid solubility of propofol and the detection of diffusion of propofol back into the circulation, it has been considered that this, at least in part, represented a similar distribution process to that described for other drugs. It is also possible, at least in some species, that metabolism may contribute to the loss of propofol during passage through the lungs. Substantial dose dependent clearance of propofol has been detected during passage through the lungs in sheep during prolonged administration (Mather et al., 1989). The lack of detectible quantities of propofol in the lungs at post-mortem in that study suggested that lung metabolism contributed substantially to the clearance. Specific examination of the activity of microsomal fractions from the lung in man and rats, however, has not found evidence of glucuronidation of propofol (Le Guellec et al., 1995).

It is possible that propofol induced changes in cardiac output could also influence the shape of the arterial concentration curve. The effect of changes in cardiac output on first-pass pulmonary arterial concentration curves entering the lung has

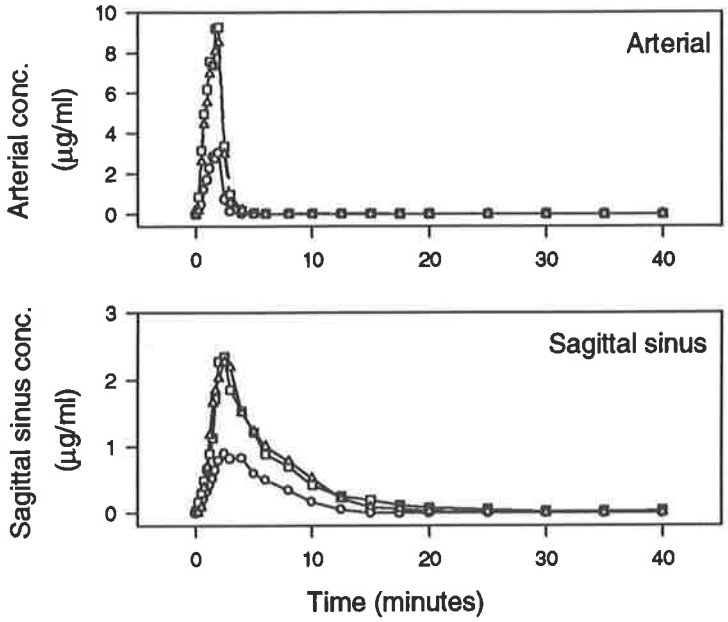


**Figure 5-6** Changes in arterial and sagittal sinus concentrations of propofol normalised for dose following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100mg (squares) and 200mg (triangles).

previously been examined for tracers such as indocyanine green (Upton and Huang, 1993), with findings that pulmonary arterial peak heights were inversely related to cardiac output. Thus, it is possible that drug induced cardiac depression might underlie the higher peak concentrations after the larger doses of propofol administered in this study. This is unlikely in these studies, however, as the large increase in normalised peak concentrations after the 100 mg and 200 mg doses compared to the 50 mg dose (figure 5-6), would require that increasing the dose of propofol from 50 mg to 100 mg decreased cardiac output 3 fold, and that increasing the dose to 200 mg produced no further decrease. This does not fit the pattern of cardiovascular effects previously described for propofol. Although propofol has been identified as causing myocardial depression in isolated myocardial preparations (Azari and Cork, 1993; Azuma et al., 1993), cardiac output changes are usually minimal *in vivo* across a range of species including man, dogs, pigs, rabbits and rats, probably because of a complex interaction between drug effects on the myocardium and peripheral vasculature, and baroreceptor reflexes (Aun et al., 1993; Grounds et al., 1985; Vohra et al., 1991; Wouters et al., 1995; Pensado et al., 1994; Coetzee et al., 1989; Blake et al., 1994; Belo et al., 1994; Carmichael et al., 1993; Diedericks et al., 1993). Data in sheep are limited, however, a study of the haemodynamic effects of general anaesthesia with administration of propofol alone found no significant change in cardiac output (Runciman et al., 1990), and minimal changes in cardiac output were found following a similar dose regimen in chapter 9.

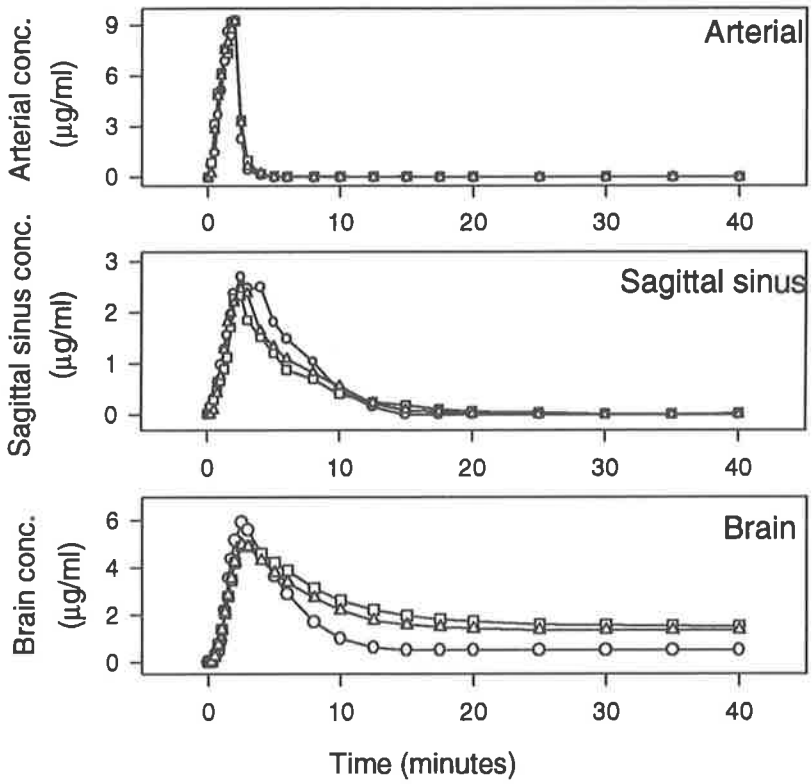


**Figure 5-7** Changes in arterial, sagittal sinus and brain concentrations of propofol normalised for peak arterial concentration following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100 mg (squares) and 200 mg (triangles).

It therefore appears likely that the haemodynamic effects of propofol did not contribute significantly to the observed changes in relative peak arterial concentrations, particularly as blood pressure changes after all doses in the current study were minimal.

Pulmonary extraction of propofol following rapid administration of sub-anaesthetic doses can therefore produce unexpectedly low brain concentrations, but it would appear to be of little significance following doses used to induce true anaesthesia. A differentiation between the processes of diffusion and metabolism cannot be made from the current study; this would require frequent measurement of cardiac output as well as and sampling from the pulmonary artery over a prolonged period post-infusion, to compare the mass of propofol entering and leaving the lung.

### 5.4.1.2 Cerebral kinetics

The influence of variable brain input of propofol was removed by normalising for the magnitude of the peak arterial concentrations. Fortunately this resulted in arterial curves which were essentially superimposed, so the concentration dependence of the cerebral kinetics could be examined. The similar time-courses of the

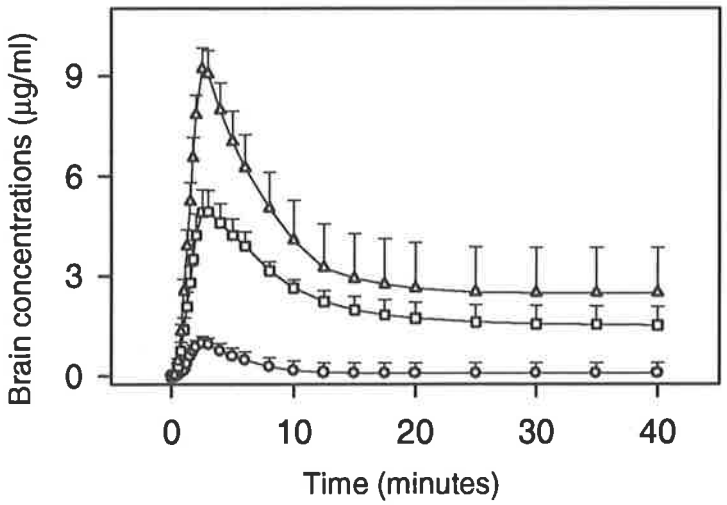


**Figure 5-8** Changes in brain concentrations of propofol following propofol i.v. over 2 minutes from time = 0 in doses of 50 mg (circles), 100 mg (squares) and 200 mg (triangles). Data presented as mean & sem.

normalised sagittal sinus and brain concentration curves revealed by this analysis confirms that brain uptake of propofol is fundamentally linear (figure 5-7). There is, however, a relatively more rapid elution from the brain after the 50 mg doses and this may reflect propofol influencing its own distribution. Uptake and elution of highly lipid soluble drugs such as propofol into well-perfused organs is frequently assumed to be predominantly flow-limited and hence any changes in organ blood flow induced by a drug can potentially alter its own organ distribution. Such a phenomenon has been described in a regional pharmacokinetic study of the brain uptake of ketamine and midazolam (Bjorkman et al., 1992). In the current study, CBF in the time period immediately after cessation of administration of propofol was relatively unchanged from baseline after the 50 mg dose, but reduced to similar levels after the 100 mg and 200 mg doses (figure 5-1). If propofol uptake and elution from the brain is flow-limited then this may explain the relatively rapid rate of elution from the brain after the 50 mg dose, and the similar rates of elution after the 2 higher doses (figure 5-7).

### 5.4.1.3 Concentration-effect relationships

The hysteresis between arterial drug concentrations and cerebral effect is the classical picture associated with disequilibria due to organ drug uptake following rapid drug administration, and this is confirmed by the close relationship between the time-course of calculated brain propofol concentrations and effects on both CBF and depth of anaesthesia (figures 5-9, 5-10). This relationship suggests that propofol concentrations in the areas of brain from which effluent venous blood

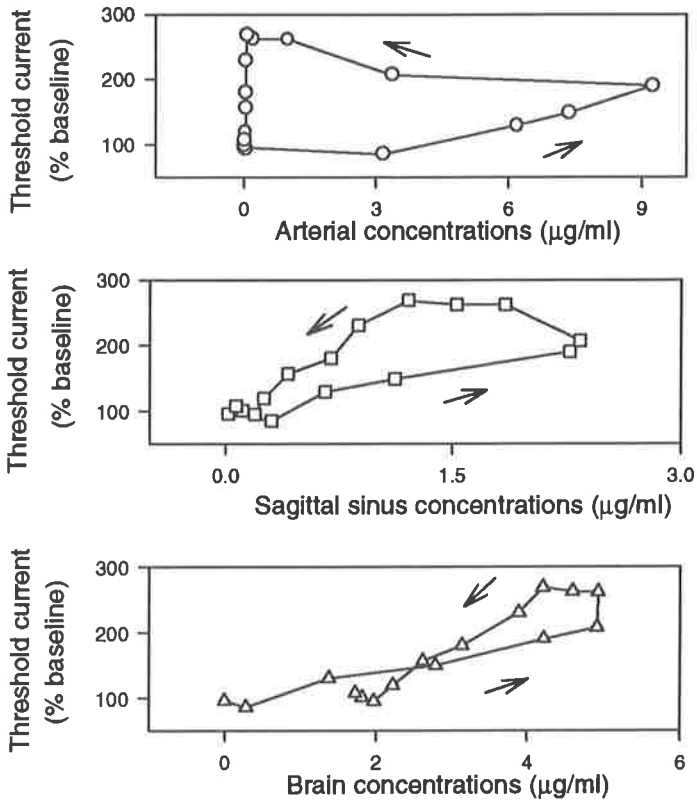


**Figure 5-9** Relationship between propofol concentrations and threshold current (% baseline) following 100mg propofol i.v. over 2 minutes from time = 0. Data presented as mean only.

drains through the sagittal sinus are in pseudo-equilibrium with concentrations at the drug's sites of action, and that the rate of drug distribution into the brain rather than any drug receptor interaction explains the hysteresis between blood propofol concentrations and cerebral effects previously described (Kanto and Gepts, 1989).

Baseline CBF was assumed to be 40 ml/min in all animals when brain concentrations were calculated (from data in chapter 4), but errors in this assumption would not alter the presence or absence of the observed hysteresis because errors in the magnitude of CBF would influence only the magnitude of calculated brain concentrations, but not the time-course (Upton, 1994). As it is this time-course which is critical to the relationship between calculated concentrations and effects displayed in figures 5-9 and 5-10, only changes in the slope, but not the shape, of the curves would be induced by variations in baseline CBF.

For mass balance calculations to accurately reflect brain concentrations for the duration of the experiment, it is necessary that the area and mass of brain from which effluent blood is collected remain constant. The close relationship between CMR and CBF after propofol administration demonstrated in the current and previous studies, and the relationship between brain concentrations and CBF in the

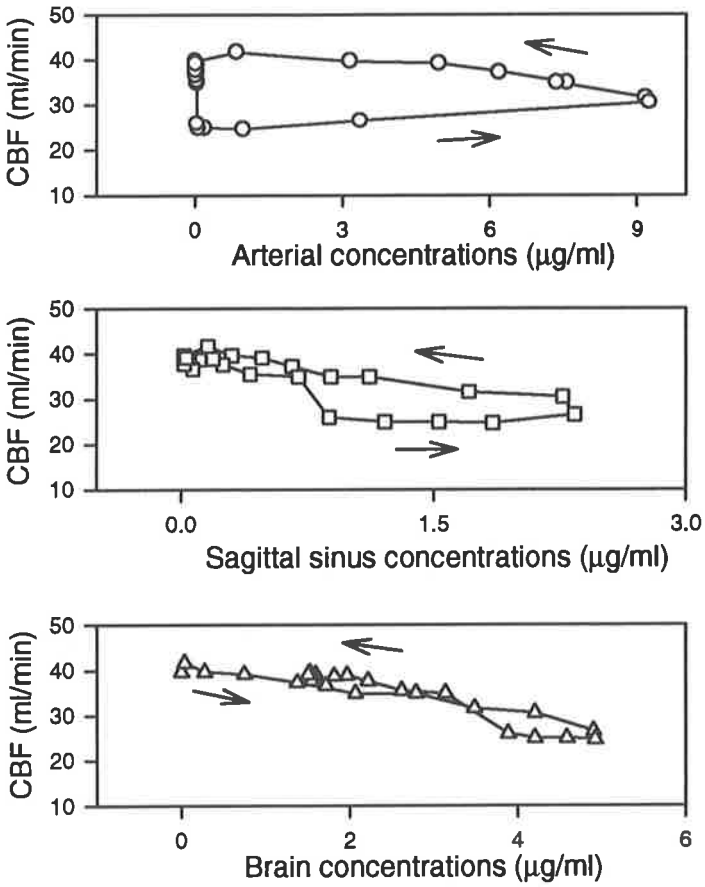


**Figure 5-10** Relationship between propofol concentrations and CBF following 100mg propofol i.v. over 2 minutes from time = 0. Data presented as mean only.

current study, both support the concept that CBF changes remain coupled to propofol induced decreases in CMR. It has previously been demonstrated that there is little regional variation in the magnitude of propofol induced decreases in CMR (Cavazzuti et al., 1991), suggesting that regional variations in CBF are unlikely to contribute to errors in calculations of brain concentrations.

Possible explanations for the return to baseline levels of current threshold while a significant quantity of propofol remained in the brain (figure 5-9) include a lower limit of brain concentration before an effect on threshold current is detectable or a change in drug-receptor interaction with time. The first phenomenon appears most likely, particularly as brain drug concentrations of approximately 1 µg/ml following the 50 mg dose produced little change in current threshold from baseline. A change in drug-receptor interaction or "acute tolerance" is possible; a phenomenon previously claimed for other i.v. anaesthetic agents (Toner et al., 1980). This has previously been specifically examined with propofol by repeated administration of propofol and comparing blood concentrations corresponding to a measure of

recovery from anaesthesia after single and repeated administration (Fassoulaki et al., 1994). While there was no significant difference in blood concentrations at recovery between groups, limitations of this study include the interval between doses (ranged between 24 and 72 hours, which is outside the generally accepted time-frame of acute tolerance), sampling of systemic venous blood, and a single measurement of recovery (such as ability of an animal to move out of a defined area). The preparation used in the current study is ideally suited to further examination of this phenomenon, using prolonged administration of propofol and simultaneous calculation of brain concentrations and cerebral effects. This will be examined in a later chapter of this thesis.

### 5.4.2 PHARMACODYNAMICS

#### 5.4.2.1 CBF and CMR

Although most studies on the cerebral effects of propofol conclude that CBF and CMR remain coupled and frequently assume that CBF follows changes in CMR, there are limited data on the comparative time-courses of these variables after propofol administration, to a degree because of the difficulties of frequent measurement of CBF and CMR. Furthermore, results in many of these studies have been potentially influenced by concomitant changes in cerebral perfusion pressure and the use of vasoactive inhaled anaesthetic agents (Van Hemelrijck et al., 1990; Stephan et al., 1987; Vandesteene et al., 1988; Artru et al., 1992; Pinaud et al., 1990; Eng et al., 1992). This may partially explain why CBF decreases have usually exceeded decreases in CMR. Similar propofol induced changes in CBF and CMR, however, have been demonstrated when mean arterial pressure has been maintained with peripheral vasoconstricting agents (Ramani et al., 1992).

The findings in the current study of parallel changes in both CBF and CMR over time and during rapidly changing depths of anaesthesia lend support to the hypothesis of CMF-CBF coupling. In addition, the stability of oxygen extraction over time reported here, a variable which usually changes only with uncoupling of CMR and CBF (Yamauchi et al., 1993), adds weight to these findings.

These data also support the hypothesis that the effects of propofol on CBF and CMR are a result of direct depression of synaptic and electrical activity. While a dose dependent effect on both variables has been demonstrated, there was a similar magnitude of maximal decrease in both variables at the two higher doses, with the 200 mg dose only increasing the duration of decrease below baseline. This is consistent with the concept of drug induced depression of synaptic and electrical activity, but preservation of the 50% of CMR which comprises basal cellular metabolic activity (Drummond and Shapiro, 1990).

In this study significant hypotension occurred only after the 200 mg dose. The relative lack of hypotension was probably because of a combination of the relatively slow bolus administration rate (Stokes and Hutton, 1991), the preservation of normal cardiovascular baroreceptor reflexes in an awake preparation, and because the relatively low vascular volume in sheep limbs limited venous pooling. This stability of MAP, and the close relationship between brain propofol concentrations and CBF demonstrated in figure 5-10, further support the hypothesis that the CBF changes which accompany propofol administration are not related to perfusion pressure changes. Indeed, other studies have shown apparent preservation of autoregulation with propofol (Strebel et al., 1995; Matta et al., 1995). It therefore seems that CBF remains pressure independent over a wide range of values of MAP.

Respiratory support was not possible when using this preparation, and propofol induced respiratory depression therefore had the potential to influence CBF. Hypoxaemia has previously been shown to influence CBF only when haemoglobin saturation falls below 80-90% (Michenfelder, 1990) and, because arterial oxygen content did not change significantly, was unlikely to have affected recorded CBF values. There were, however, dose dependant significant increases in $PaCO_2$ which may have affected CBF in the current study. Hypercarbia has been shown to increase CBF in sheep both during halothane anaesthesia and in the awake animal (see chapter 4) and previous work in other animals suggests that the CBF response to $CO_2$ is preserved during propofol administration (Eng et al., 1992; Fox et al., 1992). The experimental design in the current study did not permit control of $PaCO_2$ because it involved rapid changes in conscious state from awake to fully anaesthetised. It was not possible to accurately determine the effect of hypercarbia on the time-course of CBF changes. Probably because $CO_2$ induced changes in CBF are a result of increases in peri-vascular hydrogen ion concentrations (Kontos et al., 1977), a lag between changes in $PaCO_2$ and changes in cerebrovascular tone and CBF has been recorded (Ludbrook et al., 1992). Although this lag may be relatively brief in the awake animal, accurate compensation for hypercarbia cannot be made during periods of rapidly changing CBF, such as seen in the current study. There is some evidence for an effect of hypercarbia on CBF. There was a trend towards more rapid recovery of CBF compared to CMR after drug administration was ceased; a time period when significant hypercarbia occurred (figure 5-3). Hypercarbia, which differentially increases CBF rather than CMR (Eklof et al., 1973; Cohen et al., 1964) may underlie this trend, but any effect was minimal as there was no significant change over time in either the ratio of CMR and CBF or oxygen extraction. Accurate determination of the isolated effects of specific drug doses on CBF and the maximum propofol induced depression of CBF would require a separate protocol involving intubation and ventilatory support to maintain

normocarbia during drug administration via infusion, and therefore would only be possible at relatively deep planes of anaesthesia and probably with pharmacological support of systemic arterial pressure.

#### 5.4.2.2 Depth of anaesthesia

The use of a withdrawal response to a noxious stimulus is a concept similar to those previously used to determine anaesthetic effect of drugs discussed in chapter 3, and provided a functional measurement of sedative/anaesthetic effects somewhat analogous to that required in clinical practice. Baseline stability, and the consistency of responses both within and between animals, with this technique has previously been demonstrated in chapter 3. This allowed detection of significant changes in the threshold current after administration of the two larger doses of propofol. Indeed, changes were detected before sedative effects of propofol were revealed by observation of subjective measures such as reduction in spontaneous movements, eye closure and postural changes. After the 50 mg dose, there were minimal changes in CBF, CMR and threshold current and no visual evidence of change in behaviour of the sheep, suggesting that this dose has little sedative/anaesthetic effect in these animals.

Although it is difficult to equate an electrical current delivered to a sheep with stimuli associated with surgery and anaesthesia, experience with sheep during anaesthesia for surgery suggests that an increase in current threshold of 200-300% (an increase achieved at a dose somewhere between the 100 mg and 200 mg doses in the current study) corresponds to a depth of anaesthesia sufficient to allow laryngoscopy and endotracheal intubation without the use of muscle relaxants. Thus, the observed maximal decreases in CBF and CMR of approximately 35% recorded in the current study at these depths of anaesthesia are consistent with the published work referred to previously. This technique therefore appears valuable because it allows measurement of the degree of propofol induced sedation or light anaesthesia corresponding to the CBF and CMR changes induced by different drug doses. Other methods of measurement of propofol induced depth of anaesthesia such as electroencephalography are relatively impractical in an awake or lightly sedated animal, but do allow measurement of propofol induced suppression of cerebral electrical activity at deep planes of anaesthesia when any withdrawal response has been ablated. When this has been achieved with propofol, changes in EEG recorded depth of anaesthesia were associated with decreases in CBF and CMR, as in the current study (Ramani et al., 1992). Furthermore, in this study an isoelectric EEG, a state of complete suppression of cerebral electrical activity probably representing maximal drug induced depression of cerebral excitatory activity, was demonstrated to correspond to a 50% decrease in CMR. A similar value was found in the current study during deep planes of anaesthesia (figure 5-3).

This similarity in findings supports the hypothesis that this technique of recording changes in response to a noxious stimulus is an accurate index of drug induced depression of cerebral excitatory activity and depth of anaesthesia.

Although application of any stimulus may induce agitation and thus alter CBF, it is unlikely that this occurred in the current study. Validation of this method in chapter 3 revealed no change in behaviour of the animals unless measurements were made almost continuously (approximately every 10 seconds). Furthermore, in the pre-baseline period at the start of each experiment, CBF measurements were commenced prior to commencement of application of the electrical stimulus and no change in CBF was noted.

In summary, this study demonstrated that there was marked disequilibrium between propofol concentrations in the blood and brain after rapid administration of this drug to sheep, and that the time-course of the effects of propofol on CBF and anaesthetic effect were closely related to the time-course of propofol concentrations in the brain, but not the arterial blood. There is evidence that brain uptake and elution of propofol is a complex process that may be significantly affected by such factors as lung drug uptake and drug induced CBF changes, confirming that dose regimens using conventional compartmental pharmacokinetic modelling based on the concentration of propofol in blood may fail to accurately predict propofol brain concentrations and anaesthetic effects following rapid administration. It may therefore be appropriate to consider the time-course and determinants of brain concentrations of induction agents when devising dose regimens of short acting intravenous induction agents. The results from this study also support previous findings that propofol administration in sheep produces a dose dependant decrease in CBF independent of changes in MAP, and that CBF-CMR coupling is preserved during sedation as well as during deep planes of anaesthesia.

# CHAPTER 6. PROLONGED ADMINISTRATION OF PROPOFOL: CONCENTRATIONS AND EFFECT

## 6.1 INTRODUCTION

From the data presented in the previous chapter it is evident that there is a prolonged disequilibrium between the time-courses of concentrations of propofol in the arterial blood and the brain after rapid i.v. administration of propofol. The findings from this study, however, raised some points for further discussion.

Firstly, calculations of propofol concentrations in the brain revealed that significant quantities of the drug remained in the brain 20 minutes following commencement of administration (figure 5-8), at which time the concentrations measured in sagittal sinus blood were minimal (figure 5-5). While this may represent a very low rate of elution from the brain at this time, an alternate explanation is loss of propofol via a route other than the sagittal sinus. Such undetected loss would produce a systematic overestimation in the calculated time-course of brain concentrations.

The second point relates to the relationship between brain concentrations and measured depth of anaesthesia. While a close relationship, without hysteresis, was demonstrated, it was evident that threshold current had returned to baseline while significant quantities of propofol appeared to have remained in the brain (figure 5-9). As has been discussed, this could relate to the insensitivity of the method of measurement of depth of anaesthesia (little change in threshold current was produced by a peak brain concentration of 1 μg/ml in figure 5-4). However, a variable relationship between brain concentrations and depth of anaesthesia over time is also possible. Such a phenomenon, generally termed "acute tolerance", has been extensively debated in the literature, with published papers claiming to have demonstrated both its existence (Toner et al., 1980; Cockshott et al., 1992; Cockshott et al., 1990) and its absence (Hudson et al., 1983; Adam et al., 1982; Kay and Stephenson, 1981). Support for each opinion has wavered over the years, to a degree because of debate over the significance and understanding of regional distribution of drug after rapid i.v. administration, but it is evident that simultaneous measurements of the time-course of brain concentrations and central nervous system effects would provide strong evidence for or against such a phenomenon.

A question also remained regarding the changes in CBF recorded during administration of propofol. As discussed in chapter 5, it was not possible to determine absolute changes in CBF using spontaneously breathing animals because of the likely influence of hypercarbia on CBF and the rapid rate of change of both CBF and ventilatory depression. This could be accomplished by slower administration of propofol, allowing compensation for hypercarbia to be included in

the analysis because of the slower rate of change of both ventilatory depression and CBF.

To address these issues it was decided to administer propofol by slow continuous infusion over a prolonged period while recording effect parameters and taking blood samples as in the studies in the previous chapter. The aims of this chapter were therefore to:

- study the relationship between brain concentrations of propofol and effect on depth of anaesthesia over time to examine the issue of acute tolerance

- compare the quantity of drug measured entering and leaving the brain over a prolonged period to confirm the mass balance methodology

- further examine the effects of propofol on CBF during prolonged administration.

## 6.2 METHODS

### 6.2.1 ANIMAL PREPARATION

Six animals were prepared according to the general method described in chapter 2. A Doppler probe and a sagittal sinus catheter were placed under general anaesthesia according to the methods described in chapter 4.

### 6.2.2 STUDY DESIGN

Studies were commenced at least 1 week after surgery to allow wound healing. For each study, sheep breathing room air remained in their metabolic crates with their weight partially supported by a sling. Cerebral blood flow, mean arterial pressure and an index of depth of anaesthesia were measured in each study as described in chapters 2, 3 and 4.

In each study, after the baseline variables were recorded for 3 minutes and baseline arterial blood samples taken, propofol was administered intravenously at a constant rate of 10 mg/min for 45 minutes using a syringe pump (Model 33, Harvard Apparatus Ltd, Kent, England). All variables were recorded for 90 minutes after commencement of drug administration. Blood samples were taken from the arterial and sagittal sinus catheters at the following times: 0, 2, 4, 6, 8, 10, 12.5, 15, 17.5, 20, 23, 27, 30, 35, 40, 45.5, 47, 49, 51, 53, 55, 57.5, 60, 62.5, 65, 68, 72, 75, 80, 85, 90 minutes after the commencement of administration of propofol. One millilitre of blood from samples taken at each time point was placed in Eppendorf tubes to which heparin (25 I.U.) had been added and then frozen and stored below -5 $^{o}$C for later drug assay using the method described previously in chapter 4. One millilitre samples from both catheters at the time points of 0, 6, 10, 15, 20, 30, 45.5 minutes were sealed in gas-tight heparinised syringes for later gas tension analysis using a gas analyser (CIBA Corning 278, MA, USA).

### 6.2.3 DATA HANDLING AND STATISTICAL ANALYSIS

For CBF measurements, the Doppler output was normalised to a baseline of 40 ml/min and averaged at the time points at which blood samples were taken. To remove the effect of hypercarbia on CBF, a correction for carbon dioxide induced increases in CBF was made using the regression analysis from $CO_2$ induced CBF changes from awake animals in chapter 3 to calculate the CBF change attributed to hypercarbia. As blood gas analysis was only made at seven time points during drug administration, interpolation was made for $CO_2$ tension by curve fitting the $CO_2$ vs time data. Mass balance calculations for the calculation of brain concentrations were performed as described in chapter 4. For measurement of depth of anaesthesia, current threshold values at each time point in individual animals were expressed as the percent increases from the average baseline values, to remove the influence of variations in baseline values discussed in chapter 3. All data at each time point for all animals were then pooled and expressed as mean and standard error of the mean (sem). Changes in all variables over time were examined using repeated measures analysis of variance. The relationship between brain concentrations of propofol and depth of anaesthesia was examined both by regression analysis on the data from individual animals and from examination of the pooled data from all animals.

To examine whether all propofol entering the brain had been collected from the sagittal sinus, the mass of drug entering the brain during the 90 minute period of the study was calculated in each animal. For each time interval for which blood was sampled over the 90 minutes of the study, the area under the curve (AUC) of the arterial concentrations was calculated using the trapezoid rule. The mass of drug during each time interval was then determined from the product of the AUC and the mean CBF during that time period. The total mass of drug entering the brain over 90 minutes was then determined from the sum of these values. The mass of drug leaving the brain via the sagittal sinus was similarly calculated in each animal from the product of the AUC of the sagittal sinus concentrations over time and the CBF. These data were pooled and the masses entering and leaving the brain compared using a t-test.

## 6.3 RESULTS

### 6.3.1 PHARMACODYNAMICS

During administration of propofol, CBF gradually decreased from 40 ml/min to reach a minimum of approximately 32 ml/min towards the end of the infusion (figure 6-1). This was accompanied by a small increase in mean arterial pressure from 100 to 120 mmHg, but this was not statistically significant ($p = 0.74$, figure 6-2). There was a simultaneous increase in carbon dioxide tension from 31 to 39.8 mmHg ($p < 0.0001$), figure 6-3), but no significant change in haemoglobin saturation

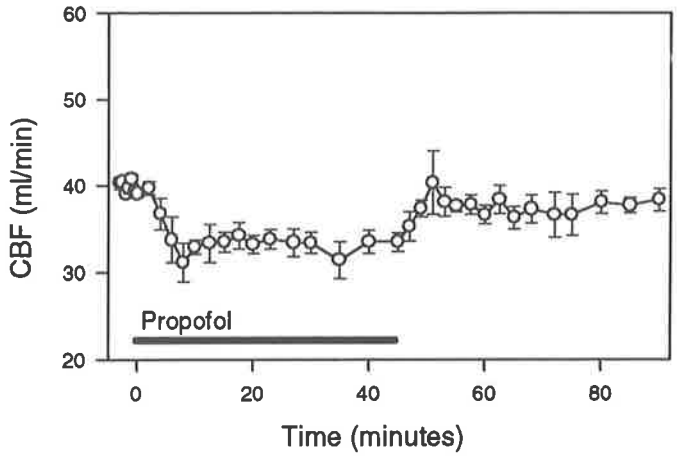


**Figure 6-1** Changes in CBF following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0. Data presented as mean & sem.

in arterial blood (100% to 99.5%, p=0.43). A second order regression equation provided a good fit of the time-course of measured $CO_2$ tensions ($r^2$ = 0.95, figure 6-3). The calculated CBF changes following removal of the hypercarbia component are displayed in figure 6-4.

During administration of propofol, there was a gradual increase in threshold current (figure 6-5). The point at which no response was elicited to the maximum current which could be delivered was reached prior to the cessation of administration of propofol, and therefore measurements of depth of anaesthesia could not be achieved beyond this point.

### 6.3.2 PHARMACOKINETICS

The concentrations of propofol in arterial and sagittal sinus blood are displayed in figure 6-6A. Arterial concentrations initially increased gradually but began to decrease prior to cessation of administration of propofol. The gradient between arterial and sagittal sinus concentrations was initially large and then gradually decreased during the course of the infusion until pseudoequilbrium was evident after 30 minutes. Following cessation of the infusion, there was a very rapid decrease in arterial concentrations, with approximately only 0.1 μg/ml present within 5-6 minutes of cessation of administration. There was a slower decrease in sagittal sinus concentrations, reflected in the relatively slow elution from the brain apparent from calculated brain concentrations. At the end of the study period (90 minutes), concentrations in the arterial and sagittal sinus blood were 0.06 and 0.09 μg/ml respectively, and approached the limit of detection of the assay method used. At this point, brain concentrations had reached 0.38 μg/ml, from a peak of 4.9 μg/ml at 30 minutes.

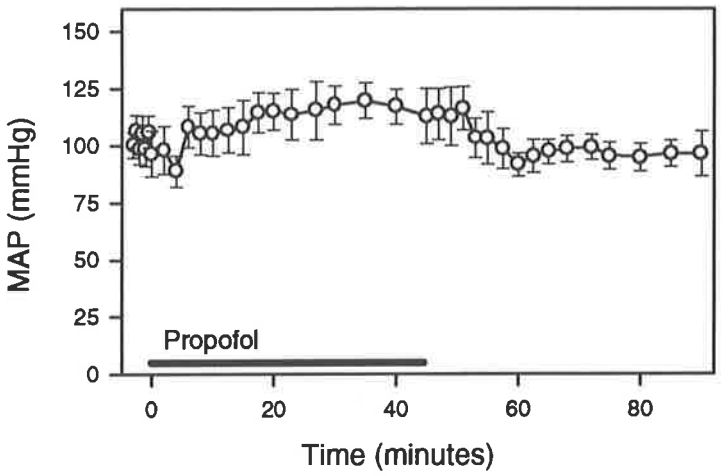


**Figure 6-2** Changes in mean arterial pressure following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0. Data presented as mean & sem

### 6.3.3 CONCENTRATION-EFFECT RELATIONSHIPS

The relationship between concentrations of propofol in arterial and sagittal sinus blood and depth of anaesthesia were not linear, with the recorded threshold current increasing at a greater rate than concentrations in the blood towards the end of the infusion (figure 6-7). The relationship between the calculated brain concentrations of propofol and depth of anaesthesia is displayed in figure 6-8. There was minimal change in current threshold until a brain concentration of approximately 1 μg/ml was reached, after which there appeared to be a linear relationship. When only data in the range of brain concentrations between 1 and 5 μg/ml was considered, there was a linear relationship between brain concentrations and threshold current in all animals, with regression analysis in individual animals producing $r^2$ values of 0.90, 0.90, 0.55, 0.87, 0.97 and 0.93. Regression analysis of pooled data revealed a linear relationship, with an $r^2$ of 0.99.

### 6.3.4 MASS BALANCE

The mean mass of drug entering the brain was 2523.4 mg (sem 780.3) and the mean mass leaving via the sagittal sinus was 2331.8 mg (sem 558.3). These were not statistically different when compared using a paired t-test ($p = 0.60$).

## 6.4 DISCUSSION

### 6.4.1 CEREBRAL BLOOD FLOW

While CBF initially decreased by approximately 25% in the first 10 minutes of propofol administration, it then remained relatively constant at that level despite both concentrations of propofol in the brain and depth of anaesthesia increasing further. Figure 6-4 reveals the contribution of propofol induced hypercarbia to this plateau, and that, if artificial ventilation were used to maintain normocarbia,

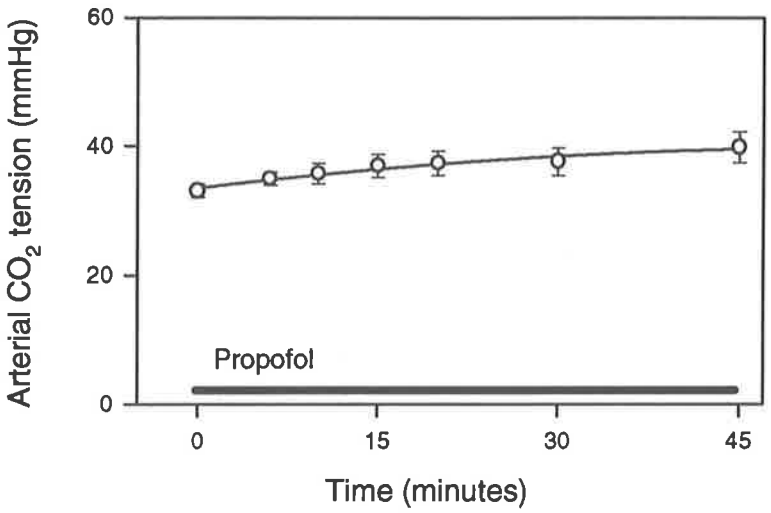


**Figure 6-3** Changes in arterial $CO_2$ tension following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0. A second order regression provided a close fit of the data ($r^2$=0.95). Data presented as mean & sem.

increasing depth of anaesthesia would have been accompanied by a further decrease in CBF. While the effect of propofol on the CBF response to changes in $CO_2$ tension has not been specifically examined in the current studies, this correction is likely to provide an accurate estimation of the isolated effect of propofol on CBF for the following reasons. Firstly, cerebral blood flow values were corrected using data on CBF responses to $CO_2$ obtained in awake sheep from the same flock (chapter 3) and secondly, previous studies have shown that propofol has minimal effect on the CBF response to $CO_2$ (Fox et al., 1992; Jansen et al. 1993; Eng et al., 1992). Lastly, the lack of change in haemoglobin saturation and the minimal increase in blood pressure (within the autoregulatory range which is unaffected by propofol) would not have affected CBF (Strebel et al., 1995; Matta et al., 1995).

It was apparent that maximal effects of propofol on CBF had not occurred at the point of loss of response to a noxious stimulus at 23 minutes. At this point, corrected CBF had decreased by 37% and decreased further to reach a value of 50% for the last 10 minutes of the infusion. These data are consistent with those previously described for thiopentone, a drug believed, like propofol, to affect CBF indirectly by its effects on cerebral metabolism. Michenfelder (1974) administered increasing and massive doses of thiopentone to dogs and found that maximal CBF decreases of approximately 50% (also measured using a venous outflow technique) occurred at doses which greatly exceeded the dose to induce anaesthesia. Currently, there are relatively few data examining changing depth of anaesthesia with propofol and CBF, but the published data is consistent with the findings for

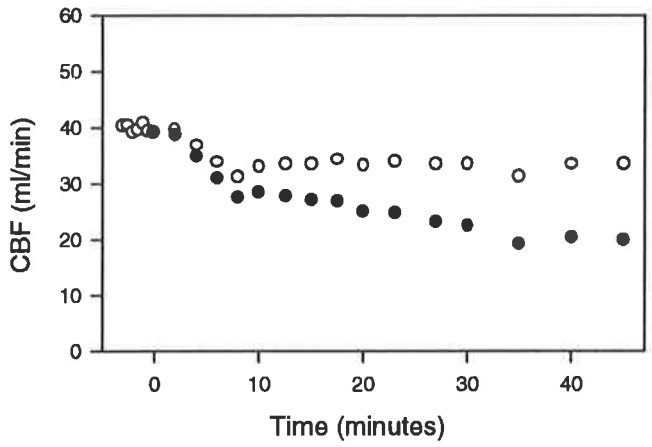


**Figure 6-4** Changes in CBF following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0. Open circles represented mean recorded values (as in figure 6-1), and closed circles represent these values corrected for the concurrent level of hypercarbia.

thiopentone and those described in the current study. In man, propofol administration in doses that achieve clinical anaesthesia (no response to noxious stimuli) has been shown to produce decreases in CBF of the order of 30-40% when perfusion pressure has been maintained (Eng et al., 1992; Fox et al., 1992). In other species, administration of increasing doses of propofol has produced decreases in CBF of up to 60%, but these doses greatly exceeded those necessary to induce anaesthesia (Ramani et al., 1992; Artru et al., 1992). With the current sheep preparation, studies using much larger doses of propofol would be needed to determine whether the 50% reduction calculated from the data in current studies is indeed the maximal effect. These studies would be significantly more complex, however, as it is likely that animals would require cardiovascular support (as did those in Michenfelder's study) to avoid the effects of hypotension on CBF, and ventilatory support to prevent hypoxaemia.

### 6.4.2 PROPOFOL CONCENTRATIONS

The time-course of propofol concentrations in arterial and sagittal sinus blood (figure 6-6) demonstrated an initial large concentration gradient across the brain, which decreased during the course of the infusion. A similar large gradient was evident throughout the brief and rapid administration of propofol in the studies reported in chapter 5, but the persistence of this gradient in the current study suggests uptake of large quantities of propofol over time. Although similar data are not generally available in the published literature, the arterio-venous gradient of propofol across the brain has been reported previously following brief administration in man (Peacock et al., 1995). Propofol was administered at 6 or 12 mg/kg/min until onset of loss of consciousness was achieved (an average period of 5 and 2.5

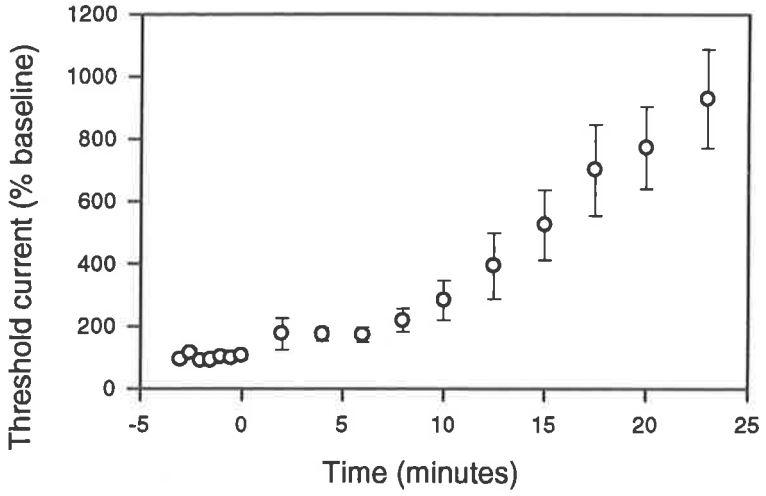


**Figure 6-5** Changes in threshold current following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0. Data presented as mean & sem.

minutes respectively) while simultaneous arterial and jugular bulb samples were taken. There were large and persistent arterio-venous gradients throughout the infusion periods, and the lack of convergence of arterial and venous concentration curves at the point when loss of consciousness was detected was evidence that significant uptake was still occurring at that point. Because no CBF measurements were possible, however, the time-course of brain concentrations could not be accurately determined. In contrast, the prolonged administration in the current study was associated with eventual convergence of arterial and sinus concentrations by 30 minutes, suggesting a state of pseudo-equilibrium between blood and brain had been achieved. This persisted for the period between 30 and 45 minutes; the arterial and sagittal sinus concentrations being essentially identical during this period. Elution of propofol from the brain began very rapidly once administration was ceased, with arterial concentrations decreasing below those in the sagittal sinus within 30 seconds. This reduction in arterial concentrations probably relates to the large and rapid redistribution of propofol from the blood previously reported (Kanto and Gepts, 1989), and will be examined in detail in the analysis of the systemic pharmacokinetics of propofol in the following chapter. It is interesting to examine the ratio of drug concentrations in brain and arterial blood at this point of pseudo steady state. At the point of peak brain concentrations, when both arterial and brain concentrations were relatively stable, the arterial and brain concentrations were 1.47 and 4.90 µg/ml respectively, producing a brain-whole blood ratio of 3.3, a value which is comparable to the value of 2.5 obtain previously in rats (Shyr et al., 1995).

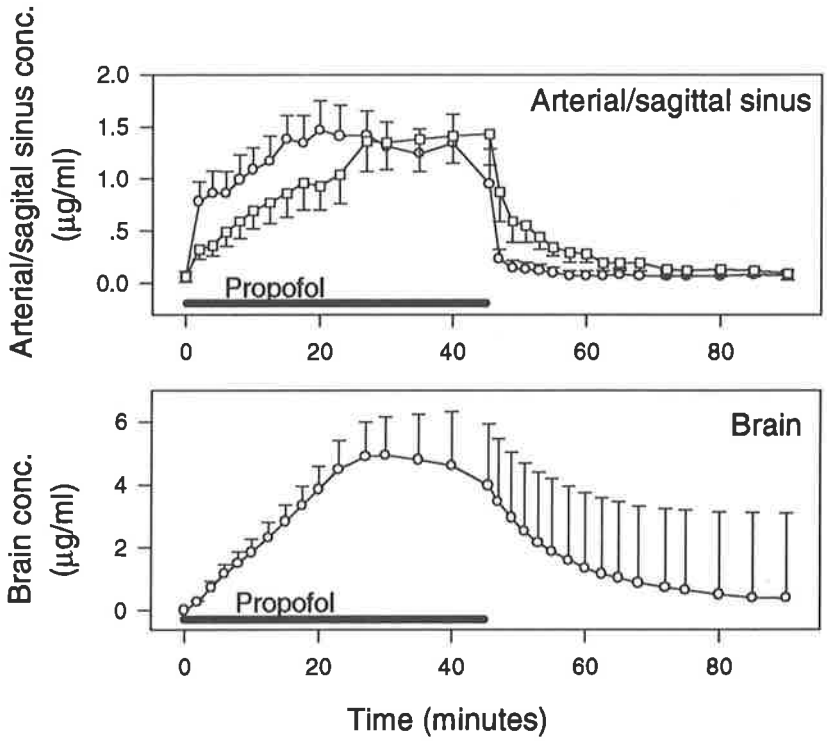


**Figure 6-6** Changes in propofol concentrations in arterial (circles) and sagittal sinus (squares) blood and the brain following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0. Data presented as mean & sem.

### 6.4.3 ACUTE TOLERANCE

Acute tolerance is a phenomenon first described for anaesthetic induction agents in 1951 (Brodie et al., 1951), findings which were apparently confirmed a few years later (Dundee et al., 1956). Both studies found that venous (or "arterialised" venous) concentrations of thiopentone were higher at the point of emergence from anaesthesia than at the point of onset, and concluded that this might represent an acute change in the biophase-effect relationship rather than a pharmacokinetic phenomenon. Descriptions of the variation in the time-course of concentrations of thiopentone at different sampling sites, including the jugular system, revealed in more recent work, has led to the recognition that regional pharmacokinetic variations might underlie this phenomenon (Barrett et al., 1984a; Barratt et al., 1984b). An alternate approach to this problem used simultaneous measurements of the time-course of arterial concentrations of thiopentone and EEG measurement of central nervous system depression in man during administration of thiopentone by infusion (Hudson et al., 1983). The chosen dose rate was sufficient to induce clinical anaesthesia and marked EEG (spectral edge) changes within approximately

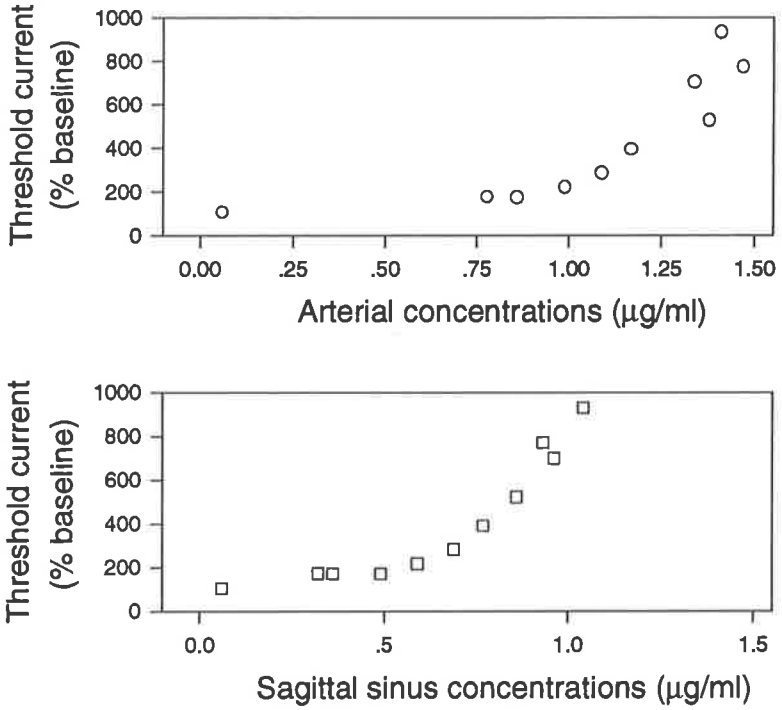


**Figure 6-7** The relationship between mean propofol concentrations in arterial (circles) and sagittal sinus (squares) blood and mean threshold current following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0.

4 minutes. There was no hysteresis between effect changes (EEG) and arterial concentrations, and this relationship did not change when thiopentone was repeatedly administered over 1 hour. This suggests that there is no evidence of acute tolerance of electrical activity to thiopentone over this time-frame. Interestingly, this close relationship between blood concentrations and cerebral effect also suggests that, for thiopentone, there is very rapid equilibration between arterial blood and brain. This contrasts with the findings of slow equilibration with the brain found for propofol in man (Peacock et al., 1995) and in the current study. This apparent difference in the rate of equilibrium with the brain also means that the type of analysis conducted by Hudson et al. (1983) for thiopentone is not possible for propofol, as the prolonged disequilibrium between blood and brain apparent from the current study means that differentiation between acute tolerance and a pharmacokinetic effect is not possible with a study of such a design. This difference in uptake also highlights the importance of knowledge of regional pharmacokinetics in determining dose strategies, especially considering the fact that examination of the systemic pharmacokinetic parameters of both agents would suggests few differences (Wood, 1982).

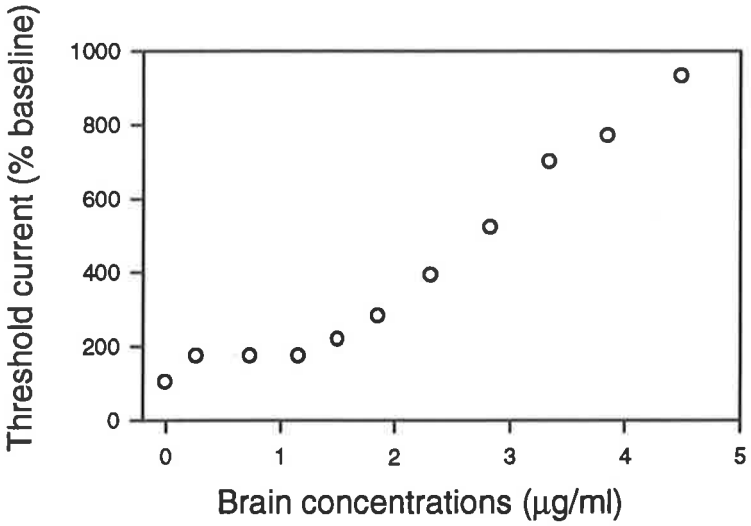


**Figure 6-8** The relationship between mean propofol brain concentrations and mean threshold current following propofol i.v. at a rate of 10 mg/minute for 45 minutes from time = 0. A linear regression line provided a close fit of the data between concentrations of 1 and 5 μg/ml ($r^2$=0.99).

There are generally fewer data on acute tolerance for propofol, with various authors claiming that this does, and does not, exist. Two studies have found that the concentration in blood at which patients awake after rapid administration of propofol is independent of both dose and the number of repeated doses (Adam et al., 1982; Kay and Stephenson, 1981), suggesting no tolerance occurred. This was supported by a study in rats where blood concentrations at awakening remained constant despite increasing bolus doses and repeated administration over 3 days (Fassoulaki et al., 1994), and a more recent study which found no evidence of tolerance with repeated administration of propofol to children (Setlock et al., 1996). In contrast, two studies claimed to have demonstrated acute tolerance following evidence of a higher concentration in blood on awakening following infusion administration compared to rapid bolus (Cockshott et al., 1992; Cockshott et al., 1990). These latter results, however, might well be explained by prolonged uptake into the brain, and disequilibrium between blood and brain concentrations demonstrated in man (Peacock et al., 1995), and in the studies in chapter 5 and the current chapter.

While most studies have attempted to solve the issue of acute tolerance using indirect methods, simultaneous measurement of brain concentrations and an index of anaesthetic effect can directly examine this. It is evident from the studies presented in this chapter that acute tolerance does not occur in sheep over a time period of approximately 20 minutes. The relationship between brain concentrations and threshold current is linear, in the range of concentrations between 1 and 4

μg/ml (figure 6-8), representing administration for 20 minutes. An acute change in the relationship between the concentration of drug in the brain and effect (acute tolerance) would produce a decreasing slope of the concentration-effect curve over time, and this is not present. Although the data in chapter 5 demonstrated that threshold current returns to baseline while there was propofol remaining in the brain (figure 5-9), the data from the current study demonstrates that this simply represents the insensitivity of the algesimetry method used to measure depth of anaesthesia. In both studies, concentrations in the brain of greater than 1 μg/ml were necessary to induce a measurable change in the threshold current. This, of course, does not exclude tolerance occurring over a longer time-period, but acute tolerance generally refers to the time-frame of the few minutes during induction. In addition, study of tolerance which develops over a longer period does not present the same methodological problems, as, in this context, the use of measurements of blood concentrations and effects only at times of pseudo-equilibrium or the use of repeated drug administration are satisfactory methods of study.

### 6.4.4 MASS BALANCE

From the very low concentrations of propofol in sagittal sinus blood by 45 minutes after the end of the infusion (figure 6-6) it is clear that elution from the brain was approaching completion. This allowed an opportunity to examine the validity of the methodology used to calculate mass balance.

Accurate application of mass balance principles for the calculation of the time-course of drug concentrations in a region requires that any drug not detected leaving the region remains in the region and, most commonly, venous blood draining a given region is sampled to detect exiting drug. There are a number of potential sources of error with these assumptions, however, as was discussed in chapter 1. Firstly, blood at the site of sampling must drain from a consistent region for the duration of the study. Secondly, a significant proportion of drug should not be lost through other routes such as direct diffusion or lymphatic drainage. Thirdly, drug should not be metabolised in the organ.

The majority of these conditions appeared to be satisfied with this sheep preparation. The pattern of arterial supply and venous drainage of the brain was discussed in chapter 4, where it was demonstrated that contamination by extra-cerebral blood was minimal, and it appeared likely that no major flow redistribution occurred with propofol. Therefore, very little blood borne drug leaving the regions of the brain studied here would escape detection. While examination for drug loss in the lymphatic system (Huang et al., 1991) or by direct diffusion (Huang, 1991) has been considered necessary in previous studies using mass balance, the lack of lymphatic drainage from the brain and its encasement by the bony skull suggested that these routes would be unlikely to contribute significantly to loss of drug from the

brain. Also, while diffusion into the 25% of the brain from which blood is not collected is possible, the previously reported homogeneous distribution of propofol in the brain (Shyr et al., 1995) suggests that minimal transfer between regions would occur.

One possible route of drug loss unique to the brain is the cerebro-spinal fluid (CSF). There are no data available on the distribution of propofol into the CSF, but it is unlikely that significant quantities would be lost to collection in sagittal sinus blood in the time frame of induction of anaesthesia. The CSF has a low volume, and the rate of formation of approximately 0.1 ml/min reported in sheep is very low compared to brain volume or CBF (Payne et al., 1986). In addition, concentrations in the CSF of drugs used in anaesthesia are generally low. For example, one hour after oral administration of temazepam, concentrations in the CSF were only approximately 10% of those in plasma in man (Moffat et al., 1995), representing a very small fraction of the administered dose. A study of the rate of appearance of morphine in the CSF following intravenous administration in sheep found that only 0.008% of the administered dose appeared in the CSF, even under steady state conditions (Payne et al., 1986). Similarly, the concentration of thiopentone measured in CSF, even at steady state, was only approximately 15-40% of that in the serum (Airey et al., 1982). Although there are no data on the distribution of propofol into the CSF, the combination of low rate of formation and the fact that concentrations are unlikely to exceed those in the brain means that propofol content in the CSF is unlikely to add significant error to mass balance calculations.

Loss of propofol via metabolism in the brain has not previously been reported. Extrahepatic metabolism of propofol has been suggested from its high clearance, which exceeds liver blood flow, and has been demonstrated in the sheep lung (Mather et al., 1989) where specific enzyme systems which act on phenols are known to exist. Enzymes for sulphation of phenols have been identified in cerebral tissue in some species, including man (Hwang et al., 1995; Hurd et al., 1993), and there is evidence that these types of enzymes systems can actively metabolise drugs in the brain (Baranczyk Kuzma et al., 1993), but it is not known whether metabolism of propofol occurs to any significant extent in the brain. This could be examined by assay of brain homogenate for metabolites of propofol, as has been previously reported in the sheep lung (Mather et al., 1989).

Despite these theoretical issues, a significant contribution of this process to removal of propofol from the brain is not supported by the present data. The calculated brain concentration had returned almost to baseline (0.38 $\pm$ 2.7 $\mu$g/ml ) 45 minutes after drug administration and the quantity of drug detected in the sagittal sinus was not statistically different from that delivered to the brain in the cerebral arterial supply. These data therefore demonstrate that loss of propofol via the non vascular

routes discussed above is negligible, and errors in mass balance calculations are likely to be minimal. It is important to note that the process of elution had not been completed at the time the study was ceased, as drug was still detectible in the sagittal sinus blood, and a greater proportion of drug may have been detected in effluent blood (approximately 93.5% of drug delivered to the brain was accounted for at 45 minutes) if the study had been extended. The very slow rate of elution, however, and the resulting low concentrations of propofol in sagittal sinus blood would make accurate measurements of propofol impossible for an extended period.

In studying the mass balance of propofol in the brain over time, it is interesting to examine the proportion of administered drug entering the brain. Of a total of 450 mg administered over 45 minutes, only approximately 2.5 mg entered the brain, representing only 0.56% of the total dose. While some loss of drug occurs in the lung, as outlined in chapter 5, this small proportion probably relates to the amount of blood flow from the heart that reaches the brain. The mass of drug reaching the left side of the heart is ejected into the aorta and is initially distributed according to the distribution of blood flow. The flow of blood to the brain in the sheep is approximately 40 ml/min (chapter 3), representing approximately 0.67% of the 6 L/min average cardiac output of a sheep at rest (Huang, 1991). This figure is comparable to the 0.56% of drug delivered to the brain. This small proportion of total dose relates to the small brain mass of the sheep and will therefore vary in different species. For example, in man CBF is normally approximately 15-20% of cardiac output (Doberstein and Martin, 1996), and hence a much higher proportion of administered drug would be delivered to the brain. The fundamental principles of distribution, however, are likely to be constant across species. Thus, relative blood flow to the brain is likely to provide a major contribution to drug delivered to the brain following rapid injection intravenously. The impact of cardiac output changes on drug concentrations in the brain will be examined in a later chapter of this thesis.

In summary, the data presented in the current study demonstrates that there is a constant relationship between the concentration of propofol in the brain and depth of anaesthesia over at least 20 minutes, findings which are not consistent with the supposed development of acute tolerance with propofol previously reported. In addition, the mass balance methods used in these pharmacokinetic studies are supported by the findings of almost complete recovery of propofol entering the brain through sampling blood in the sagittal sinus. Lastly, it was apparent that correction of CBF values for respiratory induced hypercarbia revealed similar propofol induced decreases in CBF with onset of anaesthesia as have previously been demonstrated in other species. This study also provides insight into the systemic and cerebral pharmacokinetics of propofol; these will be examined in detail in a subsequent chapter.

# CHAPTER 7. THE EFFECT OF RATE OF ADMINISTRATION ON BRAIN CONCENTRATIONS OF PROPOFOL

## 7.1 INTRODUCTION

Limitations to the insight into mechanisms underlying drug disposition that may be obtained from the systemic pharmacokinetics of a drug have previously been discussed. The published literature on the effects of altered rates of administration of propofol at induction provides an excellent example of the confusion which may result when methods are used in contexts in which assumptions underlying their validity are incorrect. For example, the early studies of slow rates of injection of propofol at induction found evidence of a reduced incidence of cardio-respiratory depression (Dundee et al., 1986; Gillies and Lees, 1989) and a low rate of coughing and breath holding when compared to the more established induction agent, thiopentone. In addition, it became evident that along with this reduction in side effects there was an apparent dose sparing effect. An administration rate of 50 mg/min was shown to reduce the total dose administered at the point of onset of anaesthesia (loss of response to a verbal stimulus) to 1.4 mg/kg, from the 2.6 mg/kg recorded following administration at a rate of 200 mg/min in a matched group of patients (Stokes and Hutton, 1991). Time to induction was also increased at the slower rate. In this study there was also a trend to a reduction in the incidence of hypotension at the slower administration rate, but this was not statistically significant. Discussion on the mechanism behind this phenomenon centred around the effect of administration rate on the biophase concentrations and the physico-chemical properties of propofol, but remained speculative in the absence of data on the determinants of the brain uptake of propofol. This speculation was only confused by the fact that, in the same study, very rapid administration (over 20 seconds) appeared to also reduce induction dose requirements, findings supported by another study which reported a better success rate of induction of anaesthesia with 2 mg/kg administered over 5 seconds, compared to 60 seconds (Rolly et al., 1985). While the findings of dose reduction with slower rates of administration have been also found in man by other workers, (Peacock et al., 1992; Peacock et al., 1990), a satisfactory explanation has not yet been provided. A study in rats using a range of administration rates and using EEG to assess onset of anaesthesia extended these findings to suggest that an optimal rate of administration existed, with excessively slow or fast administration leading to an increase in dose requirements to achieve a specific endpoint (Larsson and Wahlstrom, 1994). Again, only speculation on the mechanisms behind this observation was possible.

Attempts at explaining this phenomenon using systemic compartmental models have not provided satisfactory answers, and have occasionally produced contradictory statements (Wilder-Smith and Borgeat, 1992). It was therefore

decided to use a regional pharmacokinetic approach to examine this problem. The specific aim of this chapter was to examine the effect of fast and slow administration rates on the time-course of concentrations of propofol in the blood and the brain, and on blood pressure.

## 7.2 METHODS

### 7.2.1 ANIMAL PREPARATION

Animals were prepared according to the general method described in chapter 2. A Doppler probe and a sagittal sinus catheter were placed under general anaesthesia according to the methods described in chapter 4.

### 7.2.2 STUDY DESIGN

Studies were commenced at least 1 week after surgery to allow wound healing. For each study, sheep breathing room air remained in their metabolic crates with their weight partially supported by a sling. Cerebral blood flow and mean arterial pressure were measured in each study as described in chapters 2 and 4.

In each study, after the baseline variables were recorded for 3 minutes and baseline arterial blood samples were taken, 100 mg of propofol was administered intravenously at a constant rate over either 0.5 or 5 minutes using a syringe pump (Model 33, Harvard Apparatus Ltd, Kent, England). All variables were recorded for 40 minutes and blood samples were taken at frequent intervals from the arterial and sagittal sinus catheters. One millilitre of blood from samples taken at each time point was placed in Eppendorf tubes to which heparin (25 I.U.) had been added and then frozen and stored below -5 $^{o}$C for later drug assay using the method described previously in chapter 2.

Each administration rate was administered to 5 different sheep but, because of probe or catheter failure, a total of 9 sheep were studied. Three sheep received both administration rates in random order, 3 sheep received 100 mg over only 0.5 minutes, and 3 sheep received 100 mg over only 5 minutes. In sheep receiving more than 1 dose, at least 48 hours was allowed between studies.

### 7.2.3 DATA HANDLING AND STATISTICAL ANALYSIS

To allow a comparison of the result of a total of 3 administration rates, the data from the current study were analysed along with the study reported in chapter 5, where 100 mg was administered over 2 minutes. This then gave complete data sets of 100 mg of propofol administered over 0.5, 2 and 5 minutes (rates of 200, 50 and 20 mg/min).

In general, data on CBF, MAP and blood concentrations were handled and analysed as for the studies in chapter 5. Brain concentrations were calculated as described in chapter 5. For the 3 different administration rates, the data at each

time point for all animals were pooled and expressed as mean and standard error of the mean (sem). To examine the effect of administration rate on depth of anaesthesia achieved, the peak brain concentrations and the AUC of the brain concentration-time curves (calculated using the trapezoid rule) from the 3 groups were compared using analysis of variance. To examine the effect of administration rate on blood pressure, the peak decreases in MAP in each group were compared using analysis of variance.

To allow a comparison with published data of the dose administered at the point of onset of a given endpoint (usually loss of response to a verbal command) following different administration rates, the data were used to mimic titration to onset of anaesthesia. This was done by arbitrarily assigning the point of onset of 'true' anaesthesia as a particular brain concentration of propofol. It is difficult to equate loss of response in man to the algesimetry paradigm used in these sheep studies and so, for the purposes of this analysis, brain concentrations of both 1 μg/ml and 2 μg/ml were used separately as a surrogate for "onset of anaesthesia". The time-points at which these brain concentrations were reached following the three administration rates was then determined from the brain concentration-time curves, providing the time to "onset of anaesthesia". Knowing the administration rate in each case, the dose administered up to this point was then calculated.

## 7.3 RESULTS

### 7.3.1 PROPOFOL CONCENTRATIONS

The drug concentrations following administration of 100 mg of propofol at the 3 rates are displayed in figure 7-1. In all cases, peak arterial concentrations were reached soon after cessation of administration of the dose, and these concentrations then rapidly decreased towards zero. After administration rates of 200, 50 and 20 mg/min, peak brain concentrations of 6.47 ± 0.98, 4.93 ± 0.65 and 4.84 ± 0.69 μg/ml were reached at 1.5, 3 and 5.5 minutes respectively (figure 7-2). The AUC of brain concentrations were 73.7 ± 15.2, 54.4 ± 4.4 and 67.7 ± 11.9 respectively. Neither peak brain concentrations or the AUC of brain concentrations were significantly different when analysed using analysis of variance ($p = 0.30$ and $p = 0.55$ respectively).

The effects of administration rate of equal doses of propofol can most clearly be compared by the data displayed in figures 7-4 to 7-6. Figure 7-4 demonstrates the large increase in arterial concentrations with the most rapid rate of administration, and figure 7-5 demonstrates the minimal effect of administration rate on depth of anaesthesia. The effect of administration rate on time to peak brain concentration is demonstrated in figure 7-6, with a linear relationship apparent between the two variables.

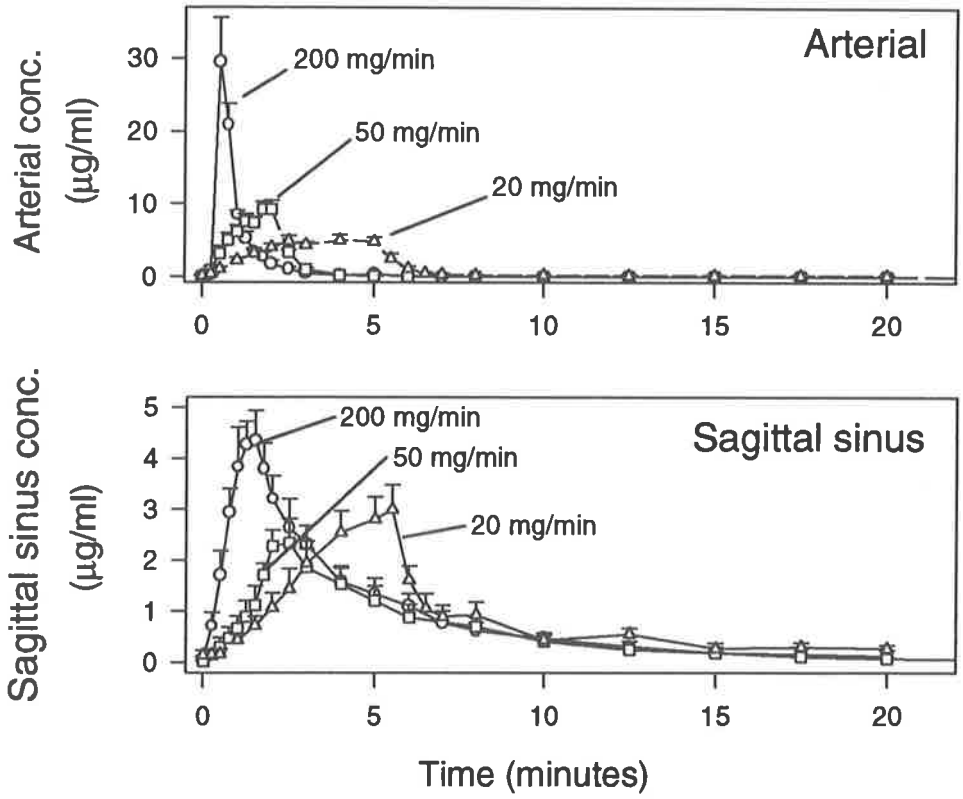


**Figure 7-1** Changes in arterial and sagittal sinus concentrations following propofol 100 mg i.v. at rates of 200 mg/min (circles), 50 mg/min (squares) and 20 mg/min (triangles). Data presented as mean & sem.

### 7.3.2 EFFECTS ON BLOOD PRESSURE

Mean arterial pressure decreased to reach a minimum of 94.7 ± 8.6, 89.8 ± 8.9 and 100.0 ± 3.5 mmHg after administration rates of 200, 50 and 20 mg/min, respectively (figure 7-3A), values which were not statistically different when compare using analysis of variance ($p = 0.66$). While this potentially could be explained by the variation in the mean baseline readings prior to administration between groups, display of the values as a percent of baseline revealed the similar magnitude of MAP changes after all three administration rates (figure 7-3B).

### 7.3.3 TITRATION TO EFFECT: EFFECTS ON DOSE AND TIME TO ONSET OF ANAESTHESIA

The results of examination of the titration to effect process are displayed in figure 7-7, along with the published results from similar studies in man. There was a decrease in the dose administered by the time a fixed brain concentration was reached as the administration rate decreased, and this was relatively unaffected by the brain concentration chosen to represent onset of anaesthesia.

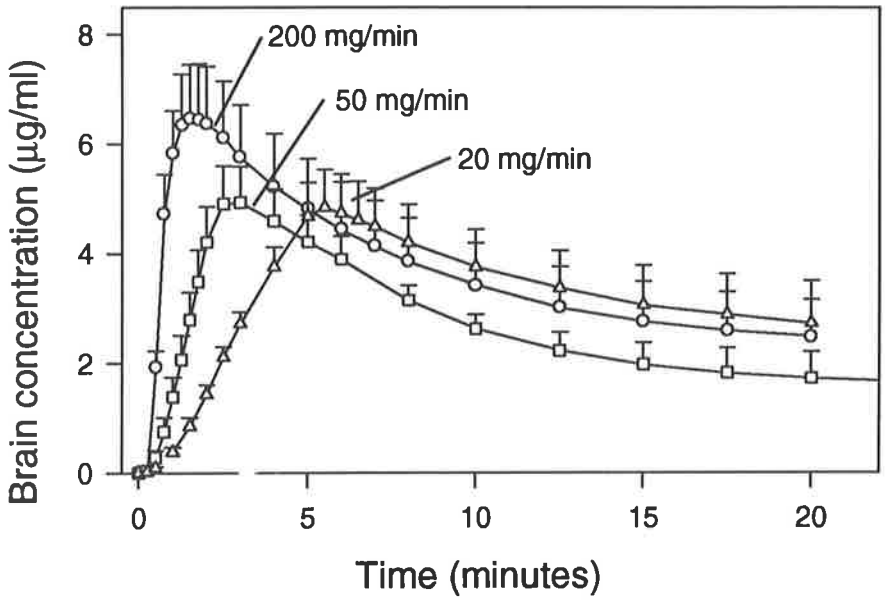


**Figure 7-2** Changes in brain concentrations following propofol 100 mg i.v. at administration rates of 200 mg/min (circles), 50 mg/min (squares) and 20 mg/min (triangles). Data presented as mean & sem

## 7.4 DISCUSSION

The range of dose rates in the current study (20, 50 and 200 mg/min) were deliberately chosen to allow a comparison with the published work showing a dose sparing effect. The papers examining effect of rate of administration discussed in the introduction of this chapter have used rates of administration between 50 and 200 mg/min, comparable to the rates used in the current study. There are fewer data on administration rates used in actual clinical practice. This was specifically assessed in a study at the Royal Adelaide Hospital, Adelaide, South Australia examining rates of administration of propofol at routine induction (Ooi, 1995). The mean duration of administration was 44 seconds, representing an administration rate of approximately 250 mg/min.

### 7.4.1 EFFECTS OF ADMINISTRATION RATE ON PROPOFOL CONCENTRATIONS

It is obvious from the data on brain concentrations in the current study that the different rates of administration had minimal effect on the magnitude of drug uptake by the brain, as both the peak brain concentration achieved and the 'amount' of anaesthesia (AUC of brain concentrations over time) were similar following all 3 dose rates (figure 7-5). Decreasing the administration rate did, however, decrease the rate of rise of brain concentrations during the induction phase, thereby slowing the induction of anaesthesia (figure 7-2). Even slower and faster rates of

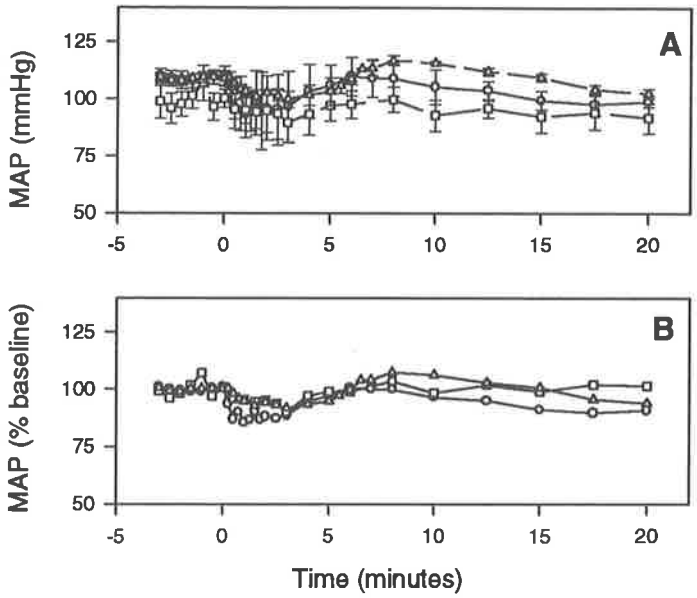


**Figure 7-3** Changes in MAP following propofol 100mg i.v. at administration rates of 200 mg/min (circles), 50 mg/min (squares) and 20 mg/min (triangles). Data presented as mean & sem of both absolute values in mmHg (A) and as % baseline (B).

administration were not included in the current study, but peak brain concentrations are probably significantly affected at these extremes. In the study presented in chapter 6, a very slow administration rate (10 mg/min over 45 minutes) was used, and produced a peak brain concentration almost identical to that following the much faster rates of administration in the current study (figure 6-6). The total dose administered to achieve that peak concentration was 450 mg, however, obviously much greater than the 100 mg used in the current study. A dose of 100 mg would have achieved much lower concentrations. This is not a dose regimen of practical value for induction of anaesthesia and, from the range of administration rates examined in the current study, it would appear reasonable to conclude that peak brain concentrations (and therefore depth of anaesthesia) are relatively independent of the rate at which the drug is administered at clinically used rates of administration at induction.

This is clearly not the case with concentrations in arterial blood, with an increase in administration rate from 50 to 200 mg/min producing a three-fold increase in peak arterial concentrations (figure 7-1). The potential impact of high blood concentrations on the cardiovascular system has been discussed previously in chapter 1. The increased rate of hypotension associated with induction with propofol compared to thiopentone has been related to the vasodilatory effect on the peripheral vasculature (Pagel and Warltier, 1993; Stephan et al., 1986) rather than myocardial depression (Cork et al., 1991). A direct effect of propofol on vascular

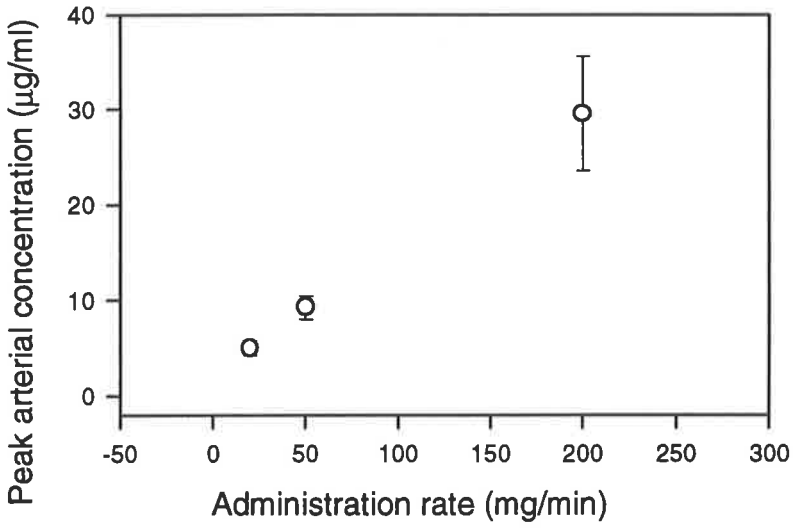


**Figure 7-4** The relationship between administration rate of 100 mg of propofol and the peak arterial propofol concentration. Data are presented as mean & sem.

smooth muscle has been proposed, although the predominant mechanism is as yet unclear (Petros et al., 1993; Chang and Davis, 1993), and so concentrations in the blood, and therefore close to vascular smooth muscle, are likely to influence the degree of hypotension. Myocardial depression may also induce hypotension following propofol administration but the impact of blood concentrations on this effect is not fully understood. As with the brain, this relates to a regional pharmacokinetic phenomenon. With administration of thiopentone and lignocaine, myocardial rather than arterial concentrations are a clear determinant of myocardial depression (Huang et al., 1993; Upton et al., 1996a) and so an understanding of the relationship between blood and myocardial concentrations is necessary to determine optimal dose regimens. These data for propofol are not yet available.

There are data to support the concept that minimising blood concentrations will reduce the risk of hypotension in man. Slower rates of administration have been associated with a smaller fall in blood pressure at induction of anaesthesia (Peacock et al., 1990; Gillies and Lees, 1989; Dundee et al., 1986) and it is clear that these slower administration rates produce lower propofol concentrations in venous blood. It is important to note that, despite reports of marked cardiovascular depression associated with propofol administration (Mackay, 1996), the differences in blood pressure recorded with different rates of administration have been relatively small. This probably reflects the ethical requirements of using relatively healthy subjects for these studies. As venous samples generally reflect the quantity of drug leaving that particular body region, and therefore give little indication of arterial concentrations (Major et al., 1983), it is difficult to understand the exact relationship

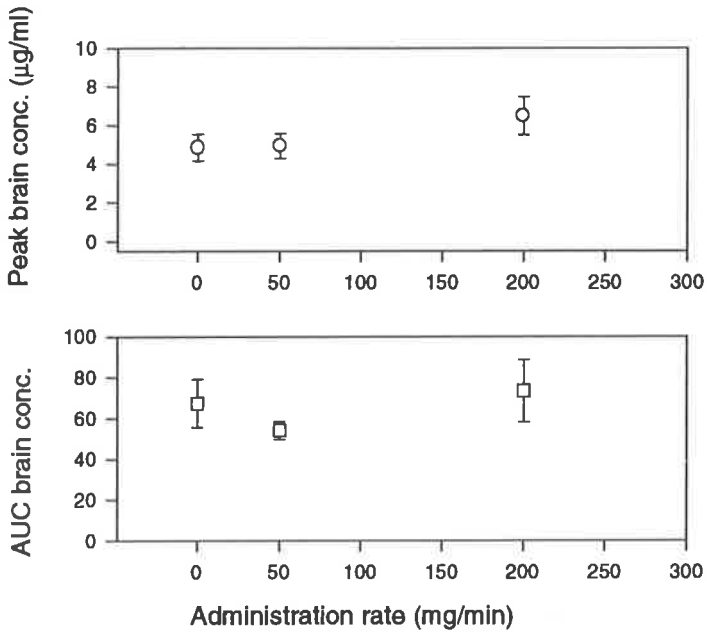


**Figure 7-5** The relationship between duration of administration of 100 mg of propofol and both the peak brain propofol concentration (A) and the AUC of brain concentrations (B). Data are presented as mean & sem.

between blood concentrations and effects on the cardiovascular system. Clearly what is needed are myocardial pharmacokinetic studies for propofol, similar to those presented here for the brain, to gain an understanding of the relationship between concentrations in blood and heart and effects on myocardial performance and blood pressure. Only with these data can true optimal dose regimens be determined; these studies are now underway.

### 7.4.2 EFFECTS OF ADMINISTRATION RATE ON BLOOD PRESSURE

Blood pressure changes using the sheep preparation have been minimal following all doses of propofol in this series of studies, and there was no evidence of a reduction in the incidence of hypotension at slower administration rates. As was discussed in chapter 5, this is probably a result of a combination of the relatively young age and general good health of the sheep, and their posture. In man, the elderly are at greater risk of hypotension with propofol (Dundee et al., 1986), which probably reflects the presence of age associated myocardial disease and perhaps effects on the baroreceptor reflex system. The use of sheep aged between 1 and 2 years (roughly equivalent to a human age of 10-20 years, considering sheep live approximately 6 years) is therefore less likely to demonstrate an effect of administration rate on hypotension. The posture and anatomy of the sheep probably also minimised hypotension. The small vascular volume of the limbs means that peripheral pooling of blood following drug induced vasodilatation is

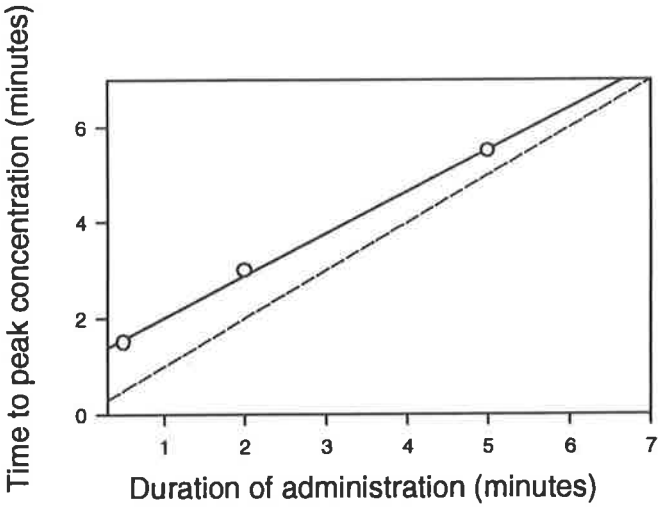


**Figure 7-6** The relationship between duration of administration of 100 mg of propofol and the time to peak brain propofol concentration (circles). Data are presented as mean only. The dashed line represents the time administration was ceased.

minimal, thus the effect on both preload and afterload is small. It would be possible to further examine the cardiovascular impact of altered dose regimens of propofol in the sheep. Clinical settings of cardiovascular compromise can be modelled using techniques such as pharmacological impairment of myocardial contractility with doxorubicin (Blake et al., 1991) and induction of hypovolaemia using venesection, but these studies are outside the scope of this thesis.

### 7.4.3 EFFECTS OF ADMINISTRATION RATE ON ADMINISTERED DOSE AND ONSET TIME

In examining the simulation of titration to effect, brain concentrations of 1 and 2 μg/ml were chosen to represent an endpoint to compare to human studies which use the endpoint of loss of response. While it is evident from the data in chapter 5 that 1 μg/ml does not alter current threshold significantly, the choice of these concentrations allowed examination of whether the level chosen altered the findings of dose reduction. The consistency of dose reduction with decreasing administration rate for both brain concentrations demonstrated that the dose reduction was minimally affected by the different concentrations selected. In reality, the data in figure 6-8 suggests that a loss of response to a painful stimulus occurs at approximately 4-5 μg/ml. Therefore, a concentration in the range of 2-3 μg/ml probably best equates to the loss of response to a verbal stimulus used in the studies cited.

It is difficult to directly compare these brain concentrations of propofol to man because of the lack of human data. There is, however, some indirect evidence of the sensitivity of the human brain to propofol. Blood concentrations above 2 μg/ml

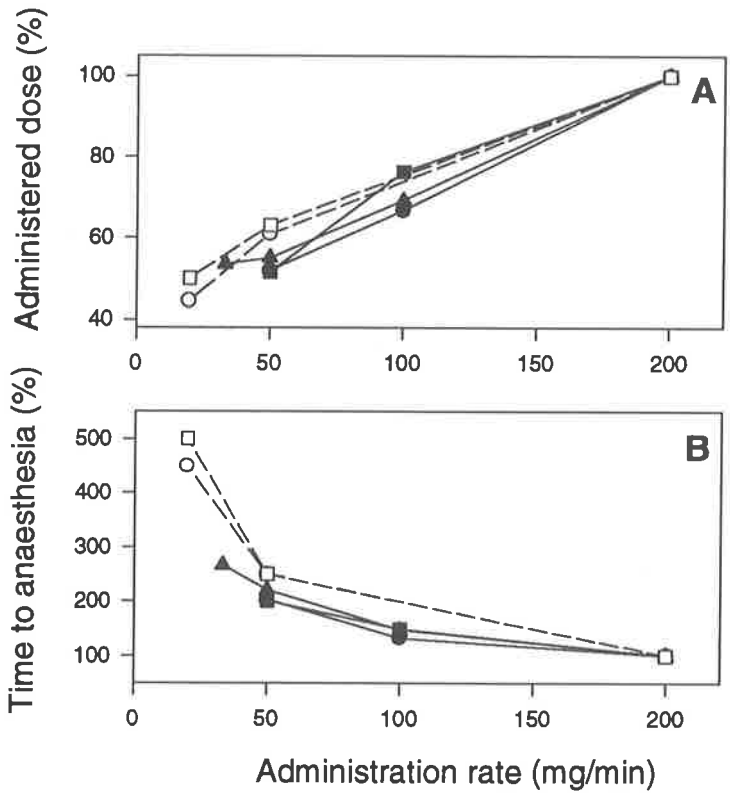


**Figure 7-7** The effect of administration rate on (A) administered dose at the point of onset of anaesthesia and (B) the time to onset of anaesthesia. The studies in this chapter are represented by open symbols, and three published papers examining this phenomenon in man by filled symbols (Stokes and Hutton, 1991 (circles); Peacock et al, 1990 (squares) and Peacock et al, 1992 (triangles). Onset of anaesthesia was defined as loss of response to voice in man in the three cited studies, and a brain propofol concentration of 1 μg/ml (open circles) or 2 μg/ml (open squares) in the studies in sheep presented in this chapter.

in man are associated with a relatively low response to verbal stimulus (Wessen et al., 1993; Kay et al., 1986; Adam et al., 1983; Shafer et al., 1988). Although some of these studies were not conducted at steady-state, if the blood-brain partition coefficient of approximately 3 demonstrated in the studies in chapter 6 and in rats (Shyr et al., 1995) is assumed, this equates to a brain concentration of approximately 6 μg/ml, approximately twice the values for sheep used earlier in this study. Because of the inaccuracies involved in estimating depth of anaesthesia between the two species, however, these values are very approximate and techniques of measuring brain concentrations in man using mass balance principles with concomitant CBF measurements or tissue biopsy intra-operatively in neurosurgery would be needed to provide a more exact comparison.

Despite these problems, it is interesting to compare the dose reduction with previously published work; this comparison appears in figure 7-7. The findings of the three cited studies in man are very similar, with an almost 50% reduction in

dose administered at the point of onset of loss of verbal contact as administration rate decreased. The dose reduction in sheep is very similar (approximately 40%), demonstrating that, whatever the mechanism, it occurs in both man and sheep. Furthermore, the increased onset times show similar comparable increases. The sheep data presented here also show that this dose-saving effect extends to an even slower administration rate to that described in man, but is associated with an extremely long induction period.

Having shown that similar phenomena occur, the mechanism seems evident from the pharmacokinetic data. The 'dose-sparing' is in fact an illusion, as all three administration rates produced very similar brain concentrations. In figure 7-6 one can see convergence of the times at which peak brain concentrations were reached and the time administration was ceased, as administration rates decreased. Thus, by administering propofol more slowly this reduction in 'lag time' means more efficient titration to effect. Rapid administration means that more drug has been administered at the point of onset of anaesthesia, but will be followed by a much deeper plane of anaesthesia. This, of course, cannot be detected by the use of a single end-point for "onset of anaesthesia" used in the cited studies, and this has led to the confusion over drug uptake. In fact this explanation has been raised previously (Wilder Smith and Borgeat, 1992) and termed 'overshoot'. While it would indeed appear to be the correct explanation, the application of systemic compartmental pharmacokinetics used by these authors could not be used to effectively support this argument.

In conclusion, it is evident from the studies presented here that, within the rates of administration used in clinical practice, the magnitude of brain uptake of propofol is not affected by rate of administration. The observed apparent dose-sparing effect of slow administration is an illusion related to improved titration to effect. While these studies have been performed in sheep, the general close agreement between the effects of propofol on the brain in sheep and in man demonstrated in chapter 5, and the similarities between species in relative changes in dose requirements associated with altered administration rates, suggest that these phenomena are similar in sheep and man. It seems likely, therefore, that there exists an optimal rate of administration that will induce anaesthesia at a reasonable rate without high blood concentrations which are believed to be associated with the risk of hypotension. In sheep, administration at 50 mg/min produced a reasonably rapid increase in brain concentrations, without very high arterial concentrations, and would appear to represent a suitable administration rate. The data in man suggest that this may also be close to the optimal rate for clinical practice.

# CHAPTER 8. A COMPARTMENTAL ANALYSIS OF THE PHARMACOKINETICS OF PROPOFOL IN SHEEP

## 8.1 INTRODUCTION

A compartmental pharmacokinetic approach describes the pattern of the time-course of drug concentrations in the systemic blood in terms of rates of movement between hypothetical compartments, which have no real anatomical or physiological identity. A typical example is shown in figure 8-1. Some of the limitations of this approach, such as poor description of concentrations immediately after rapid administration, influence of sampling times and sites on the values of calculated volumes and rate constants, poor prediction of concentrations at anatomical sites, and inability to account for physiological or pathophysiological changes were discussed in chapter 1.

Despite these limitations, it is this compartmental approach which has almost exclusively been used with propofol, both in animals and man (Smith et al., 1994; Kanto and Gepts, 1989; Cockshott, 1985), with 2 or 3 compartment models providing the best fit of the time-course of concentrations in blood. In chapter 1, some inconsistencies between the predictions of these models and observed phenomena were highlighted, suggesting that this compartmental approach may be quite inadequate for application to the induction phase of anaesthesia. The studies presented in this thesis provide data sets on both blood and brain concentrations of propofol following administration at a range of rates and doses, thereby allowing the applications and limitations of the compartment approach for propofol to be effectively analysed. The aims of this study were therefore to construct simple compartment and effect compartment models of the pharmacokinetics of propofol, based on the time-course of measured arterial and brain concentrations, and to examine their fit to the measured concentrations and their ability to accurately predict concentrations in the blood and brain under conditions of altered dose or administration rate.

## 8.2 METHODS

### 8.2.1 PHARMACOKINETIC DATA

For the purposes of this analysis, pharmacokinetic data from the studies reported in chapter 5 were used. In all cases, mean concentrations in arterial blood and in the brain were used for curve fitting or for comparison with data simulated using the model.

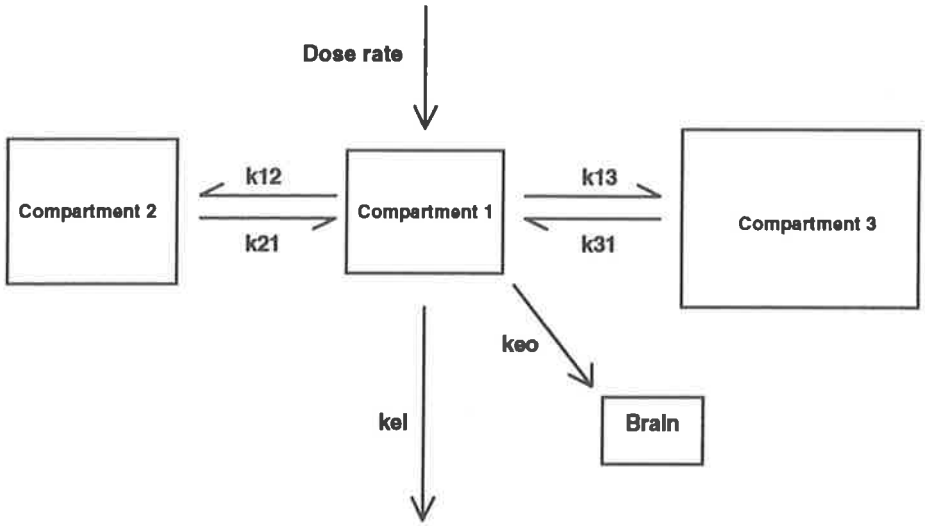


**Figure 8-1** The 3 compartment pharmacokinetic model commonly used for propofol, with an "effect" compartment thought to represent the relevent "biophase", ie brain.

### 8.2.2 MODEL STRUCTURE

The basic structure of a three compartmental model with an additional effect compartment is displayed in figure 8-1. For one compartmental analysis, the rate of propofol administration entering a central

compartment (1) was described by dose rate, with the rate constant $k_{el}$ describing the rate of elimination from that compartment. For two compartmental analysis, a second compartment (2) was added, with the rate constants $k_{12}$ and $k_{21}$ describing the rate of distribution into and out of that compartment respectively. For three compartment analysis, a third compartment (3) was added, with the rate constants $k_{13}$ and $k_{31}$ describing the rate of distribution into and out of that compartment respectively. For effect compartment modelling, a rate constant ($k_{eo}$) described the rate of distribution from the central compartment into the brain.

### 8.2.3 EQUATION SOLVING

The models were implemented as sets of differential equations (see below) and were solved using the "Scientist" modelling package (Scientist for Windows, Version 2, Micromath, Salt Lake City, USA). Curve-fitting using a least squares algorithm was performed using the "Scientist" modelling package. The best fit was judged by the maximisation of the "Model Selection Criteria" (MSC) of this package, which is the Akaike Information Criterion scaled to normalise for data sets of different magnitudes. Note that, unlike the Akaike Information Criterion, the higher the value of the Model Selection Criteria the better the fit.

### 8.2.4 EQUATIONS OF THE MODEL

The equations of the models were written in differential form suitable for "Scientist". The conventions used are common to many programming languages, but note that

an apostrophe after a variable indicates a differential with respect to time (thus C' is dC/dt) and that the "pulse" function is used to generate a square wave input. Annotations are preceded by "//", and symbols are explained in tables 8-1 and 8-2. Equations for a 1, 2 and 3 compartment model are described below, as well as equations for addition of an effect compartment.

| Variable | Description |
|---|---|
| t | Time |
| C1 | Arterial concentrations |
| Ce | Effect compartment (brain) concentration |

**Table 8-1** Variables of the model

| Parameter | Description |
|---|---|
| $V_1$ | Volume of the central compartment |
| $k_{el}$ | Rate constant for elimination from the central compartment |
| $k_{12}$ | Rate constant, central compartment ($V_1$) to first peripheral compartment ($V_2$) |
| $k_{21}$ | Rate constant, first peripheral compartment ($V_2$) to central compartment ($V_1$) |
| $k_{13}$ | Rate constant, central compartment ($V_1$) to second peripheral compartment ($V_3$) |
| k31 | Rate constant, second peripheral compartment ($V_3$) to central compartment ($V_1$) |
| $k_{eo}$ | Rate constant, central compartment ($V_1$) to effect compartment (brain) |
| R | Partition coefficient between arterial blood and the brain |

**Table 8-2** Parameters of the compartment model for propofol in sheep

### 8.2.4.1 Equations used

8.2.4.1.1 Administered dose
(100 mg over 2 minutes in this example)

```
//Dose - units are mg, min
dose1 =100
start1=0
tau1 = 2
doserate = pulse(dose1,start1,tau1)
```

8.2.4.1.2 Simple compartment models

```
//1 compartment model
V1*C1' = doserate - kel*V1*C1
//Initial conditions
t=0
C1=0
```

**//2 compartment model**
V1*C1' = doserate + k21*A2 - kel*V1*C1 - k12*V1*C1
A2' = k12*V1*C1 - k21*A2
//Initial conditions
t=0
C1=0
A2=0

**//3 compartment model**
V1*C1' = doserate + k21*A2 + k31*A3 - kel*V1*C1 - k13*V1*C1 - k12*V1*C1
A2' = k12*V1*C1 - k21*A2
A3' = k13*V1*C1 - k31*A3
//Initial conditions
t=0
C1=0
A2=0
A3=0

8.2.4.1.3 Effect compartment models

**//1 compartment model + effect compartment**
V1*C1' = doserate - kel*V1*C1
Ce' = keo*(C1-Ce)
Cereal = R*Ce
//Initial conditions
t=0
C1=0
A2=0
A3=0
Ce=0

**//2 compartment model + effect compartment**
V1*C1' = doserate + k21*A2 + k31*A3 - kel*V1*C1 - k13*V1*C1 - k12*V1*C1
A2' = k12*V1*C1 - k21*A2
Ce' = keo*(Cc-Ce)
Cereal = R*Ce
//Initial conditions
t=0
Cc=0
A1=0
A2=0
Ce=0

## 8.2.5 MODELLING OF DATA

### 8.2.5.1 Simple compartment modelling

8.2.5.1.1 Effect of administration rate

Arterial concentrations recorded following administration of 100 mg of propofol over 0.5, 2 and 5 minutes, and 450 mg over 45 minutes were individually fitted to simple 1, 2 and 3 compartment models as described above. The model providing the best fit was determined using the MSC as described above.

The accuracy of the model and parameters derived from the slow administration rate at predicting the arterial concentrations following administration at faster rates was examined by using these data to simulate administration of propofol (100 mg) over 0.5, 2 and 5 minutes. These predicted values were then graphed with the measured values.

8.2.5.1.2 Effect of dose

To examine the accuracy of the model and parameters derived from any given administration rate at predicting the outcome of administration of different doses at the same rate, the model and parameters from the best fit of propofol 100 mg over 2 minutes were used to simulate the arterial concentrations of 50 mg and 200 mg over 2 minutes. This administration rate was chosen because data for three doses at the same rate were available for analysis. These predicted values were then graphed with the measured values.

### 8.2.5.2 Effect compartment modelling

Hysteresis between blood concentrations and both brain concentrations and depth of anaesthesia was recognised from the data presented in previous chapters. To examine the efficacy of effect compartment modelling at accounting for this hysteresis, an effect compartment was added to the simple compartment model and parameters which had provided the best fit of the arterial concentrations.

8.2.5.2.1 Effect of administration rate

The effect compartment model was fitted to the observed brain concentrations (calculated using mass balance principles) following administration of propofol 100 mg over 0.5, 2 and 5 minutes, and 450 mg over 45 minutes.

To examine the accuracy of an effect compartment model and parameters derived from any given administration rate at predicting brain concentrations following administration at a different rate, the model and parameters from the best fit to propofol administered over 45 minutes were used in a simulation of the administration of propofol (100 mg) over 0.5, 2 and 5 minutes. These predicted values were then graphed with the measured values.

8.2.5.2.2 Effect of dose

To examine the accuracy of the effect compartment model and parameters derived from any given administration rate at predicting the outcome of administration of different doses at the same rate, the model and parameters from the best fit of propofol 100 mg over 2 minutes were used to simulate the arterial and brain concentrations of 50 mg and 200 mg over 2 minutes. These predicted values were then graphed with the measured values.

### 8.2.6 CALCULATION OF CONVENTIONAL COMPARTMENTAL PARAMETERS

While inter-compartment rate constants are easily determined using the above analysis and differential equations, the pharmacokinetics of propofol are also commonly expressed in terms of exponential functions. For comparison of the parameters calculated in the current analysis and published parameters, calculation of these parameters is required. The mathematics for this are described in full elsewhere (Wagner, 1975). The terminology used is described in table 8-3, and the equations used in the current analysis are described below.

| Variable | Description of term |
|---|---|
| t | Time |
| $C_1$ | Arterial concentrations |
| Cl | Clearance |
| Vdss | Volume of distribution at steady state |
| V1 | Initial volume of distribution |
| $\alpha$ | Hybrid rate constant |
| $\beta$ | Hybrid rate constant |

**Table 8-3** Terminology used in exponential equations

**One compartment model**

$C_1 = C_0.e^{-k.t}$

**Equations for calculation of parameters**

$Cl = V_1 . k_{el}$

$t_{1/2\ elim} = \ln 2/k_{el}$

**Two compartment model**

$C_1 = A.e^{-\alpha.t} + B.e^{-\beta.t}$

**Equations for calculation of parameters**

$\alpha = 1/2.[(k_{12} + k_{21} + k_{el}) + \sqrt{\{(k_{12} + k_{21} + k_{el})^2 - (4.k_{12}.k_{el})\}}]$

$\beta = 1/2.[(k_{12} + k_{21} + k_{el}) - \sqrt{\{(k_{12} + k_{21} + k_{el})^2 - (4.k_{12}.k_{el})\}}]$

$t_{1/2\ \alpha} = \ln 2/\alpha$

$t_{1/2\ \beta} = \ln 2/\beta$

$Cl = V_1 . k_{el}$

$Vd_{ss} = V_1 . [(k_{12} + k_{21})/k_{21}]$

## 8.3 RESULTS

### 8.3.1 SIMPLE COMPARTMENT MODELLING

#### 8.3.1.1 Effect of administration rate

For arterial concentrations following propofol administration over 0.5, 2 and 5 minutes, the best fit of data was achieved with simple 1 compartment models, although the parameters for each dose rate were all different (table 8-4). Two and three compartment models showed very poor estimation of parameters for the second and third compartments and very slow distribution into and out of the central

| Dose regimen | V1 (L) | $K_{el}$ ($min^{-1}$) | $K_{12}$ ($min^{-1}$) | $K_{21}$ ($min^{-1}$) | Model selection criteria |
|---|---|---|---|---|---|
| | | | | | |
| 100 mg/0.5 min | 3.06 (0.53) | 1.51 (0.35) | | | 1.31 |
| 100 mg/2 min | 4.23 (0.37) | 1.35 (0.16) | | | 3.09 |
| 100 mg/5 min | 4.58 (0.43) | 0.98 (0.10) | | | 2.75 |
| 450 mg/45 min | 8.91 (3.35) | 0.86 (0.32) | 0.39 (0.33) | 0.21 (0.12) | 3.14 |

**Table 8-4** Parameters for the models providing the best fit of arterial concentrations following administration at different rates. Values are expressed as mean and SD, and the accuracy of the fit is represented by the model selection criteria. A one compartment model best described administration over 0.5, 2 and 5 minutes, and a 2 compartment model best described administration over 45 minutes.

compartment, and thus collapsed to a one compartment model (figure 8-2). For administration over 45 minutes, while one and three compartment models could be fitted to the data, a 2 compartment model provided the best fit (table 8-4, figure 8-2).

The significance of these differences in parameters and structure of the models at different dose rates is demonstrated by the use of the 2 compartment model and parameters derived from the administration over 45 minutes to simulate propofol administration at faster rates. Figure 8-3 shows the arterial concentrations predicted by that model and the measured concentrations from experimental data presented previously. It is apparent that while the simulation provided a reasonable fit of arterial concentrations for administration over 45 minutes, at faster administration rates this model performed very poorly.

#### 8.3.1.2 Effect of dose

The arterial concentrations at different doses predicted by the model and the measured concentrations are displayed in figure 8-4. Predicted concentrations closely matched measured concentrations for the 100 mg and 200 mg doses, but predicted concentrations greatly exceeded measured concentrations after the 50 mg dose.

### 8.3.2 EFFECT COMPARTMENT MODELLING

#### 8.3.2.1 Effect of administration rate

Brain concentrations were well fitted by an effect compartment added to the one compartment model for the faster rates of administration, and the two compartment model for administration over 45 minutes. There were, however, large differences

| Dose regimen | $K_{eo}$ ($min^{-1}$) | $t_{1/2}$ effect compartment (min) | R | Model selection criteria |
|---|---|---|---|---|
| 100 mg/0.5 min | 0.084 (0.038) | 8.24 | 4.18 (1.50) | 1.45 |
| 100 mg/2 min | 0.070 (0.009) | 9.99 | 4.72 (0.47) | 2.90 |
| 100 mg/5 min | 0.061 (0.007) | 11.41 | 4.50 (0.39) | 3.22 |
| 450 mg/45 min | 0.109 (0.006) | 6.35 | 3.45 (0.07) | 3.12 |

**Table 8-5** Parameters for the effect compartment providing the best fit of brain concentrations following administration at different rates. Values are expressed as mean and SD, and the accuracy of the fit is represented by the model selection criteria.

in the $k_{eo}$ and blood-brain equilibrium half-life (calculated as the natural log of 2 divided by $k_{eo}$), as well as some variation in the partition coefficient between blood and brain, for different dose rates. These values and the model selection criteria are displayed in table 8-5. The measured and predicted brain concentrations are displayed in figure 8-5.

The significance of these differences in effect compartment parameters at different dose rates is demonstrated by the use of the 2 compartment model with an effect compartment derived from the administration of propofol over 45 minutes to simulate propofol administration at faster rates. Figure 8-6 shows the brain concentrations predicted by that model and the measured concentrations from experimental data presented previously. It was apparent that, while the simulation provided a reasonable prediction of brain concentrations for administration over 45 minutes, at faster administration rates this model performed very poorly.

#### 8.3.2.2 Effect of dose

The brain concentrations predicted at different doses by the model and the measured concentrations are displayed in figure 8-7. Predicted concentrations closely matched measured concentrations for the 100 mg and 200 mg doses, but predicted concentrations greatly exceeded measured concentrations after the 50 mg dose.

### 8.3.3 CALCULATION OF CONVENTIONAL COMPARTMENTAL PARAMETERS

The systemic parameters calculated using the formulae described previously are presented in table 8-6.

## 8.4 DISCUSSION

### 8.4.1 SYSTEMIC MODELLING

From the systemic compartmental analysis conducted in the current study, it is apparent that both the structure and values of parameters of a model which would

| Parameters | 100 mg/0.5 min | 100 mg/2 min | 100 mg/5 min | 450 mg/45 min |
|---|---|---|---|---|
| V1 (L) | 3.06 | 4.23 | 4.58 | 8.91 |
| Vdss (L) | | | | 25.45 |
| Cl (L/min) | 4.62 | 5.71 | 4.48 | 7.66 |
| $\alpha$ | | | | 1.32 |
| $\beta$ | | | | 0.14 |
| t1/2$\alpha$ (min) | | | | 0.52 |
| t1/2$\beta$ (min) | | | | 5.10 |
| t1/2 elimination (min) | 0.46 | 0.51 | 0.71 | |

**Table 8-6**. Conventional pharmacokinetic parameters for the compartmental models in exponential form.

provide a good fit of the time-course of concentrations in arterial blood were critically dependant on the administration rate of propofol. At the very rapid rates of administration used in the studies presented in chapter 7 (over 0.5 - 5 minutes), the best fit of arterial concentrations was achieved with a single compartment model. While 2 and 3 compartment models also provided a fit of the data, the fit (as judged by the Model Selection Criteria) was inferior, although the curves for all models were almost superimposed (figure 8-2).

Compartment modelling in general did not provide a very good fit of the data at the most rapid rates of propofol administration. The concentrations following administration over 0.5 minutes were relatively poorly fitted by a one compartment model. The model selection criteria of 1.31 was low compared to the values achieved when propofol was administered more slowly, and there was a large underestimation of peak arterial concentrations evident in figure 8-2. Both the model selection criteria and the visual fit improved as administration rate decreased. As administration over approximately 0.5 minutes is commonly used in clinical anaesthetic practice (Ooi, 1995), it is apparent that propofol administration at induction of anaesthesia based on compartmental pharmacokinetic parameters predicts arterial concentrations poorly .

In addition, the large differences in the values of parameters between the one compartment models derived at different rapid rates of administration suggests that these inaccuracies may be compounded if dosing based on the model derived from one administration rate were used for administration at another rate. For example, the initial volume of distribution ($V_1$) derived from the arterial concentrations following administration over 5 minutes was almost 50% greater than that derived from data from administration over 0.5 minutes. Therefore, if a loading dose aimed

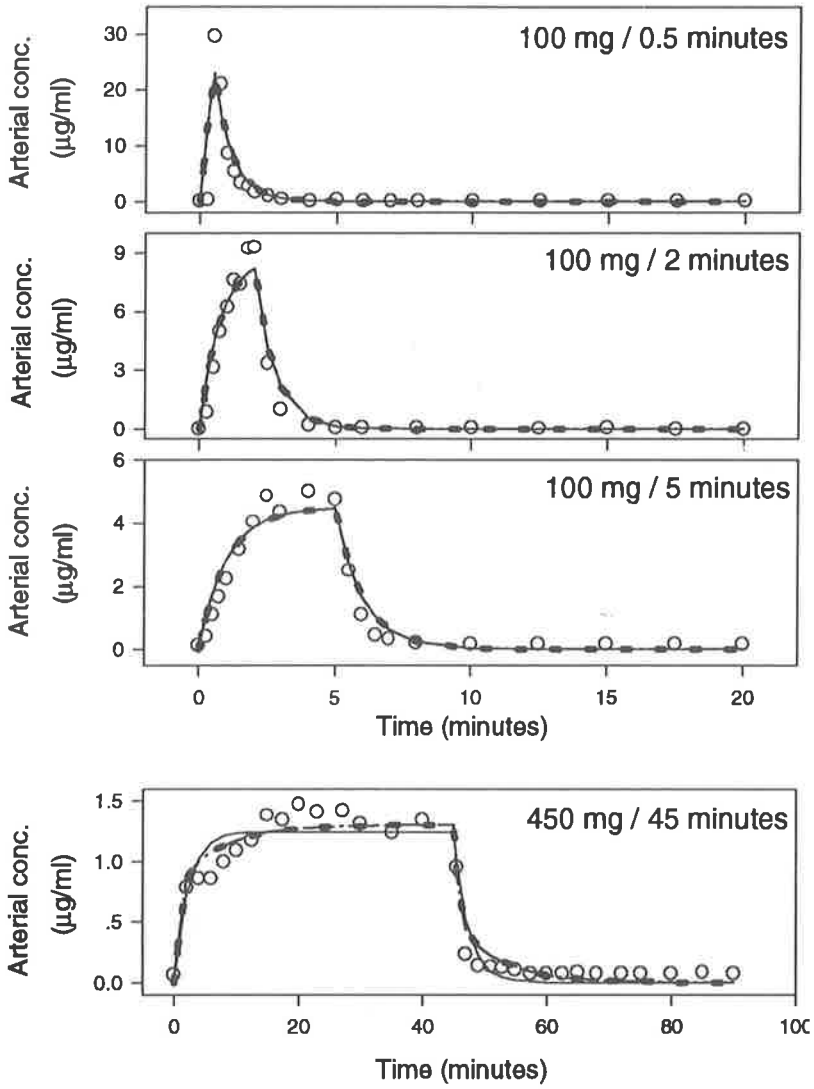


**Figure 8-2** Measured arterial concentrations (symbols) and fits to individual data sets using one (solid lines), two (dashed/dotted lines) and three (dashed lines) compartment models. The two and three compartment models are essentially superimposed in the cases of rapid propofol administration, and only minor differences between models are apparent following slow administration in the bottom graph.

at achieving a certain concentration in the blood were administered over 0.5 minutes using the parameters from the 5 minute data set, this would result in a much higher concentration than anticipated. This poor performance of conventional pharmacokinetics during rapid drug administration was in fact highlighted nearly 20 years ago (Chiou, 1979), but appears still not to be widely appreciated by all.

The significance of the discrepancies between models derived from different administration rates was apparent when arterial concentrations from slower administration rates were used for modelling. One and two compartment models produced detectible differences in fit to the data (figure 8-2), with the best fit

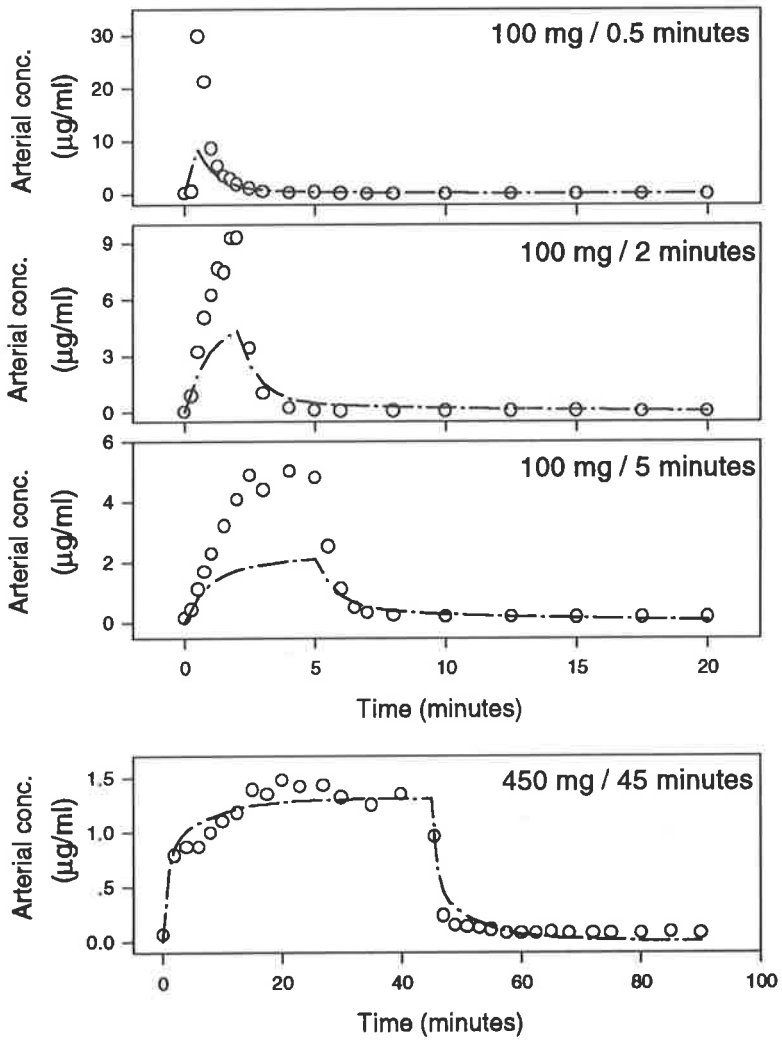


**Figure 8-3** Measured arterial concentrations (symbols) and arterial concentrations predicted for the faster administration rates using the two compartment model and parameters fitted to the arterial concentrations following propofol administered over 45 minutes (lines).

achieved with a 2 compartment model. This model produced a value of $V_1$ which was approximately double that derived from the models of faster rates. This different model structure and values of parameters resulted in an extremely poor prediction of concentrations at faster administration rates (figure 8-3). It is therefore evident that this model could not be used to devise accurate dose regimens involving rapid rates of administration, such as at induction.

While models and parameters cannot therefore be used accurately across administration rates, it is also apparent that they are not accurate across doses. Although the model and parameters derived from a dose of 100 mg over 2 minutes could be used to accurately predict arterial concentrations following 200 mg administered at the same rate, measured concentrations following 50 mg over 2

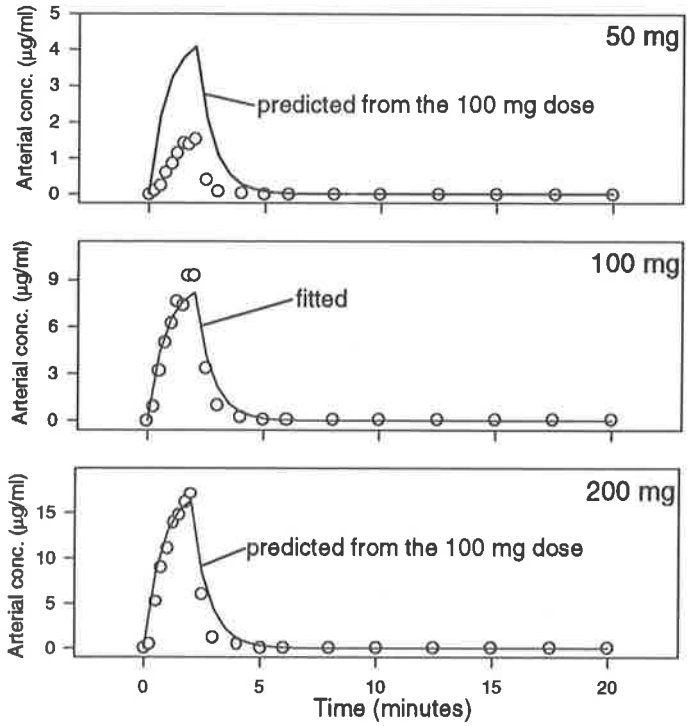


**Figure 8-4** Measured arterial concentrations (symbols) and predicted arterial concentrations for the 50 mg and 200 mg doses (lines) using the one compartment model and parameters fitted to the arterial concentrations following propofol 100 mg administered over 2 minutes (middle panel).

minutes were approximately half those predicted by the model (figure 8-4). This relates to lung uptake or metabolism of propofol and was discussed in chapter 5, and is an example of a physiological process not accounted for by mathematical modelling using a simple compartment approach. This issue of lung uptake was also addressed by Chiou (1979), but seems to have escaped widespread appreciation.

In the light of these data it is interesting to look at the compartmental pharmacokinetic data available in the literature. The most extensive data come from man, where compartmental pharmacokinetic modelling of blood concentrations of propofol has frequently been performed, and then used to determine dose regimens.

#### 8.4.1.1 Model structure and parameters

In both animals and man, the general structure of the models is that of three compartments, although two compartments have provided the best fit of data when relatively short duration of sampling has been used (Adam et al., 1983). While parameter values vary in the very large number of studies which have been performed, a general pattern is evident (Smith et al., 1994; Kanto and Gepts, 1989).

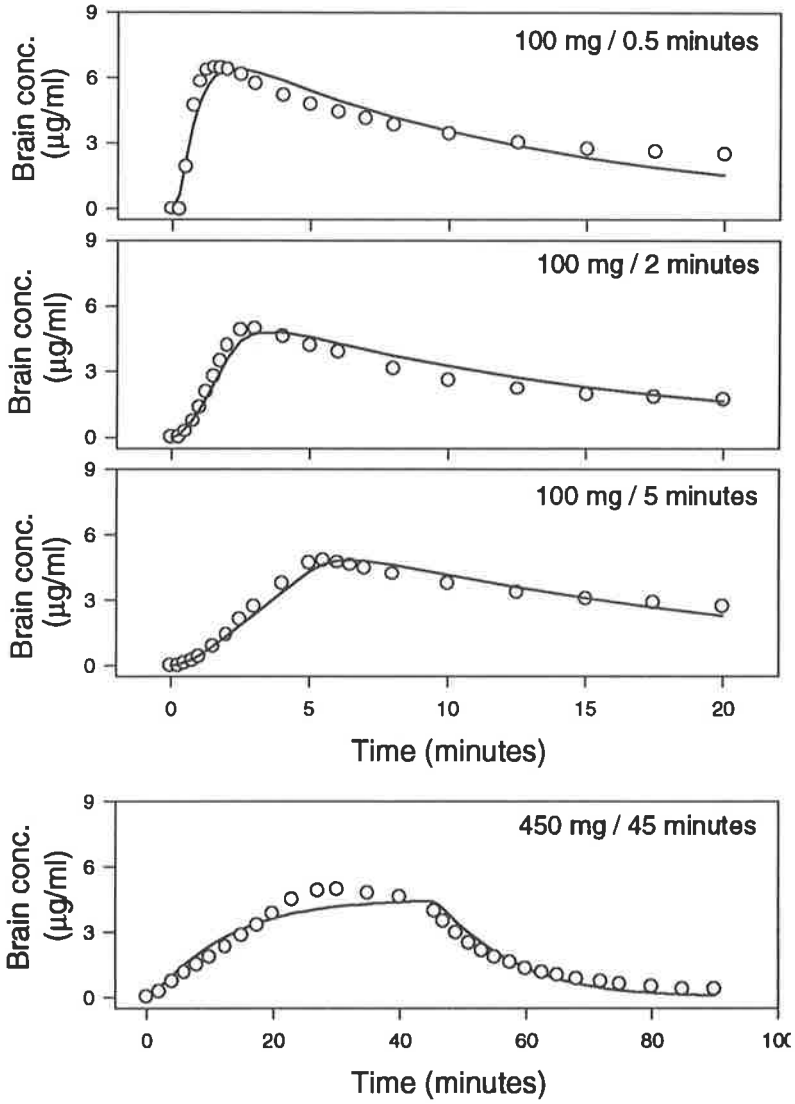


**Figure 8-5** Measured brain concentrations (symbols) and predicted brain concentrations (effect compartment, lines) fitting individual data sets using the compartment model which best fitted the arterial concentrations, but with an effect compartment model added.

In an attempt to summarise the available data, table 8-7 presents the range of values described in man.

The general pattern is therefore of a very rapid early distribution ($t_{1/2\alpha}$ = 1-3 minutes) from a central compartment that is larger than the blood volume (which is approximately 6 L in man). This is consistent with the model parameters in table 8-4, although it is apparent that early distribution in the sheep is very rapid and that the initial volume of distribution is generally closer to blood volume (approximately 4 L in these sheep) than the published human values. These differences may relate to methodology. Firstly, the delay between administration and first sampling times in most human studies may underestimate the rate of initial distribution. Furthermore, the general use of venous sampling in man and the known prolonged differential between propofol concentrations in the arterial and venous blood (Major

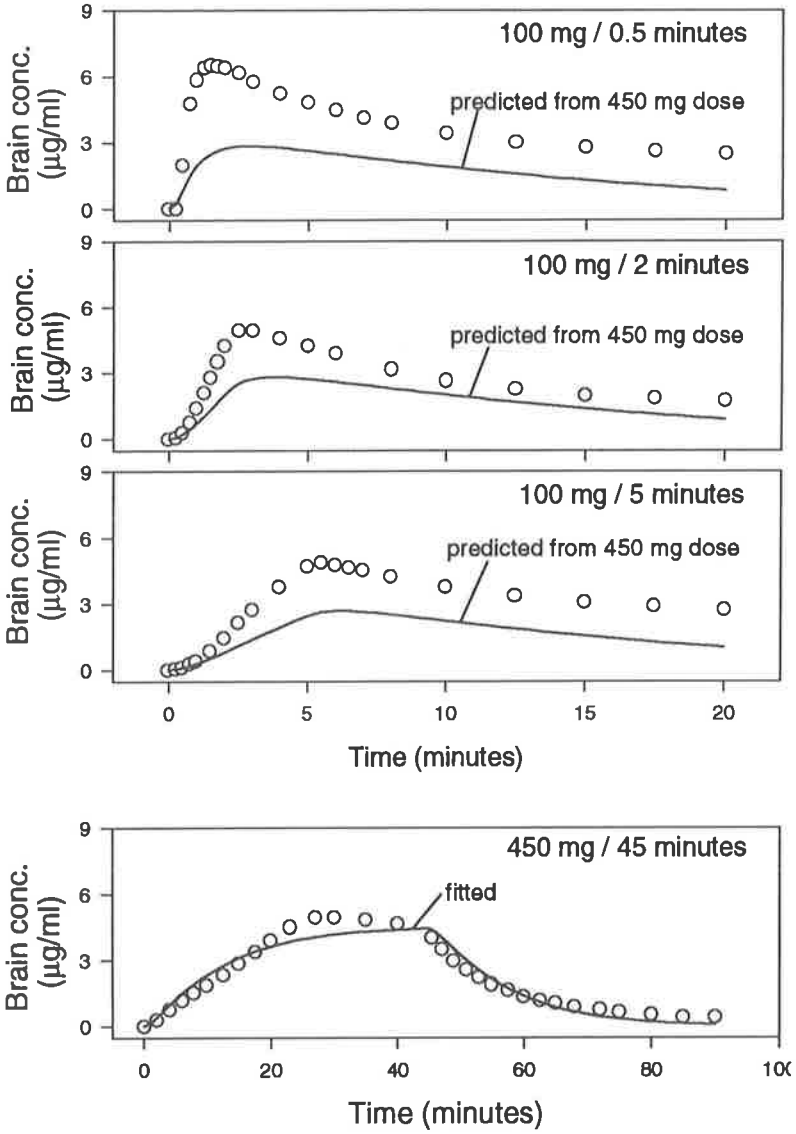


**Figure 8-6** Measured brain concentrations (symbols) and predicted brain (effect compartment) concentrations (lines) derived from the two compartment model with an effect compartment fitted to the brain concentrations following propofol administered over 45 minutes.

et al., 1983) is likely to produce an overestimation of the initial distribution volume. This was apparent in a recent review of performances of five commonly used compartmental pharmacokinetic models of propofol in man, where the only model derived from arterial rather than venous samples had the lowest $V_1$ (Vuyk et al., 1995). The finding in this paper that measured arterial concentrations were persistently higher than those predicted by models derived from venous concentrations is therefore not surprising. In another study (Schuttler et al., 1986), compartmental modelling of propofol was performed using data derived from simultaneous sampling of arterial and venous blood. The predominant difference between the models was a halving of $V_1$ when arterial samples were used.

Distribution to the second and third compartments is considerably slower, with the very slow distribution ($t_{1/2\gamma}$ = 3-30 hours) to the third compartment believed to represent very slow redistribution into muscle and fat (Smith et al., 1994). The need for prolonged sampling to delineate this third phase of distribution is obvious. The second compartment only became significant during slow administration of propofol in sheep, but distribution to this compartment was much slower, as judged by the $t_{1/2\beta}$ of 2.4 minutes compared to 0.6 minutes for $t_{1/2\alpha}$. This is consistent with the pattern for man in table 8-7.

The published values for clearance of propofol are high, and usually exceed liver blood flow (approximately 1-1.5 L/min (Guyton, 1981)). This high clearance, and findings such as the formation of propofol metabolites even during the an-hepatic phase of liver transplantation (Veroli et al., 1992), suggests extra-hepatic metabolism occurs. Clearance figures in the sheep in this analysis (4-8 L/min) are relatively higher, considering the reported values of liver blood flow in sheep are similar to those in man (Runciman, 1982). The possible contribution of the lung has been discussed in chapter 5, but it also possible that other sites of metabolism may exist. Such a phenomenon has previously been described, for example, in the hindquarters of sheep for drugs such as pethidine (Upton et al., 1991c).

#### 8.4.1.2 Model applications

Evaluation of the performance of compartmental models, such as in the paper cited above, is usually restricted to prolonged administration, without addressing the period immediately after administration. The known morbidity associated with propofol use at induction of anaesthesia, and the association between blood concentrations and side effects described above, suggests this phase deserves more attention. Careful examination of the literature reveals some of the problems with the compartmental approach during this phase which support the data in this thesis.

In one study using computer controlled delivery of propofol, it was revealed that model parameters from propofol administration at a rapid rate could not be accurately extrapolated to different administration rates (Tackley et al., 1989). Initially, parameters derived from a study of rapid (over 20 seconds) bolus administration of propofol were used to predict the time-course of blood concentrations following rapid bolus administration and then slower infusion. This approach produced predicted concentrations which were initially higher than those actually achieved in the early stages of drug administration, and this overestimation is consistent with the patterns of changing model parameters with changing administration rate revealed in the analysis in the current chapter (table 8-4). Because of these poor predictions, the authors then adopted parameters derived from slower administration for the remainder of the study, with improvement in their

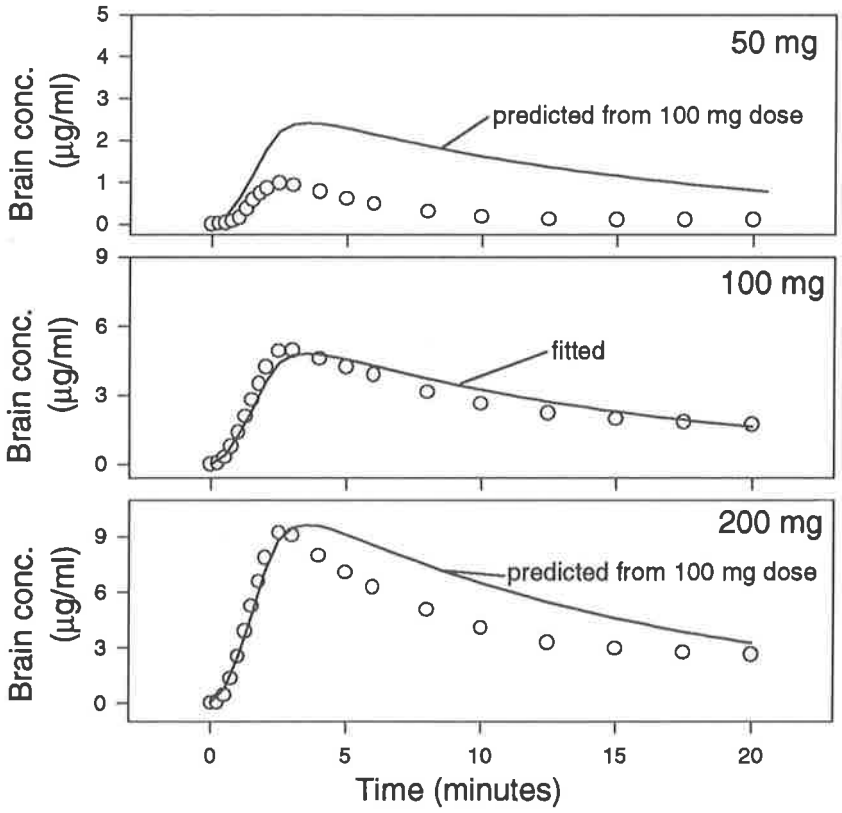


**Figure 8-7** Measured brain concentrations (symbols) and predicted brain (effect compartment) concentrations (lines) using the two compartment model with an effect compartment fitted to the brain concentrations following propofol (100 mg) administered over 2 minutes.

predictions. This is further evidence of the need to tailor the model to the infusion rate, as was revealed in figure 8-3.

The importance of matching model parameters and administration rates was also evident in a study in man examining the effect of altered administered rates on model parameters (Adam et al., 1983). The authors reported no significant change in model parameters over administration rates of 5, 20 and 40-50 seconds, yet there appeared to be changes in $V_1$ at different administration rates. As administration rate decreased, $V_1$ changed from 29.9 (SD 6.7) to 35.2 (SD 8.7) to 43.7 (SD 8.2) litres. Although the authors reported that parameter values did not change significantly (no statistical analysis was reported), application of analysis of variance (ANOVA) to these data reveals that these changes are statistically significant ($p = 0.026$). This pattern of increasing $V_1$ with increasing administration rate is very similar to that shown in the data in this thesis (table 8-4). In this paper, the authors went on to use their model and parameters to simulate blood concentrations following infusion administration. Although the accuracy of these predictions was not tested, their findings of changes in parameters with changing administration rate, the similar findings in this thesis, and the experience of Tackley

| Parameters | Approximate range of reported values in healthy adults |
|---|---|
| $V_1$ (L) | 8-30 |
| $Vd_{ss}$ (L) | 140-850 |
| Cl (L/min) | 0.9-2.3 |
| $t_{1/2\alpha}$ (min) | 1.3-2.9 |
| $t_{1/2\beta}$ (min) | 24-70 |
| $t_{1/2\gamma}$ (min) | 200-1800 |
| $k_{el}$ ($min^{-1}$) | 0.07-0.12 |
| $k_{12}$ ($min^{-1}$) | 0.06-0.25 |
| $k_{21}$ ($min^{-1}$) | 0.01-0.06 |
| $k_{13}$ ($min^{-1}$) | 0.02-0.04 |
| $k_{31}$ ($min^{-1}$) | 0.002-0.003 |

**Table 8-7** Published pharmacokinetic parameters for propofol in man. Data taken from (Smith et al., 1994; Vuyk et al., 1995; Kanto and Gepts, 1989).

et al., 1989 discussed previously, would suggest systematic errors were likely to exist.

This inaccuracy was also evident in the comparison of performance of five common compartmental models of propofol referred to earlier (Vuyk et al., 1995). These models were used to determine the dose regimen predicted to produced step wise increases in arterial concentrations of propofol (although, as was discussed previously, these were models derived from venous blood samples), and their performance evaluated by comparing measured and predicted concentrations. The dose regimens generally involved rapid bolus administration followed by slower infusion for 15 minutes. Four of the models performed similarly, but systematically underestimated arterial concentrations by 15-25% (see previous discussion). One model was outstandingly different, consistently producing concentrations that were approximately half those actually measured. The significant difference between this model and the other four was that this model was derived from blood concentrations measured after rapid bolus administration (over 0.5 minutes) while the others were derived following slower rates of administration that were much closer to that used to evaluate their performance in this study. The authors of this paper suggested that the pharmacokinetics of propofol may alter with administration rate, and indeed hypothesised that factors such as the effects of propofol on the cardiovascular system might alter drug disposition. The analysis presented in this chapter supports this concept, but it is interesting to note that the data in the current chapter would suggest that model parameters derived from a rapid bolus should

overestimate rather than underestimate blood concentrations. Although this discrepancy is not readily explained, examination of the original study from which the model was derived (Kirkpatrick et al., 1988) reveals that the first venous samples were first taken two minutes after the drug was administered. The initial distribution of propofol may therefore not have been revealed, meaning that the model was in fact not a good description of the true initial time-course of propofol concentrations in the critical mixing phase at these very rapid rates of administration. The use of very frequent arterial samples in the studies in this thesis largely overcame this.

### 8.4.2 EFFECT COMPARTMENT MODELLING

The addition of an effect compartment to the existing compartmental models provided a very good fit of the time-course of brain concentrations at each rate of administration, as shown in figure 8-5. However, again it was apparent that even this modification of a compartmental approach still produced administration rate dependent parameters. At rapid rates of administration, variation in parameters to provide a good fit of brain concentrations was not great, with blood-brain equilibrium half-lives lying between 8.2 and 11.4 minutes and the partition coefficient (R) between blood and brain fairly constant at between 4.2 and 4.7. The slower infusion administration, however, produced a much shorter half-life of only 6.4 minutes and a small decrease in R. The poor predictions from mismatching models and administration rates was again evident from figure 8-6, with parameters from slow administration greatly underestimating measured brain concentrations when propofol was administered rapidly. As with systemic modelling alone, predictions across doses were affected by lung uptake at low doses but the 200 mg dose was also relatively poorly predicted by parameters from the 100 mg dose (figure 8-7). It is possible that the prolonged depression of CBF, a factor not accounted for by compartmental pharmacokinetics, that occurred following the 200 mg dose, might explain this finding. Such a phenomenon, causing an unexpectedly rapid rate of elution, would suggest a diffusion limitation to exchange between the brain and the blood. This discrepancy can only be the subject of speculation in the absence of data on the determinants of brain uptake of propofol.

Effect compartment modelling is usually an attempt to minimise the poor correlation between blood concentrations and measured effect (Schnider et al., 1994), and is performed because concentrations at the effect site are unavailable. As previous studies in this thesis have clearly shown that effect site (brain) concentrations and the effect of propofol on depth of anaesthesia are closely correlated, there was an opportunity to examine the efficacy of this approach in relation to propofol by comparing the time-course of brain concentrations to the effect compartment concentrations.

There are in fact relatively few published data available in relation to this approach for propofol, as this type of study has not previously been performed. In support of the findings, however, simultaneous measurement of brain and blood concentrations of propofol in rats have found partition coefficients of between 2.5 and 3.5 (Shyr et al., 1995; Simons et al., 1991a), figures comparable with the values of between 3.5 and 4.7 found in the current analysis. Only one group has reported effect compartment analysis during the period of induction of anaesthesia, when hysteresis between blood concentrations and effect are maximal. In this study (Schuttler et al., 1986), cited previously, systemic compartmental modelling was performed on blood concentrations from venous and arterial sampling, and the relationship between blood concentrations and the effect of propofol on EEG median frequency used to determine the blood-brain equilibrium half-life. The value of 2.9 SD 2.2 minutes reported, demonstrates significant delay between blood concentrations and effect, but comparison with the figure from the current study is difficult. Firstly, the exact rate of administration is not reported; only that administration was based on previous pharmacokinetic parameters and designed to produce a slowly decreasing blood concentration. Assuming that this implies a relatively slow rate of administration, the figure of approximately 3 is not greatly different from the value of 6.4 derived from the 45 minute infusion administration in sheep. The second problem is that it is not reported whether arterial or venous concentrations were related to effect. As the time-course of venous concentrations is generally delayed when compared to arterial concentrations, the use of these venous blood concentrations is likely to produce a lower value for the equilibrium half-life. Thus there may be little difference in arterial blood-brain equilibrium half-lives in the two studies. Thirdly, as was discussed in chapter 1, EEG may not necessarily provide an accurate measurement of depth of anaesthesia nor correlate with the time-course of drug concentrations in the brain. Lastly, it is not possible to examine for a rate dependent effect on equilibrium half-life as the administration rate was low, and variable.

There are data available for other drugs that affect the central nervous system, such as thiopentone. A previous analysis of the disequilibrium between arterial and brain concentrations of sodium thiopentone in sheep found an equilibrium half-life of between 0.57 and 0.74 minutes, a value relatively constant across three doses (Upton et al., 1996b). This very small value reflects the rapid equilibrium for sodium thiopentone between blood and the brain, and is supported by studies relating blood concentrations of sodium thiopentone and EEG effects which have found little evidence of hysteresis (Hudson et al., 1983) and have reported equilibrium half-lives of between 0.8 and 1.3 minutes (Stanski et al., 1984). All three doses in the study of Upton et al., 1996 were administered at the same rate, and so a rate

dependent effect on equilibrium half-life could not be examined, but the rapid rate of equilibrium of this drug suggests that any rate effect would be minimal.

The importance of effect site concentrations, or at least effect compartment modelling, is emphasised by these findings. From the marked differences in effect compartment equilibrium half-lives between sodium thiopentone and propofol, it is obvious that the time-course of effects following similar rates of administration will be very different, with a much slower onset of anaesthesia with propofol. This is not revealed by the similar systemic compartmental parameters for the two agents (Reilly, 1994), the figures most readily available to anaesthetists. Personal experience, from discussions with clinical anaesthetists and attendance at clinical meetings, would suggest is not generally appreciated amongst clinical anaesthetists. One could hypothesise that this lack of appreciation of the difference between the pharmacokinetics of these agents may underlie some of the problems of hypotension at induction. An improved appreciation by anaesthetists of the true pharmacokinetic behaviour of propofol (slow onset) might lead to the use of slower rates of administration, improved titration to effect, lower administered doses at induction, and potentially a lower incidence of hypotension.

There may be a precedent for this situation. Midazolam, a relatively new intravenously administered benzodiazepine, was released for general use with systemic pharmacokinetic parameters which suggested a rapid clearance and short systemic half-life when compared to diazepam, which was to that point the standard intravenously administered sedative agent (Wood, 1982). In addition, the relative potencies of these agents were thought to be similar. There were, however, a number of incidents of excessive sedation and resulting morbidity associated with the introduction of this agent (Benjamin, 1990), suggesting that the clinical picture of effects differed significantly to the published pharmacokinetics. Later effect compartment modelling revealed that in fact midazolam has a much slower rate of uptake and elution from the brain than diazepam, with relative equilibrium half-lives of 4.8 and 1.6 minutes respectively (Buhrer et al., 1990). Furthermore, it was revealed in the same study that midazolam was approximately five times as potent on a brain concentration basis as diazepam. The differences between the agents is now appreciated more widely by clinicians, and the use of appropriate dose regimens of this agent now appears to be associated with a similar incidence of CNS related adverse events as diazepam (Benjamin, 1990).

In summary, the data presented in this chapter revealed that, during and following the period of rapid administration (over 5 minutes or less) of propofol, compartmental pharmacokinetics poorly describe the time-course of propofol in the blood. In particular, the pharmacokinetic models that provide the "best fit", and the parameters for use in these models, will differ, depending on the rate of

administration of the drug in the studies in which blood samples are taken. As a result of this, very large errors in anticipated blood concentrations and cerebral effects are likely when dose regimens are calculated using models and parameters derived from different rates of administration to that to be used. While addition of an effect compartment to these type of models improves the prediction of the time-course of cerebral effects, the accuracy of even this approach still remains administration rate dependent.

# CHAPTER 9. THE EFFECTS OF REDUCED CARDIAC OUTPUT ON BRAIN UPTAKE OF PROPOFOL

## 9.1 INTRODUCTION

The limitations of the compartmental pharmacokinetic modelling in predicting blood concentrations of propofol after rapid administration have been outlined in chapters 1 and 8.

An alternate approach was proposed over thirty years ago (Crawford, 1966). It was suggested that initial passage of a drug be considered as a "slug" passing through the circulatory tree, so that initial distribution of a drug would depend on the distribution of the circulation. In particular, distribution to the brain was considered to be dependent on the proportion of cardiac output (CO) it received. While this concept appears in some anaesthetic textbooks, it is often not appreciated by clinicians and has rarely been studied for intravenous anaesthetic agents.

From the work of Crawford in 1966, it could be postulated that increasing the percentage of CO directed to the brain would increase the initial distribution of propofol to the brain, and therefore increase cerebral effects produced by a given dose. Alternatively, this might allow a reduction in administered dose to achieve the same cerebral effect. This redirection of CO could be achieved by: (1) increasing CBF without changing CO or (2) decreasing CO without changing CBF, and these will be discussed in turn.

(1) Cerebral blood flow can be increased by pharmacological agents and, as has been demonstrated in chapter 4, by other factors such as increasing arterial carbon dioxide tension through decreases in ventilation. These changes potentially could increase propofol uptake into the brain at induction. Many drugs which increase CBF also increase CO, however, and thus the nett effect on drug distribution may be minimal. Pilot studies in the sheep preparation used for the studies in this thesis revealed that vasodilators such as hydralazine, sodium nitroprusside and glyceryl trinitrate all produced dose dependent parallel increases in both CBF and CO.

(2) An alternative approach is to produce a relative increase in CBF by decreasing CO. The studies in chapter 4 revealed that metaraminol, an alpha agonist which causes vasoconstriction, does not affect CBF but this drug can produce a reflex decrease in CO. Pilot studies showed a 25-30% decrease in CO following administration of the dose regimen used in chapter 4, suggesting that this dose regimen would successfully produce a redirection of CO to the brain.

The specific aims of this study were therefore to examine the effect of a relative increase in the percentage of CO directed to the brain, using administration of

metaraminol, on the time-course and magnitude of propofol concentrations in the blood and brain following rapid administration.

## 9.2 METHODS

### 9.2.1 ANIMAL PREPARATION

Animals were prepared according to the general method described in chapter 2. A Doppler probe and a sagittal sinus catheter were placed under general anaesthesia according to the methods described in chapter 4. In addition, a pulmonary artery thermodilution catheter was inserted at the time of sheep preparation under general anaesthesia, for measurement of cardiac output. Following insertion of 2 catheters into the right atrium via the jugular vein (for drug administration and injection of cold saline), a "Swan-Ganz" style triple lumen catheter (Biosensor International, Singapore) with a thermistor at its tip was inserted into the jugular vein and passed into the pulmonary artery under radiographic control until the tip was seen to be in the pulmonary artery. Correct placement was confirmed by the detection of a pulmonary arterial waveform. It was then secured in place using a plastic plate along with the other catheters.

### 9.2.2 STUDY DESIGN

Studies were commenced at least 1 week after surgery to allow wound healing. For each study, sheep breathing room air remained in their metabolic crates with their weight partially supported by a sling. Cerebral blood flow and mean arterial pressure were measured in each study as described in chapters 2 and 4. In addition, cardiac output was measured intermittently using a thermodilution technique by rapidly injecting 10 ml of 0.9% saline at 0 $^{o}$C into one of the right atrial catheters. The time-course of temperature change measured in the pulmonary artery was recorded, and the cardiac output calculated, using a cardiac output computer (Abbott Critical Care Systems, Model 33). The cardiac output at any given time point was taken as the mean of three consecutive readings.

#### 9.2.2.1 Control study

In each study, after the baseline values of CBF, CO and MAP were recorded and baseline arterial blood samples were taken, 100 mg of propofol was administered intravenously at a constant rate over 2 minutes using a syringe pump (Model 33, Harvard Apparatus Ltd, Kent, England). Mean arterial pressure and CBF were recorded continuously for 20 minutes, and CO was recorded at 2, 5, 10 and 20 minutes after commencement of propofol administration. Blood samples were taken at frequent intervals from the arterial and sagittal sinus catheters. One millilitre of blood from samples taken at each time point was placed in Eppendorf tubes to which heparin (25 I.U.) had been added and then frozen and stored below -5 $^{o}$C for later drug assay using the method described previously in chapter 4.

#### 9.2.2.2 Metaraminol study

In each study, after baseline values of CBF, CO and MAP were recorded and baseline arterial blood samples were taken, an infusion of metaraminol (Merck Sharp and Dohme, Granville, NSW, Australia) was commenced at a rate of 0.5 mg/min. After 3 minutes, the CO was again recorded and propofol immediately commenced using the same dose regimen as in the control study. Metaraminol was continued during administration of propofol and both drugs were ceased 2 minutes after commencement of propofol administration. Blood pressure and CBF were measured continuously for the duration of the study, and CO measurements made at 2, 5, 10 and 20 minutes following commencement of propofol administration.

Five different sheep were used, and each received both propofol alone and propofol plus metaraminol on separate occasions in random order.

### 9.2.3 DATA HANDLING AND STATISTICAL ANALYSIS

In general, data on CBF, MAP and blood concentrations were handled and analysed as in the studies in chapter 5. Data at each time point for all animals in each group were pooled and expressed as mean and standard error of the mean (sem). Brain concentrations were calculated using mass balance principles. Changes in parameters over time were analysed using repeated measures analysis of variance. To examine the effect of co-administration of metaraminol on propofol concentrations, the peak propofol concentrations and the AUC of the concentration-time curves (calculated using the trapezoid rule) for arterial blood and the brain from the 2 groups were compared using a paired t-test.

To examine the effect of the change in arterial concentrations on brain concentrations, the brain concentrations in the metaraminol group were normalised by the ratio of the peak arterial concentrations in the control and metaraminol groups - a similar analysis was performed in chapter 5. To examine the effect of initial change in CO on brain concentrations, the concentrations in the metaraminol group were normalised by the ratio of the CO immediately prior to commencement of administration of propofol in the metaraminol and control groups.

## 9.3 RESULTS

### 9.3.1 CONTROL STUDY

In the control study, similar results were achieved to those following administration of the same dose regimen in chapter 5, with a rapid increase and decrease in arterial concentrations and a slower uptake and elution to and from the brain (figures 9-1 and 9-2). Cardiac output was also measured in this group of animals and changed significantly over time ($p=0.04$), decreasing from a baseline of 7.3 ±

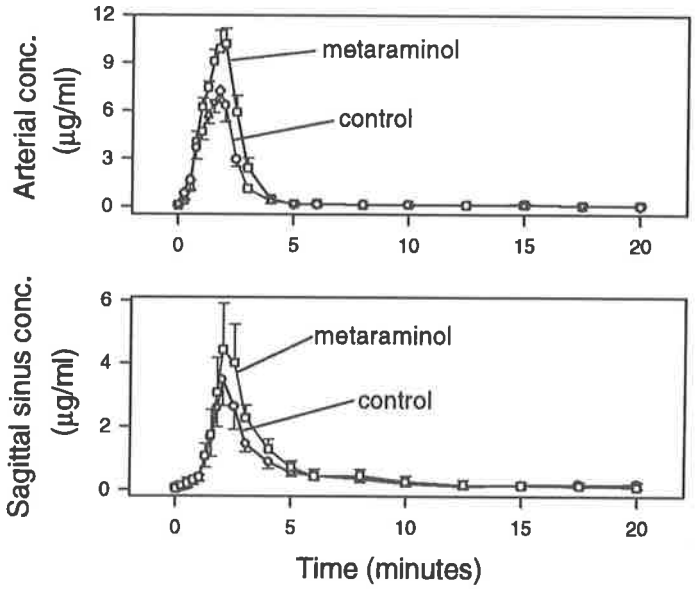


**Figure 9-1** The time-course of arterial and sagittal sinus concentrations following administration of 100 mg of propofol over 2 minutes in the control group (circles) and metaraminol group (squares). Data are presented as mean & sem.

0.8 L/min to 5.9 ± 0.4 L/min at 10 mins. There were minimal changes during the period of propofol administration, however (figure 9-3).

### 9.3.2 METARAMINOL STUDY

Administration of metaraminol prior to administration of propofol produced no change in CBF ($p=0.17$), but there was a significant change in MAP, increasing from 102 to 130 mmHg ($p<0.0001$). Cardiac output was initially similar to the control group (8.2 ± 1.4 L/min vs 7.3 ± 0.8), but decreased by 31% to 5.0 L/min prior to administration of propofol (figure 9-3). Following commencement of propofol there was a large and statistically significant increase in CO ($p=0.002$), which reached a level exceeding the baseline value at 2 minutes. There was then a slow decrease over time, but CO remained above the baseline value at the time of completion of measurements (figure 9-3).

#### 9.3.2.1 Effect of metaraminol on propofol concentrations

An initial reduction in the cardiac output produced an increase in arterial, sagittal sinus and brain concentrations (figures 9-1 & 9-2). A statistical comparison of the peak arterial and brain concentrations using a paired t-test, however, revealed no significant difference between the 2 groups ($p=0.17$ and $p=0.075$ respectively, figure 9-4). A comparison of the AUC of brain concentrations vs time did reveal a significant difference between the groups for the period of the first 5 minutes ($p=0.03$), but not for the period of the first 10 minutes ($p=0.072$) or the first 20

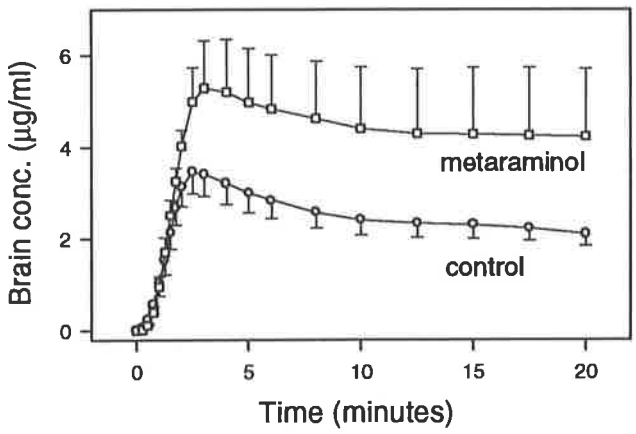


**Figure 9-2** The time-course of brain concentrations following administration of 100 mg of propofol over 2 minutes in the control group (circles) and metaraminol group (squares). Data are presented as mean & sem.

minutes (p=0.14) (figure 9-5). A comparison of the AUC of arterial concentrations vs time revealed a significant difference between the groups for the period of the first 10 minutes (p= 0.014), but not for the period of the first 20 minutes (p=0.08) (figure 9-6).

When propofol concentrations in the metaraminol group were normalised by the ratio of peak arterial concentrations, the magnitude and time-course of concentrations were very similar in both groups (figure 9-7). A similar finding was revealed when propofol concentrations in the metaraminol group were normalised by the ratio of the CO values prior to commencement of propofol administration (figure 9-8).

## 9.4 DISCUSSION

The technique of drug redirection by decreasing CO was selected because of the decrease in CO and lack of change in CBF following metaraminol administration revealed in pilot studies and the studies in chapter 3. The use of direct myocardial depressant drugs was also considered when designing these studies. Beta blockers were rejected, despite their absence of effects on CBF (Bunegin et al., 1987), because of the potential risk of hypotension when administered with propofol. These studies could also have been performed under anaesthesia with halothane, as deep halothane anaesthesia produces myocardial depression. Volatile anaesthetic agents may increase CBF, however, particularly at the higher concentrations needed to anaesthetise sheep, and this would defeat the purpose of co-administration.

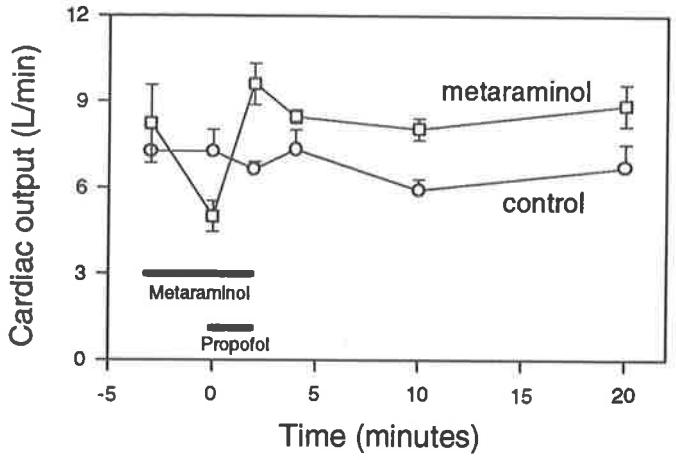


**Figure 9-3** Changes in cardiac output following administration of 100 mg of propofol over 2 minutes in the control group (circles) and metaraminol group (squares). In the metaraminol group, metaraminol was administered from -3 to 2 minutes. Data are presented as mean & sem.

The use of metaraminol avoided hypotension (and in fact induced hypertension), did not alter CBF, and produced a 30% initial reduction in CO. The delayed large CO increase after propofol administration was unexpected, however, but was poorly described because of the infrequent CO measurements and methodological problems with thermodilution techniques when CO is changing very rapidly. The mechanism behind this is unclear. Metaraminol, an alpha receptor agonist, produces an increase in SVR and a reflex decrease in heart rate and eventually CO (Saunders, 1991), and was chosen for these studies on this basis. The cardiovascular effects of propofol were discussed in chapter 1, and an interaction between these two agents causing an increase in CO is not obvious. A direct effect on the heart is unlikely as propofol has a mild negative inotropic effect (Azuma et al., 1993; Azari and Cork, 1993), and metaraminol has minimal beta receptor activity. A reflex response to a decrease in SVR is also unlikely because of the sustained increase in MAP. In addition, *in vitro* studies of propofol have demonstrated no effect of propofol on the aortic smooth muscle response to phenylephrine, another alpha agonist (Mimaroglu et al., 1994). This phenomenon has not previously been reported in the literature and warrants further investigation using more extensive cardiovascular monitoring.

Conventional compartmental pharmacokinetics applied to propofol usually assumes initial mixing of a drug after rapid administration to be instantaneous; thus the initial concentrations in the blood relate to the volume of the central compartment (Kanto and Gepts, 1989). It was, however, CO and not compartment volumes which was considered critical to this process in the alternate proposal

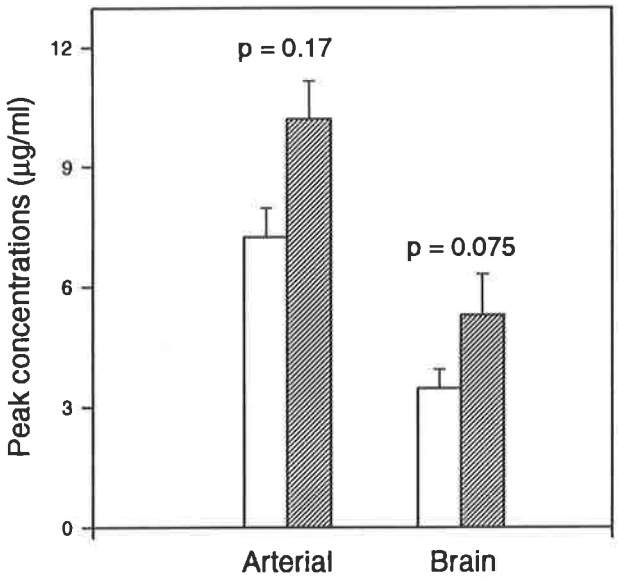


**Figure 9-4** A comparison of the peak arterial and brain concentrations achieved after administration of 100 mg of propofol over 2 minutes in the control group (unshaded) and metaraminol group (shaded). Data are presented as mean & sem.

mentioned earlier (Crawford, 1966). The effect of CO on early distribution of substances in the blood is, in fact, well recognised. In clinical medicine the "gold standard" method of CO measurement relies upon the changing time-course of arterial concentrations of a dye such as indocyanine green (ICG), or changing temperature with changing CO (Runciman et al., 1981), the method used in the current studies for CO measurement. The application of this principle to pharmacokinetics has been less commonly made. This was specifically examined in a study which looked at the relationship between CO and peak pulmonary arterial concentrations of ICG (Upton and Huang, 1993), finding an inverse relationship between the two and concluding that this may be a major factor in consideration of dosage when administering drugs rapidly.

This concept has more recently been extended. Specific examination of the process of "first pass" of drugs and the vascular mixing process has been undertaken using models based on the time-course of arterial concentrations of substances such as indocyanine green (ICG) (Krejcie et al., 1996). It is evident that these models can provide a more accurate insight into this phase of drug administration. For example, the use of a model for thiopentone developed using examination of the early distribution of both thiopentone and ICG revealed that the decreasing dose requirements for thiopentone with increasing age are in fact not related to a change in initial volume of distribution (Avram et al, 1990). Cardiac output changes with age may provide an alternative explanation. Similar analysis

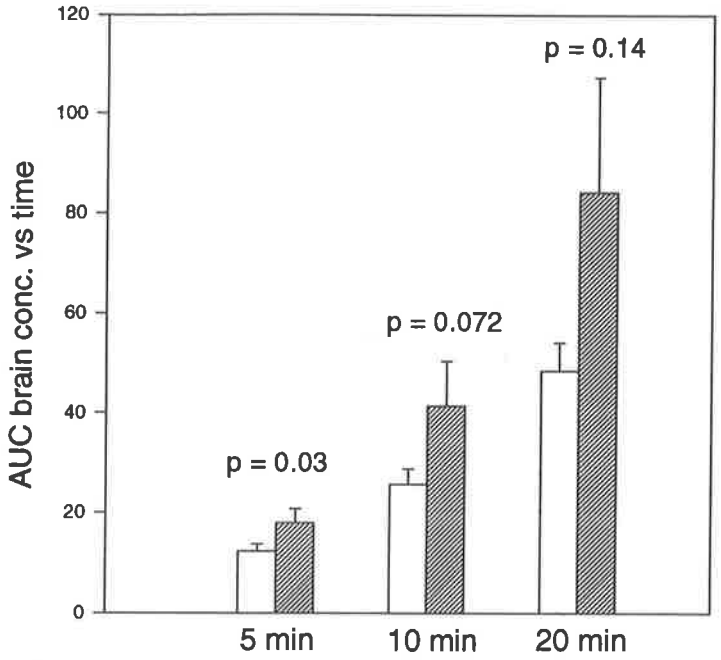


**Figure 9-5** A comparison of the area under the curve (AUC) of the brain concentration vs time curves following administration of 100 mg of propofol over 2 minutes in the control group (unshaded) and metaraminol group (shaded). The AUC is calculated for the time periods of 5, 10 and 20 minutes from commencement of administration of propofol. Data are presented as mean & sem.

for alfentanil found that distribution of alfentanil into tissues (intercompartmental clearances) was largely determined by CO (Henthorn et al, 1992).

The data presented in this chapter support the concept that the pharmacokinetics of propofol are extensively influenced by CO during rapid intravenous administration.

Firstly, the initial 30% decrease in CO induced by metaraminol resulted in an increase in arterial concentrations of approximately 40%, consistent with the effect of CO on indicators used to measure CO in the clinical setting. Furthermore, when the arterial concentrations in the metaraminol group were normalised for CO, the arterial curves may, essentially, be superimposed (figure 9-7). This is consistent with the previous findings that pulmonary concentrations of ICG were inversely proportional to CO (Upton and Huang, 1993). Secondly, it is also evident that the metaraminol induced changes in brain concentrations were secondary to these CO induced changes in arterial concentrations. Normalisation for both CO and peak arterial concentrations produced almost identical time-courses of propofol uptake into the brain, as can be seen by the superimposed brain concentration curves in figures 9-7 and 9-8.

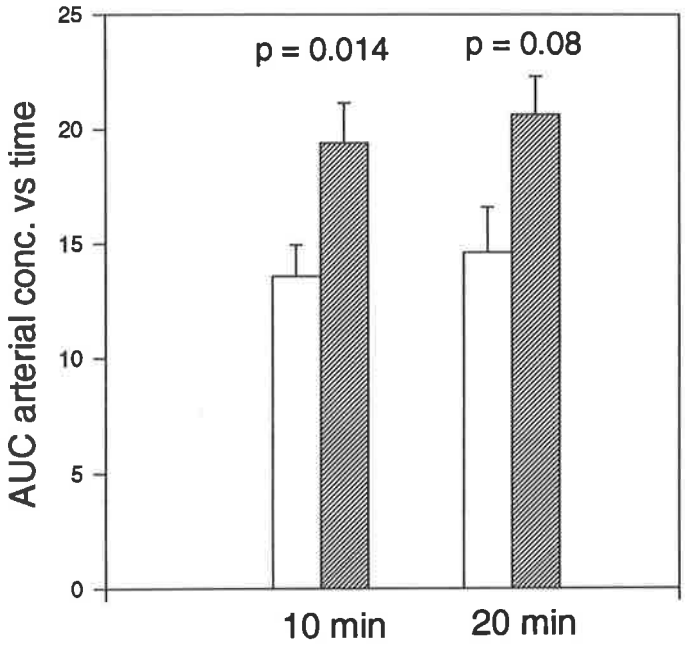


**Figure 9-6** A comparison of the area under the curve (AUC) of the arterial concentration vs time curves following administration of 100 mg of propofol over 2 minutes in the control group and metaraminol group. The AUC is calculated for the time periods of 10 and 20 minutes from commencement of administration of propofol. Data are presented as mean & sem.

It is interesting to also examine the elution phase from the brain. During this phase, CO increased rapidly in the metaraminol study until it exceeded CO in the control study, thus reversing the redistribution of blood flow originally created. This may explain the divergence, during elution, of the curves of normalised brain concentrations revealed in figures 9-7 and 9-8. In addition, this may explain the fact that brain concentrations in the two studies were statistically significantly different only during the uptake phase (equivalent to induction of anaesthesia), although the increased variance in concentrations over time apparent in all studies in this thesis is also a likely contributing factor.

Some conventional compartmental pharmacokinetic theorists have argued that the explanation behind the findings presented in this chapter is metaraminol induced systemic vasoconstriction, thus decreasing the volume of the blood (and presumably the central compartment) and increasing blood concentrations. The concept of considering the initial passage of a drug as a dynamic phenomenon relating to the mixing of drug with a flowing stream of blood doesn't support this argument. While metaraminol has an effect on the peripheral vasculature, its effects on CO are likely to be large compared to any effects on the volume of arterial blood in the vascular path between the injection point (right atrium) and the brain. This vasoconstrictive effect, however, potentially might influence systemic and brain propofol concentrations later in time. This is not apparent for the arterial

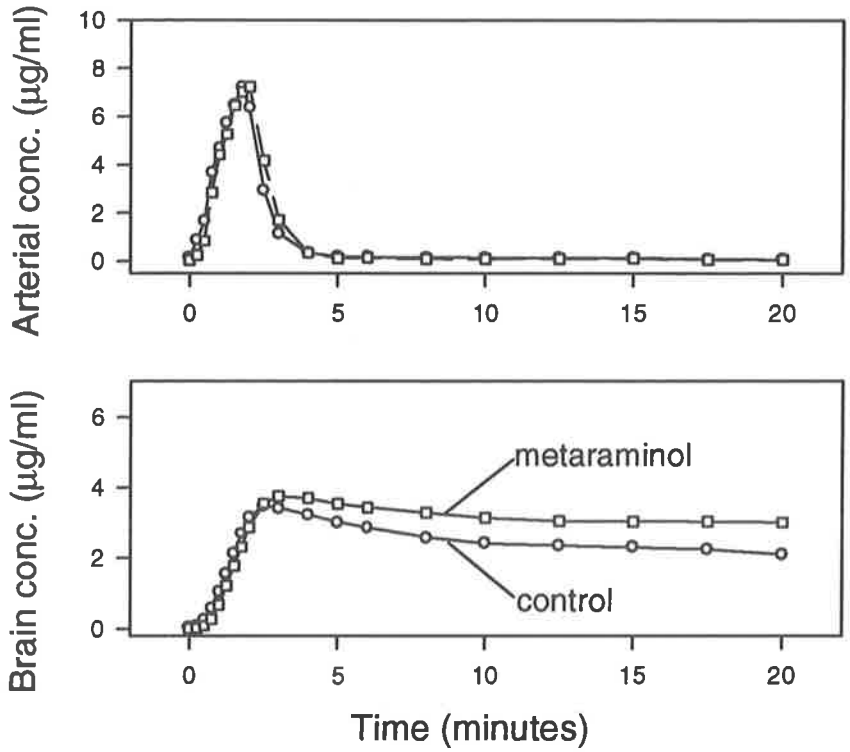


**Figure 9-7** Arterial and brain concentrations normalised for peak arterial concentration following administration of 100 mg of propofol over 2 minutes in the control group (circles) and metaraminol group (squares). Data are presented as mean only.

concentrations in figure 9-7, however, because minimal recirculation occurs and concentrations approach the limits of detection after 5 minutes.

The effects of changes in CO on propofol distribution have not previously been directly measured, but there is indirect evidence of the influence of CO on both dose requirements and effect of propofol at induction. Age is well known to affect dose requirements of propofol at induction, with recommended doses per body mass increasing in children (Smith et al., 1994) and decreasing in the elderly (Dundee et al., 1986; Robinson et al., 1985). The conventional compartmental pharmacokinetic explanation is that of changing central volume of distribution (Saint-Maurice et al., 1989), a volume which is theoretical and does not correspond to any anatomical compartment. An alternate explanation based on the data presented in this chapter might be that these dose changes simply reflect differing levels of CO with age. Young children have a high relative CO (Brown and Kisk, 1992), and a decrease in CO with age after the age of 30-40 years is well described (Ganong, 1995). The fact that their blood volumes also differ is largely irrelevant for the induction process as has already been discussed. To logically extend this concept, it could be suggested that doses of propofol at induction be determined

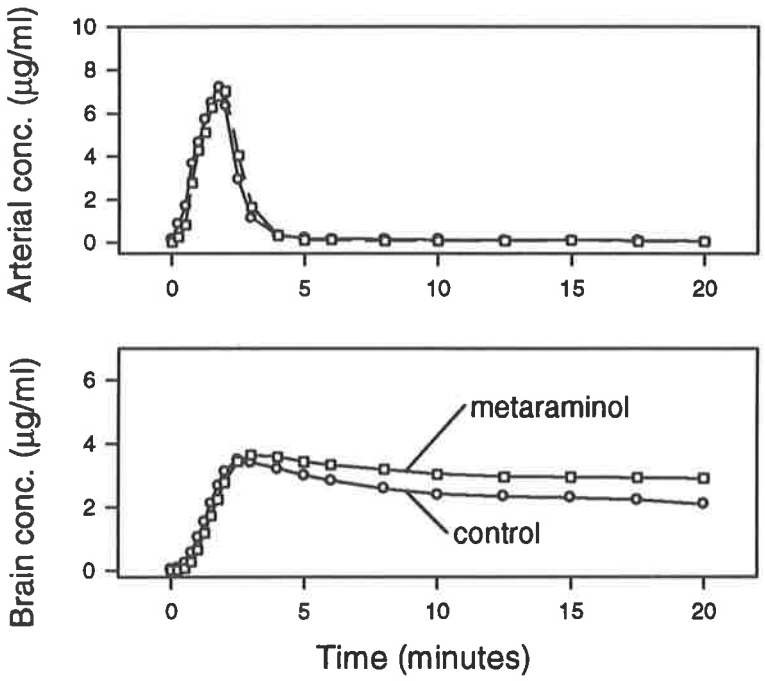


**Figure 9-8** Arterial and brain concentrations normalised for CO (immediately prior to propofol administration) following administration of 100 mg of propofol over 2 minutes in the control group (circles) and metaraminol group (squares). Data are presented as mean only.

based on estimations of CO rather than body mass. While dosing based on body mass produces acceptable results, it may be that this is simply a means of estimating CO. Indeed, as CO is usually most closely related to body surface area (Ganong, 1995) it may be more appropriate to consider the induction dose of propofol in terms of estimated CO based on calculated surface area.

Use of such a basis for the choice of induction dose is likely to be most valuable in circumstances where body mass and CO correlate most poorly. The example of young children has already been mentioned, and induction doses for obese patients are another example. Traditionally an estimation of lean body mass rather than total mass is made when calculating induction doses in such patients. Rather than removing the small influence of poorly perfused tissues, this may be a way of more accurately estimating true CO.

Anxiety is not uncommon immediately prior to induction of anaesthesia in the unpremedicated patient, and these states have been associated with increases in CO of up to 100% (Ganong, 1995; Gaffney et al., 1988). It is obvious this will have a major influence on the induction dose with propofol. While it is possible to compensate for such high CO states by increasing the administered dose, this may produce large increases in arterial concentrations. An alternate approach might be

to prevent this CO increase, or to return it towards normal. The use of premedication may therefore not just reduce dose requirements at induction by additive effects of drugs in the brain, but also by a pharmacokinetic mechanism.

It is also possible that a similar mechanism underlies some of the potential dose-sparing effects of co-administration of drugs such as midazolam and fentanyl (Short and Chui, 1991; Ben Shlomo et al., 1990; Tzabar et al., 1996). Many of these types of studies have necessarily been performed in unpremedicated young subjects, a group likely to have significant anxiety related increases in CO immediately prior to induction. The efficacy of co-induction may therefore relate to an anxiolytic effect and a normalisation of a raised CO immediately prior to induction as much as synergism at receptor sites in the brain. This mechanism more readily explains synergism between midazolam and fentanyl (Ben Shlomo et al., 1990), drugs whose receptor sites in the brain are mechanistically quite separate. Indeed, drugs with no central nervous system activity may act as co-induction agents. Esmolol, a beta blocker, has been found to provide a large dose-sparing effect on propofol requirement during induction of anaesthesia, a phenomenon consistent with a CO lowering effect (Johansen et al., 1995). While no explanation could be offered for these findings, it is likely that this study was similar to the one presented in the current chapter. A similar finding was revealed in a recent study when dexmedetomidine, an alpha-2 agonist which decreases sympathetic outflow, was co-administered with thiopentone and produced both a reduction in dose requirements at induction and a change in distribution of thiopentone (Buhrer et al., 1994). While dexmedetomidine has some sedative action, the authors discussed the impact of such co-administration on CO and thiopentone distribution.

Conventional pharmacokinetic analysis cannot account for this effect, and examination of blood concentrations alone may not reveal any evidence of propofol redistribution. While there were initial suggestions of increased blood concentrations of propofol after pre-treatment of unpremedicated patients with fentanyl (Cockshott et al., 1987), later studies found no effect (Gill et al., 1990). In this later study, the use of venous rather than arterial sampling, and blood sampling commencing at 2 minutes following rapid administration of propofol, probably did not provide sufficient sensitivity for detecting changes in blood concentrations during the initial mixing phase in the blood as a result of CO changes. It has been suggested that co-administration of midazolam and propofol does not significantly affect venous blood levels of either agent (Teh et al., 1994), but in this study these drugs were administered during maintenance of anaesthesia, removing the potential anxiolytic effect on CO during the induction process. Thus, these findings cannot be used to argue for or against an effect of co-induction on pharmacokinetics during induction. The implications of these observations for co-

induction warrants further study. If effects on CO are shown to be important then, while it may have a major dose-sparing role to play during induction of patients with high CO, its advantages may be minimal when CO is normal or low.

In conclusion, the current study has demonstrated that CO is an important determinant of distribution of propofol to the brain, and that co-administration of a drug which reduces CO will produce a dose-sparing effect. It is therefore important to consider the estimated CO when administering propofol rapidly, and strategies to reduce CO towards normal may be useful to minimise dose requirements at induction of anaesthesia.

# CHAPTER 10. GENERAL DISCUSSION, CONCLUSIONS AND FUTURE DIRECTIONS

Drugs are usually administered systemically with the intention of delivering a sufficient quantity to the therapeutic site of action, or biophase, in order to produce the desired therapeutic response. However, measurement of distribution to and from the biophase is not usually possible. Thus, the pharmacokineticist is forced to infer the rate of delivery to and from the biophase from the drug concentrations in the systemic blood. The conventional compartmental approach to this generally allows a mathematical description of the time-course of drug concentrations in blood and, under most circumstances, this approach is quite satisfactory. Firstly, drugs are usually administered relatively slowly and for prolonged periods, thus minimising the disequilibrium between blood and the biophase and providing a reliable relationship between concentration in the blood and desired effect. Secondly, most drugs have relatively high therapeutic indices, and so errors in estimation of the dose delivered to the biophase are well tolerated.

Outside these circumstances, a systemic approach to pharmacology becomes inadequate. This is certainly the case for the induction of anaesthesia, as rapid injection rates (e.g. over less than 60 seconds) are used with sedative/hypnotic drugs with low therapeutic indices. The perceived need for this speed of injection lies in the risks of respiratory impairment and aspiration of gastric contents during the period of "light anaesthesia", in the desire for rapid achievement of specific therapeutic effects on the brain such as a decrease in CMR, CBF or anticonvulsant activity, and in the pressures of time in the workplace. While this need for haste might be questioned in many circumstances of elective surgery, rapid induction **is** necessary in a number of clinical settings, and so rapid drug administration is inevitable. This may contribute to the recognised incidence of adverse events at induction outlined in chapter 1.

Sodium thiopentone has been the most popular intravenous induction agent for the last 50 years, and its pharmacokinetics have most commonly been described using the compartmental approach based on blood concentrations, as discussed above. In clinical practice, anaesthetists have come to understand its effects at induction by trial and error, with little useful contribution to understanding being made by predictions of compartmental pharmacokinetic methods.

Following the introduction of propofol in the late 1970's, it initially appeared similar to thiopentone, but with a shorter duration of sedative/hypnotic effect. Its systemic compartmental pharmacokinetic parameters were quite similar to those of thiopentone, a similar volume was required to be injected to achieve induction, and its central nervous system effects appeared little different. Slowly it became

evident, however, that there were significant differences between these drugs. Firstly, the incidence of hypotension at induction was greater than with thiopentone when administered by conventional intravenous bolus, although the exact mechanism still remains unclear. Secondly, its effects on the brain were different to those of thiopentone, with questions about its effect on coupling of CBF and CMR and more potent anticonvulsant effects during electroconvulsant therapy. Thirdly, it became evident that slow administration was not associated with the same problems that can occur with this rate of administration of thiopentone, and that this slow administration appeared to reduce the risk of hypotension. Fourthly, a number of phenomena were observed with altered conditions of administration at induction, such as dose-sparing with either slower rates of administration or co-administration with small doses of other sedative/hypnotic agents.

As these differences have become apparent, there has been a great deal of speculation in the literature about the cerebral pharmacokinetics and pharmacodynamics of propofol. Conventional pharmacokinetic theory cannot readily explain many of these phenomena, however, and study of the pharmacodynamics has frequently been limited by available methodology. Regional pharmacokinetic techniques allow direct measurement of the distribution of drugs from the blood to specific organs, thus potentially providing answers to the issues raised in relation to propofol pharmacokinetics at induction. In addition, measurements of variables such as CBF and oxygen extraction across the brain can provide insight into the effects of propofol on the brain. The invasive nature of these techniques have largely precluded their use in man, but a chronically instrumented sheep preparation has previously been used in regional pharmacokinetic and pharmacodynamic studies in organs such as the heart and liver. It was therefore adapted to allow studies of the cerebral pharmacokinetics and pharmacodynamics of propofol. The development of this preparation and the findings of the subsequent studies are summarised below.

## 10.1 GENERAL DISCUSSION OF EXPERIMENTAL STUDIES

### 10.1.1 Chapter 3. A method for the frequent measurement of the antinociceptive effects of drugs

Measurement of depth of anaesthesia was important to the studies in this thesis, but is a parameter which has traditionally been difficult to quantify, with most techniques having significant limitations. The adaptation of an algesimetry paradigm, using a pulsed, ramped electrical current as a noxious stimulus and leg lift as a response, proved successful. The studies in this chapter demonstrated stable baseline measurements, and that changes in electrical current threshold following administration of anaesthetic agents were measurably different from

baseline and were dose dependent. Therefore, an index of depth of "anaesthesia" was successfully developed for use in sheep.

### 10.1.2 Chapter 4. Development of a cerebral blood flow method for studies of cerebral pharmacokinetics and pharmacodynamics in unrestrained sheep.

It was important to measure CBF in the studies in this thesis for three reasons. Firstly, some doubt existed about the effects of propofol on CBF, particularly during the induction phase of anaesthesia. Secondly, it was thought that propofol induced changes in CBF might alter propofol distribution to the brain, as has been demonstrated for other anaesthetic agents (Bjorkman et al., 1992). Thirdly, measurements of CBF were necessary for calculation of propofol brain concentrations. Existing methods, however, were inadequate for the proposed studies, and so a new method using a Doppler flow probe implanted on the sagittal sinus was developed.

Angiography and dye studies confirmed the vascular anatomy of the sheep, and good correlation between measured Doppler shift and flow was achieved. Later studies examining the behaviour of flow in response to perturbations such as changes in blood pressure and carbon dioxide tension found measured flow to respond in a manner similar to that previously reported for CBF. It was apparent from this work that this was a successful method for accurately and continuously measuring CBF, and suitable was for the requirements of the studies planned in this thesis.

### 10.1.3 Chapter 5. The relationship between brain and blood concentrations of propofol, and cerebral effects after rapid intravenous injection in sheep.

The relationship between the time-course of concentrations in blood and brain, and its effects on the brain were examined after rapid administration. The dose dependent effects on depth of anaesthesia, CBF and cerebral metabolism found in these studies, and the close matching of changes in CBF and CMR, support the use of propofol in neuroanaesthesia. It was revealed that systemic concentrations were markedly affected by non-linear lung uptake, particularly at low doses. There was also a marked delay between changes in arterial and brain concentrations of propofol, with cerebral effects correlating well with brain, but not blood concentrations. This confirmed the hypothesis that brain concentrations of propofol determined cerebral effects, but also suggested that conventional compartmental pharmacokinetic analysis based on concentrations of propofol in the blood would poorly predict the time-course of cerebral effects, including depth of anaesthesia, at induction of anaesthesia. The return of depth of anaesthesia to baseline before efflux of propofol from the brain was complete raised the question of acute changes

in drug-receptor interactions (or "acute tolerance"). This was considered further in chapter 6.

### 10.1.4 Chapter 6. Prolonged administration of propofol: concentrations and effect

In addition to the information provided by the studies in the preceding chapter, study of propofol pharmacokinetics and pharmacodynamics during slower administration allowed the issues of acute tolerance, validity of mass balance techniques, and CBF effects to be further examined. These studies revealed that the effects of propofol on CBF, once correction for drug induced hypercarbia was performed, were similar in magnitude to those previously described for thiopentone. The constant relationship over time between brain concentrations and depth of anaesthesia clearly demonstrated that acute tolerance does not occur during the time frame of induction of anaesthesia, and that previous suggestions of this phenomenon were a result of the disequilibrium between blood and brain concentrations and the inadequacies of compartmental pharmacokinetic methods during rapid drug administration. Lastly, the almost complete recovery from the sagittal sinus of drug entering the brain confirmed the validity of the mass balance techniques used in this thesis to calculate brain drug concentrations.

### 10.1.5 Chapter 7. The effect of rate of administration on brain concentrations of propofol

These studies were specifically designed to examine the mechanism behind the phenomenon of apparent dose-sparing observed with slower rates of administration of propofol at induction. This phenomenon had raised questions about the determinants of the brain uptake of propofol, but the current level of pharmacokinetic knowledge was unable to provide answers. It was evident from the studies presented in this chapter that administration rate had minimal effect on the quantity of propofol distributed to the brain, although faster rates did slightly increase the rate of uptake. Arterial concentrations, however, were markedly affected by rate, with the fastest rates increasing arterial concentrations 6-fold. Although the increased arterial concentrations did not have a significant effect on blood pressure, data from administration of propofol to those with cardiovascular impairment would suggest that arterial concentrations should be minimised in these subjects. The theoretical analysis of titration to effect performed on the data from these studies also revealed that dose-sparing is an illusion related to the use of a single anaesthetic endpoint. Thus, there appears to be an optimal administration rate of propofol. Excessively fast administration only increases concentrations in the blood and the risk of hypotension, while excessively slow administration unduly prolongs induction of anaesthesia.

### 10.1.6 Chapter 8. A compartmental analysis of the pharmacokinetics of propofol in sheep

In this chapter, data from chapters 6 and 7 were analysed using the conventional compartmental and effect compartment pharmacokinetic techniques which have featured in the pharmacokinetic analysis of propofol in the published literature. This allowed a comparison of the drug concentrations predicted by this type of analysis and those directly measured. It was revealed that both the structure and parameter values of these models fitted to arterial and brain concentrations were dependent on the rate of administration. Therefore, drug concentrations predicted by the models were highly inaccurate unless the administration rate used matched that which was used to derive the model. In addition, factors such as non-linear lung extraction could lead to inaccurate predictions of models when dose, but not administration rate, was changed. These findings clearly demonstrated the marked limitations of the conventional compartmental pharmacokinetic analysis used with propofol to date for the induction phase of anaesthesia.

### 10.1.7 Chapter 9. The effects of reduced cardiac output on brain uptake of propofol

Published studies demonstrating dose-sparing with "co-induction" of propofol and other anaesthetic agents have invoked synergism as the likely explanation for this phenomenon. However, a regional pharmacokinetic explanation related to altered drug distribution to the brain with changes in CO could also account for this phenomenon. This was examined using the sheep preparation by altering the distribution of cardiac output with metaraminol. The resulting initial decrease in cardiac output (or relative increase in CBF) produced a redistribution such that the brain concentrations of propofol were significantly higher in the metaraminol treated group. This demonstration of effective "co-induction" using drugs which do not alter conscious state supported the concept that this phenomenon may be related to pharmacokinetics and not drug-receptor interaction. This is further supported by the effectiveness of co-induction with beta-blockers in man (Johansen et al., 1995). The implications of these findings are that co-induction may be an effective tool for minimising induction doses in high cardiac output states, such as with anxious unpremedicated patients, but may be of minimal benefit, or even be potentially harmful, when used in low CO states.

## 10.2 IMPLICATIONS OF EXPERIMENTAL STUDIES, AND FUTURE DIRECTIONS

The findings in this thesis of poor performance of compartmental pharmacokinetic methodology when describing rapid propofol administration is not surprising. Despite the prevalence of the application of this approach to circumstances such as induction, there is an increasing awareness of its limitations, and of the need for alternatives. Indeed, this was the subject of a recent editorial in one the major

anaesthesia journals (Fisher, 1996). An effective alternative is the use of physiological models, which simulate "real life" and can account for many of the variables involved in drug distribution, such as non-linear lung extraction and the influence of CBF and cardiac output. Development of such a model is a logical extension of the studies in this thesis, and has already been commenced. Further data must be acquired, however, before thoroughly "grounded" models can be developed. For example, the issue of flow or diffusion limitation of cerebral uptake and elution of propofol must be resolved.

In addition, it is important to confirm the findings of this thesis in man. Although the basic physiological and pharmacological processes behind many of the findings presented in this thesis are likely to be common to other vertebrate biological systems, there may be minor differences between species. For example, significant lung extraction may not exist in man (there are currently insufficient data to determine this), and properly conducted studies examining extraction across the lung together with pulmonary blood flow need to be performed. Newer technologies such as continuous thermodilution pulmonary artery catheters now make these studies possible. While the invasive nature of some of the other techniques performed in sheep prevent their application to man, transcranial Doppler technology (for continuous measurement of CBF), and catheters for placement in the jugular bulb (to access cerebral venous blood) are now available, and it is proposed to use these to examine the cerebral pharmacokinetics of propofol and other drugs in man.

Some relatively simple studies may also provide supporting data for the findings of this thesis. Co-induction is one such example. Small doses of propofol tend to be anxiolytic, in a similar manner to small doses of midazolam. A study using co-induction of propofol using small doses of propofol as the co-administered agent might therefore be valuable, as synergism at a receptor level would then be excluded. A finding of effective dose-sparing using this technique would strongly support a pharmacokinetic (anxiolysis producing a lower cardiac output) effect of co-induction rather than the receptor synergism which appears currently to be generally accepted.

The findings in this thesis also strongly suggest that this type of regional pharmacokinetic approach should be considered during the development phase of any new drug which is intended for rapid administration. Some of the answers to problems or questions related to propofol administration are now only becoming available nearly twenty years after its release into clinical practice, and one can only speculate whether some of the recognised morbidity associated with its use might have been prevented if this knowledge had been available earlier. This pattern of a slow increase in awareness of the relevant pharmacokinetics and optimal drug

administration appearing only after commercial release seems to be very similar to that evident with the release of midazolam.

Dissemination of the information on propofol distribution and the principles of dose regimens gained from the types of studies in this thesis is also critical. Despite the appearance of editorials such as the one cited previously (Fisher, 1996), alternate approaches to pharmacokinetics and dose strategies are still not common, and pharmacokinetic teaching of anaesthetists remains almost exclusively restricted to conventional compartment models. A recent survey of anaesthetists in the State of South Australia conducted by this Department, for example, found that most anaesthetists had little knowledge of pharmacokinetics beyond the conventional compartmental perspective taught to them in training (Chong, 1995). Furthermore, most anaesthetists recognised that conventional pharmacokinetic compartment theory was not adequate for use in decision making about dose regimens at induction, and they relied more commonly on clinical experience. While that experience is undoubtedly useful, it probably reflects an informal understanding of some of the principles of pharmacokinetics revealed by approaches such as regional pharmacokinetic studies or physiological modelling. An example might be the reduction in induction dose in patients with severe cardiovascular disease which is commonly used by experienced anaesthetists (unlike in 1945 (Bennetts, 1995)), and often overlooked by those training in anaesthesia. As the data in this thesis show, the explanation for this probably lies in cardiac output dependent pharmacokinetics and a low cardiac output state in these patients, but without an understanding of the mechanisms behind these phenomena it is difficult to pass this knowledge on to those in training. An active program of teaching in this area is necessary, and teaching aids, such as computer simulators based on the types of data gained from the studies in this thesis, are currently being developed.

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