Total Intravenous Anaesthesia in dogs: Development of a Target Controlled Infusion (TCI) scheme for propofol

A thesis presented for the degree of Doctor of Philosophy

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Summary

The aim of this work was to develop a propofol (PPF) based Total Intravenous Anaesthesia (TIVA) technique to be used in dogs by veterinary surgeons in practice. As PPF is a poor analgesic agent, this work also looked into the development of a co-infusion scheme for the potent alpha$_2$-adrenoceptor agonist medetomidine (MED) and its active enantiomer dexmedetomidine (DEX). The study was divided into 4 phases.

In phase one, canine PPF pharmacokinetic (PK) parameters, derived from previous studies reported in the literature, were incorporated to a Target Controlled Infusion (TCI) system. This approach, comprising computer software incorporated into a syringe driver, which will deliver PPF to a predicted blood concentration, is widely used in humans. The performance of the system was investigated in 6 greyhounds and 10 mixed-breed dogs undergoing routine dental work by comparing predicted with measured PPF concentrations in venous blood samples obtained during and following TCI PPF anaesthesia. The optimal induction target was 3 μgml^{-1}, and an adequate depth and a satisfactory quality of anaesthesia were achieved with mean maintenance targets of between 3.4 and 4.5 μgml^{-1} of PPF. The performance of the TCI system was considered clinically acceptable as the Median Prediction Error (MDPE%), a measure of bias, and the Median Absolute Performance Error (MADPE%), a measure of the accuracy, were -12.47 and 28.47 respectively, in the greyhounds and 1.56 and 24.79 respectively, in the mixed-breed dogs. The system was easy to use and the quality of anaesthesia was judged to be adequate for dental work.

Phase 2 illustrated the inhibitory effect of MED and DEX on PPF metabolism at the level of the cytochrome P450 in rat and canine hepatic tissue and highlighted, therefore, the possible effect on the metabolism dependant performance of the TCI system.

Before designing an infusion scheme for MED and studying its possible effect on PPF PK *in vivo* (phase 4), the purpose of phase 3 was to characterise cardiovascular and respiratory effects of MED administered IV to dogs anaesthetised with a TCI of PPF, and to assess its suitability for use in a TIVA regime. Eighty dogs, ASA 1 or 2, aged 0.5 to 8 years, were randomly allocated into 8 groups of 10 dogs according to the dose of MED administered (Groups 1-8: 0 (saline, 0.9%, 1 ml), 0.01, 0.03, 0.1, 0.3, 1, 3, 10 μgkg^{-1} MED, respectively). Following premedication, anaesthesia was induced with a PPF target blood concentration of 3 μgml^{-1} and maintained with a target concentration of 3.5 μgml^{-1}. Cardiovascular and respiratory parameters were recorded for 15 min post induction and before saline (group 1) or MED (groups 2-8) was injected slowly over 1 min. Medetomidine induced a dose-dependent reduction in heart rate (HR) and increase in systolic arterial blood pressure (ABP). At the time of maximum observed effect (2 min post MED

injection), the ED_{50} for ABP and HR were 2.05 and 0.187 μgkg^{-1} respectively, while the ED_{95} (doses of MED inducing 95% of the maximum effect) values were estimated to be 18.1 μgkg^{-1} and 3.1 μgkg^{-1}, respectively. The no effect doses for MED were 0.01 μgkg^{-1} for HR, and 0.1 μgkg^{-1} for ABP. Minimal respiratory effects were observed in all groups except the group receiving 10 μgkg^{-1} of MED where, by the end of the recording period (20 min post MED injection), 8 of 9 spontaneously breathing dogs became apnoeic after MED administration.

Phase 4 was designed to develop and assess a stepped infusion scheme for MED and DEX in the TCI PPF anesthetised dog using MED PK parameters from O. Vainio (V1 = 470 $mlkg^{-1}$, K12 = 0.0954, K21 = 0.0438, K10 = 0.0489); to observe the possible PK and PD (pharmacodynamic) interactions between PPF and the 2 $alpha_2$-adrenoceptor agonists during co-infusion; to determine the minimum blood PPF infusion target (MIT) necessary to prevent purposeful movement during supramaximal noxious stimulation (tetanic twitch for 5 sec at the level of the 4^{th} and 5^{th} coccygeal vertebrae) with and without a co-infusion of MED or DEX and to confirm the DEX minimum analgesic blood concentration of 0.85 $ngml^{-1}$. Six female beagle dogs, 7.3 (± 2.3) years old, were anaesthetised on 4 occasions, following a randomised cross over design: PPF TCI with either co-stepped infusion of saline (PS), MED (blood target of 1.7 $ngml^{-1}$, PM), low DEX (blood target of 0.85 $ngml^{-1}$, PLD) or high DEX (blood target of 1.7 $ngml^{-1}$, PHD). The co-infusion was started 25 min after the start of anaesthesia (instrumentation period), while the MIT determination was conducted 15 min after the last step of the co-infusion. Venous blood samples were taken at specific times for determination of the PPF, MED and DEX plasma concentrations.

The performance of the TCI system for PPF in the dog was only clinically acceptable in the PS and PLD treatments with MDPE% values of 18.85 and 25.94 respectively, and MDAPE% values of 18.85 and 35.80 respectively. In this study the use of DEX 0.85 $ngml^{-1}$ had a similar PPF sparing effect to the equivalent MED blood concentration of 1.7 $ngml^{-1}$, but with less effects on ABP, as well as on the performance of the TCI for PPF in the dog. Therefore, it could be concluded that DEX was more advantageous than MED given by infusion in PPF anaesthetised dogs. The study also confirmed the validity of the PK of MED from the previous study. The study redefined specific PK parameters for DEX, although the MED PK parameters could also be used. The study indicated that DEX blood concentrations as low as 0.85 $ngml^{-1}$ decreased the measured PPF blood concentrations necessary to maintain anaesthesia during noxious stimulation by about 38%. However, although this study supported the suitability of the co-infusion of DEX during PPF anaesthesia in the dog, and the analgesic/sedative effects of DEX were present at the lowest blood concentrations with well maintained respiratory parameters, the CV effects were marked with a decrease in HR and CO and an increase in systolic and mean ABP. Further studies are therefore necessary to establish if a lower blood concentration of DEX will provide analgesia while preserving the CV system.

TABLE OF CONTENTS

LIST OF CHAPTERS

CHAPTER 5:

PHARMACODYNAMIC AND PHARMACOKINETIC PROPERTIES OF MEDETOMIDINE AND DEXMEDETOMIDINE INFUSIONS IN DOGS ANAESTHETISED WITH PROPOFOL ADMINISTERED BY TCI. 115

LIST OF TABLES

LIST OF FIGURES

DEDICATION

A mes parents, ma sœur, Sooze et Talis

DECLARATION

I, Thierry Beths, do hereby declare that the work carried out in this thesis is original, was carried out by myself or with due acknowledgement, and has not been presented for the award of a degree at any other University

The following paper was published:
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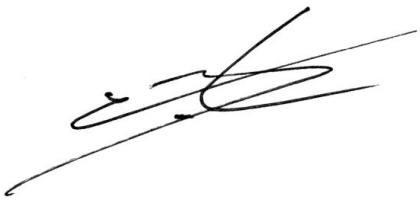
Signed: 

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ABBREVIATIONS

ABP	Arterial Blood Pressure
CO	Cardiac Output
CRI	Constant Rate Infusion
CV	Cardiovascular
CYP	Cytochrome P450
DEX	Dexmedetomidine
ECG	Electrocardiogram
ED	Effect Dose
HR	Heart Rate
IC	Inhibition Concentration
IM	Intra Muscular
IV	Intra Venous
IPPV	Intermittent Positive Pressure Ventilation
h	hour
LEV	Levomedetomidine
kg	kilogram
MED	Medetomidine
µg	microgram
µl	microlitre
µM	micromolar
mg	milligram
ml	millilitre
mM	micromolar
min	minute(s)
MIT	Minimum Infusion Target
ng	nanogram
PD	Pharmacodynamic
PK	Pharmacokinetic
PPF	Propofol
RR	Respiratory Rate
sec	second(s)
TCI	Target Controlled Infusion
TIVA	Total Intravenous Anaesthesia

CHAPTER 1:

GENERAL INTRODUCTION AND REVIEW OF THE LITERATURE

In the last decade, medical anaesthesia has seen the rapid growth of new techniques to provide total intravenous anaesthesia (TIVA) (Mirakhur and Morgan, 1998), resulting in the development of target controlled infusion (TCI) systems for anaesthesia with the hypnotic agent propofol (PPF) (Milne and Kenny, 1998a). In veterinary anaesthesia PPF is frequently used to induce anaesthesia in dogs, but its use as a maintenance agent is relatively uncommon, partly because of the lack of a 'user friendly' technique for infusion of the drug (Morgan, 1983).

The overall aim of this project was to develop and evaluate a technique for TIVA in dogs which would be suitable for use in veterinary practice. This involved the development of a system for the administration of the hypnotic, PPF, by TCI and the evaluation of the alpha$_2$-adrenoceptor agonists medetomidine (MED) and dexmedetomidine (DEX) as analgesic supplements.

To examine a possible source of pharmacokinetic (PK) interaction between these agents, isolated hepatic microsomal preparations were used to study the metabolism of PPF alone and in the presence of MED and DEX since these have been shown to have some potential inhibitory effect on the hepatic metabolism of other agents such as ketamine.

In view of the potential of some alpha$_2$-adrenoceptor agonists to produce significant haemodynamic effects, preliminary studies were undertaken to evaluate the dose relationship of these effects in order to inform the selection of suitable doses of alpha$_2$-adrenoceptor agonists for the subsequent clinical study.

Finally, a clinical study investigated the performance of infusions of MED and DEX in beagle dogs anaesthetised with PPF TCI. Meanwhile the effect of these infusions on the performance of the TCI system for PPF in the dog was assessed. In addition, the effects of these infusion schemes on cardiovascular (CV) variables were quantified in addition to their analgesic properties.

The first part of this introduction provides background information on TIVA and the development of infusion techniques as well as the concept, evaluation and use of TCI systems in humans and animals. This is followed by more detailed descriptions of PPF, MED and DEX, their pharmacokinetics, pharmacodynamics and possible PK/pharmacodynamic (PD) interactions.

1.1 Anaesthesia

The term anaesthesia is derived from the Greek *anaisthaesia,* meaning insensibility. Anaesthesia results in depression of the activity of nervous tissue locally, regionally, or within the central nervous system. General anaesthesia consists of loss of consciousness in addition to loss of

sensation. Ideally, general anaesthesia is made up of three components, namely unconsciousness, analgesia and muscle relaxation (Muir and Hubbel 1995). The anaesthetic state is induced by anaesthetic agents administered by injection or by inhalation.

1.2 The History of Intravenous Anaesthesia

Sir Christopher Wren performed the first recorded intravenous (IV) injection in a living animal at Oxford in 1656. He injected a variety of substances, including opium, into the circulation of dogs using a quill and a pig's bladder (Glass *et al*., 1991). Two centuries later, in 1853, following the development of the needle and syringe, Alexander Wood injected opiates (morphine) intravenously to patients (Glass *et al.*, 1991; Miller, 1994), but it was not until 21 years later, in 1874, that the first attempt to use IV agents to provide anaesthesia was recorded. Pierre-Cyprien Ore anaesthetised patients using chloral hydrate, but this technique was rapidly abandoned in humans because of low safety margin (Glass *et al.*, 1991). Interestingly, the use of chloral hydrate for anaesthesia of horses continued up to the late 1980s, more than one century later (Hall and Clarke, 1991a).

Meanwhile, W.F. Clarke and J.Y. Simpson were developing the use of the volatile anaesthetic agents diethyl ether (1842) and chloroform (1847) and these dominated the field for the first half of the twentieth century, being superseded by the fluorinated hydrocarbon halothane (1957), followed by methoxyflurane (1960) and then isoflurane in the 1970s (Steffey, 1995; Smith and White, 1998a).

The discovery of the barbiturates, such as thiopentone sodium (1934), heralded a new era in IV anaesthesia. However, when these drugs were used as sole agents in man, the high doses required for reflex suppression resulted in disastrous outcomes for patients, earning IV anaesthesia the reputation of being an "ideal method of euthanasia" (Halford, 1943). Although thiopentone was used as a sole agent in veterinary practice for rather longer than in humans, its small volume of distribution and long elimination half-life of several hours leads to accumulation within the body and renders it unsuitable for TIVA, for all but the shortest of surgical procedures (Morgan, 1983; Smith and White, 1998b) However, its rapid onset of action, of around 30 seconds, makes it a very useful drug to induce anaesthesia prior to maintenance with a volatile agent and it is still used for this purpose in veterinary anaesthesia (Muir *et al*., 2007) .

The concept of balanced anaesthesia was first developed in 1926 by Crile of Ohio who introduced the use of premedication before anaesthesia (Van Hemelrijck and Kissin, 1997). In 1952, Gray and Rees introduced a new concept which divided anaesthesia into three components, the so-called

'Triad of Anaesthesia', analgesia, muscle relaxation and hypnosis, each of which was achieved by use of a different drug. In 1957, Woodbridge added the abolition of autonomic reflexes to the triad, which became the tetrad of anaesthesia (Van Hemelrijck and Kissin, 1997). Although these new concepts helped in the development of IV anaesthesia, progress was still slow in this domain. Amongst the delaying factors were the lack of agents with adequate PK/PD profiles and the lack of delivery systems.

However, a major driving force pushing the development of TIVA was an awareness of the possible adverse effects to the health of theatre personnel resulting from chronic exposure to traces of gaseous or volatile anaesthetic agents (Morgan, 1983) and so, from the beginning of the 1970s onwards, rapid progress in new technologies and software led to a quick expansion of infusion techniques and tools. The syringe and an IV line gave way to the PK model-driven pump through gravity-driven devices and positive displacement pumps (volumetric) (Egan, 1996). In parallel, new agents were discovered with more appropriate PK/PD properties for IV anaesthesia, such as ketamine (1963), alphaxolone/alphadolone (1972), and etomidate (1972). However, none of these proved to be entirely satisfactory. Ketamine offered the advantage of being non-cumulative and analgesic, but its undesirable side-effects, in particular unpleasant dreams and hallucinations, precluded its wider use in adults, although it enjoyed limited popularity in children. Similarly, the steroid combination alphaxolone and alphadolone (Althesin; Saffan) does not have a cumulative effect when repeated doses are administered and recovery is rapid (Child *et al*., 1971), but it could produce severe anaphylactic reactions in some patients and this resulted in its withdrawal from the human market (Stock, 1973; Morgan, 1983; Sear, 1987; Hall and Clarke, 1991a). The imidazole derivative etomidate became the hypnotic drug of choice for critical cases in humans because of its haemodynamic stability, minimal respiratory depression and cerebral protective effects (Morgan, 1983; Aitkenhead, 1996) and in addition, its PK profile with a short elimination half-life and rapid clearance, made it ideal for administration by repeated doses and continuous infusion (Davis and Cook, 1986). However, it caused a significant and prolonged inhibition of the stress response following infusion (Davis and Cook, 1986; Aitkenhead, 1996). This precluded its use as a maintenance agent. Propofol was introduced into clinical practice in 1986 and has been described as being the most suitable drug currently available for TIVA (White, 1988). It has many of the properties of the ideal IV agent, namely rapid onset of action, short duration of clinical effect, high clearance, minimal tendency for accumulation, and minimal side-effects (Morgan, 1983; Miller, 1994).

The discovery of PPF, along with a greater understanding of its PK properties, the improvement of infusion methods and the progress in computer technology led to the introduction of modern IV infusion techniques, which became established as part of routine anaesthetic practice in man in the 1990s (Padfield, 2000a).

1.3 Techniques used to administer TIVA

Two techniques are used to maintain anaesthesia with an IV agent, namely multiple bolus injections or continuous infusion (Smith and White, 1998a). Administering the agent by multiple bolus injections has the advantage of being very simple, but results in the administration of a large total drug dose and slow recovery (Miller, 1994; Smith and White, 1998c; Padfield 2000b). In addition, the quality of the anaesthesia tends to be very poor as the drug plasma level is inconsistent (Smith and White, 1998c). By eliminating the peaks and troughs in plasma concentration which occur with the multiple bolus technique, continuous infusion results in a better quality of anaesthesia and a decrease in the total drug dose delivered (Gepts, 1998). Infusions can be controlled manually or by a computer, but in either case an infusion pump is required.

1.3.1 Anaesthetic Infusion Systems

The most common and the easiest technique to use is the constant rate infusion (CRI) or rate-controlled infusion (Smith and White, 1998c). Unfortunately, with such a system, the plane of anaesthesia is very difficult to control and with time, drug plasma level and side-effects increase (Smith and White, 1998c). As an alternative to keeping the rate of infusion constant, the concept of keeping the blood/plasma drug concentration (target) constant has led to the development of infusion schemes such as the stepped infusion scheme (Kruger-Thiemer, 1968; Wagner, 1974; Miller, 1994). With a stepped infusion a fast initial infusion is administered to fill the volume of distribution of the central compartment, followed by a maintenance infusion which will be determined by the desired target or central compartment drug concentration and the drug's rate of clearance (Wagner, 1974). This system, which has been described in humans for PPF and other agents, is usually simplified to a minimum number of steps to make it more practical Unfortunately, like the CRI system, the stepped infusion system is very rigid and difficult to adapt to the clinical situation (Gepts, 1998; Smith and White, 1998c).

In 1968, Kruger-Thiemer described an infusion scheme known as BET for Bolus (loading dose), Elimination (steady state rate of infusion according to drug's elimination) and Transfer (exponentially decreasing rate to match the redistribution of drug from the central compartment to peripheral sites) (Kruger-Thiemer, 1968). Thirteen years later, Schwilden (1981) demonstrated the clinical application of the BET infusion scheme, interfacing a microcomputer to an infusion pump. The first drugs to be administered by this system were etomidate and alfentanil in 1983 (Schuttler *et al.*, 1983). The development of new pumps, computer systems and infusion rate control algorithms to enable the anaesthetist to vary the target plasma drug concentration followed (Alvis *et al.*, 1985). In 1988, Schuttler and colleagues described the first TCI system (Schuttler *et al.*,

1988). Eight years later, in 1996, ICI (now AstraZeneca) in a collaboration with Kenny and co-workers at the Glasgow Royal Infirmary launched the first commercial TCI system for use with PPF in humans (Milne and Kenny, 1998b; Glen, 2003). This system was developed with a Graseby 3400 syringe driver with a custom built external computer backbar which controls the syringe driver and became a fully integrated system with the computer software incorporated into the syringe pump. The associated software for the administration of PPF in humans (Diprifusor; Zeneca Ltd) consists of a PK model, a set of specific PK variables for PPF and infusion control algorithms.

1.3.2 The Concept of Target Controlled Infusion

The fundamental principles and development of the TCI (Diprifusor) system were described by Gray and Kenny (1998) and Glen (2003). A TCI system consists of a syringe driver coupled with a computer, which is programmed with the PK parameters of the specified drug in a particular species. In contrast to a conventional pump where drug is administered at a fixed rate, in a TCI system, the computer pump control algorithm calculates the infusion rate that is necessary to achieve a blood target concentration. Thereafter, the computer controls the pump to maintain the target concentration. If the target is kept constant, the infusion rate will decrease over time to match the cumulative characteristic of the agent (Egan, 2003). At any point the anaesthetist can choose to modify the target. An increase in target concentration will result in injection of a calculated bolus dose, followed by an exponentially decreasing infusion rate that will be higher than the original infusion rate. Following a decrease in target concentration the infusion will cease until the new target, as predicted by the computer, is reached. Thereafter, an exponentially decreasing infusion rate will resume, at a lower rate than previously (Egan, 2003). Although the system is dependent on very complex mathematical models, its principle remains that of the BET method, described by Kruger-Thiemer in 1968 (Egan, 2003).

1.3.3 Evaluation of Target Controlled Infusion Systems

The accuracy of a TCI system is dependent upon the PK variables that have been used to programme the device. Consequently, the system must be validated before general use in clinical practice. In summary, during infusion using a TCI system, blood samples are taken at determined set points and the target concentrations are noted. Thereafter, using mathematical formulae, the predicted and actual blood concentration values for these time points are compared. From these calculations the bias and the accuracy of the system are determined. In human studies, it has been proposed that the performance of a TCI system can be considered clinically acceptable when the bias is not greater than +/- 10-20% and when the accuracy is between 20 to 40% (Schuttler *et al.*, 1988; Glass *et al.*, 1991).

1.3.4 Use of Target Controlled Infusions

In humans, TCI has been used mainly for induction and maintenance of anaesthesia. More recently, such systems have also been used to provide either conscious sedation or peri- and post-operative analgesia (Barvais *et al*., 1996; Oei-Lim *et al.,* 1998; Milne and Kenny, 1998a; Milne and Kenny, 1998b). Although PPF is used extensively in TCI systems, studies designed to demonstrate its analgesic properties have been inconclusive (Langley and Heel, 1988; Borgeat *et al.*, 1994; Zacny *et al*., 1996; Cheng *et al.*, 2008). Consequently, the use of PPF as the sole agent for TIVA in humans has proved unsatisfactory for major procedures because the doses required to eliminate responses to surgery induce CV and respiratory depression (Smith *et al*., 1994a). As a result potent opioids such as fentanyl, alfentanil or remifentanil are commonly co-infused with PPF to improve reflex suppression (Smith and White, 1998d). However, a feature of this combination is the necessity to control ventilation because of the respiratory depression caused by these drugs.

1.4 TIVA in veterinary medicine

Following the introduction of PPF to veterinary practice in the 1980s, interest in TIVA in domestic species was rekindled. Nolan and Hall (1985) and Waterman (1988) showed the possible use of PPF in horses and sheep respectively as a part of a TIVA technique while PK studies came later on for horses (Nolan *et al.*, 1996) and sheep (Mather *et al*., 1989; Correia *et al.*, 1996). Although different publications can be found in the literature regarding these species and others, it is in dogs that we will find the main PD and PK studies, leading to increased use of PPF for TIVA techniques and leading to the possible development of PPF computer-driven infusion. In 1984, Glen and Hunter published the first report on the use of PPF in the dog (Glen and Hunter, 1984). They compared the effect on histamine release of a single dose of the old formulation in Cremophor EL with a single dose IV of the lipid emulsion formulation that became the marketed preparation. Thereafter, other PD studies of PPF used for TIVA were published, such as Hall and Chambers (1987), Goodchild and Serrao (1989), Robertson *et al.* (1992), Thurmon *et al.* (1994), Muir and Gadawski (1998), and Murrell *et al.* (2005) to cite a few. In parallel, PK studies were conducted in dogs undergoing surgery (Nolan and Reid, 1993), in dogs comparing different breeds (Zoran *et al.*, 1993; Court *et al.*, 1999; Hay Kraus *et al*., 2000) and in the elderly canine patient (Reid and Nolan, 1996) or looking at drug interaction (Perry *et al*., 1991; Nolan *et al.*, 1993; Reid and Nolan, 1993; Hall *et al.*, 1994; Mandsager *et al*., 1995). All these studies and the development of computers and their association with infusion devices led eventually to computer-driven delivery of IV anaesthesia.

Computer driven PPF infusion has been used in dogs. In 2001, the anaesthesia group in Glasgow developed and assessed the performance of a TCI system for PPF in the dog (see chapter 2) (Beths *et al*., 2001). In 2004, a team from South-Africa used a TCI like system to anaesthetise four dogs with PPF while undergoing neurosurgery. In that study, they used a computer programmed using "Stelpump" software and attached to a syringe driver (Joubert *et al.*, 2004). That study did not assess the PK parameters used to programme the system. These parameters were assessed the same year by a team from China who infused PPF through the same TCI system as Joubert, but in enflurane anaesthetised dogs (Luo *et al*., 2004). In 2005, Musk and colleagues, including the author, studied the optimal target to induce dogs premedicated with acepromazine and morphine, using the TCI system developed by Beths and others in 2001 (see chapter 2).

Others have used computer driven infusion in other species with drugs other than PPF: in cats with alfentanil and in horses with alfentanil and detomidine (Daunt *et al*., 1993; Pascoe *et al.*, 1993; Ilkiw *et al.*, 1997). Unfortunately, these systems do not seem to have left the experimental milieu.

1.4.1 Alternatives to co-infusions of opioids with propofol.

Opioid infusions are a common feature of specialist veterinary anaesthetic practice, but in general veterinary practice, where it is routine for anaesthesia to be monitored by nursing staff, controlled ventilation is rarely employed other than when it is mandatory for the surgical procedure e.g. thoracotomy. Since the purpose of this work was to develop a TIVA technique for the dog, which would be practical to use in general veterinary practice, finding an alternative analgesic which could be administered while maintaining spontaneous ventilation, was fundamental to the success of the project.

Potential alternatives to the use of opioids as an adjuvant to PPF anaesthesia include ketamine, a dissociative anaesthetic agent that has few respiratory effects. However, the author and colleagues showed that although ketamine had anaesthetic sparing effects and CV stability in spontaneously breathing dogs anaesthetised with halothane, hypertension and tachycardia were commonly observed side-effects (Beths *et al*., 2000a). These CV side effects were unacceptably severe when ketamine infusions were co-administered with PPF infusion. Consequently, the search for a drug which would be PPF sparing when co-administered, while preserving spontaneous respiration, lead to the investigation of the alpha$_2$-adrenoceptor agonists, MED and its active enantiomer DEX.

1.5 Propofol

Propofol (2, 6, di-isopropylphenol) is a short acting, rapidly metabolized IV anaesthetic, which is insoluble in water, but highly lipid soluble. Initially, PPF was formulated with Cremophor EL (1977), but pain on injection and complement-mediated adverse reactions to the Cremophor necessitated a radical change in its formulation (Glen and Hunter, 1984). Since 1986, it has been marketed as a 1% emulsion containing 10% Soya bean oil, 2.25% glycerol, and 1.2% egg lecithin. Although this resolved the problems linked to the complement-mediated reactions, pain on injection is still a common side effect reported in humans (McCulloch and Lees, 1985; Kanto, 1988; Mirakhur, 1988; Scott *et al.*, 1988; Mangar and Holak, 1992).

Propofol has a molecular weight of 178 and a pKa of 11. Since it is a preservative-free product with a neutral pH value, it will support bacterial growth and endotoxin production (Berry *et al*., 1993; Quinn *et al*., 1993; Sosis and Braverman, 1993; Sosis *et al*., 1995; Aydin *et al.*, 2002; Joubert *et al.*, 2005; Strachan *et al*., 2008) and consequently aseptic conditions must be observed during its handling (Lorenz *et al*., 2002).

Propofol is licensed in dogs and cats, but its use has also been reported in other species, either as an induction agent or for TIVA (Nolan and Hall, 1985; Mama *et al.*, 1995; Correia *et al.*, 1996; Duke *et al*., 1997; Schumacher *et al.*, 1997; Machin and Caulkett, 1998; Bennett, 1998; Bennett *et al.*, 1998; Matthews *et al.*, 1999; Bettschart-Wolfensberger *et al.*, 2001a, 2001b; Hawkins *et al.*, 2003; Bettschart-Wolfensberger *et al.*, 2005; Oku *et al.*, 2005; Umar *et al.*, 2006, 2007).

1.5.1 Metabolism of propofol

Metabolism of drugs usually include biotransformation (phase I), which converts fat-soluble (lipophilic) compounds into water-soluble (hydrophilic) compounds. These undergo conjugation (phase II) before being excreted by the bile (high molecular weight) or in the urine (low molecular weight) (Chang and Kam, 1999).

Propofol metabolism occurs mainly in the liver and results in the formation of inactive metabolites excreted in the urine (Simons *et al*., 1991a; Veroli *et al*., 1992; Dawidowicz *et al*., 2000). In humans, the major pathway is a direct glucuronidation resulting in the formation of a glucuronic acid conjugate of PPF which accounts for 53-73% of the total metabolites (Benet *et al.*, 1996). Other metabolites are glucuronide and sulphate conjugates of quinol that arise from the transformation of PPF by the Cytochrome P450 enzymes (CYP) oxidative system.

CYP isoenzymes are principally located in the endoplasmic reticulum of the hepatocyte and are mainly responsible for biotransformation (oxidation, reduction, hydrolysis and hydration reactions), which prepares the drug for conjugation. The CYPs are iron-containing proteins (haemoproteins) which exhibit a spectral absorbance maximum at 450 nm when reduced and complexed with carbon monoxide. The general reaction catalysed by the CYPs can be written as follows:

$$NADPH + H^+ + O2 + RH \text{——————CYT P 450————>} NADP^+ + H_2O + ROH$$

RH = oxidisable drug substrate; ROH = hydroxylated metabolite.

The CYP isoenzymes are a superfamily whose classification and nomenclature have been derived from gene cloning and sequencing (Chang and Kam, 1999). The superfamily is divided into families which are themselves divided into sub-families, depending on the percentage of their genome the CYPs have in common. The enzyme families are described by an Arabic number, the subfamilies by a capital letter and a final Arabic number denotes individual enzymes. In the dog, only a few CYPs have been screened and identified so far, including CYP 1A1, 1A2, 2B11, 2C21, 2C41, 2D15, 3A12, 3A26 (The International Centre for Genetic Engineering and Biotechnology, www.icgeb.trieste.it/, 2001). In dogs CYP2B11 has been recognised as being responsible for the formation of inactive sulphate and glucuronide conjugates and also for the breed differences seen in PPF metabolism (Court *et al.*, 1999; Hay Kraus *et al.*, 2000).

1.5.2 Pharmacokinetics of propofol

Several groups have reported PK data for PPF in the dog with the model used to describe its PK being either biphasic or triphasic, depending on the design of the study (Cockshott e*t al*., 1992; Nolan and Reid, 1993; Reid and Nolan, 1993; Zoran *et al.*, 1993; Hall *et al*., 1994; Mandsager *et al.*, 1995).

The clinical success of PPF is partly attributable to its unique PK profile, particularly its redistribution and rapid metabolic clearance. After a single bolus injection, the plasma level of PPF decreases rapidly as a result of its redistribution from the blood and highly vascularised tissues to less well and very poorly vascularised tissues, such as muscle and fat respectively (Short and Bufalari, 1999). Its clearance is very rapid and exceeds hepatic blood flow, suggesting an extrahepatic site of metabolism. This hypothesis was corroborated when metabolites of PPF were detected during the anhepatic phase of orthotopic liver transplantation in man (Veroli *et al*., 1992). The origin of this extrahepatic metabolism is still to be determined, but the lung and kidney have been suggested (Kanto and Gepts, 1989; Veroli *et al.*, 1992; Matot *et al.*, 1993; Correia, 1994; Le Guellec *et al*., 1995; Raoof *et al.*, 1996; Kuipers *et al.* 1999; Dawidowicz *et al*., 2000; Takizawa *et al.*, 2004a; Murayama *et al.*, 2005). Recent studies confirm the importance of the role of the

kidneys in the extrahepatic PPF clearance while the lungs do not seem to be very much involved (He *et al.*, 2000; Hiraoka *et al*., 2005; Takizawa *et al.*, 2005a, 2005b; Al-Jahdari *et al.*, 2006; Chen *et al.*, 2006).

Although PPF has a relatively prolonged elimination half-life in man and in the dog (Reid and Nolan, 1993; Zoran *et al.*, 1993; Smith and White, 1998b), anaesthetised patients experience a short recovery time. Similarly, prolonged infusion of PPF has been shown to have very little effect on the recovery time in man (Smith and White, 1998b). According to Hughes and colleagues (1992), elimination half-life is a very poor predictor of recovery times and context-sensitive half-time, which is the measure of the time taken for the drug concentration in the central compartment to fall by 50% after continuous infusion, is a more relevant measure. In man, simulation data shows only a slight variation in this half-life for PPF infusions of between 0 and 9 hours. The PK of PPF is relatively unaffected by the presence of pathology affecting the hepatic (Servin *et al*., 1990) and renal systems (Kirvela *et al*. 1992; Nathan *et al*., 1993).

In summary: rapid redistribution, efficient metabolic clearance with a short context sensitive half-life irrespective of the duration of infusion, in addition to the minimal effects of hepatic and renal dysfunction on its PK parameters, make PPF the anaesthetic agent of choice for TIVA.

1.5.3 Pharmacodynamic characteristics of propofol

The reported CV effects of PPF are a transient decrease in ABP due mainly to a decrease in peripheral vascular resistance (Goodchild and Serrao, 1989; Pagel and Warltier, 1993), decreased sympathetic outflow, and myocardial depression (Coetzee *et al*., 1989). This hypotension does not usually result in reflex tachycardia, and a decrease in HR is more commonly observed. This is believed to result from resetting of the baroreceptor reflex by the agent (Cummings *et al*., 1984; Ebert *et al*., 1992a; Sellgren *et al.*, 1994). Although cardiac arrhythmias have been reported with the use of PPF in man, this is not a common finding in the dog (Watkins *et al.*, 1987; Smith *et al*., 1993; Quandt *et al*., 1998).

Respiratory depression and apnoea are the most common adverse effects associated with IV administration of PPF (Morgan and Legge, 1989; Ilkiw *et al*., 1992; Watney and Pablo, 1992; Muir and Gadawski, 1998; Quandt *et al*., 1998). However, in animals this may be minimised by injecting slowly to effect (Watkins *et al.*, 1987; Weaver and Raptopoulos, 1990; Quandt *et al.*, 1998).

The chemoreceptors responsible for central respiratory inhibition have been reported to be in the dorsomedial and ventromedial medulla (Yang *et al*., 1997). In 2001, Nieuwenhuijs and colleagues confirmed that low (sedative) doses of PPF resulted in depression of the ventilatory response which

was attributable to a central (medulla) rather than a peripheral (carotid bodies) effect. However, high doses of PPF have been shown to depress carotid body function in cats and rabbits (Ponte and Sadler, 1989) indicating that, whereas with low dose PPF the respiratory depression is mostly centrally mediated, at high doses, the carotid bodies are also involved.

Although induction and recovery from PPF have been described as being smooth and excitement free in animals (Hall and Chambers, 1987; Watkins *et al.*, 1987; Morgan and Legge, 1989; Weaver and Raptopoulos, 1990), some adverse effects have been reported: signs of pain at injection (less commonly reported in animals than in man), vomiting during recovery, excitation, paddling, muscle twitching, and opisthotonos (Davies, 1991; Cullen and Reynoldson, 1993; Smith, *et al.*, 1993; Smedile *et al.*, 1996). However, the use of a pre-anaesthetic tranquillizer, sedative, or opioid decreases the incidence of these reactions.

1.6 Alpha$_2$-adrenoceptor agonists

1.6.1 Medetomidine

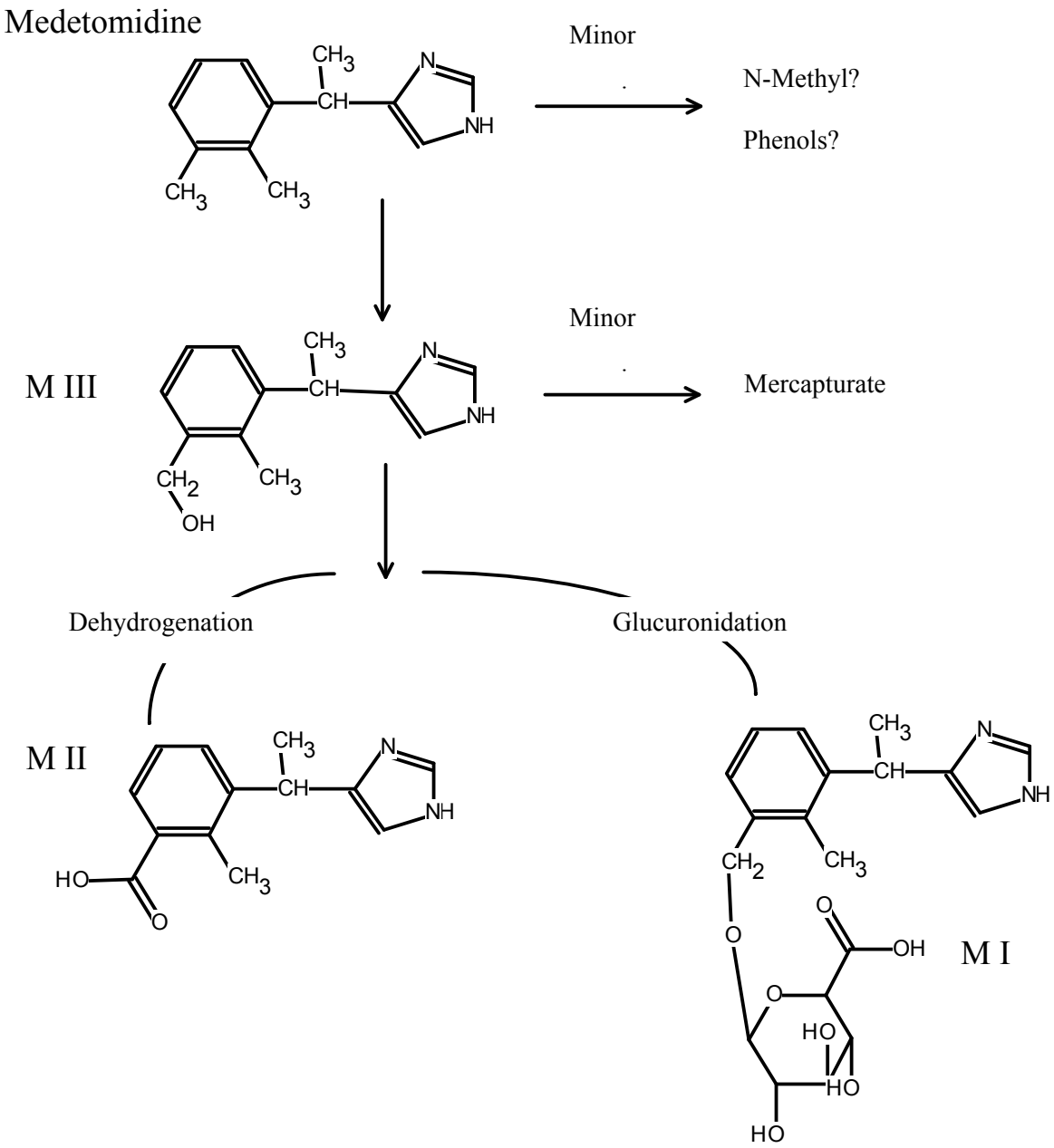
Medetomidine ((4-[2,3 dimethylphenyl] ethyl)-H-imidazole), first described in 1986, is a racemate composed of two stereoisomers: the D-stereoisomer (DEX) and L-stereoisomer (LEV) (Savola *et al.*, 1986), of which the former is the active component (Vickery *et al.*, 1988; Savola, 1989; MacDonald and Virtanen, 1991; Schmeling *et al.*, 1991).

Medetomidine has selective alpha$_2$-adrenoceptor agonist activity and an α_2/α_1 ratio of approximately 1600/1. The molecular formula of MED is $C_{13}H_{16}N_2$ with a molecular weight of 236.7 and a pKa of 7.1 (Datasheet, Abbott Laboratories, 2001). Medetomidine was introduced to veterinary medicine in the late 1980s, and in common with other alpha$_2$-adrenoceptor agonists, has been used for its sedative and analgesic properties in small animals as well as in rodents and wildlife (Moens, 2000). Similarly, MED infusions have been described in other species. In cats, Ansah and colleagues (2000) looked at the analgesic, sedative and some CV effects of increasing 3-step infusions of MED and DEX. They showed that although analgesia was dose dependent, this was not the case with sedation. They observed that increasing doses of the alpha$_2$-adrenoceptor agonist increased sedation but only to a given point. Thereafter, any increase in the plasma concentration resulted in a decrease of the level of sedation. In their equine study, Bettschart-Wolfenberger and colleagues (1999) looked at the PK parameters of MED while sedating ponies with a CRI. Thereafter, they developed an infusion scheme for MED which they used during PPF anaesthesia in ponies (Bettschart-Wolfensberger *et al.*, 2001a, 2001b, 2005). This combination provided very good anaesthesia and recovery in most of the animals studied. However some

animals experienced hypoxaemic episodes which were treated accordingly (Bettschart-Wolfensberger *et al*., 2001a). Similarly, MED has been used as an adjunct to general anaesthesia in dogs (Beths *et al*., 2000b). These workers described the use of 1 μgkg^{-1} MED during halothane anaesthesia in dogs and reported a marked anaesthetic sparing effect, with typical CV effects following the first injection and CV stability thereafter. In 2007, a study reported the sparing effect of 2 MED infusions (0.5 and 1 μgkg^{-1} h^{-1}) on desflurane concentration in anaesthetised dogs (Gomez-Villamandos *et al*., 2008).

1.6.1.1 Metabolism of medetomidine

The primary route of biotransformation of MED in the rat is hydroxylation of the methyl substituent at position 3 of the aromatic ring (Salonen and Eloranta, 1990). This reaction is thought to be catalysed by more than one CYP isoform and results in the formation of hydroxymedetomidine. In the dog, similar biotransformation occurs with both DEX and LEV, but the formation rate and relative amount of products formed differ. *In vitro*, kinetics of glucuronidation in dog liver microsomes show enantioselectivity, with LEV being faster (8 fold) than DEX (Kaivosaari *et al*., 2002). *In vivo*, DEX forms a large number of products while LEV gives a much "cleaner" profile (Salonen, personal communication, 2001). Although hydroxylation is the main pathway, some direct conjugation has also been observed (Salonen, personal communication, 2001). No enantiomer interconversion during the metabolism has been shown in dogs or any other species (Salonen, personal communication, 2001).



M1 Hydroxymedetomidine glucuronide

M II Medetomidine carboxylic acid

MIII Hydroxymedetomidine

1.6.1.2 Pharmacokinetics of medetomidine

Medetomidine is a weak organic base that is 85% protein bound. Following intramuscular (IM) injection in the dog, absorption is rapid ($t_{1/2d}$ of 3.2 min) and it is rapidly distributed to well vascularised tissues such as the brain, the lungs, the kidney, and the liver; the heart being an exception (Salonen, 1989; Salonen, 1991). Following IV injection of MED, Salonen and Kuusela showed that the PK were characterised by rapid redistribution and clearance (Salonen, 1989; Kuusela *et al*., 2000).

The elimination of MED depends mainly on biotransformation since only traces of unchanged drug are found in faeces and urine of dogs, cats or rats (Salonen, 1989; Salonen and Eloranta, 1990).

1.6.1.3 Pharmacodynamic characteristics of medetomidine

The sedative effects of the alpha$_2$-adrenoceptor agonists are principally derived from their ability to decrease the firing of the locus coereleus, an important modulator of vigilance, sited in the pons in the lower brain stem (Aghajanian and VanderMaelen, 1982). It is commonly accepted that the administration of an alpha$_2$-adrenoceptor agonist results in a biphasic haemodynamic response (Hall and Clarke, 1991b; Pypendop and Verstegen, 1998; Ebert *et al.*, 2000; Talke *et al.*, 2003), the initial phase corresponding to an increase in blood pressure and vascular resistance (Guimaraes and Moura, 2001). Although the duration of action is dependent on the alpha$_2$-adrenoceptor agonist used and its plasma concentration, this phase is usually of short duration and is followed by a relaxation of the vascular beds leading to normo- or hypotension (phase 2) (Hall and Clarke, 1991b; Pypendop and Verstegen, 1998). In common with all other alpha$_2$-adrenoceptor agonists, the administration of MED is followed by bradycardia and a decrease in cardiac output (Hall and Clarke, 1991b). Stimulation of both central and peripheral alpha$_2$-adrenoceptors is responsible for these CV changes (Hayashi and Maze, 1993; Kamibayashi and Maze, 2000) and there is no evidence of a direct action on the heart muscle (Flacke *et al.*, 1992; Day and Muir, 1993; de Morais and Muir, 1995; Khan *et al.*, 1999; Murrell and Hellebrekers, 2005). The duration of the bradycardia is dose dependent, but maximal effects in the conscious dog are obtained with doses as low as 5 μgkg^{-1} (Pypendop and Verstegen, 1998).

In anaesthetised dogs, antidysrhythmic properties have been described for MED and DEX. In halothane anaesthetised dogs with epinephrine-induced arrhythmias, Hayashi and colleagues (1991) reported that DEX, but not LEV possessed antidysrhythmic properties and suggested that this effect was mediated through stimulation of central alpha$_2$-adrenoceptors. However, two years later, Lemke and colleagues (1993a, b) demonstrated that MED (15 μgkg^{-1} given IM) had neither arrhythmogenic nor antidysrhythmic effects on halothane or isoflurane/epinephrine dysrhythmias in dogs.

Kamibayashi and colleagues (1995a) reported a role for the vagus nerve in mediating the antidysrhythmic effects of DEX on halothane/epinephrine dysrhythmias in dogs. They proposed the nucleus tractus solitarius and the dorsal motor nucleus (nuclei rich in alpha$_2$-adrenoceptors) as the main target for the alpha$_2$-adrenoceptor agonist in its antidysrhythmic role. In another study, Kamibayashi and colleagues (1995b) reported that targeting of imidazoline receptors in the central nervous system was responsible for mediating the antidysrhythmic actions of DEX in dogs.

The analgesia observed with the alpha$_2$-adrenoceptor agonists is mediated both spinally and centrally (Guo *et al.*, 1996; Zhang *et al.*, 1998a, b; Smith and Elliott, 2001; Molina and Herrero, 2006). Although it has been shown that the alpha$_2$-adrenoceptor agonists produce analgesia spinally (Maze and Tranquilli, 1991; Asano *et al.*, 2000), doubt has been cast on the existence of direct central mediation of analgesia by this class of drug (Murkin, 1991; Sabbe *et al.*, 1994; Mansikka *et al.*, 2004). According to several studies, supraspinal analgesia could be related simply to the sedative effects of the alpha$_2$-adrenoceptor agonists (Buerkle and Yaksh, 1998; Khan *et al.*, 1999; Ansah *et al.*, 2000; Xu *et al.*, 2000b).

The analgesic effects of MED have been demonstrated in various species. For example in conscious cats, MED infusion decreases the response to ear pinch, tail clamp and skin clamp in a dose dependent manner (Ansah *et al.*, 2000). In the same way, in conscious sheep, increasing doses of MED IV reduced the pain response to a pin pressing against the anterior surface of the metacarpus with gradually increasing force (Muge *et al*., 1994).

Similarly, in the conscious dog, several studies have demonstrated the dose dependent analgesic properties of MED injected IV or IM using different kind of noxious stimuli such as toe pinching, electrical stimulation or tail clamping (Raiha *et al*., 1989; Vainio *et al.*, 1989; Kuusela *et al*., 2000; Kuo and Keegan, 2004). However, the comparison of analgesic duration between studies is difficult, since they often differ in terms of dose, injection site and the noxious stimulus used as a test for analgesic effect. In two studies in dogs using toe pinching and IV injection, the analgesia lasted between 75 and 90 min after a single dose of 20 μgkg^{-1} (Kuo and Keegan, 2004) while a 40 μgkg^{-1} dose showed some effect up to 90 minutes (Kuusela *et al.*, 2000).

Medetomidine, like the other alpha$_2$-adrenoceptor agonists, has a relatively mild depressant effect on ventilation. In dogs, a decrease in respiratory rate is relatively common, with occasional irregular breathing. Although cyanosis has been reported in up to 33% of dogs sedated with MED, this is thought to be due to the decrease in cardiac output (CO), which slows down the blood flow through the tissues, leading to increased oxygen extraction (Bergstrom, 1988; Clarke and England, 1989; Vainio, 1990). The cyanosis is therefore considered to result from venous desaturation (England and Clarke, 1989; Sap and Hellebrekers, 1993) and alteration in arterial blood gas

parameters is minimal (Vainio and Palmu, 1989; England and Clarke, 1989; Venugopalan *et al.*, 1994; Cullen, 1996).

1.6.2 Dexmedetomidine

Dexmedetomidine has the same chemical characteristics as MED. Because of its appealing characteristics of sedation, anxiolysis, analgesia, haemodynamic stability and minimal respiratory depression, its use in medical anaesthetic practice as an adjunct to general anaesthesia is increasing (Aho *et al*., 1992a; Peden and Prys-Roberts, 1992; Talke *et al.*, 1997; Hall *et al.*, 2000; Venn *et al.*, 2002; Paris and Tonner, 2005; Tanskanen *et al.*, 2006). Although it is not yet commercially available for use in veterinary medicine in every country, some studies have been undertaken to compare the CV, respiratory, sedative and analgesic effects of DEX with MED and LEV, in the dog and cat (Ansah *et al.*, 1998; Kuusela *et al.*, 2000, 2001a). In 2001, Kuusela concluded that the use of DEX alone may have some CV benefits over administration of the racemic mixture (Kuusela *et al.*, 2001a).

1.6.2.1 Pharmacokinetics of dexmedetomidine

In the blood, DEX is approximately 97% protein bound (Karol and Maze, 2000). Kuusela *et al.* (2000) reported the pharmacokinetics of DEX in the dog and found there to be no difference between the PK parameters of MED and DEX in this species. In common with MED, DEX is highly lipophilic and is rapidly distributed to tissues with a distribution half-life ($t_{1/2\alpha}$) of about 4 minutes. It is characterised by a short elimination phase with a mean elimination half-life ($t_{1/2}$) of approximately one hour (Kuusela *et al.*, 2000).

In humans, DEX is thought to be eliminated almost exclusively by metabolism, as no unchanged DEX has been detected in the urine of male patients (Karol and Maze, 2000; Venn *et al.*, 2002). In human patients with severe renal impairment, the PK profile of DEX was unchanged when compared with a control group (De Wolf *et al.*, 2001). As the elimination of MED in the dog is also mainly by metabolism (Salonen, 1989), it is likely that the same holds are true for DEX in the dog, although evidence to support this has not yet been published

1.6.2.2 Pharmacodynamic characteristics of dexmedetomidine

Kuusela and colleagues (2000) compared the clinical effects of MED and DEX in dogs and reported little difference in their CV effects. Like MED, DEX has no direct effect on the myocardium (Flacke *et al*., 1992). Similarly, equipotent IV doses of DEX and MED have minimal effects on ventilation in dogs (Kuusela *et al.*, 2000). Following IV injection of 10 μgkg^{-1} of DEX in dogs, a decrease in pH (7.3) was recorded, with no significant change from baseline in $PaCO_2$ or PaO_2 (Kuusela *et al*., 2000, 2001a, 2001b).

The analgesic effects of DEX and MED have been compared in dogs (Kuusela *et al.*, 2000, 2001b)and cats (Ansah *et al.*, 2000) with no difference in the level of analgesia being detected, although in the study of Kuusela (2001b), a slight increase in length of action was noted with equipotent doses of DEX compared with MED.

1.7 Drug interactions

Combinations of intravenously administered drugs may result in drug-drug interactions, which can be synergistic, antagonistic or additive (Benet *et al.*, 1996). These interactions can be explained by three mechanisms, namely, physicochemical, PK and PD. Physicochemical interactions usually occur when two incompatible drugs are mixed together (i.e. pH difference).

1.7.1 Pharmacokinetic interaction

Although PK interactions can take place during the different PK phases of absorption, distribution, elimination or biotransformation, the effects resulting from the interactions during biotransformation are generally more pronounced (Benet *et al.*, 1996). Drug metabolism occurs mainly in the liver and can be studied *in vitro* as well as *in vivo*. *In vitro* studies on PK interaction are preferable as they are time- and cost-effective with minimal risk attached. They offer the opportunity to investigate specific biotransformation pathways under strictly controlled conditions and to investigate the ability of a drug to inhibit the metabolism of other drugs. For these reasons *in vitro* studies form part of the screening processes used by pharmaceutical companies in the selection of new drugs (Gibson and Skett, 1994a; Lin and Lu, 1998; Venkatakrishnan *et al*., 2003).

For *in vitro* studies, liver preparations are required which are either physiological preparations (whole perfused liver), biochemical preparations (sub-cellular fractions) or a compromise between the two (liver slices, cubes or cells) (Venkatakrishnan *et al.*, 2003).
Sub-cellular fractions offer the advantage of being easy to produce with a high reproducibility (Gibson and Skett, 1994b). Unfortunately, their *in vivo* relevance is not as good as a perfused liver. The sub-cellular fraction consists mainly of microsomes containing the CYP enzymes which are involved in phase 1 metabolism and which are most commonly responsible for metabolism-based interactions (Benet *et al.*, 1996). Propofol, as well as MED and its enantiomers are potential CYP inhibitors through different mechanisms (Gepts *et al.*, 1988; Kharasch *et al.*, 1991; Pelkonen *et al.*, 1991; Janicki *et al.*, 1992; Kharasch *et al.*, 1992; Baker *et al.*, 1993; Chen *et al*., 1995a, 1995b; Rodrigues and Roberts, 1997; McKillop *et al*., 1998; Miller and Park, 1999; Naoya *et al.*, 1999;

Gemayel *et al.*, 2001; Lejus *et al.*, 2002; Inomata *et al.*, 2003; Yang *et al.*, 2003; Osaka *et al.*, 2006; Yamazaki *et al.*, 2006; Lennquist *et al.* 2008).

1.7.2 Pharmacodynamic interactions

Pharmacodynamic interactions result in modification of the response of the body to drugs due to alteration of the receptor sensitivity of one drug by another, or by the production of additive or inhibitory effects, due to actions at different sites in an organ, or by different mechanisms.

1.7.2.1 Propofol-medetomidine

The combination of MED premedication and PPF induction either with or without PPF maintenance has been used in species other than the dog, including goat, cat, ostrich, rabbit and horses (Ko *et al*., 1992; Langan *et al*., 2000; Akkerdaas *et al.*, 2001; Amarpal *et al*., 2002; Bettschart-Wolfensberger *et al.*, 2005).

In the dog, when used as part of the premedication with or without anticholinergic drugs, MED decreases the induction and maintenance doses of PPF (Manners, 1990; Davies, 1991; Vainio, 1991; Cullen and Reynoldson, 1993; Sap and Hellebrekers, 1993; Hall *et al*., 1994; Thurmon *et al*., 1994, 1995; Bufalari *et al*., 1996, 1997; Hellebrekers *et al*., 1998; Bufalari *et al.*, 1998; Scabell *et al*., 1999; Redondo *et al*., 1999; Kuusela *et al*., 2001a; Vaisanen *et al*., 2002; Ko *et al.*, 2006). Bradycardia is a common feature, but the effect on blood pressure is more ambiguous with an increase in some individuals (Vainio, 1991; Thurmon *et al*., 1994; Hellebrekers and Sap, 1993; Bufalari *et al.*, 1996) and a decrease in others (Ko *et al.*, 2006). Although the PD changes are significant, the PK effects of the alpha$_2$-adrenoceptor agonist on PPF during infusion are not (Hall *et al*., 1994).

1.7.2.2 Propofol-dexmedetomidine

TIVA regimes comprising PPF and DEX have been described in humans (Dutta *et al*., 2001; Larson and Talke, 2001; Peden *et al*., 2001). Although no unwanted side effects were reported in the first two of these studies, Peden *et al.* (2001) reported important adverse effects including sinus arrest and 24 hours duration of post-operative dizziness, possibly due to a high infusion rate. The use of DEX as a premedicant followed by PPF for induction and maintenance of anaesthesia has been described in dogs (Proctor *et al.*, 1992; Kuusela *et al*., 2001a, 2003) and apart from some bradycardia, no adverse effects were reported.

1.8 Purpose of this Study

The aim of this study was to develop a TIVA technique for the dog, based on co-infusion of MED or DEX with TCI PPF, which would have minimal effects on the CV and respiratory systems, thus providing a practical scheme for use in veterinary practice.

The development of the PPF TCI system is described in chapter 2. Furthermore the suitability of the suitability of the PK parameters used to programme the TCI system is assessed in the context of acceptable clinical performance.

Chapter 3 examines the possible interaction at the level of the hepatic CYP enzyme between PPF and MED or its enantiomers *in vitro*. Both MED and DEX are potential inhibitors of hepatic microsomal oxidative metabolism and might therefore modify the PK parameters of PFF; a factor which may influence the accuracy of the model evaluated in chapter 2.

Significant CV effects of the alpha$_2$-adrenoceptor agonists have been demonstrated and while much information exists about these effects in conscious dogs, there is no data reporting the CV effects in dogs anaesthetised with PPF. Since the aim of the study was to devise an infusion system for MED or DEX which would provide adequate analgesia to supplement PPF infusion in dogs, while keeping the CV effects to a minimum, a dose response study to determine optimal infusion rates is described in chapter 4.

Chapter 5 looks at the design of infusion schemes for MED and DEX, in the light of the results of chapters 3 and 4, using the PK software PK-SIM. Thereafter the predictive performance of these infusion schemes is tested in beagle dogs anaesthetised with TCI PPF. In addition the effects of these infusion schemes on CV variables are quantified as are their analgesic effects. The possible influence on the performance of the PPF TCI system resulting from the co-infusion with MED or DEX is also evaluated.

TIVA is still a developing field. Advances in technology render infusion devices more precise, more sophisticated and safer. New infusion models are developed incorporating not only PK parameters but also PD information. The industry is developing new drugs and modifying others to respond better to the needs of TIVA and become closer to the “ideal” agent with short onset of action, short context sensitive half-life undisturbed by the length of infusion, quick recovery, no cumulation and with no or little PD effect. The effect of these developments and the impact of the studies described in this thesis on the future of TIVA in the dog are developed in chapter 6.

CHAPTER 2:

DEVELOPMENT OF A TARGET CONTROLLED INFUSION SYSTEM FOR PROPOFOL IN THE DOG.

2.1 Introduction

The general concept of target controlled infusion (TCI) has been described in Section 1.3.2. With this mode of administration the amount of drug delivered by an infusion pump to achieve a desired target blood concentration of drug is dependent on the pharmacokinetic (PK) parameters of the drug incorporated in the pump software. Acceptable performance of such systems can be expected only if the PK parameters in the pump provide a good description of the distribution and elimination of a particular drug in the species to be studied.

The development of a TCI system for propofol (PPF) in the dog required the modification of a system originally designed for the administration of PPF to human patients (White and Kenny, 1990; Glen 1998; Gray and Kenny, 1998). This consisted of three distinct phases: the selection of appropriate PK parameters for the dog to programme the infusion control software; determination of suitable targets for induction and maintenance of anaesthesia in the dog; and evaluation of the performance of the system (Glass *et al.*, 1991; Varvel *et al.*, 1992; Coetzee *et al*., 1995; Vuyk *et al*., 1995; Glen, 1998).

2.1.1 Determination of pharmacokinetic parameters

Different methods have been described to determine PK parameters for use in computerised infusion system such as the TCI system (Vuyk *et al.*, 1995). Some groups have used PK parameters derived from a population having the same characteristics as those patients for which the system is being developed (Coetzee *et al.*, 1995; Vuyk *et al*., 1995; Schuttler and Ihmsen, 2000). Alternatively a population-based PK parameter set has been used which allows adjustments of the PK data to accommodate the characteristics of individual patients (Maitre *et al.*, 1987; Oei-Lim *et al.*, 1998; Slepchenko *et al.*, 2003). A more recent method consist in using computer simulation to compare predicted blood concentration of the drug with actual blood concentrations in patients and to redefine more precisely the PK parameter set that will be used thereafter in the TCI system (Vuyk *et al.*, 1995; Wietasch *et al.*, 2006). Glen (1997, personal communication) used computer simulation (PK-SIM; Specialised Data Systems, Jenkintown, PA, USA) to determine the PK parameter set used to programme the TCI system used in this study. A review of reported PK parameters for PPF in the dog, derived from bolus and/or infusion studies, had demonstrated that there was wide variation between individual studies (Cockshott *et al*., 1992; Nolan and Reid, 1993; Nolan *et al.*, 1993; Reid and Nolan, 1993; Zoran *et al.*, 1993; Hall *et al*., 1994; Mandsager *et al*., 1995). Nolan and Reid (1993) used a standardised administration scheme of PPF of 4 $mgkg^{-1}$ for induction of anaesthesia followed by an infusion of 0.4 $mgkg^{-1}min^{-1}$ for 60 min to maintain anaesthesia in association with 67% nitrous oxide in six beagle dogs undergoing body surface surgery, and reported the blood PPF concentration. Glen used this administration scheme for PPF

and PK variables from selected published studies as a basis for computer simulation. Thereafter, he compared the predicted blood concentrations with the actual blood concentrations obtained by Nolan and Reid. None of the published PK models provided an accurate prediction of the measured profile. Parameters derived from the studies described by Nolan *et al.* (1993) and Reid and Nolan (1993) underpredicted the measured profile while those of the study by Cockshott and colleagues (1992) underpredicted the early part of the measured profile but over- predicted the later part. The slope of the predicted 'Cockshott' profile indicated greater accumulation of PPF than was evident from the measured profile, suggesting the actual rate of elimination of PPF was greater than that predicted by the model. Thereafter, an iterative approach was used whereby empirical adjustments were made firstly to k_{10} (elimination clearance) to alter the slope of the predicted profile, and then to V_1 (central compartment) to alter the "gain" of the model.

At each step computer simulation was used to monitor the impact of each change before a final model was defined. The final model parameters were incorporated in the prototype TCI system evaluated in this study.

2.1.2 Optimisation of blood propofol targets

In humans, blood levels of PPF required for induction and maintenance of anaesthesia are variable, depending on adjuvant therapy, patient health status and the severity of the surgical procedure for which the patient is anaesthetised (Dixon *et al*., 1990; Shafer, 1993).

Struys *et al.* (1998) showed a significant reduction in the induction target of PPF required when patients were premedicated with diazepam, and Chaudhri and colleagues (1992) reported that 90% of patients premedicated with temazepan, another benzodiazepine, could be intubated at a blood PPF concentration of 5 μgml^{-1}. A similar PPF sparing effect has been demonstrated when opioids are used for premedication. Kazama *et al*. (1998) showed that the PPF blood concentration required to allow intubation in 50% (Cp_{50}) of healthy patients was 19.6 μgml^{-1}, but this was decreased by 34.7 and 46.7% when fentanyl (blood concentration of 1 and 3 $ngml^{-1}$, respectively) was included in the anaesthetic protocol.

Studies in dogs have shown that a variety of drugs and drug combinations used for premedication have a sparing effect on PPF used by bolus injection to induce anaesthesia, to a greater or lesser degree (Geel, 1991; Bufalari *et al*., 1996; Stegmann and Bester, 2001; Sano *et al*., 2003; Gomez-Villamandos *et al.*, 2005, 2006; Braun *et al.*, 2007). Similarly, Bufalari and colleagues (1998) used a variety of drugs for premedication prior to administering a constant rate infusion PPF of 0.11 $mgkg^{-1}sec^{-1}$ to dogs and showed that depending on the premedication used, the amount of PPF used for induction of anaesthesia varied between 2.2 and 6.6 $mgkg^{-1}$.

Struys *et al.* (1998) demonstrated that the amount of PPF required to induce hypnosis in humans decreased with age. Age has also been shown to influence the induction dose of PPF in the dog. Reid and Nolan (1996) described the PK profile of PPF given as a bolus to induce anaesthesia in geriatric dogs and concluded that elderly patients need a lower induction dose than younger animals.

In humans, to maintain anaesthesia and prevent reaction to skin incision in 50% of patients, targets of 4.2 to 15.2 μgml^{-1} have been reported, with this variation being dependent on whether or not an analgesic adjuvant was used (Davidson *et al*., 1993; Taylor *et al.*, 1993; Smith *et al.*, 1994b; Andrews *et al.*, 1997; Kazama *et al.*, 1997; Schnider *et al*., 1998). Several studies in animals have also demonstrated the effect of an analgesic adjuvant on the PPF blood levels necessary for reflex suppression in a variety of surgical procedures. Correia *et al.* (1996) demonstrated that PPF blood concentrations ranging between 2.98 and 7.1 μgml^{-1} abolished reaction to skin incision in sheep infused with PPF and premedicated with acepromazine and papaveretum. In dogs, also premedicated with acepromazine and papaveretum undergoing similar surgery, Nolan and Reid (1993) showed that blood PPF concentrations between 3.5 and 5.8 μgml^{-1} were necessary to suppress the response to surgery. Similarly ketamine has been shown to reduce the infusion rate of PPF required to maintain anaesthesia in sheep undergoing superficial skin surgery (Correia *et al.* 1996), ponies undergoing castration (Flaherty *et al*., 1997) and cats subjected to a variety of noxious stimuli (Ilkiw *et al.*, 2003).

In humans, inter-individual PK and pharmacodynamic (PD) variation is a well-recognised factor influencing the range of PPF blood concentrations necessary to induce and maintain anaesthesia (Spelina *et al*., 1986; Schuttler *et al.*, 1988; Shafer *et al.* 1988; Chaudhri *et al.*, 1992; Struys *et al.*, 1998; Schuttler and Ihmsen, 2000; Kazama *et al*., 2000, 2001). Inter-individual variations for PPF have also been demonstrated in a study of beagle dogs anaesthetised with a zero rate infusion of PPF (Nolan and Reid, 1993). The authors observed a wide variation in the PPF blood concentration between the dogs. In addition, in this species, inter-breed differences have also been reported (Robertson *et al.*, 1992; Zoran *et al.*, 1993; Court *et al.*, 1999; Hay Kraus *et al.*, 2000). Greyhounds required more PPF for induction and all the recovery stages were delayed in this breed compared with mixed-breed dogs (Robertson *et al.*, 1992). Heart rate (HR) and body temperature also varied between the groups. In another study in greyhounds and mixed-breed dogs, Zoran and colleagues (1993) showed that PK parameters such as volume of distribution and clearance were significantly different between the two groups. They also found slower recovery times in the greyhound group and reported higher PPF blood concentrations at extubation than in the mixed-breed group.

However, clinical practice in human patients has demonstrated that despite inter-individual variability in PK and PD, anaesthesia can be achieved effectively and safely with a TCI system, as the titration to a desired depth of anaesthesia is simple to achieve with such systems (Struys *et al*., 1997; Russell, 1998; Li *et al.*, 2005).

2.1.3 Evaluation of the performance of the TCI system

Evaluation of the predictive performance of a TCI system is carried out by comparing the PPF concentrations predicted by the system with the measured PPF concentrations either in venous or arterial blood samples taken at various time points during anaesthesia, over a range of target concentrations, according to the methodology described by Varvel and colleagues (1992). This approach has subsequently been used by many authors (Coetzee *et al.*, 1995; Vuyk *et al.*, 1995; Short *et al.*, 1996; Glen, 1998; Oei-Lim *et al.*, 1998; Swinhoe *et al*., 1998; Varvel, 2002; Slepchenko *et al*., 2003; Li *et al.*, 2005; Ko *et al.*, 2007; White *et al*., 2008). This methodology is based on the calculation of the percentage prediction error (PE) as the difference between measured and predicted values expressed as a percentage of the predicted value. Using values of PE% derived at each measurement point, a number of indices of performance in an individual subject are calculated. The median prediction error (MDPE%), provides a measure of bias to indicate whether measured concentrations are systematically above or below targeted values. The median absolute prediction error (MDAPE%) measures inaccuracy and gives information on the typical size of the difference between measured and targeted concentrations.

Two other indices are wobble and divergence, both of which reflect time related changes. Wobble measures the total intra-individual variability in performance error, and divergence describes any systematic time-related changes in measured concentrations away from or towards the targeted concentration. A positive value indicates a widening of the gap between the predicted and measured concentrations over time, while a negative value indicates that the measured concentrations converge on the predicted values.

Although no reference values are reported for divergence or wobble, it has been suggested that the performance of a TCI system is clinically acceptable if the bias (MDPE%) is not greater than ±10-20% and the inaccuracy (MDAPE%) falls between 15% and 40% (Glass *et al.*, 1991; Swinhoe *et al.*, 1998; Egan, 2003; Li *et al.*, 2005).

2.1.4 Goal of the study

To determine canine PPF PK parameters to use to program the TCI system and to furthermore assess the suitability of these in the context of acceptable clinical performance.

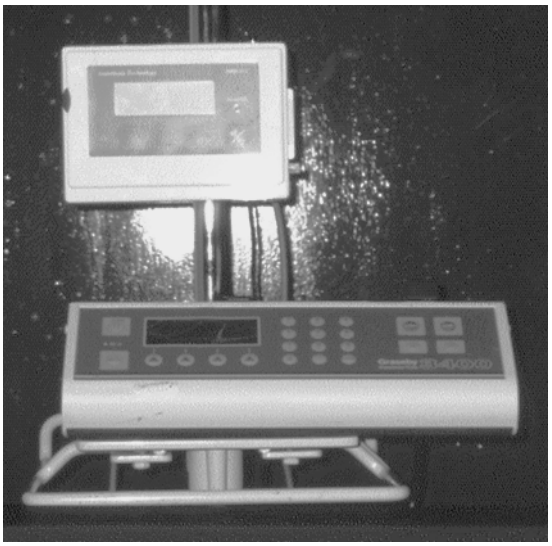
2.2 Materials and methods

2.2.1 Animals

This study was approved by the Clinical Research Sub-Committee of the Faculty of Veterinary Medicine of the University of Glasgow. Sixteen dogs, ten mixed-breeds (dogs 1-10) and six greyhounds (dogs 11-16) attending the Glasgow Small Animal Hospital for routine dental work were included in the study. They were ASA 1 and owner consent was obtained for inclusion in the study.

2.2.2 TCI Equipment

The infusion system consisted of a custom-built external computer containing TCI software linked via a serial port to a Graseby 3400 infusion pump (White and Kenny, 1990).



Based on the work of Glen (1997, personal communication), the following PK parameters for PPF in the dog were incorporated in the TCI control software:

$V_1 = 780$ mlkg^{-1}

$k_{10} = 0.07$ min^{-1}

$k_{12} = 0.0365$ min^{-1}

$k_{21} = 0.0312$ min^{-1}

$k_{13} = 0.0049$ min^{-1}

$k_{31} = 0.0011$ min^{-1}

2.2.3 Anaesthetic protocol

Dogs were premedicated with acepromazine (ACP; Cvet) 0.03-0.05 $mgkg^{-1}$ and methadone (Martindale Pharmaceuticals, Essex, UK) 0.1 $mgkg^{-1}$ or pethidine (Martindale Pharmaceuticals, Essex, UK) 2 $mgkg^{-1}$, injected together intra-muscularly, 30 to 40 min before induction of anaesthesia. Anaesthesia was induced with PPF (Rapinovet; Schering-Plough Ltd, Hertfordshire, UK), given through a cannula (Biovalve, Vygon, Cirencester, UK) preplaced in a cephalic vein, using the prototype computer-driven TCI system described. In order to programme the system for each individual, the dog's weight, age and a target blood PPF concentration were entered prior the start of the procedure.

Initially induction targets were set at 8 and 15 $\mu g\ ml^{-1}$ for dogs 1 and 2, respectively. Targets of 12 $\mu g\ ml^{-1}$ and 6 $\mu g\ ml^{-1}$ respectively were set for dogs 3 and 4. Thereafter the induction target concentration was set at 3 μgml^{-1} for all remaining dogs with the exception of three dogs, one mixed-breed and two greyhounds, which had initial target concentrations of 2 - 2.5 μgml^{-1} (Table 2.4). The end point of induction of anaesthesia was defined as when the animal assumed lateral recumbency and tolerated intubation of the trachea with no tongue movement or resistance. If this end point was not reached within 3 min, the target concentration was increased by a further 1 $\mu g\ ml^{-1}$ (dogs 5 and 8) and by 0.5 μgml^{-1} (dogs 10, 11, 13 and 15) at 2 min intervals until induction of anaesthesia was complete.

The target concentration was maintained initially as that at which intubation was achieved, and was altered in increments of 0.5 to 1 $\mu g\ ml^{-1}$ as necessary to maintain a satisfactory depth of anaesthesia. Depth of anaesthesia was assessed subjectively by recording the dog's response to palpebral stimulation, eye position, pulse rate, arterial blood pressure (ABP) and respiratory rate (RR). Criteria indicating inadequate anaesthesia were sudden tachycardia >10% resting HR, sudden tachypnoea > 20% resting RR, increase in systolic ABP to more than 15 mmHg above baseline, somatic responses such as swallowing, evidence of a palpebral reflex or somatic movement. Signs indicative of excessive depth of anaesthesia were a mean ABP of less than 60 mmHg, a HR lower than 30 bpm and apnoea for more than 30 sec.

The ECG (Kontron Micromon 7141, Kontron Instruments Ltd, England) was monitored continuously and the pulse and RR were recorded every 5 min. Indirect ABP (Dinamap; Critikon 1846 SX, Critikon Inc, Tampa, Fl, USA) with an appropriate cuff placed over the pedal artery was monitored in all patients and recorded every 5 min. Dogs breathed an O_2/N_2O (1:2) mixture throughout anaesthesia, delivered via an appropriate non-rebreathing system at fresh gas flows of 150 to 400 $mlkg^{-1}min^{-1}$, depending on the system used. In the event of apnoea occurring during

maintenance of anaesthesia, dogs were ventilated at 12 breaths min^{-1} until spontaneous respiration resumed. Nitrous oxide was switched off 5-10 min before the end of the procedure and the PPF infusion was terminated at the end of the procedure. Time of extubation was taken as the interval from the end of infusion of PPF to that point when stimulation of the tongue or pharynx elicited a swallowing reflex.

For each individual animal, infusion time, total volume of PPF infused and the number of target adjustments made during the infusion were noted and the infusion rate calculated. The dose of PPF at induction was calculated using computer simulation (PK-SIM, Specialised Data Systems, Jenkintown, PA, USA) using the target input profile up to the point of successful endotracheal intubation. Total infusion rate was derived from the total amount of PPF infused, body weight and the total duration of infusion. Maintenance infusion rate was derived using the difference between the total amount infused and the amount required at induction for the time period going from the start to the end of the infusion.

2.2.4 Blood sampling

Blood samples (1.5-2 ml) for PPF analysis, were collected from a cephalic cannula into fluoride oxalate tubes (Sarstedt). Samples were taken before anaesthesia, at the point of induction, then 2, 5, 7 min after induction, at 20 min intervals throughout anaesthesia, at the end of the infusion period, 2, 5, 7 min later, and at the time of extubation. Additional blood samples were taken 2, 5 and 7 min after any alteration in the target concentration.

2.2.5 Blood propofol analysis

The analyses were performed by Dr A.M. Monteiro from the Institute of Comparative Medicine of the University of Glasgow.

A stock solution of PPF (100 $\mu g ml^{-1}$) was prepared from pure compound (Tocris Cookson Inc., Bristol, UK) using methanol (HPLC grade, Rathburn Chemicals Ltd, Walkerburn, Scotland) as the solvent, and this was used for all subsequent dilutions. A stock solution of Thymol, the internal standard (100 $\mu g ml^{-1}$), was also prepared in methanol from pure compound, and was further diluted to two working solutions of 1 and 5 $\mu g ml^{-1}$. The HPLC mobile phase consisted of a mixture of methanol, water and trifluoroacetic acid (TFA) in a 70:30:0.1 (volume:volume:volume) ratio, respectively. Propofol external standard was prepared from the stock in distilled water. Tetram methylamonium hydroxide (TMAH) 7.5% solution in propan-2-ol (Rathburn Chemicals Ltd, Walkerburn, Scotland) was prepared prior to use. Phosphate buffer (0.1M) was prepared in distilled water from sodium dihydrogen orthophosphate (BDH Chemical Ltd, Poole, England).

The samples were stored at 4 ^{o}C until PPF was analysed, within three weeks of collection, by high performance liquid chromatography (HPLC) using fluorescence detection as described by Plummer (1987). Propofol was extracted from blood following a method described by Plummer in 1987 (Plummer 1987). Spiked samples were used to assess the percentage recovery of PPF from blank blood samples for each assay and to construct a calibration curve, by reference to external standards. The external standard consisted of PPF 2 μgml^{-1} in distilled water. Spiked samples, prepared with 0.5 ml blank blood in Tris buffer and PPF or methanol, contained concentrations of PPF from 0, 0.1, 0.5, 1, 5, 10 μgml^{-1} (S1-S6).

From the other samples, 0.5 ml was pipetted into appropriate tubes. Samples and spikes were then treated identically. In spikes S1-S3, 50 µl of Thymol (1 μgml^{-1}) was added while 50 µl of Thymol (5 μgml^{-1}) was added in S4-S6 and the other samples. Thereafter, 0.5 ml phosphate buffer (0.1 M) and 6 ml cyclohexane were added. The tubes were then mixed for 15-20 min on a slow rotary mixer. Four ml of the cyclohexane were removed from each tube, and placed in fresh tubes containing 50 µl of TMAH (7.5%). The cyclohexane was then evaporated to dryness under a stream of nitrogen in a dri-block (Techne, Cambridge, UK) at room T°. Spikes S1-S3 were reconstituted in 50 and 200 µl of methanol and distilled water respectively. Spikes S4-S6 and samples were resuspended in 100 µl of methanol and 400 µl of distilled water. Chromatography was carried out using a Shimadzu HPLC system (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). The samples were chromatographed on Nemesis column C18 (Phenomenex, Cheshire, UK) connected to a variable wavelength fluorescence spectrophotometer (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). The excitation wavelength of the detector was set at 276 nm and the emission wavelength fixed at 310 nm. The results were integrated on a PC (Reeve Analytical L2700, Glasgow, Scotland). One external standard was injected after the injection of two spikes or samples. The injection volume was 20 µl and the analysis time was about 12 min. The chromatography was always started and finished with the injection of an external standard.

The peak heights resulting from the injection of external standard solutions was used as a reference to calculate the recovery of PPF from the spikes. The recoveries were then used to obtain a calibration curve, from which the concentration of the samples was obtained. The limit of quantification was 10 $ngml^{-1}$. Each sample was analysed in duplicate.
The mean recovery was 90.2%, (± 9.17) with a coefficient of variation of 10.09%.

2.2.6 Data handling

The target and predicted concentrations were noted at each sampling time point. Data were logged by the TCI system and an additional written record kept. The data from each animal were downloaded onto a Microsoft Excel 97 spreadsheet. For each blood sample, the percentage error (PE%) of the predicted concentration of PPF in the blood was calculated as follows, PE% = {(measured concentration – predicted concentration) / predicted concentration} * 100 (Varvel *et al.*, 1992). For each animal, the median prediction error (MDPE%), a measure of bias, and the median absolute prediction error (MDAPE%) a measure of inaccuracy were calculated as follows: MDPE% = median$\{PE_{ij}, j=1,\ldots,N_i\}$, where N_i is the number of PE values obtained for the i^{th} subject; MDAPE% = $\{/PE/_{ij}, j=1,, Ni\}$, where N_i is the number of /PE/ values obtained for the i^{th} subject (Varvel *et al*., 1992; Coetzee *et al*., 1995). Group median MDPE% and MDAPE% values were calculated from the values obtained in individual animals to describe the overall performance of the TCI System (Varvel *et al.*, 1992; Coetzee *et al*., 1995).

For each individual, divergence was calculated as the slope of the linear regression equation of /PE/ against time (Varvel *et al.*, 1992; Coetzee *et al*., 1995; Swinhoe *et al.*, 1998).Wobble was calculated as follows for each subject: in the i^{th} patient., $wobble_i$ = median $\{/PE_{ij} – MDPE_i/, j = 1,\ldots, N_i\}$ (Varvel *et al.*, 1992; Coetzee *et al*., 1995). Divergence and wobble for the population were estimated using the median value for the whole population (n = 16) (Varvel *et al.*, 1992; Coetzee *et al*., 1995).

2.2.7 Statistical analysis

Student t-test was performed to compare the two groups of dogs, mixed-breed and greyhound, for the age, weight, infusion time, volume of PPF, average infusion rate, number of adjustments, extubation time, predicted and actual PPF blood concentration at extubation time, induction target and predicted PPF blood concentration at intubation. A p value < 0.05 was considered as a significant difference. Mean results are presented ± 1 standard deviation. A Kruskall Wallis test was used to compare the 2 groups of dogs mixed-breed and greyhound, for the MDPE% and MAPE%.

2.3 Results

The 16 dogs (10 mixed-breeds and 6 greyhounds) comprised 4 males (3 mixed-breeds and 1 greyhound) and 12 females (7 mixed-breeds and 5 greyhounds). The mixed-breeds were between 2.5 and 10 years old (mean, 7.2 ± 2.4) and the greyhounds between 7 and 12 years old (mean, 8.30

± 1.9). They weighed between 9 and 38 kg (mean, 21.1 ± 9.4) and between 27.5 and 30 kg (mean, 28.8 ± 0.9) respectively (Table 2.1). Although no significant difference was observed between the 2 groups for the age ($p > 0.05$) the mean weight in the greyhound group was greater ($p < 0.05$).

No significant differences were reported for the intubation and extubation times (Table 2.2). Although no significant difference is shown between the groups for the infusion time and the total amount of PPF (Tables 2.2 and 2.3, respectively), mean maintenance and total infusion rate (Table 2.3) were significantly lower in greyhounds ($p = 0.03$).

Although the dose at induction for dogs 1, 2 and 3 was about 2 to 5 times the dose used for the other mixed-breed dogs, no significant difference was observed between this group and the greyhounds for this parameter (Table 2.3).

Mixed-breed	Age (year)	Weight (kg)
1	4	9
2	9	21
3	9	27.5
4	9	15
5	7	14
6	7	31
7	7	13.5
8	2.5	14.5
9	10	38
10	7.5	27
Means	7.2	21.1[a]
SD	2.4	9.4
Greyhound		
11	12	27.5
12	7	28
13	7	29
14	8.5	29
15	8	30
16	7	29
Means	8.3	28.8[a]
SD	1.9	0.9
Total		
Means	7.6	23.9
SD	2.2	8.3

Table 2.1

Age and weight of mixed-breed and greyhound dogs, undergoing dental surgery, anaesthetised with a target controlled infusion system of propofol.

a = significant difference between mixed breed and greyhound dogs ($p = 0.03$).

Mixed-breed	Induction time (min)	Infusion time (min)	Extubation time (min)
1	7	33	nr
2	3	33	10
3	2	27	6
4	2	31	5
5	4	57	10
6	1	51	12
7	3	49	7
8	5	38	7
9	2	35	9
10	5	41	13
Means	3.4	39.5	8.8
SD	1.8	9.8	2.7
Greyhound			
11	4	44	8
12	2	70	11
13	4	75	2
14	3	37	15
15	5	39	7
16	3	36	6
Means	3.5	50.2	8.2
SD	1.0	17.6	4.5
Total			
Means	3.5	43.5	8.5
SD	1.5	13.8	3.4

Table 2.2

Induction, infusion and extubation time (min) in mixed breed dogs and greyhounds undergoing dental surgery and anaesthetised using a target controlled infusion system for propofol.

nr = not recorded

Mixed-breed	Dose of PPF at induction ($mgkg^{-1}$)	Maintenance infusion rate ($mgkg^{-1}min^{-1}$)	Total infusion rate ($mgkg^{-1}min^{-1}$)	Volume of PPF (ml)
1	14.4	nr	nr	nr
2	14.8	nr	nr	nr
3	10.5	nr	nr	nr
4	5.5	0.25	0.41	19
5	4.1	0.31	0.36	28.5
6	2.5	0.27	0.31	49
7	3	0.26	0.30	20
8	3.8	0.3	0.36	20
9	2.8	0.22	0.29	38
10	3.4	0.28	0.33	36
Means	6.5	0.27[a]	0.34[a]	30.1
SD	4.9	0.03	0.04	11.4
Greyhound				
11	2.7	0.24	0.28	33.5
12	2.3	0.19	0.22	43
13	3.7	0.21	0.25	55
14	3	0.28	0.34	36
15	4	0.24	0.31	36
16	3	0.19	0.26	27
Means	3.1	0.22[a]	0.28[a]	38.4
SD	0.6	0.03	0.04	9.6
Total				
Means	5.2	0.25	0.31	33.9
SD	4.1	0.04	0.05	11.1

Table 2.3

The amount of propofol (PPF) needed for induction ($mgkg^{-1}$), the maintenance infusion rate ($mgkg^{-1}min^{-1}$), the total infusion rate ($mgkg^{-1}min^{-1}$) and the total volume of PPF (ml) in greyhounds and mixed-breed dogs undergoing dental surgery and anaesthetised using a target controlled infusion system for PPF.

a = significant difference between mixed breed and greyhound dogs ($p = 0.03$).

nr = not recorded

2.3.1 Optimisation of blood propofol targets

Induction, intubation and average maintenance target values as well as the number of target adjustments made with the TCI during the anaesthesia are reported in Table 2.4. A target concentration of 8 μg ml^{-1} of PPF did not produce conditions satisfactory for intubation in dog 1. At 6 min, the target concentration was increased to 12 μgml^{-1} at which point endotracheal intubation was successful. Thereafter the target was set at 6 μgml^{-1} and altered according to clinical requirement between 6 and 8 μg ml^{-1}. Recovery was smooth and uneventful.

For dog 2 the target concentration was set initially at 15 μgml^{-1} in order to hasten the onset of anaesthesia. However, immediately following the initial bolus of PPF, although induction of anaesthesia was smooth and rapid, apnoea occurred. Following endotracheal intubation the target concentration was decreased to 5 μg ml^{-1} and intermittent positive pressure ventilation (IPPV) was carried out until spontaneous breathing resumed after 9 min. Cardiovascular (CV) parameters were well maintained throughout. Thereafter the target concentration was set at 5 μg ml^{-1} for maintenance of anaesthesia and recovery was smooth.

For dogs 3 and 4, in which initial targets were 12 and 6 μg ml^{-1} respectively, apart from apnoea (for 10 and 17 min, respectively), induction of anaesthesia was satisfactory.

In the remaining dogs, only 1 mixed-breed dog and 2 greyhounds had an initial target concentration lower than 3 μgml^{-1}. Endotracheal intubation in 2 of these 3 animals was unsuccessful by 3 min and the target concentration was increased by 1 increment to 2.5-3 μg ml^{-1}, after which intubation was carried out easily. Apart from dogs 1-4 and dog 13, post induction apnoea was not a feature. Time to induction varied from 1 to 7 min (mean 3.5 ± 1.8). With target concentrations of 3 μgml^{-1}, although induction was slow, excitation was never observed and dogs drifted to sleep in a calm controlled manner. Compared with dogs 1-4, dogs 5-16 were maintained on lower predicted target concentrations throughout the procedure (range 2.5-4.9 μg ml^{-1}) while the measured blood concentration ranged from 1.5 to 6.8 μgml^{-1}. Amongst the 9 dogs which had an induction target of 3 μgml^{-1} (dog 5-9 and 13-16), only 2 had more than 1 target adjustment during the maintenance of anaesthesia, and 4 did not need one at all. Although some muscle twitching occurred in 2 of 6 mixed-breed dogs and 3 of 6 greyhounds, the depth of anaesthesia, although light, was considered satisfactory for the dental procedure. Alteration in depth of anaesthesia was easily achieved when necessary by increasing or decreasing the target concentration in increments of 0.5 μgml^{-1}. Increments of 1.0 μg ml^{-1} (dogs 6 and 7), when used to increase the target concentration, were associated with transient apnoea (10 min for dog 6 and 3 min for dog 7). It was noted that targets in excess of 6.5 μg ml^{-1} were associated with occasional muscle twitching and apnoea following the

small bolus of PPF which followed an increase in target concentration. The mean maintenance target concentration was significantly lower in greyhounds ($p = 0.01$).

2.3.2 Evaluation of the performance of the TCI system

The measured and the predicted blood PPF concentrations for each dog are reported in Appendix 1 and 2 together with PE% values. Measured and predicted blood PPF concentrations at extubation time are shown in Table 2.5.

The group median values for MDPE% and MDAPE% were 1.56% and 24.79% respectively for the mixed-breed dogs, –12.47% and 28.47% for the greyhounds and –3.05% and 27.15% for both groups combined (Table 2.6). No statistical difference (P □ 0.664) was found between the 2 groups for the MDPE% and MDAPE%. The group median values for wobble and divergence were 14.42% and 5.7% h^{-1} for the mixed-breed dogs, 11.2% and 20.7%h^{-1} respectively for the greyhounds and 11.68% and 11.4% h^{-1} for both groups combined.

Examples of results obtained from individual dogs showing the best, the median and worst performance (based on ranked MDAPE% values) are shown in Figures 2.1, 2.2 and 2.3.

Mixed-breed	Induction target (μgml^{-1})	Intubation target (μgml^{-1})	Average maintenance target (μgml^{-1})	Number of adjustments
1	8	12*	6	6
2	15	5	5	1
3	12	6	6	1
4	6	6	5	2
5	3	4	4	4
6	3	3	4	1
7	3	3	4	1
8	3	4	4	0
9	3	3	3	0
10	2.5	3	4	3
Means	5.9	4.9	4.5[a]	1.9
SD	4.5	2.8	1	1.9
Greyhound				
11	2	2.5	3.5	3
12	2.5	2.5	3	1
13	3	3.5	3.5	0
14	3	3	4	3
15	3	3.5	3.5	0
16	3	3	3	1
Means	2.8	3	3.4[a]	1.3
SD	0.4	0.5	0.4	1.4
Total				
Means	4.7	4.2	4.1	1.7
SD	3.8	2.4	1	1.7

Table 2.4

Initial induction target (μgml^{-1}), intubation target, average maintenance target (μgml^{-1}) and number of adjustments in greyhounds and mixed breed dogs undergoing dental surgery and anaesthetised using a target controlled infusion system for propofol.

* dog 1 had its target increased from 8 to 12 μgml^{-1} at 6 min.

a = significant difference between mixed breed and greyhound dogs ($p = 0.01$)

Mixed-breed	Measured blood concentration at extubation (μgml^{-1})	Predicted blood concentration at extubation (μgml^{-1})
1	nr	nr
2	2.8	2.4
3	2.2	2.7
4	2.1	2.1
5	2.1	2.4
6	1.9	1.9
7	2.2	2.2
8	2.2	2.2
9	1.2	1.7
10	2.6	2.6
Means	2.1	2.2
SD	0.5	0.3
Greyhound		
11	1.3	1.9
12	1.5	1.5
13	1.5	2.8
14	2.3	1.4
15	1.5	1.5
16	1.8	1.6
Means	1.6	1.8
SD	0.4	0.5
Total		
Means	1.9	2.1
SD	0.5	0.5

Table 2.5

Measured and predicted propofol (PPF) blood concentration (μgml^{-1}) at time of extubation in dogs anaesthetised with a PPF target controlled infusion system and undergoing dental surgery.

nr = not recorded

Mixed-breed	MDPE%	MDAPE%
1	-32.5	32.5
2	13.67	16.07
3	-19.92	19.92
4	-21.5	22.5
5	5.5	16.5
6	48.06	48.06
7	-16.57	27.08
8	38.81	38.81
9	-2.38	60.83
10	17.42	17.42
Median	1.56	24.79
Range	-32.5 – 48.06	19.92 – 60.83
Greyhound	**MDPE%**	**MDAPE%**
11	-29.72	29.72
12	-21.23	21.23
13	-31.71	31.71
14	62.25	62.25
15	-3.71	12.00
16	19.67	27.22
Median	-12.47	28.47
Range	-31.71 – 62.25	12.00 – 62.25
Total		
Median	-3.05	27.15
Range	-32.5 – 62.25	12.00 – 62.25

Table 2.6

MDPE% (bias) and MDAPE% (inaccuracy) values for each individual, each group and for the whole group.

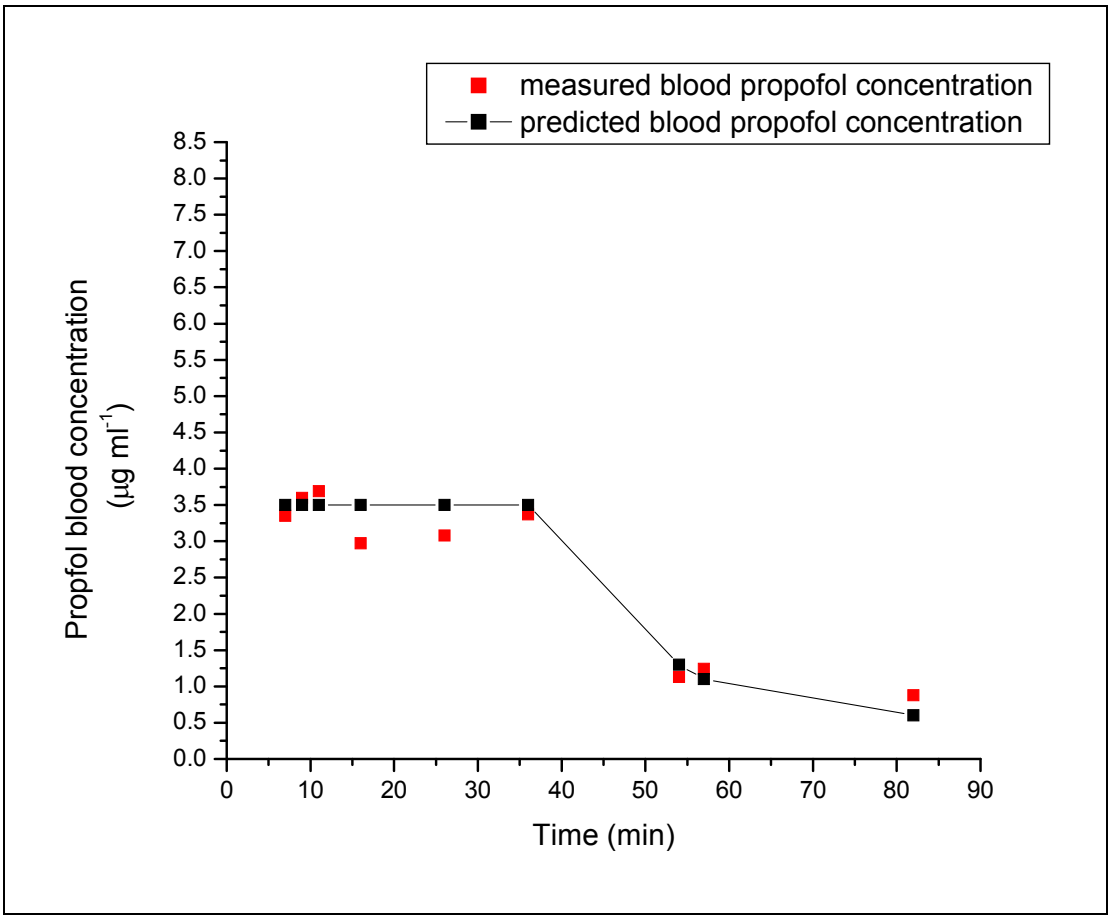


Figure 2. 1

Comparison between measured and predicted propofol blood concentrations in a dog (15) anaesthetised with a propofol target controlled infusion system and undergoing dental surgery, showing the best fit based on ranked MDAPE% (accuracy) values.

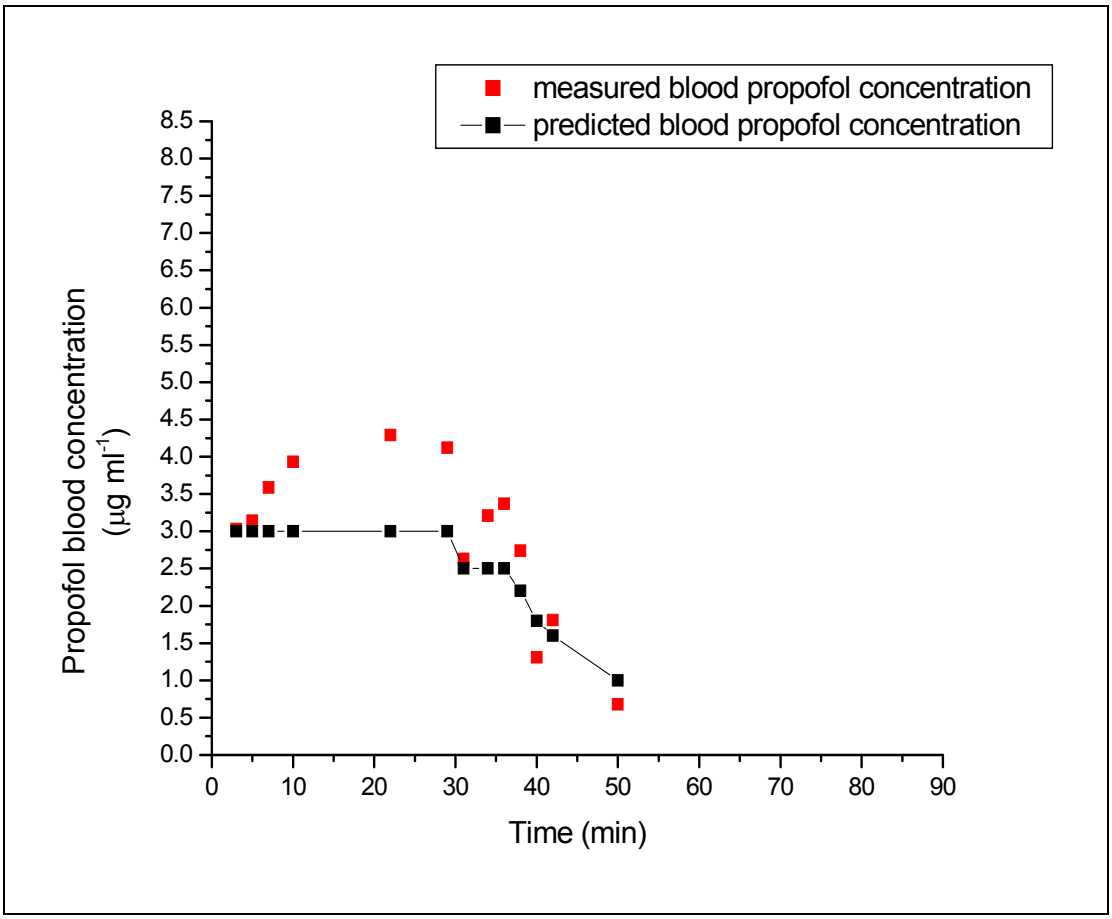


Figure 2.2

Comparison between measured and predicted propofol blood concentrations in a dog (16) anaesthetised with a propofol target controlled infusion system and undergoing dental surgery, showing the median fit based on ranked MDAPE% (accuracy) values.

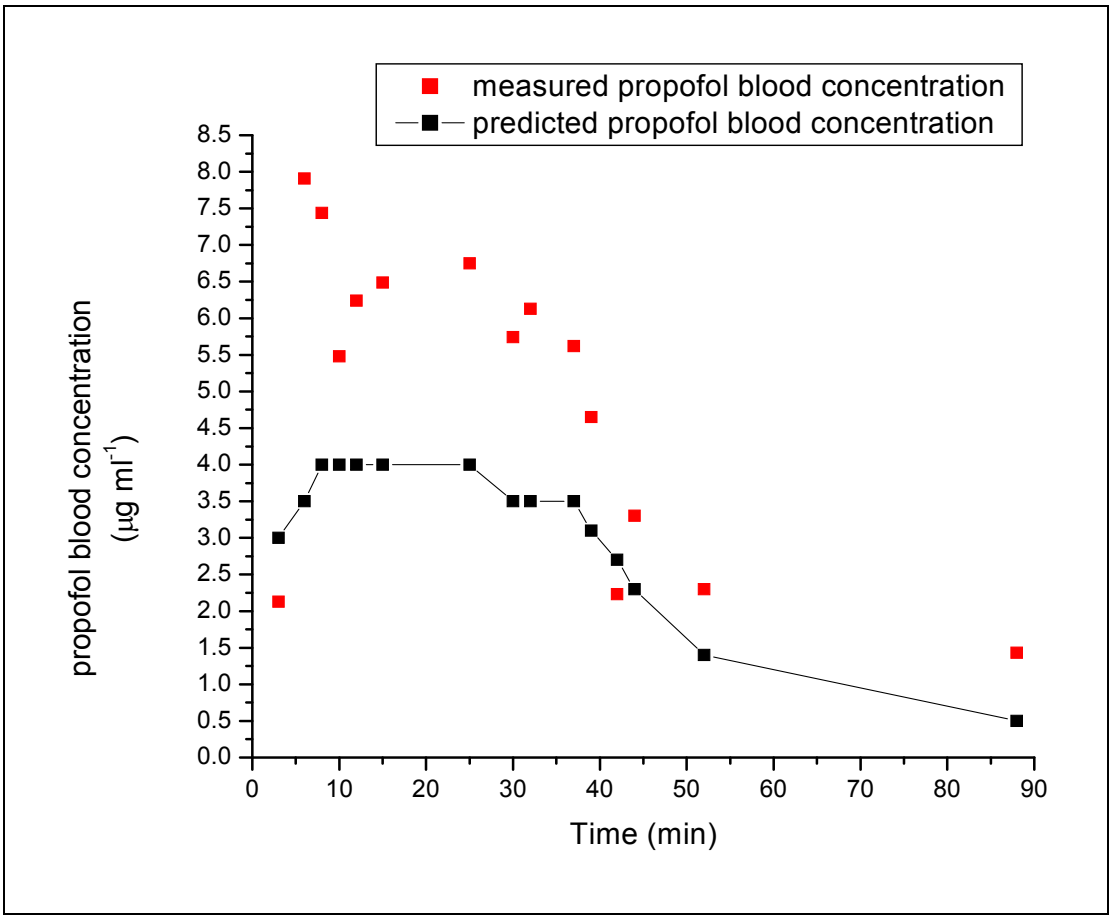


Figure 2.3

Comparison between measured and predicted propofol blood concentrations in a dog (14) anaesthetised with a propofol target controlled infusion system and undergoing dental surgery, showing the worst fit based on ranked MDAPE% (accuracy) values.

2.4 Discussion

2.4.1 Propofol target concentrations for induction

Targets of between 4 and 14 $\mu g ml^{-1}$ have been reported as being necessary for induction of anaesthesia with PPF using a target controlled infusion system in humans (Doyle *et al.*, 1993; Russell *et al*., 1995; Short. *et al.*, 1996). However, the dose of PPF required to induce anaesthesia in healthy, unpremedicated dogs by bolus injection is greater (5.95 $mg kg^{-1}$) (Watkins *et al.*, 1987) than that reported in healthy unpremedicated human patients (2 $mg kg^{-1}$) (Rolly *et al.*, 1980). Although these induction doses looked different, using the PK-SIM simulator software (PK-SIM; Specialised Data Systems, Jenkintown, PA, USA), and the appropriate PK parameters, it was noticed that the plasma concentrations predicted from these induction doses were very similar: 8.7 (man) and 7.7 (dog) $\mu g ml^{-1}$ (see appendix 3 and 4). For that reason and also to minimise the chance of the dogs becoming anxious and struggling should induction of anaesthesia be prolonged, it was decided to use a target of 8 $\mu g ml^{-1}$ for the first dog. This produced an unacceptably prolonged induction time and only after the target was increased by 50% was intubation successful. Thereafter it was necessary to alter the infusion rate six times in order to maintain a satisfactory plane of anaesthesia for a dental procedure which could be classified as a very mild surgical procedure. Although this may have been attributed to inexperience with the use of the technique, retrospectively it might also have resulted from the light sedation observed in this dog after premedication. Moreover, the target concentration during the procedure could not be reduced below 6 $\mu g ml^{-1}$ which also suggests that poor sedation could have contributed to the unsatisfactory anaesthetic conditions produced in this dog.

Based on the unacceptably slow induction in the first dog, the induction target for the second dog was set at 15 $\mu g ml^{-1}$ and although this was followed by very rapid induction of anaesthesia, prolonged apnoea occurred. This was not surprising since apnoea has been recorded as the commonest side effect of induction of anaesthesia with PPF given by intravenous bolus in dogs and cats (Morgan and Legge, 1989). Targets were subsequently decreased progressively in order to find a target which would allow intubation of the trachea, without causing apnoea, and a target of 3 $\mu g ml^{-1}$, used initially in dog 5, seemed to satisfy these criteria. Dogs 5–9 received this target for induction of anaesthesia and the quality of induction was very good. However an attempt to decrease the target further, to 2.5 $\mu g ml^{-1}$ in dog 10, proved unsatisfactory in that intubation could not be performed until the target concentration was increased to 3 $\mu g ml^{-1}$.

It was decided to include a group of greyhound dogs in this study and to reduce the target concentration to 2 $\mu g\ ml^{-1}$ in the first instance to take account of the findings of Robertson *et al.* (1992) and Zoran *et al*. (1993) who reported that all recovery phases following infusion and single

bolus injection of PPF, respectively, were delayed in greyhounds compared with mixed-breed dogs. However, the first dog to receive a target of 2 μg ml^{-1} required an increase to 2.5 μg ml^{-1} before intubation could be performed successfully and accordingly subsequent targets were increased. Four of the six greyhound dogs received targets of 3 μg ml^{-1} and the quality of induction and intubation were remarkably consistent with those of the mixed-breed dogs, which suggested that 3 μg ml^{-1} was the optimum initial concentration for both mixed-breed dogs and greyhounds.

More recently, using the same TCI system for PPF as in this study, Musk and colleagues compared four induction targets (2.5, 3, 3.5 and 4 μgml^{-1}) in 80 dogs premedicated with acepromazine and morphine (Musk *et al.*, 2005). Although clinically, the two higher targets had a 100% success for intubation at 3 min, compared with 80 and 65% for the 3 and 2.5 μgml^{-1} groups respectively, there was no statistical difference between the groups. In the 2 higher target groups, they also observed an increase in the incidence (35 and 45%, respectively) and duration of apnoea (10 and 40% respectively of apnoeic dogs in group at 5 min) as well as a decrease in ABP (post-induction). From the results of the present study and those from Musk and colleagues, it seems that a target of 3 μgml^{-1} is optimal in dogs premedicated with acepromazine (0.03-0.05 mgkg^{-1}) and an opioid.

2.4.2 Induction doses

The induction dose of PPF was not significantly different between the two groups ($p = 0.06$) although there was a tendency towards lower doses in greyhounds. As the first four dogs received a higher dose than any other dogs, and the anaesthetist was unfamiliar with the TCI system, the analysis was repeated with the exclusion of these dogs from the results. This resulted in a mean induction dose (± SD) of 3.3 (± 0.6) mgkg^{-1} for the mixed-breed dogs and a p value between the 2 groups of 0.717. The mean for the induction dose for the two groups together was then 3.2 (± 0.6) instead of 5.5 (± 4.1) mgkg^{-1}.

In studies where the PPF induction followed a premedication similar to the one used in this study (acepromazine and an opioid), a dose between 3.3 and 4 mgkg^{-1} of PPF is necessary to induce anaesthesia in less than one min (Bufalari *et al.*, 1998; Lerche *et al.*, 2000). These infusion doses are very similar to the one described in this study (with the first four cases excluded). The TCI system, although it did not decrease the induction dose of PPF, with a longer induction time of 3.4 (± 1.6) min, decreased the incidence of apnoea: 1/12 in the actual study (5/16 with first four dogs) compared with 4/6 and 6/15 in the studies of Bufalari and Lerche, respectively (Bufalari *et al.*, 1998; Lerche *et al.*, 2000). Although the decrease in the incidence and duration of apnoea with slower injection speed of PPF has also been shown in man (Peacock *et al.*, 1990; Stokes and Hutton, 1991; Passot *et al.*, 2002), a study in dogs by Murison (2001) receiving 4 mgkg^{-1} of PPF

over 4 and 30 sec showed different results: longer injection time increased the incidence as well as the duration of post-intubation apnoea.

Struys *et al.* (1998) demonstrated that the amount of PPF required to induce hypnosis in humans decreased with age and this has also been shown to be the case in the dog. Reid and Nolan (1996) described the PK profile of PPF given as a bolus to induce anaesthesia in non-premedicated geriatric dogs and concluded that these should receive an induction dose of 5 $mgkg^{-1}$ which is less than the manufacturer's recommended dose of 6.5 $mgkg^{-1}$ (Datasheet: Propofol; Abbott Animal Health). These authors defined geriatric dogs as those over eight years, and on that basis six of the sixteen dogs used in this study could be classed as such.

Amongst these 6 dogs, one dog (Dog 11) had an induction target of less than 3 $\mu g\ ml^{-1}$ and two dogs (Dogs 9 and 14) had targets set at 3 $\mu g\ ml^{-1}$. Dog 11 required an increase in target to allow intubation and a target of 3 $\mu g\ ml^{-1}$ produced optimum intubating conditions and a good quality induction in Dogs 9 and 14. It is interesting to note that the remaining three geriatric dogs (Dogs 2, 3 and 4) received the highest induction targets, namely 15, 12 and 6 $\mu g\ ml^{-1}$ respectively, which represented an increase in target by factors of five times, four times and double, respectively, over that recommended as a result of this study. Apart from apnoea, the quality of induction in these dogs was good and CV parameters (HR and mean ABP) were well maintained, thus demonstrating the safety of the TCI system. This would tend to suggest that, while as a general rule, it is safer to reduce the target concentration for induction of anaesthesia in healthy geriatric dogs, this might not be necessary in some cases where the dogs are healthy. However the numbers on which these statements are based were small.

2.4.3 Propofol target concentrations for maintenance

When mean values obtained in the two groups of dogs were compared, the average target concentration used for maintenance was lower in the greyhound group and this was reflected in lower total and maintenance infusion rates in this group.

The overall mean maintenance target of 4.1 ± 1 μgml^{-1} is similar to values seen in humans (Davidson *et al.*, 1993; Taylor *et al.*, 1993), although dogs require more PPF for induction and maintenance of anaesthesia (Watkins *et al.*, 1987). Although PK differences are most likely to be responsible for the greater dose requirement in dogs, the effect of the premedication should also be considered. The use of an opioid analgesic in combination with a sedative/tranquillizer for premedication is common to both medical and veterinary clinical practice, but the choice of sedative component differs. Benzodiazepines which are frequently used in humans, can cause excitement in some animals (Hall and Clarke, 1991b) and as a consequence are used less frequently

than the phenothiazine tranquilliser acepromazine which has a more predictable sedative effect. The combination of acepromazine and either methadone or pethidine could be expected to have produced a moderately profound sedative effect. If these dogs had been premedicated with a combination of a benzodiazepine and methadone or pethidine, which would have produced less sedative effect, then the required target may have been higher. This would be consistent with clinical experience in humans (Chaudhri *et al.*, 1992; Kazama *et al.*, 1998; Olmos *et al*., 2000) and animals (Geel, 1991; Bufalari *et al*., 1998) which has shown that the type of premedication used has a strong influence on the amount of PPF required for induction and maintenance of anaesthesia.

The dogs in this study were all categorised as American Society of Anesthesiologists (ASA) Class 1 and further work will be required to define appropriate PPF target settings in dogs with significant systemic disease. However, since the end of this study, the TCI system developed in this chapter is used frequently in the clinic (Flaherty, personal communication, 2007) for "routine" but also for more demanding cases such as porto-systemic shunt (Musk and Flaherty, 2007).

The inexperience of the anaesthetist with the system resulted in the use of higher induction and maintenance targets in the first four cases. This has also been observed in human patients, where inexperienced anaesthetists (Russell *et al*., 1995; Russell, 1998), although they found the system easy to use compared to a manual technique, tended to use higher targets to induce and or maintain anaesthesia.

The TCI system was found to be easy to use in dogs, principally because of the similarity of use between this equipment and a vaporiser, allowing the depth of anaesthesia to be changed rapidly. Any increase in surgical stimulation will require an increase of the depth of anaesthesia (an increase of the target concentration) and/or an increase in the amount of analgesia provided. In this study, the dental procedure was associated with minimal painful stimulus and consequently where it was considered necessary to increase the depth of anaesthesia this was achieved by increasing the target concentration. However increasing the target in increments of 1.0 $\mu g\ ml^{-1}$ and/or increasing the target to 6.5 $\mu g\ ml^{-1}$ or more was associated with apnoea. Consequently it was decided to limit increases in target to 0.5 $\mu g\ ml^{-1}$ increments and to try to keep maintenance targets below 6 $\mu g\ ml^{-1}$ when spontaneous breathing was desirable.

2.4.4 Maintenance infusion rate

The average maintenance infusion rate of 0.25 $mgkg^{-1}min^{-1}$ with TCI was somewhat less than the rate of 0.4 $mgkg^{-1}min^{-1}$ found by Nolan and Reid (1993) when using a manual infusion system of PPF to anaesthetise a group of beagle dogs. They used a zero order infusion compared with a decreasing order infusion which would normally be used in clinical practice; however the extra PPF

required would be offset by the fact that their dogs might have been less heavily premedicated than the dogs in the present study. Surgical stimulus was skin incision, compared with dental surgery in the present study, and the depth of anaesthesia was similar in both studies. TCI in the dog being more economical in terms of PPF consumption than manual infusion is in contrast to findings in humans, where some early studies have demonstrated a higher consumption of PPF with the target controlled system (Russell *et al*., 1995; Struys *et al*., 1997; Servin, 1998). In these studies, the flexibility of the TCI system appears to have encouraged the use of deeper anaesthesia in the TCI group, despite the fact that the patients were anaesthetised for procedures where minimal surgical stimulus was required. In these studies, TCI system was a new technique for the investigators which might have contributed for a lack of titration downwards as they would have done with manual infusions, mainly towards the end of the procedure (Servin, 1998). More recently, a study in man comparing target versus manually controlled PPF infusion with experienced anaesthetists still showed a higher PPF usage in the TCI group (Breslin *et al.*, 2004), while in inexperienced anaesthetists, no difference was found in the amount administered and blood concentrations of PPF (Rehberg *et al.* 2007).

2.4.5 Side effects

A number of adverse effects have been described for PPF in humans (Scott *et al.* 1988; Bevan, 1993; Nathanson *et al.*, 1996; van den Berg *et al*., 2001) and dogs (Davies, 1991; Smith *et al*., 1993; Smedile *et al.* 1996). These include signs of pain at injection, vomiting during recovery, excitation, paddling, muscle twitching, and opisthotonos. The incidence of side-effects (apnoea not included) with conventional administration of PPF in dogs varies between studies from 7.5% up to 20% (Hall and Chambers, 1987; Morgan and Legge, 1989; Davies, 1991; Smith *et al*., 1993; Zoran *et al.*, 1993; Quandt *et al.*, 1998; Tsai *et al.* 2007). In this study only four dogs (dog 1, 5, 7 and 13) showed some paddling and/or muscle twitching for a short period of time during anaesthetic induction and maintenance or recovery from anaesthesia. No other adverse effects were observed in these dogs. Although this still compares well with the literature, the incidence is high (25%). Although different explanations have been proposed regarding the origin of these movements (Saravanakumar *et al.*, 2005), it is known that they do not originate from the cortex (Borgeat *et al*., 1991). A systematic review of seizure-like activity with PPF in man revealed a predominance of these phenomena during induction, recovery or delayed after anaesthesia (Walder *et al.* 2002). These authors related this observation to 2 issues: PPF plasma concentration is stable during maintenance which implies that the seizure-like activity phenomena tend to occur during changes of blood and brain tissue levels of PPF; during maintenance, the level of consciousness is also stable, resulting in less cerebral excitation that may serve as a promoter for seizure. An interesting observation is that in the actual study, the muscle movements were observed at time when the target PPF plasma concentration was changed, either during the induction and maintenance of

anaesthesia (dog 13) or at the end of the procedure and during the recovery period (dogs 1, 5, 7 and 13).

2.4.6 Extubation time

The different PK observed between greyhounds and other breeds (Robertson *et al.*, 1992; Zoran *et al.*, 1993; Hay-Kraus *et al.*, 2000) might explain why, in the present study, greyhounds received a lower infusion rate (0.28 $mlkg^{-1}min^{-1}$) than the mixed-breed dogs (0.34 $mlkg^{-1}min^{-1}$) since the PPF target was altered in accordance with clinical requirement. The mean measured blood concentration of PPF at extubation was also lower in greyhounds than in mixed-breed dogs. However, the extubation times in the two groups were not significantly different. These results are different from the studies from Robertson and colleagues (1992) and Zoran and colleagues (1993) where the greyhounds had a delayed recovery times compared with the mixed-breed dogs. In the present study, the PPF target was titrated depending on the patient's reaction. As a result, the greyhounds received less PPF than the mixed-breed dogs for a similar infusion time and a similar surgical stimulation. In the studies of Robertson *et al.* (1992) and Zoran *et al.* (1993) the greyhounds and the mixed-breed dogs received the same infusion protocol for PPF. Thus the delayed recovery observed in greyhounds by these authors may have resulted from relative overdosage.

The mean measured PPF concentration at extubation for the two groups combined in the present study was 1.94 (± 0.5) $\mu g\ ml^{-1}$ and is similar to the value of 2.3 $\mu g\ ml^{-1}$ reported by Reid and Nolan (1993) in beagles given a manual infusion.

2.4.7 Evaluation of the performance of the TCI system

Variation of measured concentrations of PPF in blood compared with the target concentration is inevitable during TCI and this is illustrated in Figures 2.1 to 2.3 which demonstrate this in the best, the average and the worst case. The principal source of this error is likely to be related to differences between the PK parameters used to programme the system and the elimination and clearance of PPF in individual animals. In addition, PK variability relating to haemodynamic changes occurring during anaesthesia as a result of the use of adjunctive drugs (Swinhoe *et al*., 1998) may have contributed. In order to evaluate the predictive performance of computer-controlled infusion pumps Varvel *et al.* (1992) described the use of two performance indices, MDPE%, a measure of bias, and the MDAPE%, a measure of inaccuracy. A negative bias indicates that measured values are lower than predicted by the TCI system and a positive bias is associated with measured values greater than predicted. The tendency in the group as a whole was for negative bias (MDPE -3.05%) with a greater figure in the greyhound group (MDPE -12.47%) and a small positive bias in the mixed-breed dogs (MDPE 1.56%) as a consequence of a range of values that demonstrated positive or negative bias in an equal number individual animals.

The rationale for splitting the dogs into a mixed-breed group and a greyhound group was that lower values for total body clearance of PPF have been described in greyhounds compared with mixed-breed dogs (Zoran *et al.*, 1993), and this could affect the performance of the TCI system in this breed. With a reduction in PPF clearance, an increase in positive bias might be expected. In fact there was a trend towards a greater degree of negative bias in the greyhound group although this was statistically insignificant and the accuracy (MDAPE%) for all dogs and for each group taken separately was similar.

The results obtained in the two groups of dogs depend on the choice of the parameters used to programme the TCI system. But for V1 (central compartment) and k_{10} (elimination rate constant), the parameters are the same as those described in beagles (Cockshott *et al*., 1992). Caution in interpreting possible differences between Greyhounds and mixed-bred dogs is also required in view of the smaller number of animals studied and the fact that this was not a randomised comparative study.

In human studies, it has been proposed that the performance of a TCI system can be considered clinically acceptable when the bias (MDPE%) is not greater than +/-10-20% and when the accuracy (MDAPE%) is between 20 to 40% (Schuttler *et al.*, 1988). The median values obtained in our two groups together or taken separately fall within these ranges. Furthermore, this study showed, with the relatively small degree of wobble and divergence observed in the two groups, that the bias and inaccuracy remained fairly constant with time. We conclude that the PK model selected for TCI infusion of PPF in dogs was clinically acceptable in both mixed-breed dogs and greyhounds. However, while an evaluation of predictive performance is important when comparing different systems, measurement of bias and accuracy are of little clinical value when individual PK variation between animals is likely to be a feature. Accordingly there will always be the necessity to titrate the target concentration of PPF to effect in each individual dog.

2.5 Conclusions

The objectives of this study were, firstly, to devise an optimum target concentration regime for induction and maintenance of anaesthesia and, secondly, to assess the predictive performance of the prototype system by comparing the PPF concentrations predicted by the system with the measured PPF concentrations in venous blood samples taken at various time points during anaesthesia. An initial PPF target setting of 3 μgml^{-1} proved an optimum starting point for induction of anaesthesia and maintenance was achieved with target concentrations in the range of 3-6 μgml^{-1}. The system proved acceptable in terms of predictive performance and once it was obvious that lower targets for induction of anaesthesia in the dog were adequate to produce a stable plane of anaesthesia and avoid prolonged apnoea, the anaesthetist quickly became familiar and confident with the technique. The anaesthetist can alter the depth of anaesthesia rapidly and effectively with no apparent adverse effects on the CV or respiratory systems.

The total number of dogs studied with TCI is small and further studies will be required to look in more detail at the influence of breed, age, physical condition, premedication and supplementary analgesic agents on the target PPF concentrations required for particular diagnostic and surgical procedures.

CHAPTER 3:

PHARMACOKINETIC INTERACTION BETWEEN PROPOFOL AND THE ALPHA$_2$-ADRENOCEPTOR AGONIST MEDETOMIDINE AND ITS ENANTIOMERS - *IN VITRO* STUDY IN RAT AND DOG TISSUES

3.1 Introduction

During propofol (PPF) based total intravenous anaesthesia (TIVA), other drugs are commonly co-infused in order to produce satisfactory surgical conditions. Combinations of intravenously administered drugs may result in drug-drug interactions (see chapter 1). Briefly, pharmacokinetic (PK) interactions mostly take place at the level of biotransformation, in the liver (Benet *et al.*, 1996). To study these possible interactions, either *in vitro* or *in vivo* studies can be undertaken. Because of risk, cost and time efficiency as well as offering the advantage of investigating the ability of a drug to inhibit the metabolism of other drugs, *in vitro* studies are preferred (Venkatakrishnan *et al.*, 2003). They require the use of a liver preparation amongst which, sub-cellular fractions, consisting in the main of cytochrome P450 (CYP) microsomal enzymes, offer the advantage of being easy to produce with a high reproducibility (Gibson and Skett, 1994a).

Microsomal preparations have been used to demonstrate that PPF can be a CYP inhibitor in man as well as in animals (Gepts *et al.*, 1988; Janicki *et al.*, 1992; Baker *et al.*, 1993; Correia, 1994; Chen *et al.*, 1995a, 1995b; McKillop *et al.*, 1998; Miller and Park, 1999; Naoya *et al.*, 1999; Gemayel *et al.*, 2001; Lejus *et al.*, 2002; Inomata *et al.*, 2003; Yang *et al.*, 2003; Osaka *et al.*, 2006). It has also been demonstrated that the metabolism of PPF can be either enhanced by some drugs such as ketamine (Chan *et al.*, 2006) or inhibited by agents such as fentanyl, ketoprofen, enalapril, oxazepam and chloramphenicol in man (Le Guellec *et al.*, 1995) and 1-amynopyrine (Correia, 1994) or PPF itself (Yamazaki *et al.*, 2006) in rats.

Medetomidine (MED) and its enantiomers dexmedetomidine (DEX) and levomedetomidine (LEV) are imidazole derivatives and have been reported as highly potent CYP inhibitors during *in vitro* and *in vivo* studies in man with alfentanil (Kharasch *et al.*, 1991) and ketamine (Kharasch *et al.*, 1992) metabolism as well as in rats with different substances (Pelkonen *et al.*, 1991) and more recently in fish (Lennquist *et al.*, 2008). In human tissues, the highly inhibitory effects of MED and its enantiomers have been demonstrated on a specific CYP, CYP2D6 (Rodrigues and Roberts, 1997).

In microsomal preparations from rats, Correia (1994) reported the inhibitory effect PPF and amiopirine had on each other illustrating either true competitive inhibition or feedback inhibition. Medetomidine and its enantiomers are both potential CYP inhibitors. To the author's knowledge, no studies have yet described the interaction between PPF and the racemate MED or its enantiomers, DEX and LEV, in any species. The potential effect of these alpha$_2$-adrenoceptor agonists on the metabolism and PK profile of PPF is of particular relevance since the predictive performance of the target controlled infusion (TCI) system for PPF is dependent on the accuracy of the PK parameters used to programme the infusion pump. Consequently, it was considered relevant

and timely to study the potential for metabolic interaction between MED and PPF, which might influence their administration when used for TIVA *in vivo*.

3.1.1 Goal of the study

To study *in vitro* the possible inhibitory effect of MED and its enantiomers on canine and rat hepatic cytochrome P450.

3.2 Materials and Methods

3.2.1 Animals

Ten healthy adult male Wistar rats, weighing about 400-450 grams, were used for the preparation of hepatic microsomes. Canine hepatic microsomes were prepared from eight healthy beagles (four males and four females), aged 1-3 years and weighing around 20 kg, which had been euthanized using lethal injection (pentobarbitone sodium). These rats and dogs were not killed for the purpose of this study, but were control animals from other ongoing investigations.

3.2.2 Isolation of hepatic microsomes

3.2.2.1 Rats

Following the methods described by Rutten *et al*. (1987) and Correia (1994) for the isolation of the microsomes, the rats were killed by cervical dislocation. After opening of the body cavity, the portal vein was catheterised and ice-cold normal saline was infused. Thereafter, the liver was removed, detached from the abdominal cavity and set free from any remaining connective tissue. Livers were weighed, sliced and then homogenised in a volume of KCl 1.15% equivalent to 3 times their weight in a Potter-Elvehjem glass Teflon homogeniser (6 complete passes with the pestle). The liver homogenate was then centrifuged at 9000g for 20 min (Beckman J2-21 Centrifuge). The floating fat layer was removed using a Pasteur pipette. The supernatant was decanted in Beckman Ultra-Clear tubes (California, USA). Following centrifugation at 10,500g for 75 min (Beckman L8-70M Ultracentrifuge), the supernatant (cytosolic fraction) was then discarded and the precipitate (microsomal pellets) was resuspended using 15 ml of Tris Buffer (0.1 M; pH 7.4) containing glycerol 20% (v:v), using an Ultra-turrax (Janke and Kunkel GmbH and Co, Germany). The suspension was divided in aliquots of 1 ml and stored at -70˚C until the incubation assays were performed.

3.2.2.2 Dogs

A piece of the liver was removed (about 300 grams), washed in ice-cold saline and treated as described for the rats. As the dogs were not euthanized on site, the preparation of the canine hepatic samples was delayed by one hour, the time necessary for the transportation of the liver slices. During that time, the samples were stored in dry ice.

All chemicals were purchased from Sigma-Aldrich Company Ltd, Poole, Dorset, England, unless stated otherwise. Potassium Chloride (KCl) 1.15% was made in distilled water. A 0.1 M Tris Buffer solution in glycerol 20% (v:v) with a pH of 7.4 was prepared from a mixture of Trizma® Base and Trizma® Hydrochloride.

All the instruments (tubes, homogeniser, chopping knife, and the centrifuge rotors) and solutions used for the preparation of the microsomes were stored in melting ice (0˚C) for about 30 min before use.

3.2.2.3 Protein content

The protein content of the microsome preparations was determined using the Coomassie Blue protein assay reagent calibration curve, calculated before each essay using BSA (Bovine Serum Albumin). Using this curve, the protein content of diluted (x 5000) microsomal suspensions was determined and the result corrected according to the dilution factor, in order to calculate the protein concentration in the original samples. Each measurement was performed in duplicate.
The mean (± SD) protein concentrations in the rat and canine hepatic microsomal preparations were 18.88 (± 1.55) and 17.89 (± 4.88) $mgml^{-1}$, respectively.

3.2.3 Propofol study

3.2.3.1 Incubation mixture

All solutions and ingredients were stored at 4 ˚C.

Cytochrome activation is dependent on the presence of NADPH (nicotinamide adenine dinucleotide phosphate) throughout the whole incubation period (Gibson and Skett, 1994b). NADPH was obtained by mixing $NADP^+$ (1 mM), β-Nicotinamide (0.5 M), Isocitrate Dehydrogenase (0.2 units ml^{-1}), Mg Cl (0.15M) and Trisodium isocitrate (15m M), made up to a volume of 20 ml by adding Tris Buffer (0.1 M; pH 7.4) containing glycerol 20% (v:v). This incubation mixture was prepared before each assay except for the addition of $NADP^+$ which was added immediately before the start of the incubation, to avoid early breakdown and disappearance of the freshly generated NADPH. Each assay used 1 ml of solution.

Inactivated microsomes were also prepared by heating (70-100 ˚C) the microsomal samples for 10 min (Correia, 1994).

3.2.3.2 Time course pilot study

A pilot study was undertaken to evaluate the time course of PPF degradation by hepatic microsomes from the rat and the dog, using microsome samples from six animals of each species.

Propofol degradation was investigated using six incubation times (0, 5, 10, 15, 30 and 45 min) and four different doses of PPF (2.5, 5, 7.5 and 10 $\mu g ml^{-1}$) in each microsomal preparation. Microsomes were diluted to a working protein concentration of 0.5 $mg ml^{-1}$ (Guitton *et al.*, 1998). For each dose of PPF and each animal, seven tubes in duplicate were prepared. These consisted of one tube with PPF and inactive microsomes and six tubes with PPF and active microsomes (6 incubation times). The tube with the inactive microsomes was incubated for 45 min. to assess any extra microsomal drug degradation and drug stability.

All the tubes consisted of PPF in methanol (volume between 100 and 200 µl), co-factor solution microsomes (1 ml), made up to a final volume of 1.5 ml with Tris buffer. After an incubation period of 2 min, activated or inactivated microsomes (0.5 ml) were added (final incubation volume 2 ml). Incubations were performed aerobically at 37˚C in a shaking water bath (Grant Instruments Ltd, Cambridge) with a shaking speed of 120 cycles min^{-1}. Enzyme activity was stopped by putting the samples in iced water for 5 min. Thereafter the mixtures were transferred to 10 ml reagent free plastic tubes (Sarstedt Ltd, Leicester, UK) and stored a -20˚C until drug analysis was performed, usually within one week of incubation.

3.2.3.3 Study of the effect of medetomidine and its enantiomers on propofol metabolism in rat and dog microsomes.

Standard solutions (1000 $\mu g ml^{-1}$) of MED, DEX and LEV (Orion Corporation, Orion PHARMA ANIMAL HEALTH, Turku, Finland) were prepared in distilled water and stored at 4˚C. For this study, a concentration of PPF of 2.5 $\mu g ml^{-1}$ was used. This study was performed in rat microsomal preparations (n = 6) and dog microsomal preparations (n = 6).

In the rat for MED, DEX and LEV and in the dog for MED, ten samples in duplicate were studied (PPF and inactivated cytochrome, PPF and active cytochrome with 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µM of either MED, DEX or LEV). In the dog, for DEX and LEV, an additional sample was added: 0.001 µM. The preparation and the incubation methods were the same as described for the pilot study. The incubation time was 15 min. The volume of MED, DEX and LEV added to the tubes varied between 50 and 140 µl.

3.2.3.4 Propofol analysis.

Propofol was analysed by high performance liquid chromatography (HPLC) with fluorescence detection as described in Chapter 2 (2.2.5).
The mean (± SD) percentage recovery of PPF from microsome standards (0.5-10 μgml^{-1}) was 90.14 ± 8.3% with a coefficient of variation of 9.17%.

Extraction of PPF from spikes and samples
Propofol was extracted from microsomes following the method described by Plummer (1987) (See 2.2.5).

3.2.4 Medetomidine, dex- and levomedetomidine analysis

The MED, DEX and LEV stock solutions were analysed regularly to ensure the stability of the solutions. This was done using high performance liquid chromatography (HPLC) with UV detection according to the method described by Őrn *et al.* (1990).

Chromatography was carried out using a Shimadzu HPLC system (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). The samples were chromatographed on a chiral AGP column (Chrom Tech, Sweden) connected to a UV spectrophotometer (Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan). The excitation wavelength of the detector was set at 220 nm. The mobile phase consisted in a mixture of a phosphate buffer (0.03 M, pH 5.1) and Acetonitrile (AcCN, 100%) at a ratio of 0.9/0.1. The results were integrated on a PC (Reeve Analytical L2700, Glasgow, Scotland).

3.2.5 Statistical analyses

IC_{50} (the concentration of MED, DEX or LEV inducing 50% of the maximum inhibition) and IC_{Max} (the concentration of MED, DEX or LEV inducing maximum inhibition) were calculated for PPF metabolism fitting a curve to the data using a logistical equation (Origin 6.1; Microcal Software, Inc., Northampton, USA.):

$$Y = \frac{A1-A2}{1 + (X / X_0)^p} + A2$$

Where x_0 is the centre
p is the power
A1 is the initial Y value
A2 is the final Y value
The Y value at X_0 is half way between the two limiting values A1 and A2:
$Y(X_0) = (A1 + A2)/2$

Statistical analysis consisted in repeated measures ANOVA (General Linear Model) for dose effect in each group as well as to compare IC_{50} values between MED, DEX and LEV in each species. Differences were considered significant when $p < 0.05$. One way ANOVA was used for comparison of IC_{50} values between MED, DEX and LEV between species.

3.3 Results

3.3.1 Pilot study: Effect of propofol concentration on rat and canine hepatic microsomal activity

Metabolism of PPF was rapid and was faster in the dog than in the rat. In rats, 17, 26, 19 and 32% of PPF (10, 7.5, 5 and 2.5 μgml^{-1}, respectively) was broken down after 15 min incubation. In dogs, 21, 28, 26 and 63% of PPF (10, 7.5, 5 and 2.5 μgml^{-1}, respectively) was eliminated after 15 min incubation. For both species, the speed of metabolism was inversely proportional to the initial PPF concentration (Figures 3.1 and 3.2) and the 2.5 μgml^{-1} was selected as the working PPF concentration for the dose effect study. Fifteen min incubation time was chosen for the dose-response study (see 3.3.2) as by that time a fair amount (20 – 77%) of the PPF (2.5 μgml^{-1}) had already disappeared.

3.3.2 Effect of medetomidine, dex- and levomedetomidine on rat and canine hepatic microsomal activity

The addition of MED, DEX or LEV to PPF (incubated) with rat and canine hepatic microsomes resulted in a dose-dependent inhibition of PPF metabolism (Figures 3.3 to 3.6). Propofol working solution was 2.5 μgml^{-1} and the incubation time 15 min.

In rat and dog hepatic microsomes, doses as low as 0.023, 0.07 and 0.023 μgml^{-1} of MED, DEX and LEV, respectively, induced a significant inhibition ($p < 0.05$) of PPF degradation. The IC_{50} (50% of the maximum inhibitory concentration) for MED and its enantiomers in the two species are shown in Table 3.1.

In rat hepatic microsomes, the only significant difference in the IC_{50} occurred with DEX which was higher than LEV ($p = 0.029$). The difference between MED and DEX almost reached significance ($p = 0.067$). In dog hepatic microsomes, the IC_{50} was significantly higher with DEX than with MED and LEV ($p \leq 0.002$). No significant difference was observed ($p > 0.05$) in the IC_{50} values, when comparing MED, DEX and LEV between the two species,

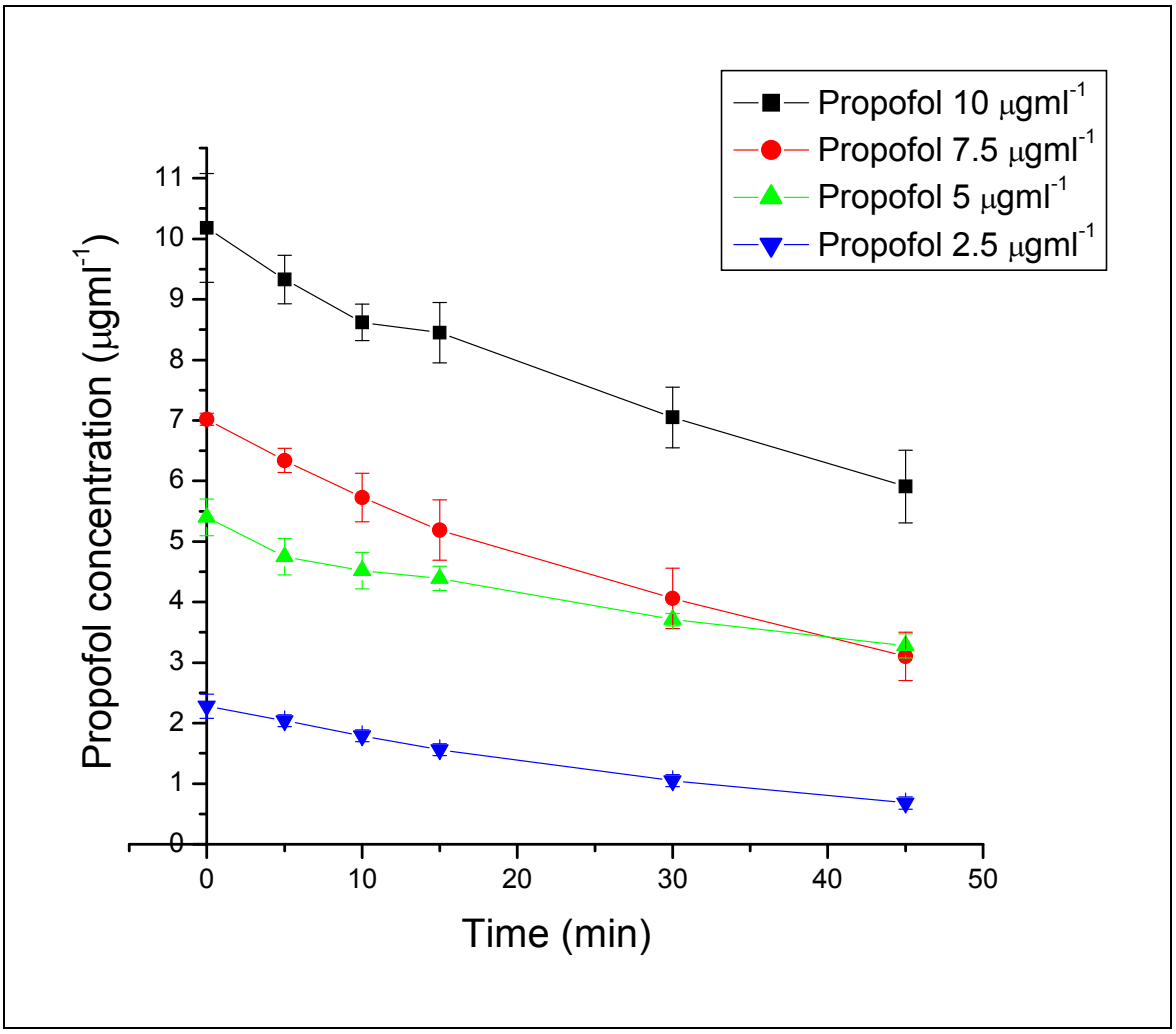


Figure 3.1

Degradation of propofol (PPF) concentrations (10, 7.5, 5 and 2.5 μgml^{-1}) over time in rat hepatic microsomes. At each time point, the mean value of two propofol determinations was calculated. Each point is the mean (± SD) PPF concentration from microsomes of 6 rats.

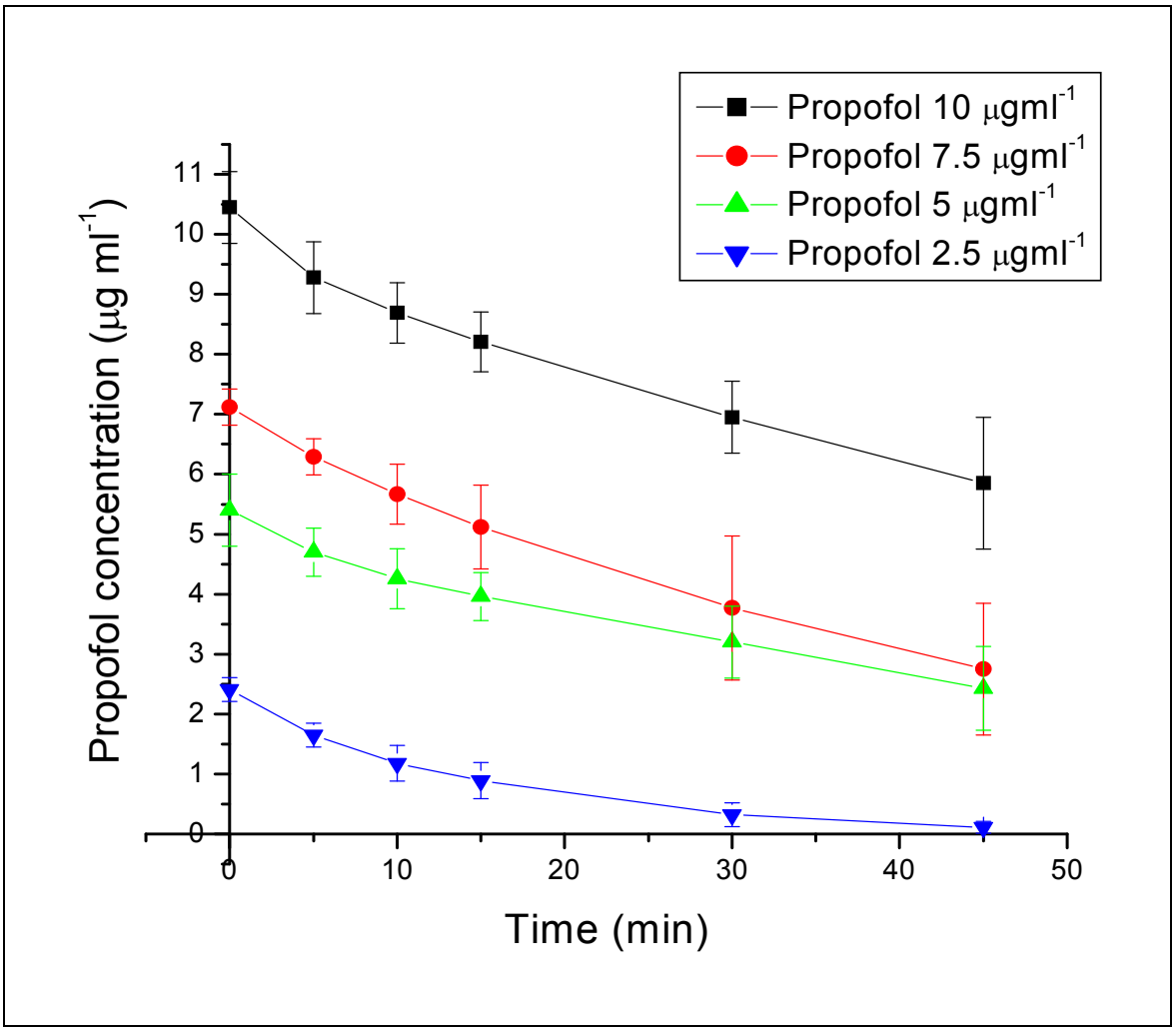


Figure 3.2

Degradation of propofol (PPF) concentrations (10, 7.5, 5 and 2.5 μgml^{-1}) over time in dog hepatic microsomes. At each time point, the mean value of two PPF determinations was calculated. Each point is the mean (± SD) PPF concentration from microsomes of 6 dogs.

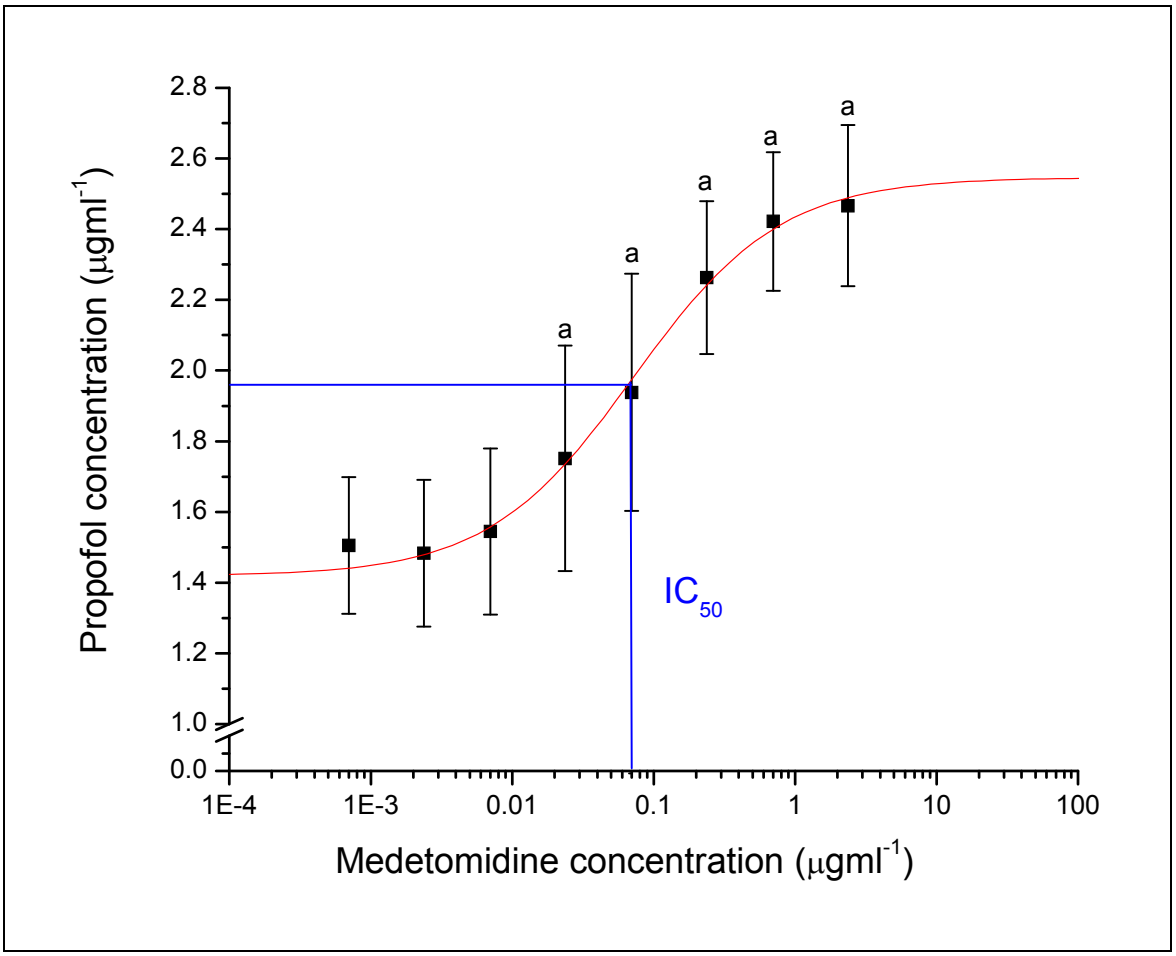


Figure 3.3

The effect of medetomidine (µgml^{-1}) on propofol (PPF) (2.5 µgml^{-1}) metabolism in rat (n = 6) hepatic microsomes over an incubation period of 15 min. IC_{50} = 0.072 (± 0.017) µgml^{-1}. Each point is the mean (± SD) PPF concentration from microsomes of 6 rats.

IC_{50} = concentration of drug necessary to inhibit PPF metabolism by 50% (as predicted by the equation, using Origin 6.1; Microcal Software, Inc., Northampton, USA).

a = significant difference from the control sample ($p \leq 0.0006$).

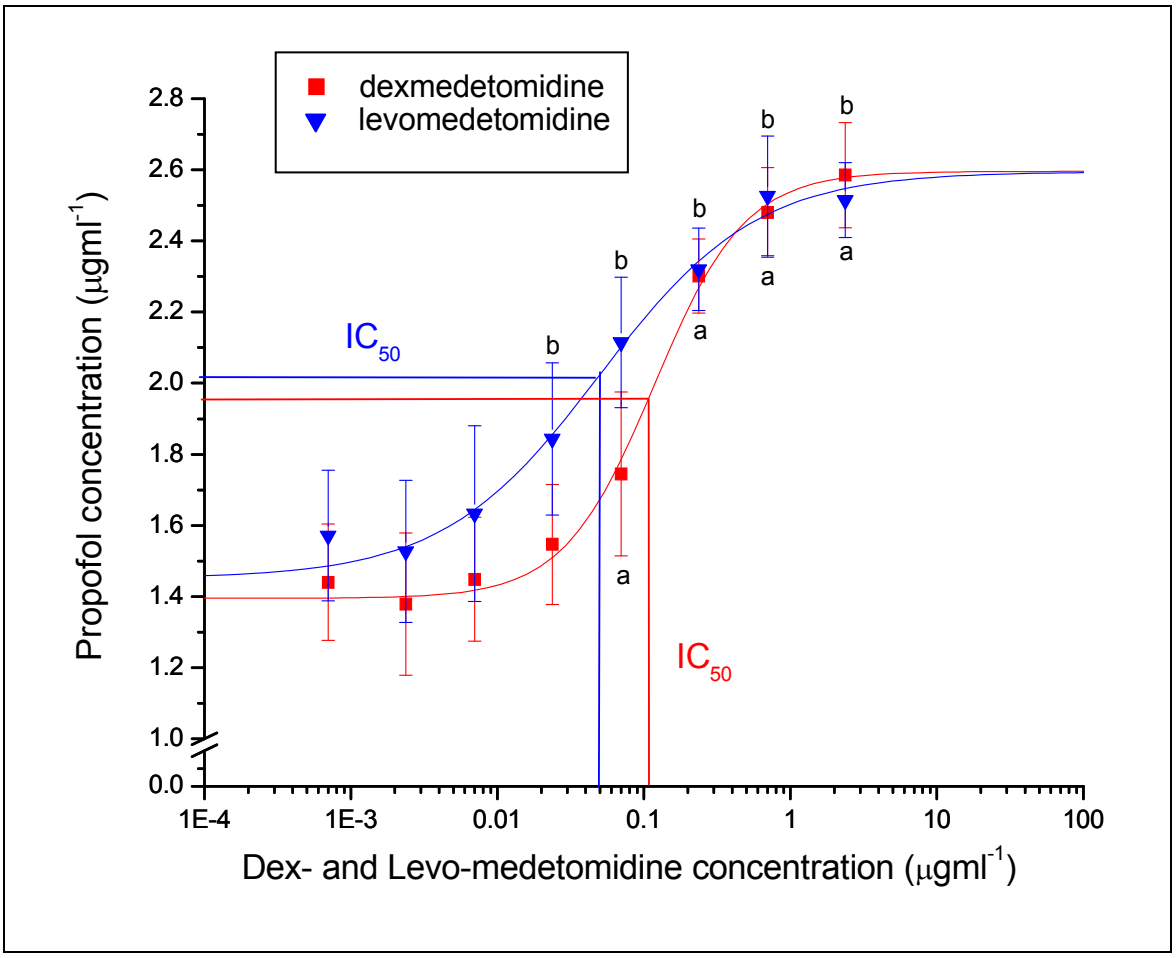


Figure 3.4

The effect of dexmedetomidine (DEX) and levomedetomidine (LEV) (µgml^{-1}) on propofol (PPF) (2.5 µgml^{-1}) metabolism in rat (n = 6) hepatic microsomes over an incubation period of 15 min. DEX and LEV IC_{50} = 0.11 (± 0.016) and 0.05 (± 0.013) µgml^{-1}, respectively. Each point is the mean (± SD) PPF concentration from microsomes of 6 rats.

IC_{50} = concentration of drug necessary to inhibit PPF metabolism by 50% (as predicted by the equation, using Origin 6.1; Microcal Software, Inc., Northampton, USA).

a = significant difference from the control sample for DEX ($p \leq 0.0036$).

b = significant difference from the control sample for LEV ($p \leq 0.0001$).

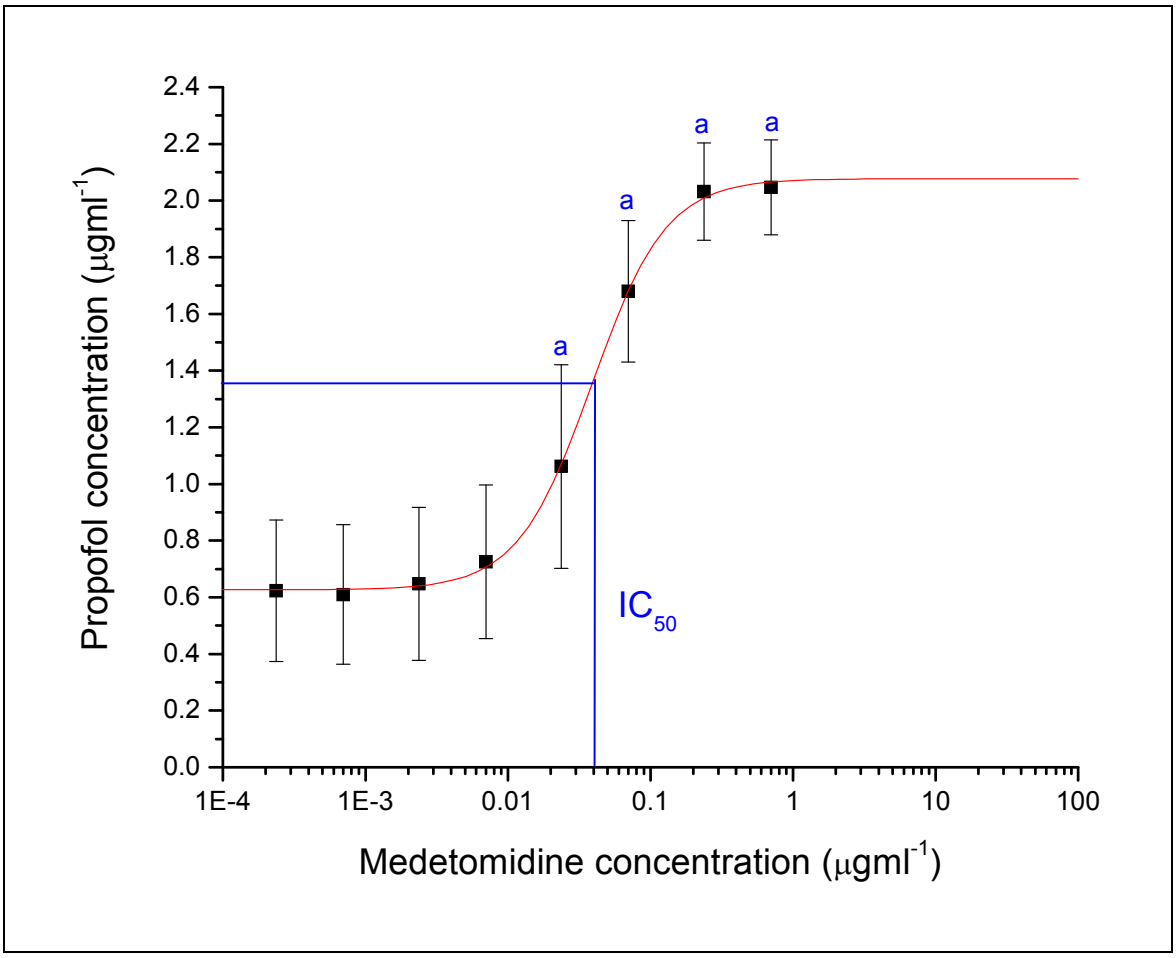


Figure 3.5

The effect of medetomidine (µgml^{-1}) on propofol (PPF) (2.5 µgml^{-1}) metabolism in dog (n = 6) hepatic microsomes over an incubation period of 15 min. IC_{50} = 0.04 (± 0.001) µgml^{-1}. Each point is the mean (± SD) PPF concentration from microsomes of 6 dogs

IC_{50} = concentration of drug necessary to inhibit PPF metabolism by 50% (as predicted by the equation, using Origin 6.1; Microcal Software, Inc., Northampton, USA).

a = significant difference from the control sample ($p \leq 0.002$).

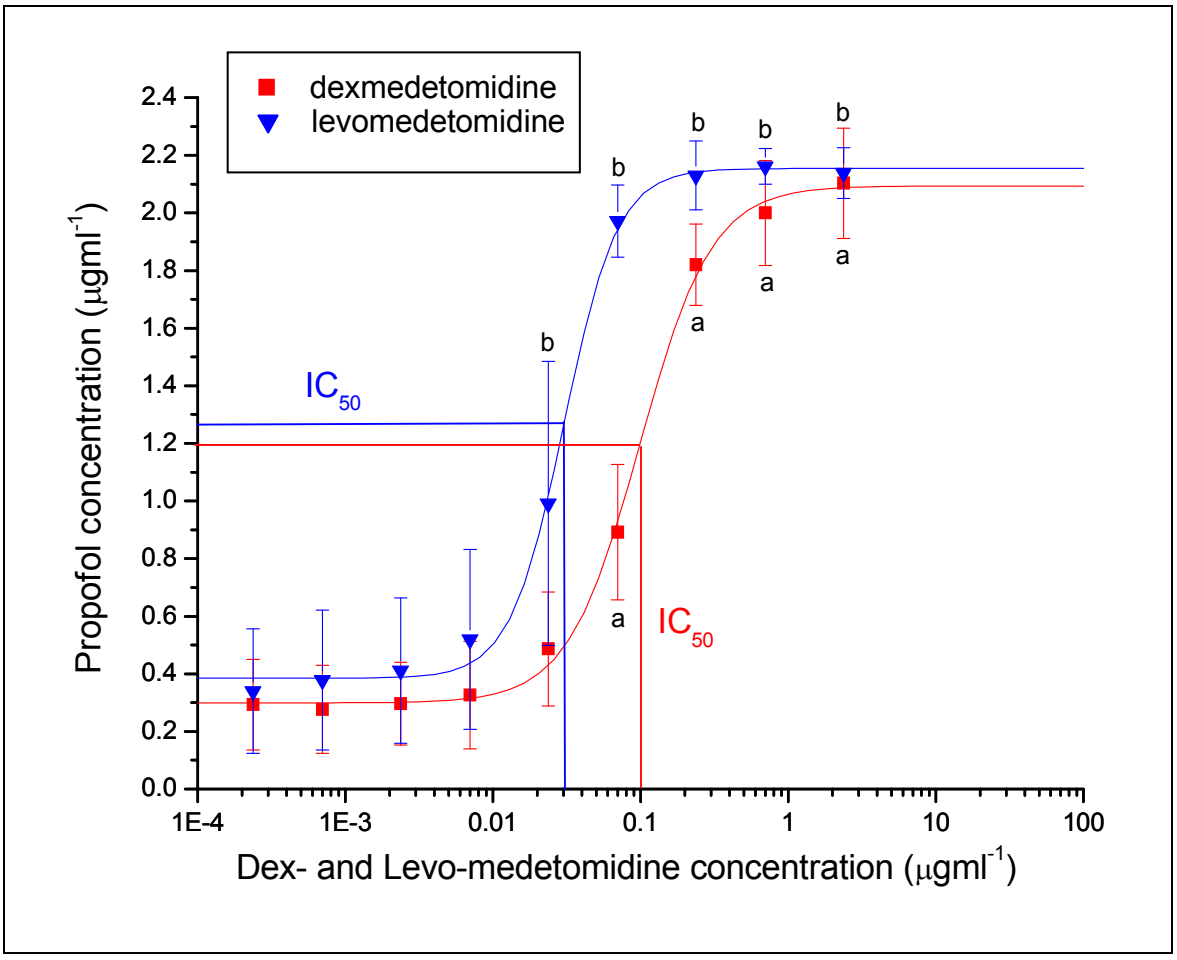


Figure 3.6

The effect of dexmedetomidine (DEX) and levomedetomidine (LEV) (μgml^{-1}) on propofol (PPF) (2.5 μgml^{-1}) metabolism in dog (n = 6) hepatic microsomes over an incubation period of 15 min. DEX and LEV IC_{50} = 0.1 (± 0.005) and 0.03 (± 0.002) μgml^{-1}, respectively. Each point is the mean (± SD) PPF concentration from microsomes of 6 dogs.

IC_{50} = concentration of drug necessary to inhibit PPF metabolism by 50% (as predicted by the equation, using Origin 6.1; Microcal Software, Inc., Northampton, USA).

a = significant difference from the control sample for DEX ($p \leq 0.0001$).

b = significant difference from the control sample for LEV ($p \leq 0.0001$).

		MED	DEX	LEV
Rat hepatic microsomes	IC_{50}	0.072 μgml^{-1} (0.3 μM)	0.11 μgml^{-1} (0.5 μM)	0.05[a] μgml^{-1} (0.21 μM)
Dog hepatic microsomes	IC_{50}	0.04[b] μgml^{-1} (0.17 μM)	0.1 μgml^{-1} (0.41 μM)	0.03[b] μgml^{-1} (0.13 μM)

Table 3.1

Comparison of IC_{50} for medetomidine (MED) and enantiomers (μgml^{-1} (and μM)) on propofol (PPF) (2.5 μgml^{-1}) metabolism in rat (n = 6) and dog (n = 6) hepatic microsomes.

IC_{50} = concentration of drug necessary to inhibit PPF metabolism by 50% (as predicted by the equation, using Origin 6.1; Microcal Software, Inc., Northampton, USA).

a = significant difference in rat hepatic microsomes from DEX ($p = 0.029$).

b = significant difference in dog hepatic microsomes from DEX ($p \leq 0.002$).

DEX = dexmedetomidine

LEV = levomedetomidine

3.4 Discussion

In the dog and man, PPF is metabolised principally by glucuronidation but also in part through CYP dependent phase 1 biotransformation (Guitton *et al*., 1998; Court *et al.*, 1999); hence the choice of microsomal preparations for this study. Microsomal preparations are simple to produce and maintain their quality for up to one year if stored at –80°C (Court, personal communication, 2002) and in this study the prepared microsomes were stored at –80°C and used within six months. However, the initial quality of the enzymes can be affected by several factors, including the presence of underlying illness in the donor and the cause of death, as well as the time between harvesting and freezing (Venkatakrishnan *et al.*, 2003).

Both rat and dog hepatic microsomes used in this study were from healthy control animals being used in another study, and were killed either by neck dislocation (rats) or with a single injection of pentobarbitone in case of the dogs. Although hepatic CYP microsomal enzymes have been induced by the chronic exposition to pentobarbital in humans (von Bahr *et al*., 1998) and in dogs (Kawalek *et al*., 2003), it is unlikely that a single injection of the pentobarbitone administered just before death would have any effect on the CYP of the dog hepatic microsomes used in this study. However, because the dogs were not killed on site, there was an inevitable delay between harvesting the dog liver samples and freezing the microsomal preparation, but this was kept to a minimum and the liver samples transported in dry ice to limit post-mortem change.

In the pilot study, PPF concentrations of 2.5, 5, 7.5 and 10 μgml^{-1} were investigated as they reflect a range of blood PPF concentrations that could be expected during the clinical use of a PPF-based TIVA technique. This was considered important because the use of supratherapeutic drug concentrations may produce drug interactions *in vitro* which would not rise *in vivo* (Chen *et al.*, 1995b).

The degradation of PPF was faster when the lowest concentration of drug (2.5 μgml^{-1}) was used, compared with those that were higher. It is possible that the lower concentration allowed a better contact with CYP enzymes and consequently faster degradation. Alternatively, since PPF itself has been shown to inhibit CYP, the higher concentrations of PPF might have decreased the enzymatic activity. In 1994, Correia showed the inhibitory effect of increasing concentrations of PPF (5-100 μgml^{-1}) on the rat hepatic microsomal enzymatic system responsible for the N-demethylation of amiopyrine. Other studies reported the inhibitory effects on the CYP for different substrates by PPF concentrations of 1-100 μgml^{-1}, 10-50 μgml^{-1}, 1-400 μgml^{-1} in rats, hamsters and humans respectively (Baker *et al.*, 1993; Chen *et al*., 1995b; McKillop *et al.*, 1998; Inomata *et al.*, 2003; Osaka *et al.*, 2006). More specifically it has been demonstrated that, in humans, the IC_{50} for PPF inhibition *in vitro* of CYP1A2, CYP2C9, CYP2D6 and CYP3A4 is 7.1, 8.75, 38 and 2.75 μgml^{-1}, respectively (McKillop *et al.*, 1998; Yang *et al.*, 2003) and of these CYP1A2 at least has been

identified as being present in the dog (The International Centre for Genetic Engineering and Biotechnology, www.icgeb.trieste.it/, 2001). Propofol concentrations of 5-10 μgml^{-1} used in this study fall within the ranges that have been shown to cause CYP inhibition in other species which lends weight to the hypothesis that CYP inhibition by PPF may have caused the decrease in degradation of PPF seen at these higher concentrations. Yamazaki and colleagues (2006) reported the inhibitory effect of PPF on its own metabolism in rat, through an action on CYP 2C11 liver microsomes.

Accordingly, PPF, 2.5 μgml^{-1} seemed the most appropriate concentration to use in the investigation of possible interaction between PPF and MED, DEX and LEV. This is slightly lower than the range of PPF blood concentrations that has been reported as necessary to induce and maintain anaesthesia in the dog, namely 3.31 to 13.79 μgml^{-1} (Simons *et al*., 1991a; Nolan and Reid, 1993; Hall *et al*., 1994; Mandsager *et al*., 1995). However, the concentration of PPF required to maintain anaesthesia depends on a variety of factors including the choice of drugs used for premedication (see Chapter 2). In Chapter 2, the PPF blood target concentration required to maintain anaesthesia during dental when the dogs were premedicated with a mixture of acepromazine and methadone or pethidine was around 4 μgml^{-1} (4.2 μgml^{-1} actual PPF blood concentration). However, MED premedication causes more sedation than acepromazine/methadone or acepromazine/pethidine and has been shown to decrease PPF induction and maintenance doses by 50 to 75% (Cullen and Reynoldson, 1993). Consequently, a PPF concentration of 2.5 μgml^{-1} was considered to be a reasonable estimate of what might be required in dogs undergoing mild to moderate surgery when anaesthesia was maintained with a TIVA technique combining PPF and either MED or DEX infusions.

Unfortunately, no information exists regarding the correlation between PPF plasma concentration and the concentration at the site of the enzyme system, namely the liver. In rats, it has been shown that the hepatic concentration was up to four times greater than the plasma concentration for the first 15 min after bolus injection and equal thereafter (Simons *et al*., 1991b). Although it could be argued that PPF, which is 98% bound to plasma protein, might be less concentrated in the liver, Servin and colleagues (1988) showed that the extent of protein binding was unlikely to influence the metabolism of a drug with high hepatic extraction such as PPF.

Although the speed of PPF metabolism can vary between species and even between breeds (Correia, 1994; Court *et al*., 2001), in this study, with the exception of when the initial PPF concentration was 2.5 μgml^{-1}, the amount of PPF metabolised at 15 min (Figures 3.1 and 3.2) seems to be the same in rat and in dog hepatic microsomes. However, with an initial concentration of 2.5 μgml^{-1}, more PPF was metabolised in dog (63%) than in rat (32%) hepatic microsomes at 15 min. Since the purpose of this work was to investigate infusion schemes in the dog, an incubation time of 15 min was chosen because a substantial percentage of the PPF was metabolised by that

time when the concentration was 2.5 μgml^{-1}. Other studies have used similar incubation times: 10 min in dog and human tissues (Court *et al.*, 1999; Hay Kraus *et al*., 2000; Court *et al*., 2001) and 15 min in rat and sheep tissues (Correia, 1994).

To estimate the speed of enzymatic reaction, the simplistic enzyme kinetic model of Michaelis-Menten can be used (Venkatakrishnan *et al.*, 2003). This model is characterised by a hyperbolic concentration (substrate)-velocity (rate of transformation) function. However the present study was not designed to estimate Vmax (the velocity) and Km (the Michaelis-Menten constant) and therefore a difference in the speed of metabolism for PPF between the two species could not be confirmed. Studies in humans have shown a great variability between liver samples from different individuals and speed of PPF degradation (Guitton *et al.*, 1998; Court *et al.*, 2001). In dogs, two studies have looked at Vmax and Km and found some variability between breeds, within a breed, and between sexes (Court *et al.*, 1999; Hay Kraus *et al*., 2000). These results, for both humans and dogs, might explain why in this study, the amount of PPF degraded by the rat hepatic microsomes was less than that found in the study published by Correia (1994). In this study hepatic microsomes from Wistar rats were used and Correia (1994) mentioned that the microsomal system from these rats seemed slower in the degradation of PPF than hepatic microsomes from Sprague Dawley rats. Other explanations for the observed difference might come from different conditions during harvesting and preparation of the liver extracts as well as during the metabolism studies, although the protocols were very similar.

Imidazole derivatives are known to be inhibitors of microsomal oxidative drug metabolism (Wilkinson *et al.*, 1974) and in this regard are often described as potent and non-selective inhibitors of CYPs (Testa and Jenner, 1981). They have been shown to inhibit a number of CYP families, including CYP1A, 2A, 2B, 2C and 3A, which confirm their non-selectivity (Kharasch *et al.*, 1991; Pelkonen *et al*., 1991; Rodrigues and Roberts, 1997). The mechanism for their inhibitory effects is believed to be a direct and reversible interaction with the ferricytochrome heme, an important constituent of the CYP enzyme system (Wilkinson *et al.*, 1974; Kharasch *et al.*, 1992; Rodrigues and Roberts, 1997). Of all imidazole derivatives, those that are N-substituted are considered to be the most potent inhibitors of CYPs (Kharasch *et al.*, 1991), with IC_{50} values in the submicromolar range. The present study confirms the work of Karrash *et al.* (1991) in that DEX and LEV are highly potent inhibitors of PPF metabolism, despite the fact that they are not N-substituated imidazole but only 4(5)-substituted imidazoles.

There was a significant difference ($p < 0.05$) in the IC_{50} values of DEX and LEV in rat and in dog hepatic microsomes; a difference which was noted in earlier studies in humans and rats (Pelkonen *et al*., 1991; Kharasch *et al.*, 1991, 1992). This difference in inhibition between chiral imidazoles is

not uncommon, for example, inhibition of adrenal steroidogenesis occurs only with the d-isomer of etomidate (Kharasch *et al.*, 1992).

Nevertheless, it is important clinically that although both DEX and LEV have been shown to be inhibitors of CYPs, only DEX has analgesic and sedative efficacy. Accordingly, LEV has no place in the overall aim of this project, which was to develop a system for the administration of the hypnotic PPF, by TCI, with co-infusion of an analgesic supplement, either the alpha$_2$-adrenoceptor agonist MED or its enantiomer DEX.

In the dog, the plasma concentrations of MED and DEX required for analgesia are believed to be around 2-9 ngml^{-1} and 0.7 ngml^{-1}, respectively (Salonen, 1991; Kuusela *et al*., 2000; Granholm, personal communication, 2003). In this study, the IC_{50} for MED and DEX in dogs was 0.04 μgml^{-1} (40 ngml^{-1}) and 0.1 μgml^{-1} (100 ngml^{-1}), respectively. These concentrations are about 4 to 20 (MED) and 143 (DEX) times higher that their reported minimum analgesic plasma concentrations, and therefore it is likely that neither MED nor DEX will have an inhibitory effect on PPF metabolism when they are co-infused with PPF at a rate derived to achieve analgesia. Moreover, as MED and DEX are 85% protein bound in the plasma, their hepatic concentration might not be as high as their analgesic plasma concentration. In a study looking at the effect of DEX on amiopyrine in rats, Pelkonen and colleagues (1991) showed that although the alpha$_2$-adrenoceptor agonist had a high inhibitor potency *in vitro* (IC_{50} = 5.2 μM), doses up to 100 μgkg^{-1} *in vivo,* corresponding to an intrahepatic concentration of more than 5 μM, had no significant inhibitory effect.

This notwithstanding, if DEX and or MED were to be present *in vivo* in sufficient concentrations to affect the metabolism of PPF through inhibition of the hepatic CYP enzyme system, the fact that this is not the sole metabolic pathway for PPF might minimize its impact. Direct glucuronidation might compensate for any decrease in hepatic CYP efficacy, as might extrahepatic metabolism, such as that demonstrated during the anhepatic phase of liver transplantation in human (Veroli *et al*., 1992).

In conclusion, this study demonstrated that *in vitro* PPF metabolism was impaired, dependent on dosage, by MED and its enantiomers DEX and LEV. However i*n vitro* studies are acknowledged as valuable qualitative but not quantitative predictors of *in vivo* interactions. Accordingly, on the basis of the results presented, it is not possible to make quantitative predictions regarding the possible effect of MED and DEX on PPF metabolism *in vivo* and subsequently on the performance of the PPF TCI system. However, the lower therapeutic potency of MED and the fact that it appears to have a greater potential for drug interaction, support the further clinical development of DEX in preference to the racemate as an adjunct to PPF TCI.

CHAPTER 4:

DOSE RESPONSE STUDY OF THE CARDIO-RESPIRATORY EFFECTS OF MEDETOMIDINE IN DOGS ANAESTHETISED WITH A TARGET CONTROLLED INFUSION OF PROPOFOL.

4.1 Introduction

Medetomidine (MED), an alpha$_2$-adrenoceptor agonist, has been on the shelves of small animal practices for more than 15 years. It is mainly used in dogs for its sedative, anxiolytic and anaesthetic-sparing effects. However, this drug, like other drugs of its class, induces significant cardiovascular (CV) side effects, mainly hypertension, bradycardia, cardiac arrhythmias and decreased cardiac output (CO) (Hall and Clarke, 1991b).

4.1.1 Cardiovascular effects of Medetomidine

4.1.1.1 Arterial Blood pressure (ABP)

The effect of the alpha$_2$-adrenoceptor agonists on arterial blood pressure (ABP) is variable. In humans, clonidine was first used as an antihypertensive agent (Kamibayashi and Maze, 2000). Dexmedetomidine (DEX), the active enantiomer of MED introduced in 1999 in the US (Nelson *et al.*, 2003), is used perioperatively and in the ICU setting because of its anxiolytic, analgesic and anaesthetic sparing properties. Although it is also used for its sympatholytic and haemodynamic stabilising effects (Paris and Tonner, 2005; Mukhtar *et al.*, 2006), studies have presented diverse results concerning its effect on ABP : both hyper- and hypotension have been described (Bloor *et al*., 1992; Talke *et al*., 1995; Dutta *et al*., 2000; Venn *et al.*, 2003).

In dogs, similar results have been reported with the use of MED. Its effect on ABP is usually described as biphasic (Hall and Clarke, 1991b; Pypendop and Verstegen, 1998; Talke, 2000). First, an increase of ABP is observed, followed by a progressive diminution, leading to a return to pre-injection values or even a reduction below baseline values. The first phase is considered to be a consequence of the stimulation of postsynaptic alpha$_2$-adrenoceptors present in the smooth muscle of the vascular bed (peripheral effect), while the second phase occurs following activation of alpha$_2$-adrenoceptors located on presynaptic terminals of the postganglionic nerve fibres innervating vascular smooth muscle (peripheral effect) and an increase in vagal activity (central effect) (Guimaraes and Moura, 2001). However, in clinical cases, the two phases are not so well defined, and the effect of MED on ABP is mixed with evidence suggesting that with increasing plasma concentration of MED, peripheral effects predominate, leading to increased ABP (MacDonald and Virtanen, 1991).

Pypendop and Verstegen (1998) carried out a dose response study (range 1–20 μgkg^{-1}) for MED given intravenously (IV) to conscious beagles. They reported that at doses of 2 μgkg^{-1} and lower, increase in the ABP was not observed, but instead a decrease from the baseline, which was not considered as hypotension (mean ABP < 60 mmHg) by the authors. Increasing doses up to 20

μgkg^{-1} resulted in a significant increase in ABP but they failed to determine a maximum effect. They concluded that small doses of MED might have more of a central effect resulting in a decrease in heart rate (HR) and ABP without the peripherally mediated hypertensive phase 1. Similar results were found in awake man with decreasing plasma concentration of DEX (Ebert *et al.*, 2000). In a more recent study using different plasma concentrations of DEX (0.075 to 0.6 $ngml^{-1}$) in awake and anesthetised patients, Talke e*t al.* (2003) eliminated the sympatholytic effect by either sympathetic denervation (awake patient) of one hand through brachial plexus blockade or by attenuation of the sympathetic nervous system activity using general anaesthesia. They also looked at the local (finger) blood flow. In both cases (awake vs. anaesthetised patients), they observed vasoconstriction in the finger, but in anesthetised patients, the general haemodynamic effect resulted in increase in systemic ABP while in the awake but arm denervated patient, the systemic ABP was decreased. They concluded their observations were consistent with the known effects of the $alpha_2$-adrenoceptor agonists: centrally mediated decrease in ABP and peripherally mediated vasoconstriction.

Studies in different species looking at the $alpha_2$-adrenoceptor subtypes (A, B and C) attribute the centrally induced sympatholytic effect to the $alpha_{2A}$-adrenoceptor (MacMillan *et al.*, 1996; Altman *et al.*, 1999; Schwartz *et al.*, 1999) and the peripheral vasopressor effect mainly to the $alpha_{2B}$-adrenoceptor (Link *et al.*, 1996; Hein *et al.*, 1999; Kable *et al.*, 2000; Paris *et al.*, 2003). Unfortunately, the repartition in the body and related effect of these receptor subtypes is not that clearly defined and 2A subtypes have also been found on the wall of the canine external carotid mediating vasoconstriction (Willems *et al.*, 2001) whereas 2B subtypes are believed to be responsible for centrally mediated hypertensive effects (Gavras *et al.*, 2001a, b).

4.1.1.2 Heart rate

Bradycardia is a common CV effect of $alpha_2$-adrenoceptor agonists that has been observed in different species; human (Maze and Tranquilli, 1991; Hall *et al.*, 2000), horse (Freeman and England, 2000), sheep (Talke *et al.*, 2000a), cat and dog (Cullen, 1996).

Medetomidine induced-bradycardia results from stimulation of both central and peripheral $alpha_2$-adrenoceptors (Kamibayashi and Maze, 2000). The mechanism of this action is not fully understood but is likely to be multifactorial, including inhibition of sympathetic tone by inhibition of noradrenaline release within the central nervous system (CNS); enhanced vagal response due to peripheral vasoconstriction; increase in the release of acetylcholine from the para-sympathetic nerves in the heart (MacDonald and Virtanen, 1991). There is no evidence to date of a direct action on the heart muscle (Flacke, *et al.*, 1992; Day and Muir, 1993; de Morais and Muir, 1995; Khan *et al.*, 1999), although prejunctional $alpha_2$-adrenoceptors might be present in the human heart (Brodde *et al.*, 2001).

Pypendop and Verstegen (1998) reported that the intensity and duration of bradycardia was dose-dependent in conscious beagles. Maximum effect on the HR was observed with a dose as low as 5 μgkg^{-1} of MED administered IV. Higher doses resulted in increase of duration of the bradycardia effects rather than in increase of intensity.

In human, as well in veterinary medicine, the $alpha_2$-adrenoceptor agonists are known for their potential arrhythmogenic effects (Moens, 2000; Talke, 2000). In humans, DEX has been reported as producing in some cases sinus pauses and even sinus arrest (Scheinin *et al*., 1998 ;Talke *et al*., 2000b). In dogs, Vainio (1989) reported the appearance of second degree atrioventricular (A-V) block after IV and IM injection of 10 to 180 $\mu gkg^{-1}$of MED. These lasted for up to 20 min in some cases. The incidence in IV and IM groups was not reported. Other groups have reported similar dysrhythmias as well as sinus pauses and A-V block of the 1st and 2nd degree (Vainio, 1990; Ewing *et al*., 1993; Thurmon *et al*., 1994; Tyner *et al.*, 1997; Kuusela *et al*., 2001a).

Medetomidine is a racemate composed of DEX and levomedetomidine (LEV). Levomedetomidine, the inactive enantiomer of MED, did not induce dysrhythmias in a study in dogs (Kuusela *et al*., 2000). This was confirmed later in a study of the sedative, analgesic and CV effects of LEV alone and in combination with DEX in dogs (Kuusela *et al*., 2001b).

The incidence of dysrhythmias seems to be also dose-dependent for MED and its active enantiomer (Kuusela *et al*., 2001a) while the method of injection (IV or IM) seems to have little effect (Tyner *et al*., 1997).

4.1.2 Respiratory effects of medetomidine

$Alpha_2$-adrenoceptor agonists such as clonidine or more recently DEX have minimal effects on ventilation in humans (Khan *et al.*, 1999), but hypercapnoea is frequently observed (Bailey *et al.*, 1991; Ooi *et al.*, 1991; Belleville *et al*., 1992).

In dogs, many studies have reported some degree of respiratory depression following clinically used doses of MED (Clarke and England, 1989; Vainio and Palmu, 1989; Vainio, 1990; Pettifer and Dyson, 1993; Hammond and England, 1994; Venugopalan *et al*., 1994; Cullen, 1996; Paddleford and Harvey, 1999). Cullen and Reynoldson (1993) reported a significant increase in arterial carbon dioxide tension ($PaCO_2$) from 38.5 to a maximum of 41.8 mmHg after 20 min in dogs given MED 30 μgkg^{-1} IM. They also reported a decrease in respiratory rate (RR) (from 23 to 13 breaths min^{-1} at 30 min), as well as a decrease in arterial oxygen tension (PaO_2) from 118.4 to a minimum of 97.9 mmHg. Such changes have also been observed by other authors (England and Clarke, 1989; Vainio, 1990; Pettifer and Dyson, 1993; Venugopalan *et al.*, 1994).

Nguyen and colleagues (1992) demonstrated that DEX, 1 μgkg^{-1} IV decreased minute ventilation in conscious dogs by 22%, while higher doses (10, 20 and 100 μgkg^{-1}) increased minute ventilation, which doubled with a dose of 100 μgkg^{-1}.

The mechanism behind the respiratory depression observed after administration of an $alpha_2$-adrenoceptor agonist is still not totally understood, but could result from activation of $alpha_2$-adrenoceptors in brainstem sites associated with cardio-respiratory control, such as the pre-inspiratory neurons of the rostral ventrolateral medulla (O'Halloran *et al.*, 2001).

Cyanosis in dogs after administration of MED has also been described by some authors (Clarke and England, 1989; England and Clarke, 1989; Sap and Hellebrekers, 1993). This sign of oxygen (O_2) desaturation of blood in the venous beds was thought to result from the reduced CO increasing the contact time between the blood and peripheral tissues, increasing the total amount of O_2 being extracted (England and Clarke, 1989; Sap and Hellebrekers, 1993). The effect of DEX on the hypoxic drive is negligible even at doses as high as 100 μgkg^{-1} (Nguyen *et al.*, 1992).

4.1.3 Perioperative use of medetomidine

The perioperative use of DEX infusion has been described in humans (Talke *et al*., 1995; Jalonen *et al*., 1997; Talke *et al*., 2000b; Paris and Tonner, 2005), in cats (Ansah *et al.*, 2000) and in dogs (Pascoe *et al.*, 2006; Lin *et al.*, 2008; Uilenreef *et al.*, 2008). Although the perioperative use of MED has been best described in horses (Bettschart-Wolfensberger *et al.*, 2001a, 2001b, 2002), only two reports have been published regarding its use in dogs. Gomez-Villamandos and colleagues (2008) determined the effect of MED infusion (0, 0.5 and 1 $\mu gkg^{-1}h^{-1}$) on desflurane requirement to maintain anaesthesia in MED premedicated dogs (2 μkg^{-1}, IV). Although no difference between the three groups was observed on the CV and respiratory parameters recorded during the minimum desflurane determination, the HR and RR decreased significantly after premedication with MED while a non-significant increase in the systolic, mean and diastolic ABPs were observed. In an earlier study, Beths and colleagues (2000b) described the halothane-sparing effect of repeated (every 20 min) IV doses of MED (1 μgkg^{-1}) in dogs undergoing orthopaedic surgery. Bradycardia and increased ABP without hypertension (systolic ABP > 150 mmHg) were observed after the first injection. Subsequent boluses did not produce additive effects on the CV system, although reduction in the inspired concentration of halothane (range from 0.9 to 1.4%) was observed.

4.1.4 Cardiovascular effects of propofol

In most species, propofol (PPF) decreases ABP while having no effect, or a negative effect, on the HR. This effect is believed to be mediated by resetting the baroreflex response to a lower HR despite the fall in ABP (Ebert *et al.*, 1992b; Sellgren *et al.*, 1994). However, more recently, it was reported that low doses of PPF in rabbits had no effect on the baroreflex response (Xu *et al.*, 2000).

4.1.4.1 Arterial blood pressure

Arterial hypotension is a common side effect of PPF injection and has been described in different species such as rabbit (Xu *et al.*, 2000) and cat (Andress *et al.*, 1995; Pereira *et al.*, 2004).

Robertson *et al.* (1992) reported that after induction of anaesthesia with PPF (4 and 3.2 $mgkg^{-1}$, respectively) in greyhounds and non-greyhound dogs sedated with acepromazine and atropine, a non-significant increase in mean ABP was observed in the greyhounds for the first 45 min during maintenance of anaesthesia (PPF, 0.4 $mgkg^{-1}min^{-1}$). In the non-greyhound dogs, a significant decrease in mean ABP was observed (from 90.2 to a maximum of 76.7 mmHg) at 30 min. In a pharmacokinetic (PK) study, Nolan and Reid (1993) induced (4 $mgkg^{-1}$) and maintained (0.4 mg $kg^{-1}min^{-1}$) anaesthesia with PPF for 60 min in seven beagles premedicated with acepromazine and papaveretum. Systolic ABP decreased slightly from 130 to 112-121 mmHg during the 60-min period. A decrease in systolic ABP was also reported after induction of anaesthesia with PPF (4 $mgkg^{-1}$) in 15 dogs premedicated with acepromazine and pethidine (Lerche *et al.*, 2000).

Dose-dependent direct myocardial depression and a decrease in preload due to venous dilation resulting in a decrease in CO are the most accepted explanations for the hypotensive effects of PPF (Cook and Housmans, 1994; Wallerstedt and Bodelsson, 1997; Ririe *et al.*, 2001; Klockgether-Radke *et al.*, 2004). In contrast to animal investigation using *in vitro* studies, an *in vivo* study in humans refutes the direct action of PPF on peripheral blood vessels as the cause of decreased ABP (Robinson *et al.*, 1997). In the same study, another hypothesis was proposed, that the hypotension would result, in part, from the inhibition of peripheral sympathetic vasoconstrictor activity.

4.1.4.2 Heart rate

The action of PPF on HR is variable. In humans, for example, the use of PPF either has minimal effects or induces bradycardia (Tramer *et al.*, 1997; Williams *et al.*, 1999; Kleinsasser *et al.*, 2000; Olmos *et al.*, 2000; Kanaya *et al.*, 2003). In pigs, intravenous injection of 2.5 and 5 $mgkg^{-1}$ of PPF in Cremophor EL (2% propofol in 16% Cremophor and 8.66% of Ethyl alcohol) resulted in significant tachycardia (Glen, 1980), while in rabbits, PPF (2.5, 5 and 10 $mgkg^{-1}$, IV) had no effect on the HR, but injection of 20 $mgkg^{-1}$ resulted in a significant decrease (Xu, *et al.*, 2000). In cats,

different effects were again described, but a decrease in HR is more commonly reported (Brearly *et al.*, 1988; Andress *et al.*, 1995; Pereira *et al.*, 2004).

Nolan and Reid (1993) reported no significant effect on the HR in dogs premedicated with acepromazine and papaveretum and induced with PPF 4 $mgkg^{-1}$ followed by an infusion of 0.4 $mgkg^{-1}min^{-1}$. In their study with greyhounds and non-greyhound dogs anaesthetised with PPF after ACP and atropine premedication, Robertson and colleagues (1992) observed no effect on the HR in the non-greyhound group while a significant decrease was observed in the greyhounds. Decrease of HR has also been described in other studies in dogs (Brussel *et al.*, 1989; Lerche *et al.*, 2000; Whitwam *et al.*, 2000).

4.1.4.3 Respiratory effects

In humans, respiratory depression after injection of PPF is a well described side effect. Goodman *et al.* (1987) studied the ventilatory effects of PPF in 14 healthy patients and observed a decrease in the breathing rate, increase in end-tidal CO_2 and a decrease in the ventilatory response to CO_2. This was after bolus induction, but ventilatory depression has also been observed during manual or TCI-driven infusions (Russell *et al.*, 1995; Taylor and Kenny, 1998).

During a study on the anaesthetic activity of ICI 35868 (PPF in Cremophor EL), Glen (1980) observed dose-dependent respiratory depression in mice, rats, rabbits, pigs and cats. These effects were also described using the emulsion formulation of PPF in cats (Brearly *et al.*, 1988), horses (Flaherty *et al.*, 1997) and in dogs (Robertson *et al.*, 1992; Ilkiw *et al.*, 1992; Muir and Gadawski, 1998; Quandt *et al.*, 1998).

The effect of PPF on observed respiratory function is not fully understood. Two different sites of action of the drug are usually proposed; peripheral sites (peripheric chemoreceptors of the carotid bodies) and central sites (central chemoreceptors located in the medulla) (Teppema *et al.*, 1997; Yang *et al.*, 1997; Nieuwenhuijs *et al.*, 2001).

4.1.5 Propofol and medetomidine premedication (cf section 4.1.3)

Different groups have studied the CV effects of the combination of MED and PPF in dogs. Vainio (1991) reported on a study in ten beagles, premedicated with 40 μgkg^{-1} of MED IM. Anaesthesia was induced with PPF 4 $mgkg^{-1}$ (IV) and maintained with PPF infusion (0.15 $mgkg^{-1}min^{-1}$). An increase in the mean ABP after induction of anaesthesia was observed, which lasted about 15 min. Thereafter the ABP decreased to values below pre-PPF. Meanwhile, HR increased at induction and remained above pre-PPF values throughout the study period. In another study (Thurmon *et al.*, 1994), six beagles were premedicated with MED (30 μgkg^{-1}) and atropine (0.044 $mgkg^{-1}$) IM.

Anaesthesia was induced 10 min later with 2 $mgkg^{-1}$ of PPF (IV) and maintained with an infusion of PPF (0.165 $mgkg^{-1}min^{-1}$). Arterial blood pressure increased after induction of anaesthesia for 10 min, before decreasing slightly, but stayed at values higher than baseline. Heart rate decreased after induction with a maximum at 5 min then rose again to values above baseline for most of the infusion period. Although there were some differences, the main trends in these two studies are an increase in the ABP observed at induction followed by a decrease after 10-15 min and a HR staying above pre-propofol baseline. Atropine administration was the main difference between these 2 studies and resulted in a HR twice higher and systolic arterial ABP around 150 mmHg for the whole study period for the Thurmon *et al.*'s study.

In another study on 20 dogs undergoing ovario-hysterectomy, Hellebrekers and Sap (1997) used MED 40 μgkg^{-1} (IM) for premedication, PPF (2 $mgkg^{-1}$ IV) for induction and repeated PPF boluses (mean of 0.06 $mgkg^{-1}min^{-1}$ IV) for maintenance. They reported an increase in HR and ABP. The values were back to baseline by 70 min. More recently, Seliskar and colleagues (2007) induced and maintained anaesthesia with PPF in medetomidine (40μgkg^{-1}, IM) premedicated dogs with PPF. An increase of HR and mean ABP from baseline values was also observed. While the ABP stayed high throughout the procedure, the HR when back to baseline values.

When PPF induction in MED premedicated dogs is not followed by PPF anaesthesia, the CV effects are different. Without atropine, Cullen and Reynoldson (1993) observed after MED 30 μgkg^{-1} (IM) and PPF 3 $mgkg^{-1}$ (IV), an increase in mean ABP, which was higher than baseline for the whole period of the recordings (60 min). Regarding the HR, it increased briefly after induction then went below baseline values during the recordings. When compared with Vainio's (1991) studies, PPF infusion causes a decrease in ABP while keeping the HR above baseline in the MED premedicated dogs. In a more recent study, dogs were administered MED 1 μgkg^{-1} IV 45 sec before induction with PPF (3.7 $mgkg^{-1}$ IV). The HR decreased while no change in the ABP was observed at 1 min post intubation up to 5 min followed by a decrease below baseline lasting till the end of the recording period (17 min) (Ko *et al.*, 2006). With atropine (0.02 $mgkg^{-1}$) added to MED (10 μgkg^{-1}, IM), Bufalari *et al*. (1996) observed after PPF 2.2 $mgkg^{-1}$ IV higher ABP than baseline for the whole period of the recordings (60 min), as in the Thurmon *et al*. study (1994) where atropine was also used. Similarly, the HR stayed slightly above baseline for the whole procedure.

In summary, in the 4 studies where atropine was not used in the premedication, induction with PPF resulted in an increase in HR and ABP (Vainio, 1991; Cullen and Reynoldson, 1993; Hellebrekers and Sap, 1997, Seliskar *et al*. 2007). When the induction was then followed by PPF for maintenance (Vainio, 1991; Hellebrekers and Sap, 1997, Seliskar *et al.* 2007), the values for these 2 parameters tended to slowly return to values above (Hellebrekers and Sap, 1997) or equal to (Vainio, 1991) or while the HR slowly returned to values above baseline, mean ABP stayed elevated (Seliskar *et al.* 2007). In their study, Seliskar and colleagues administered 20 min after

induction a second dose of MED (20 μgkg^{-1}, IM) which might have influenced the effect observed on the mean ABP. In the Cullen and Reynoldson (1993) study, where induction was not followed by an infusion of PPF, the ABP stayed elevated while the HR was below baseline values. The addition of atropine in the premedication (Thurmon *et al.*, 1994; Bufalari *et al.*, 1996) resulted in an increase in HR and ABP followed by a decrease but with values above baseline during most of the recording period (30-60 min). In the study from Ko and colleagues (2006), the results are different from Vainio (1991), Cullen and Reynoldson (1993) and Hellebrekers and Sap (1997) as the authors were using a low dose of DEX (1 μgkg^{-1}) which might have resulted in centrally mediated sympatholytic effect resulting in decrease HR and systemic ABP (Pypendop and Verstegen, 1998). Thereafter, these effects might have been exacerbated by the addition of PPF.

The same studies but one (Hellebrekers and Sap, 1997) have also reported on the respiratory effects of PPF induction and/or maintenance on MED premedicated dogs. But for 2 studies (Ko *et al*. 2006; Seliskar *et al.*, 2007) they all reported a decrease in the breathing rate associated with an increase in the $PaCO_2$ from 36 up to a maximum of 45.8 mmHg after 60 min (Vainio, 1991); from 37.3 up to a maximum of 44.6 mmHg after 3 min (Cullen and Reynoldson, 1993); from 41.6 up to a maximum of 53.5 mmHg after 40 min (Thurmon *et al*., 1994); from 30 up to a maximum of 35 mmHg after 10 and 15 min (Bufalari *et al.*, 1996). Although Ko and colleagues (2006) reported a decrease in breathing rate, they observed no significant changes in the Et CO_2 values. Post-induction apnoea was only reported in one of these studies (Cullen and Reynoldson, 1993). In their study, Seliskar and colleagues (2007) did not report changes in respiratory rate (RR), $PaCO_2$ nor PaO_2 (dogs were breathing 100% O2) from baseline.

4.1.6 Goal of the study

Medetomidine induces significant dose-dependent CV effects in dogs (Pypendop and Verstegen, 1998) including bradycardia and hypertension. Respiratory depression has also been observed in different species, ranging from a slight decrease in minute ventilation to cyanosis. Apnoea with DEX has been described in humans, but not yet in the dog. In the development of an infusion scheme for MED as an adjunct to PPF anaesthesia, it was considered appropriate to determine if an infusion regime could be defined which would cause only minimal effects on the CV system, while reducing the amount of PPF required to maintain anaesthesia. The study described in this chapter was designed to identify the CV dose-response curve for IV doses of MED in dogs anaesthetised with TCI PPF, in order to define the no–effect dose and a dosing range which would induce minimal effects on HR and ABP.

4.2 Materials and methods

This study was approved by the Clinical Research Sub-Committee of the Faculty of Veterinary Medicine of the University of Glasgow and owner consent for inclusion in the study was acquired. Eighty dogs (46 males and 34 females) attending the Small Animal Hospital for diagnostic and/or surgical procedures were included in the study. Only dogs classified as ASA 1 or 2 and aged between 0.5 and 9 years were included in this study. The operator was unaware of the treatment and was handed a syringe containing MED at a concentration of 0, 1, 10, 100 or 1000 $\mu g ml^{-1}$ with a maximum injection volume of 2 ml. The dilutions were made every day using NaCl 0.9%.

Sixty dogs were randomly divided into six groups of ten dogs each: group 1, 2, 3, 4, 5, and 6 receiving respectively 0, 0.01, 0.03, 0.1, 0.3, and 1 $\mu g kg^{-1}$ of MED (IV). Subsequently, 20 additional dogs were included and were randomly divided into two groups of ten receiving 3 (group 7) and 10 $\mu g kg^{-1}$ (group 8) of MED (IV). Dogs were randomly allocated to a group using Excel software (Microsoft Office 2000). The random numbers were normally distributed. All dogs were premedicated 20 min to 1 h before induction of anaesthesia with acepromazine (ACP, Novartis Animal Health UK Ltd; Herts, UK) 0.02-0.03 $mg kg^{-1}$ and methadone (Martindale Pharmaceuticals, Essex, UK) 0.2 $mg kg^{-1}$ injected IM.

Anaesthesia was induced and maintained with PPF administered with a TCI system (see chapter 2). The induction target concentration was 3 $\mu g\ ml^{-1}$. If after 3 min endotracheal intubation was not possible, the target was increased by 0.5 $\mu g\ ml^{-1}$. One min later, if intubation was still not possible, the target was again increased by 0.5 $\mu g\ ml^{-1}$ and so on every 1 min until successful endotracheal intubation. The anaesthesia maintenance target concentration was set at 3.5 $\mu g\ ml^{-1}$ and stayed unchanged till the end of the study. The dogs were allowed to breathe a mixture of O_2/N_2O (1/3:2/3) through an appropriate non-rebreathing system.

ECG (bpm) (Kontron Micromon 7141, Kontron Instruments Ltd, England), indirect ABP (mmHg) (Dinamap; Critikon 1846 SX, Critikon Inc, Tampa, FL, USA) with an appropriate cuff placed over the pedal artery (first six groups) and with an Ultrasonic Doppler flow detector (last two groups) (811B Parks Medical Electronics, Aloha, Oregon, USA), oxygen saturation (SpO_2) (Nellcor N20; Nellcor Puritan Bennett Inc, CA, USA), oesophageal temperature (T °C) (Libra Medical ET 402; Libra Medical Ltd, Berks, England) and end-tidal CO_2 (Et CO_2) levels (mmHg) (Nellcor NPB 70) were monitored in all dogs. Data for HR, ABP, RR, Et CO_2 and SpO_2 were recorded every 5 min. Body temperature lower than 36°C resulted in removal of the patient from the study.

Twenty to 30 min after induction of anaesthesia, when the HR, ABP and Et CO_2 had been stable for at least 15 min, 1 ml of saline 0.9% (Group 1) or MED (0.01, 0.03, 0.1, 03, 1, 3 or 10 μgkg^{-1}; groups 2 to 8) was injected IV over 1 min, and the CV and respiratory parameters recorded 2, 5, 10, 15 and, 20 min later. In the event of apnoea (no breathing for more than 30 sec), IPPV was instigated at 6 breaths min^{-1}. The dogs were kept warm using blankets. At the end of the trial, the infusion of PPF was stopped and anaesthesia continued with either halothane or isoflurane.

4.2.1 Data Analysis

ANOVA for time and treatment (dose) effects was carried out for the ABP, HR, Et CO_2 and RR data, using a General Linear model (Minitab 13). One-way ANOVA was also used to compare the groups for body weight and age distribution. Difference were considered significant when $p < 0.05$.

The ED_{50} (the dose of MED inducing 50% of the maximum effect) and ED_{95} (the dose of MED inducing 95% of the maximum effect) were calculated for HR and ABP, fitting a curve to the data using a logistical equation (Origin 6.1; Microcal Software, Inc., Northampton, USA.):

$$Y = \frac{A1 - A2}{1 + (X / X_0)^p} + A2$$

Where x_0 is the centre

p is the power

A1 is the initial Y value

A2 is the final Y value

The Y value at X_0 is half way between the two limiting values A1 and A2:

$Y(X_0) = (A1 + A2)/2$

To determine the effect of MED on the HR at the time of maximum effect (2 min), the difference in HR at that time (2 min) and at time 0 were calculated for each dog. The mean value of these differences from 10 dogs was then obtained for each group and used with the logistical equation to make the dose response curve. An identical method was used to determine the effect of MED on the ABP at the time of maximum effect, 2 min.

4.3 Results

The 80 dogs comprised 46 males and 34 females, weighing between 7 and 45 kg (27.3 ± 9) and aged between 0.5 and 8 years old (3.7 ± 2.6). The eight groups were similar ($p > 0.05$) in age and weight. All the dogs were intubated in less than 3 min after starting the infusion of PPF. No dogs were removed from the study for hypothermia ($T < 36°C$).

No significant ($p > 0.05$) effect of MED treatment on HR was observed with doses equal to and below 0.01 μgkg^{-1} (group 2) (Figure 4.1). At 2 min, the HR in groups 3 to 8 (0.03, 0.1, 0.3, 1, 3 and 10 μgkg^{-1}) decreased significantly from baseline. In all these groups, the HR stayed clinically for some (groups 0.03, 0.1, 0.3 and 10 μgkg^{-1}) and significantly for others (groups 1 and 3 μgkg^{-1}) lower than baseline up to the end of the recording period (20 min). First and second degree AV blocks were observed in one dog in groups 0.03, 0.1 and 0.3 μgkg^{-1} and in two dogs in groups 3 and 10 μgkg^{-1}.

No significant ($p > 0.05$) effect of MED treatment on ABP was observed with doses equal to and below 0.1 μgkg^{-1} (group 4) (Figure 4.2). At 2 min, the ABP in groups 5 to 8 (0.3, 1, 3 and 10 μgkg^{-1}) increased significantly from baseline. By the time 10 min, the ABP was back to baseline values for groups 0.3 and 1 μgkg^{-1} and did not change up to the end of the recording period. Although the values for the ABP for groups 3 and 10 μgkg^{-1} decreased constantly from time 2 min, they did not reached baseline by the end of the recording period.

The Dinamap monitoring system failed to provide readings 2 min after injection in one dog from group 1 μgkg^{-1} and in all the dogs from group 3 and 10 μgkg^{-1} after MED injection. The data regarding the ABP for the last two groups (3 and 10 μgkg^{-1}) were therefore obtained with the Doppler system.

At the time of maximum observed effect (2 min post MED injection), the values for the ED_{50} and the ED_{95} were respectively 0.187 and 3.1 μgkg^{-1} for the HR and 2.05 and 18.1 μgkg^{-1} for the ABP (Figures 4.3 and 4.4).

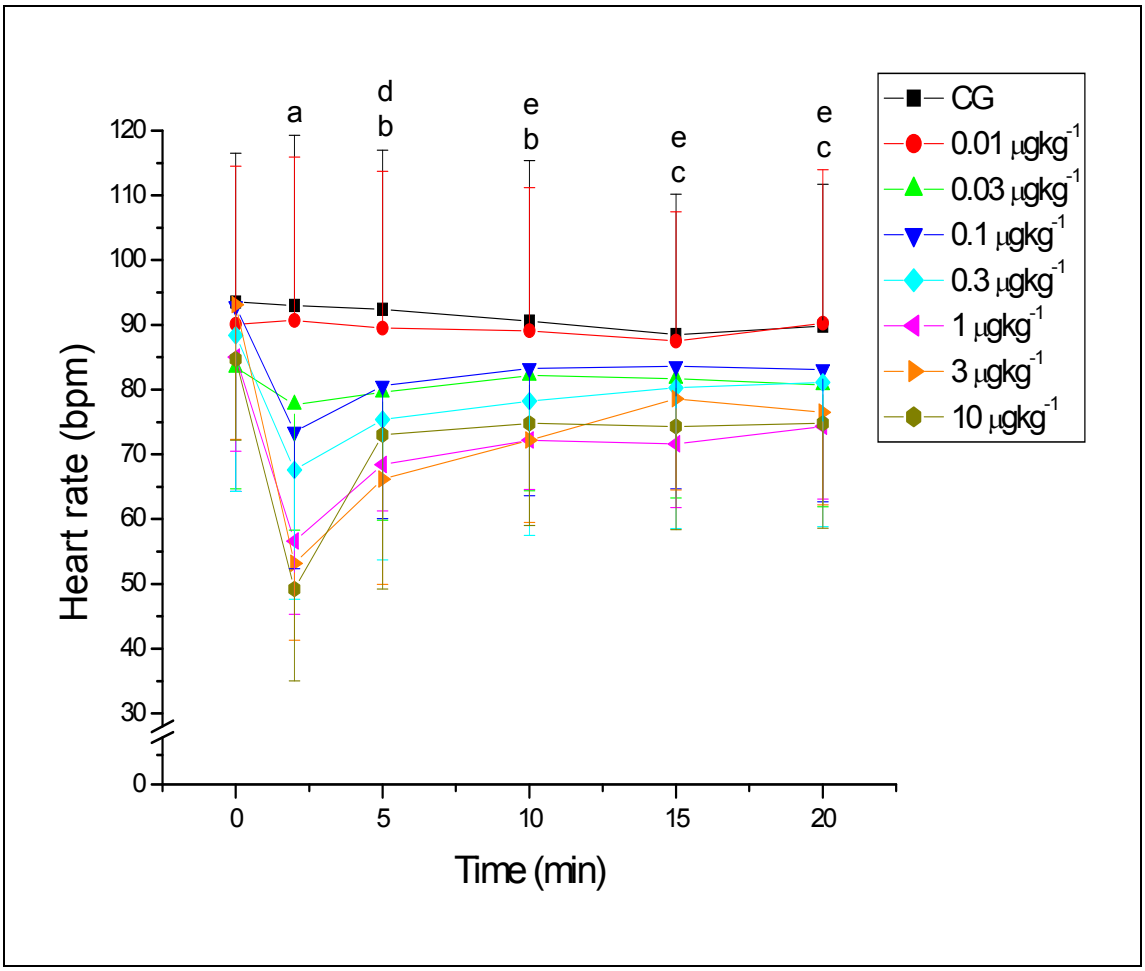


Figure 4.1

Mean (± SD) heart rate (bpm) for 8 groups (1 to 8) of 10 dogs given medetomidine (MED) (control group (CG), 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µgkg^{-1}, respectively)) from the time of MED bolus injection (time 0) to the last recording time (time 20 min).

a = significant difference in groups 0.03, 0.1, 0.3, 1, 3 and 10 µgkg^{-1} from baseline ($p < 0.0128$).

b = significant difference in group 0.3, 1 and 3 µgkg^{-1} from baseline ($p < 0.0408$).

c = significant difference in group 1 and 3 µgkg^{-1} from baseline ($p < 0.0337$).

d = significant difference in group 1 and 10 µgkg^{-1} from time 2 min ($p \leq 0.0043$).

e = significant difference in group 0.3, 1, 3 and 10 µgkg^{-1} from time 2 min ($p \leq 0.029$).

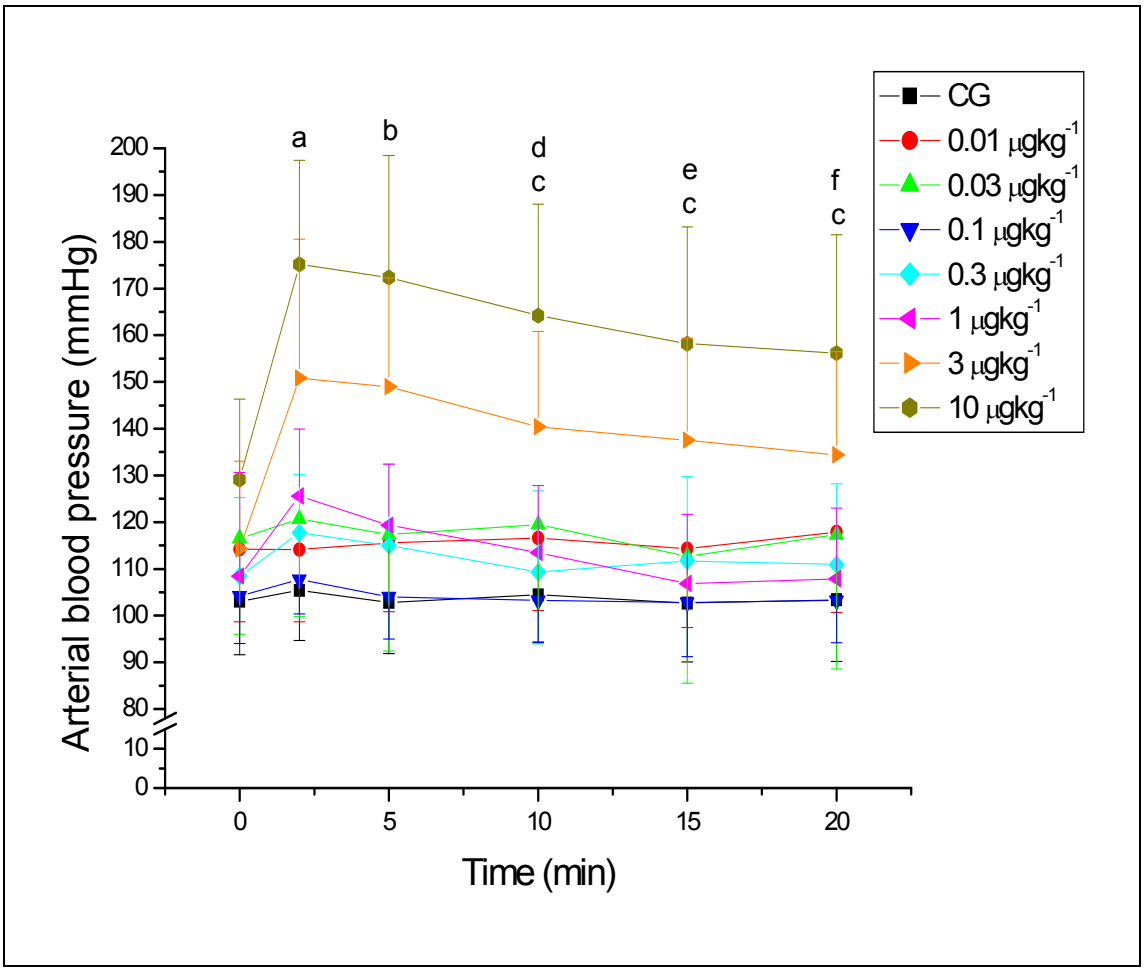


Figure 4.2

Mean (± SD) systolic arterial blood pressure (mmHg) for 8 groups (1 to 8) of 10 dogs given medetomidine (MED) (control group (CG), 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µgkg^{-1}, respectively)) from the time of MED bolus injection (time 0) to the last recording time (time 20 min).

a = significant difference in groups 0.3, 1, 3 and 10 µgkg^{-1} from baseline ($p \leq 0.0356$).

b = significant difference in group 1, 3 and 10 µgkg^{-1} from baseline ($p \leq 0.0015$).

c = significant difference in group 3 and 10 µgkg^{-1} from baseline ($p \leq 0.0001$).

d = significant difference in group 1 µgkg^{-1} from time 2 min ($p \leq 0.0003$).

e = significant difference in group 1 and 10 µgkg^{-1} from time 2 and 5 min ($p \leq 0.0336$).

f = significant difference in group 1, 3 and 10 µgkg^{-1} from time 2 and 5 min ($p < 0.0088$).

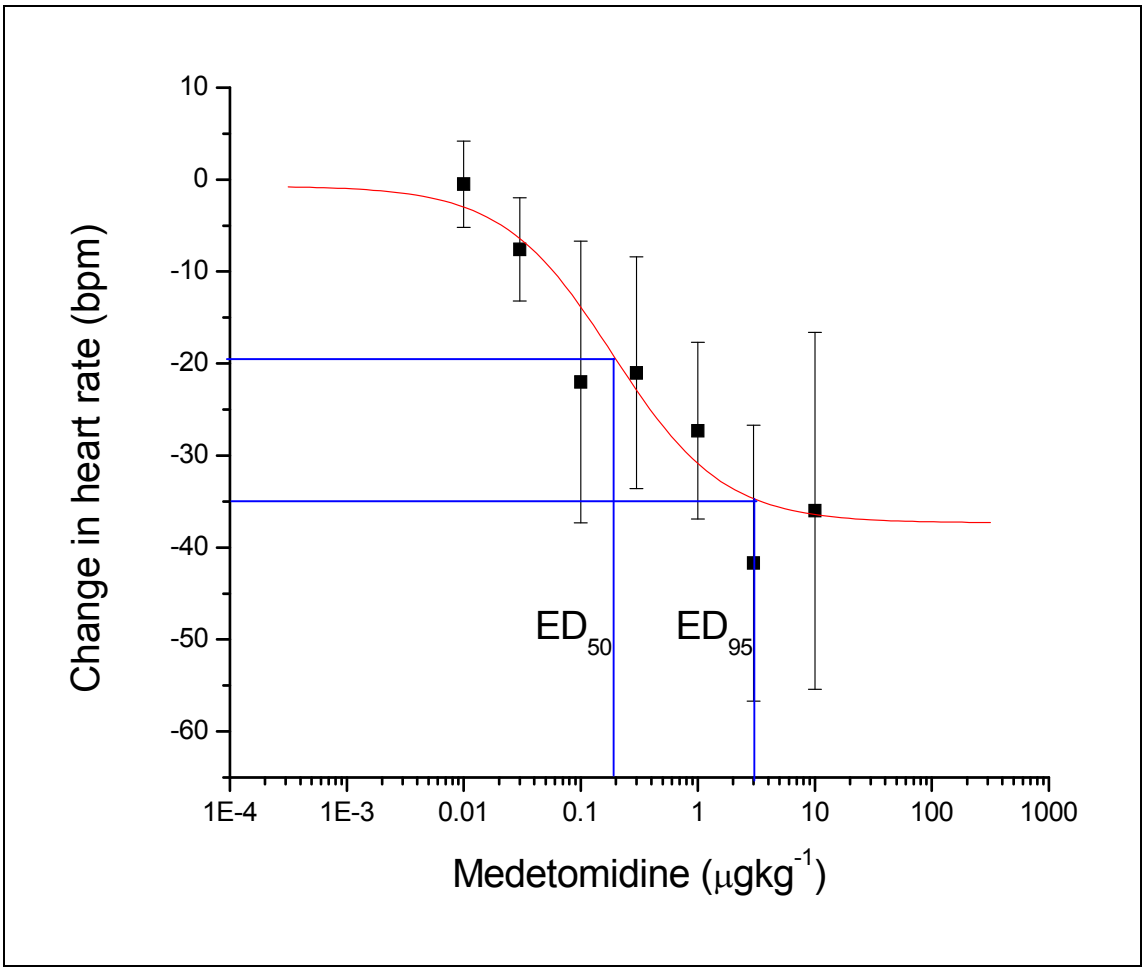


Figure 4.3

Dose response curve for the heart rate (HR) (bpm). Each point corresponds to one group (control group (CG) to 8) of dogs (n = 10) and is the mean value of change in HR taken at 2 min post medetomidine (MED) IV injection (0, 0,01, 0.03, 0.1, 0.3, 1, 3 and 10 μgkg^{-1}, respectively). ED_{50}-ED_{95} is the dose of MED that induces 50 and 95% respectively of the maximum effect on the HR (as predicted by the equation, using Origin 6.1, Origin Lab Corporation**,** Massachusetts, USA, 2000).

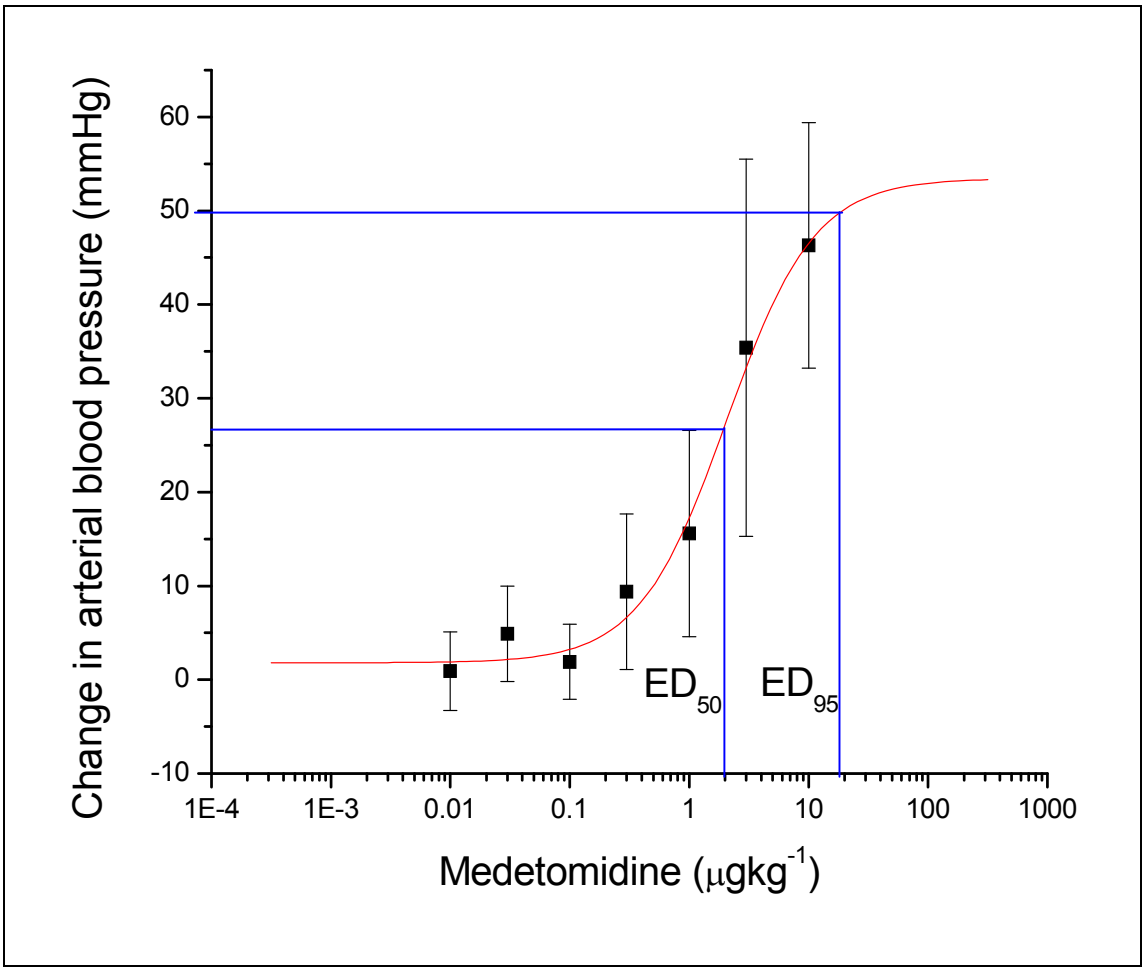


Figure 4.4

Dose response curve for the systolic arterial blood pressure (ABP) (mmHg). Each point corresponds to one group (control group (CG) to 8) of dogs (n = 10) and is the mean change in systolic ABP taken at 2 min post medetomidine (MED) IV injection (0, 0,01, 0.03, 0.1, 0.3, 1, 3 and 10 μgkg^{-1}, respectively). ED_{50}-ED_{95} is the dose of MED that induces 50 and 95% respectively of the maximum effect on the systolic ABP (as predicted by the equation, using Origin 6.1, Origin Lab Corporation**,** Massachusetts, USA, 2000).

Amongst the 80 dogs, 18 were apnoeic after induction (2, 1, 1, 3, 3, 5, 2 and 1 dogs from groups 1 to 8, respectively). One dog from groups 0.3 μgkg^{-1} and 1 μgkg^{-1}, which were not apnoeic at induction, became apnoeic before time 0 (MED injection), bringing the number of dogs requiring assisted ventilation at time 0 to 20 (2, 1, 1, 3, 4, 6, 2, 1 dogs from groups 1 to 8, respectively).

Amongst the spontaneously-breathing patients (60/80) at time 0, apnoea as a result of MED injection was observed in one dog in groups 0.3, 1 and 3 μgkg^{-1}. The resulting apnoea lasted less than 10 min. Five of the nine spontaneously breathing dogs (one being already apnoeic) from group 8 given 10 μgkg^{-1} became apnoeic after MED injection and did not breathe spontaneously for the duration of the trial. In the same group, between time 5 and 15 min, three spontaneously-breathing dogs became apnoeic, bringing the number of apnoeic patients in that group to nine. At the end of the study, the number of apnoeic dogs in the groups 0 to 3 μgkg^{-1} was 12 (1, 0, 1, 2, 1, 5 and 2, respectively). In the group that received the highest dose of MED (10 μgkg^{-1}), nine dogs were still apnoeic at the end of the study (Table 4.1).

	Groups of dogs (n = 10 per group)								Total
Medetomidine (μgkg^{-1})	0	0.01	0.03	0.1	0.3	1	3	10	
Apnoeic dogs at time 0	**2**	**1**	**1**	**3**	**4**	**6**	**2**	**1**	**20**
Number of dogs becoming apnoeic after MED injection	**0**	**0**	**0**	**0**	**1**	**1**	**1**	**8**	**11**
Number of dogs apnoeic at the end of the study	**1**	**0**	**1**	**2**	**1**	**5**	**2**	**9**	**21**

Table 4.1

Number of apnoeic dogs in the 8 groups (control group (CG) to 8) of 10 dogs at time 0, after the injection of saline (CG) or medetomidine (MED) (0.01, 0.03, 0.1, 0.3, 1, 3 and 10 μgkg^{-1}) and at the end of the study (20 min).

Figure 4.5 reports the changes in respiratory rate (RR) observed in the spontaneously breathing dogs (60/80) from the 8 groups from time 0 to the end of the recording period. At 2 min, a non significant ($p > 0.05$) drop in RR from baseline was observed in group 1 μgkg^{-1}. By 15 min, the rate was back to baseline. Although group 3 μgkg^{-1} had a RR below baseline from 2 min up to the end of the recording period, it was only significant at 5, 10 and 15 min. In the last group (10 μgkg^{-1}), at 2 min, a significant decrease in RR was recorded and stayed significantly lower than baseline up to the end of the recording period.

From the 8 groups of 10 breathing and non-breathing dogs, a non-significant ($p > 0.05$) decrease in Et CO_2 was observed in groups 1 and 10 μgkg^{-1} at 2 min post MED injection (Figure 4.6). It was followed by an increase above baseline which was maintained for the all recording period. In group 3 μgkg^{-1}, the drop in Et CO_2 recorded at 2 min was significant and was followed by an increase above baseline for the rest of the recording time. Due to a technical incident, capnography was not available for 14 dogs from groups 0.01, 0.03, 0.1, 0.3 and 1 μgkg^{-1} (2, 2, 3, 5 and 2 dogs, respectively). Eight of these 14 dogs were spontaneously breathing at time 0: 1 in group 0.01, 2 in groups 0.03 and 0.1, and 3 in group 0.3 μgkg^{-1}).

SpO_2 stayed over 97% for all patients.

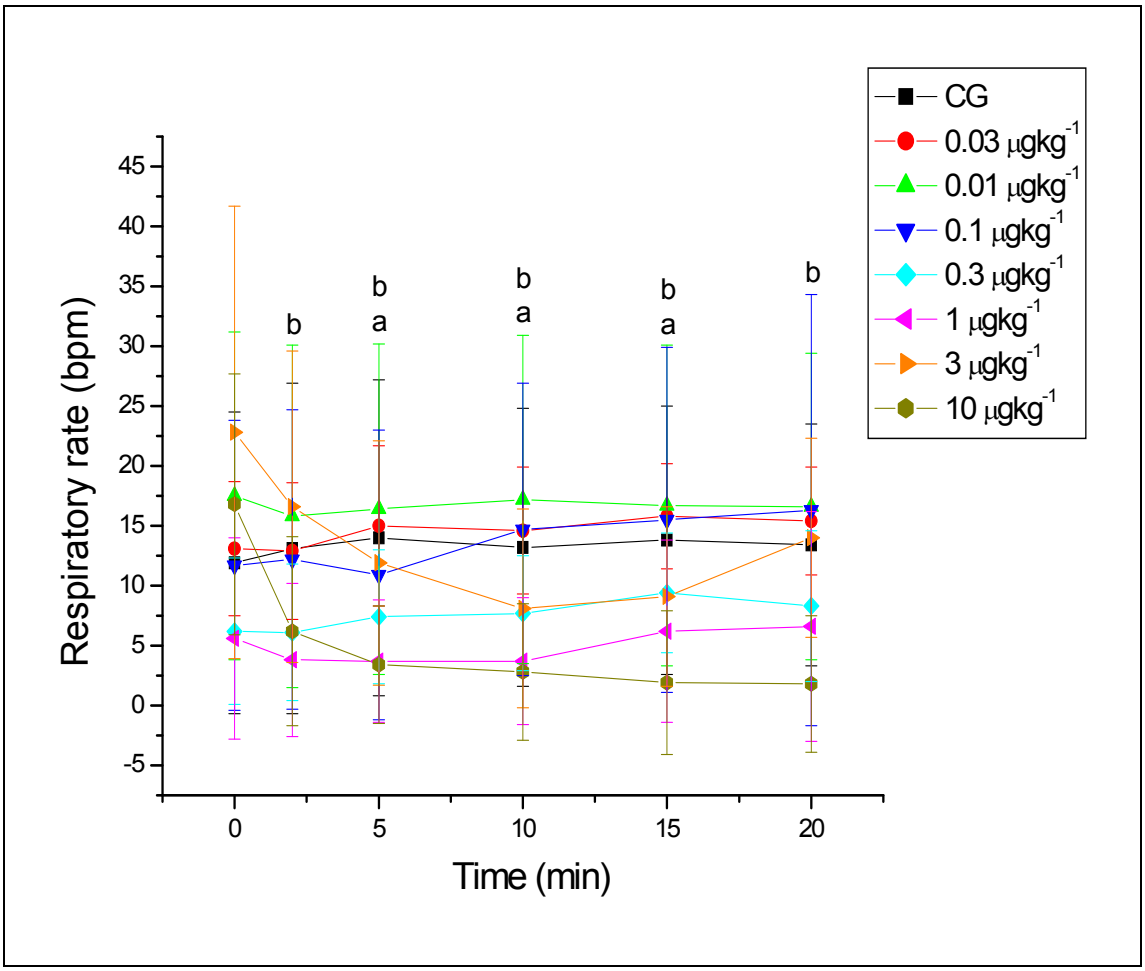


Figure 4.5

Mean (± SD) respiratory rate (bpm) for 8 groups (control group (CG) to 8) of 10 dogs given medetomidine (MED) (CG, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µgkg^{-1}, respectively) from the time of MED bolus injection (time 0) to the last recording time (time 20 min).

a = significant difference in groups 3 µgkg^{-1} from baseline ($p \leq 0.0426$).

b = significant difference in group 10 µgkg^{-1} from baseline ($p \leq 0.0002$).

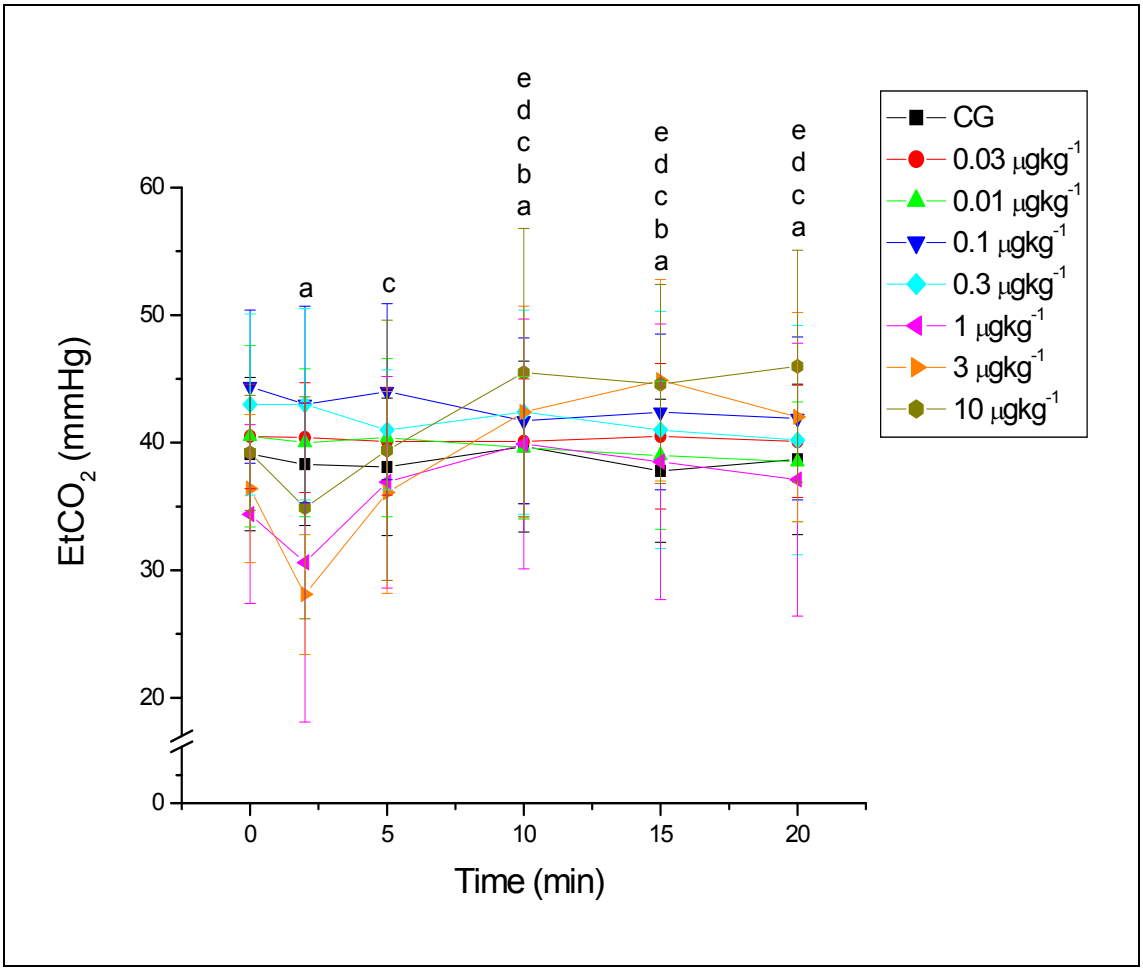


Figure 4.6

Mean (± SD) end tidal CO_2 (Et CO_2) (mmHg) for 8 groups (control group (CG) 1 to 8) of 10 dogs given medetomidine (MED) (CG, 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 µgkg^{-1}, respectively) from the time of MED bolus injection (time 0) to the last recording time (time 20 min).

a = significant difference in groups 3 µgkg^{-1} from baseline ($p \leq 0.0192$).

b = significant difference in group 1 µgkg^{-1} from time 2 min ($p \leq 0.0129$).

c = significant difference in group 3 µgkg^{-1} from time 2 min ($p \leq 0.0002$).

d = significant difference in group 3 µgkg^{-1} from time 5 min ($p \leq 0.0111$).

e = significant difference in group 10 µgkg^{-1} from time 2 min ($p \leq 0.0155$).

4.4 Discussion

In this study, a significant increase in the ABP was observed in dogs receiving MED 0.3 μgkg^{-1} or more (group 5, 6, 7 and 8). During the 20 min observation period, the ABP returned to pre-injection values for groups 5 and 6 (0.3 and 1 μgkg^{-1}) while the ABP stayed significantly higher in groups 7 and 8 (3 and 10 μgkg^{-1}). Neither hypotension (systolic ABP < 80 mmHg) nor ABP below baseline was ever observed. Previously, Pypendop and Verstegen (1998) observed an increase in ABP after injecting IV a dose of MED of 1, 5 10 and 20 μgkg^{-1} in conscious dogs. In the same study, although no increased in ABP was observed in the group receiving MED 2 μgkg^{-1}, in this group as well as in all the others, the ABP started to decrease, reaching levels below baseline.

Pypendop and Verstegen (1998) also concluded that low doses of MED (2 μgkg^{-1} and below) would have predominantly central effects and would therefore result in decreased ABP. In the study reported here, even doses as low as 0.01 μgkg^{-1} did not result in a decrease in the ABP. Only increases were observed in this study with doses of 0.3 μgkg^{-1} and greater. In the study reported here, an observation time of 20 min was possibly too short to record ABP below baseline. Although Pypendop and Verstegen (1998) showed that the "decrease in ABP" phase was already apparent in all the groups after 10 min observation period.

The importance of the integrity of the sympathetic nervous system for expression of the centrally induced sympatholytic effect of the $alpha_2$-adrenoceptor agonists has been demonstrated in humans suffering from high cervical, complete, spinal cord injury where the administration of clonidine (*per os* or IV) did not result in decrease ABP (Reid *et al.*, 1977; Kooner *et al.*, 1991; Young *et al.*, 2006). Similarly general anaesthesia in dogs (this study) created a state with decreased sympathetic tone resulting in an apparent lack of centrally induced sympatholytic effect. Therefore, only the peripheral vasopressor effect of MED is observed. In animals, autonomic denervation enhances the vasopressor response to the use of an $alpha_2$-adrenoceptor agonist (Schmeling *et al.*, 1991). The effect of anaesthesia vs. consciousness on the centrally induced sympatholytic effect of $alpha_2$-adrenoceptor agonists such as DEX has been previously demonstrated in studies in man (Talke *et al.*, 1999; Talke *et al.*, 2003) as well as in dogs (Flacke *et al.*, 1990; Flacke *et al.*, 1993).

In their study, Pypendop and Verstegen (1998) only reported the changes in the mean ABP but indicated that the systolic ABP followed a similar pattern. A similarity in the degree of increase from baseline between systolic and mean ABP was also reported later in a study in dogs with DEX (Kuusela *et al.*, 2001). Therefore, we can consider that in the Pypendop and Verstegen study, as the mean ABP, the systolic ABP must have increased by up to 27% (20 μgkg^{-1} group). The magnitude of increase in the systolic ABP in this study was similar, up to 36% (10 μgkg^{-1} group), although the doses of MED used here was 2 times less.

Pypendop and Verstegen (1998) used a group of beagles aged between 1 and 4 years while the actual study used a group of mixed breed dogs aged between 0.5 and 9 years (mean of 3.7 ± 2.6).

The baseline in systolic ABP of each group in the actual study was similar to the one reported by Nolan and Reid (1993) in beagles (8-9 months old) under similar circumstances. Although it is possible that the magnitude of the response in ABP to the injection of MED might be influenced by the breed, the groups (beagles vs. mixed-breed) seem to be similar in terms of baseline systolic ABP values.

Vickery and Maze (1989) reported no significant effect on ABP of MED (1, 3 and 10 μgkg^{-1}) injected into the right atrium in halothane-anaesthetised dogs, but the $alpha_2$-adrenoceptor agonist was injected over a 15 min period compared to 1 min and less in this study and Pypendop and Verstegen's (1998), respectively. The speed of injection being similar between the 2 studies (actual and Pypendop and Verstegen), it seems less likely to be responsible for the difference observed in the ABP as it has been previously described in humans with DEX (Talke *et al.*, 1999). One can speculate that if the speed of injection might have an effect, a difference in drug concentration could as well. Pypendop and Verstegen (1998) did not mention the concentration of MED used. In the actual study, dogs from groups 7 and mainly 8 (3 and 10 μgkg^{-1}) were more likely to receive the commercial concentration of MED (1000 μgml^{-1}) as the max volume of injection could not be higher than 2 ml. Thus in the Pypendop and Verstegen study, the concentration could only be equal or lower than the one used in group 10 μgml^{-1} from this study. This observation combined with a higher speed of injection (Pypendop and Verstegen 1998) does not make the speed of injection nor the MED concentration the likely causes for the observed difference in the trends in ABP between the 2 studies.

Propofol is contained in an intralipid solution. It has been shown that intralipid solution has some indirect positive effects on the pressor activity of phenylephrine, a pure $alpha_1$ agonist (Haastrup *et al*., 1998). Medetomidine shows some $alpha_1$ activity ($alpha_2/apha_1$ = 1620) (Virtanen *et al*., 1988; Bryant and Clarke, 1996). Dexmedetomidine has an $alpha_{2/1}$ ratio of 1620, LEV has only a ratio of 23 (Kuusela *et al*., 2001b). Both enantiomers and mainly LEV might have some $alpha_1$ effects. These effects might have been enhanced by the intralipid solution during PPF infusion, blunting the centrally induced vascular effect of MED.

In a study in enflurane-anaesthetised dogs (Flacke *et al.*, 1993), an increase in ABP was also observed even after IV DEX doses as low as 0.25 μgkg^{-1} (equivalent to about 0.5 μgkg^{-1} of MED), which rules out the intralipid solution as the principal cause of the difference in results between this study and Pypendop and Verstegen study (1998).

Nitrous oxide was also used in this work and it has some sympatho-mediated CV effects (Steffey, 1999; Talke *et al*., 2000c). The possible effects of this gas (at the concentration used in this study) on the CV system include an increase in HR as well as in CO and in mean ABP (Clutton, 1999) and therefore it could also have participated in blunting the hypotensive phase of MED injection.

Finally, in a study on autonomically denervated chloralose anesthetised dogs, Flacke and colleagues (1990) speculated that the exaggerated and sustained increase in ABP observed after DEX administration was due to the absence of a concomitant, centrally mediated, decrease in

sympathetic vasoconstrictor tone. Different studies in mice have compared the effect of DEX in normal vs. alpha$_{2A}$- adrenoceptor depleted animal. They all reported a lack of hypotensive phase after DEX injection while the observed and sustained hypertensive phase was of higher intensity than in the normal mice (MacMillan *et al*., 1996; Altman *et al*., 1999; Tan *et al*., 2002). Therefore the difference observed in the magnitude of increase in ABP between the Pypendop and Verstegen study and this study probably resulted from the anaesthesia induced decrease of sympathetic tone leading to an apparent lack of the centrally mediated sympatholytic effect of MED, thus unmasking the peripheral vasopressor effect.

The effect of MED on ABP in this study was dose-dependent, as previously indicated in conscious dogs. Pypendop and Verstegen (1998) reported a "near" maximum effect for the ABP with the highest dose (20 μgkg^{-1}), which was confirmed in this study in PPF anaesthetised dogs, with a near maximum effect (ED$_{95}$) estimated as 18 μgkg^{-1}. This result was an estimation, calculated using a programme software Origin 6.1 (Origin Lab Corporation**,** Massachusetts, USA, 2000), as the use of doses in excess of 10 μgkg^{-1} is not considered appropriate in clinical cases (Murrell and Hellebrekers, 2005). Although it is an extrapolation, the ED$_{95}$ obtained is more representative of the canine population, as mixed breed dogs were used instead of a specific breed. The results reported in this study can also be compared with those from Pypendop and Verstegen' study, as the groups of dogs in this study were comparable to a group of anaesthetised beagles in terms of baseline values for ABP (Reid and Nolan, 1993) (see above). The reliability and generalisation of the results is likely to be greater, since more groups of dogs (8 vs. 5) and twice as many dogs per group (10 vs. 5) were used. In this study, client owned dogs could be used only once and therefore no crossover design was possible which would have given more strength to the results (Myles and Gin, 2004). Equally, the instrumentation used in the Pypendop and Verstegen study (1998) did not allow such a crossover design either.

While direct arterial catheterisation may have improved the accuracy of the results obtained in this study, this was not considered justifiable in ASA 1 and 2 elective surgical patients. Moreover, several studies have shown good correlation between indirect and direct ABP monitoring in anaesthetised dogs (Sawyer *et al.*, 1991; Gains *et al*., 1995; McMurphy *et al.*, 2006). These studies concluded that while the oscillometric techniques tend to underestimate the diastolic pressure and overestimate (Sawyer *et al*., 1991; Gains *et al.*, 1995) or underestimate (McMurphy *et al*., 2006) the systolic ABP, they seem to adequately demonstrate changing trends in the ABP. Even so the difference for the systolic ABP tends to increase during hypertension when compared to direct reading (McMurphy *et al*., 2006). Similar results were described previously by Bodey and Michell, (1996), but in conscious dogs). It may be argued that peripheral vasoconstriction due to MED may render indirect methods of ABP measurement inaccurate (Kittleson and Olivier, 1983). The lack of readings for ABP from the oscillometric system in the dogs dosed with 3 and 10 μgkg^{-1} (groups 7

and 8) could effectively have resulted from peripheral vasoconstriction or from the sudden decrease in HR and appearance of A-V blocks as these systems have been shown to be unreliable during episodes of arrhythmia (Vater, 1996). Although some episodes of 1st degree A-V blocks appeared in some dogs of groups 5 and 6, it did not seem to interfere with the readings of the Dinamap. The use of a Doppler system in the last 2 groups to measure the systolic ABP allowed this problem to be overcome. Doppler methods in dogs tend to have a low bias with good precision to reading of the systolic ABP mainly during hypertension (Chalifoux *et al.*, 1985; Stepein and Rapoport, 1999; Haberman *et al*., 2006). With non-invasive methods, it is usually accepted that in the conscious dog the mean of series of 3 to 10 readings much improve the precision of the measurements. This method increases the measurement time up to 6 min (3 readings from 30 up to 120 sec per readings) (Bodey and Michell, 1996; Stepien and Rapoport, 1999; Haberman, *et al.* 2006). As the ABP was changing rapidly in the first 2 min in the dogs in this study, this would have increased the risk of variability and inaccurate measurement. Although the recording of one reading might equally have added variability to the data, in a study in anaesthetised dogs it was shown that for the systolic ABP, a single indirect measurement (oscillometric) was a good estimate of the ABP (Gains *et al.*, 1995). The same observation was reported with the Doppler system in conscious cats (Jepson *et al*., 2005).

In this study, the effect of MED on the HR was dose-dependent and confirmed the results of the study of Pypendop and Verstegen (1998). Although a maximum effect was achieved with similar doses (between 3 and 10 μgkg^{-1} and about 5 μgkg^{-1} for the actual study and the Pypendop and Verstegen study, respectively), the magnitudes were different, with 50% changes in this study while the Pypendop and Verstegen study the decrease of the HR was about 62%. Looking at the graph for the HR from their study, it appears that a 66% effect on the HR (ED_{66}) was obtained with doses of 1 and 2 μgkg^{-1}. The lack of lower doses of MED does not allow us to extrapolate an ED_{50} from those data. An interesting observation is that an estimate of the ED_{66} in this study would be around a dose of 0.6 μgkg^{-1}. It seems therefore that, in PPF-anaesthetised dogs, the effect of the $alpha_2$-adrenoceptor agonist on the HR is not as pronounced but appeared sooner by comparison with the conscious patients.

The HR in PPF-anaesthetised patients slows down (Godet *et al*., 2001; Pereira *et al*., 2004), probably due to a decrease in sympathetic outflow (Ebert *et al*., 1992b) as well as a decrease in baroreceptor sensitivity (Godet *et al*., 2001; Sato *et al.*, 2005). As for the ABP (see above), the decrease in sympathetic tone resulting from general anaesthesia will unmask peripheral effect from MED injection (Talke *et al.*, 2003). Therefore, the effect on the HR is likely to be dependent on ABP or systemic vascular resistance (SVR) and baroreflex activity. As baroreceptor sensitivity is decreased by PPF (Ebert *et al.*, 1992b; Sellgren *et al.*, 1994) changes in HR due to an increase in SVR will probably be attenuated, resulting in a decrease in magnitude in the change in HR. In

studies on alpha$_2$-adrenergic receptor subtype, it has been shown that in transgenic mice missing the alpha$_{2A}$-adrenoceptor responsible for the centrally mediated sympatholytic effect, the observed bradycardia was of lower intensity than in normal subjects (MacMillan *et al.*, 1996; Altman *et al*., 1999; Tan *et al*., 2002). In this study, it has been proposed for the ABP that general anaesthesia, by decreasing the sympathetic tone, caused an attenuation if not an elimination of the centrally mediated response. Consequently, a decreased sensitivity of the baroreflex, in addition to a lack of sympatholytic activity, is likely to have resulted in a bradycardia of lower magnitude compared with the conscious dog.

An effect on the HR was observed with a dose as low as 0.03 μgkg^{-1}, while at that dose no effect on the ABP was detected. A similar observation was made by Pypendop and Verstegen (1998), but with doses of 1 and 2 μgkg^{-1}. In a study in conscious rats using DEX IV, Bol and colleagues (1997) arrived at the conclusion that on the basis of the plasma concentration, the HR-decreasing and mean ABP-increasing effects of the alpha$_2$-adrenoceptor agonist could be separated, the decrease in HR being the most sensitive, followed by the increase in ABP.
A possible explanation for this difference in sensitivity could be a direct effect of the alpha$_2$-adrenoceptor agonist on the myocardium, but it has been shown that in the isolated canine heart the decrease in cardiac function observed after the use of DEX is not due to a direct action on the myocardium (Flacke *et al*,. 1992; Day and Muir, 1993; de Morais and Muir, 1995; Khan *et al.*, 1999; Murrell and Hellebrekers, 2005).
In isoflurane anaesthetised cats, a dose of 20 μgkg^{-1} of MED given IV resulted in no effect on the ABP while the HR was significantly reduced (Lamont *et al*., 2001). As Lamont and colleagues observed a significant increase in SVR, they attributed the decrease in HR to a baroreceptor-mediated response to an increase in SVR, as well as decreased central nervous system sympathetic outflow. In this study, it was hypothesised that the decrease in HR resulted from a stimulation of baroreceptors due to increases in SVR. It is therefore proposed that this increase in SVR resulted in a decrease in stroke volume and therefore CO. As the ABP is the product of the CO and the SVR, it is possible that with low doses of MED, the decrease in CO and the increase in SVR compensate each other and therefore no alteration in ABP is observed.

It has been shown that DEX has a higher binding potency for the alpha$_{2A}$ adrenoceptors than for the alpha$_{2B}$ (Audinot *et al*., 2002; Lalchandani *et al.*, 2004). Although it is usually accepted that the 2As subtypes are mainly responsible for the centrally mediated sympatholytic effect and that the 2Bs are responsible for the peripheral action of the alpha$_2$-adrenoceptor agonists (Link *et al*., 1996, Talke *et al*. 2003), some studies have reported that alpha$_{2A}$ receptor subtypes were also present in the wall of some arteries or veins (MacMillan *et al*., 1996; Gavras *et al*., 2001; Willems *et al.*, 2001). As general anaesthesia decreases the sympathetic tone, it is possible that low doses of DEX mainly stimulate the 2A subtypes present on walls of arteries and veins, resulting in a decrease in

CO equal in intensity to the SVR so that the ABP remains unchanged. Moreover, as sedation and analgesia are centrally mediated by the alpha$_{2A}$ adrenoceptors (Lawhead *et al*., 1992; Mizobe *et al.*, 1996; Hunter *et al.*, 1997; Lakhlani *et al*., 1997; Stone *et al*., 1997; Zhang *et al.*, 1998; Malmberg *et al.*, 2001; Mansikka *et al.*, 2004), it is hypothesised that doses of 0.03 μgkg^{-1} will induce few CV effects while still providing analgesia and a sparing effect on the anaesthetic agent. More studies need to be designed to confirm this, as it has been shown in mice that while occupancy of less than 50% of the alpha$_{2A}$ receptors is required to lower ABP, more than 50% occupancy is needed to provide sedation (Tan *et al.*, 2002).

Post-induction apnoea is the most common side effect following PPF administration (Goodman *et al.*, 1987; Watkins *et al.*, 1987; Smith *et al*., 1993). The incidence and duration of apnoea is dependent on the premedication regimen used, the speed of injection of PPF and the dose of PPF administered (Langley and Heel, 1988; Smith *et al*., 1993). In this study, although dogs received the same premedication, some appeared to be more profoundly sedated than others. This assertion is based on clinical judgement, only, as assessment of the level of sedation was not incorporated into the protocol. The more profound sedation observed in these dogs may have counted for the greater effect of the PPF induction dose on the respiratory system. When compared with other studies of the use of PPF in the dog, the 22.5% incidence of apnoea at induction observed in this study was elevated but still within reported ranges (Morgan and Legge, 1989; Cullen and Reynoldson, 1993; Smith *et al*., 1993; Nolan *et al.*, 1993; Quandt *et al*., 1998; Muir and Gadawski, 1998; Lerche *et al.*, 2000; Musk *et al*., 2005). For those dogs where the premedication had a greater effect, it is reasonable to think that in the normal clinical environment, lower induction targets would have been chosen, resulting probably in a lower incidence of apnoea.

Apnoeic episodes of short duration, separated by irregular breathing, have been described in humans with DEX infusion (1 and 2 μgkg^{-1} over 2 min) (Belleville *et al.*, 1992). This has also been described in dogs with the use of MED (IV bolus of 40 μgkg^{-1}) (Clarke and England, 1989; Short, 1991; Alibhai *et al.*, 1996). On the other hand, extended apnoea in the conscious or anaesthetised dog after the use of MED or its active enantiomer DEX has not yet been described. In this study, apnoea after MED injection was observed in one dog in groups 5, 6 and 7 (0.3, 1 and 3 μgkg^{-1}). The apnoea lasted less than 10 min in these three dogs. In the last group (10 μgkg^{-1}), eight out of the nine spontaneously breathing dogs became apnoeic and did not recover spontaneous breathing for the time of the study (20 min). These observations show with the addition of a hypnotic agent such as PPF, sub-sedative to sedative doses of MED might result in apnoeic episodes similar to the one observed with potent opioids (Hughes and Nolan, 1999). These results are in line with a study by Nguyen *et al.* (1992) in the 1.5% isoflurane-anaesthetised dog where they found that an IV injection of 20 μgkg^{-1} of DEX resulted in a significant depression of the hypercapnoeic response. Therefore precautions must be taken when combining MED or its active enantiomer with a

hypnotic agent such as PPF. This raises questions concerning the additive or synergistic effects the two agents might have on ventilation in dogs.

At time 2 min, a significant (group 7) and non-significant (groups 6 and 8) decrease of the Et CO_2 was observed. In these groups, the number of dogs breathing spontaneously was 6, 7 and 5 for groups 1, 3 and 10 μgkg^{-1}, respectively. In these dogs the RR decreased from 14 (± 7.4), 32 (± 15.2) and 22 (± 8.6) to 10 (± 7.1), 24 (± 7.6) and 12 (± 6.5) breaths per min as well as the Et CO2, from 39 (± 6.7), 35 (± 5) and 39 (± 3.8) to 35 (± 16.5), 27 (± 4.4) and 32 (± 8.1) mmHg for groups 1, 3 and 10 μgkg^{-1}, respectively. The values for the Et CO2 were back to baseline values by time 5 min for groups 1 and 3 μgkg^{-1} while they were still elevated at the end of the study for group 10 μgkg^{-1}. Cardiovascular incidents such as decrease of CO or venous air embolism are the usually described causes of abrupt decrease in the Et CO_2 readings (O'Flaherty, 1994). Other causes such as apnoea, endotracheal tube occlusion, cardiac arrest, capnometer disconnection and sampling tube occlusion have also been described (O'Flaherty, 1994). The observed decrease in the Et CO_2 values in this study could be related to the negative effect of the $alpha_2$-adrenoceptor agonist on the CO (Bergstrom, 1988; Clarke and England, 1989; Savola, 1989; Vainio, 1990; Zornow *et al.*, 1990; Hall and Clarke, 1991b; Bloor, *et al.* 1992). In a study by Lerche and Muir (2006), MED 5 μgkg^{-1} was administered IV to anesthetised dogs (isoflurane or halothane), a situation very similar at this study. No decrease in Et CO_2 was reported following the injection of the $alpha_2$-adrenoceptor agonist. Although the decreased Et CO_2 phase might have been present in that study, it is possible that the decrease in Et CO_2 was missed, as the first reading time post MED injection was 5 min, by which time in the study reported here, most the Et CO2 values in the ventilated and non-ventilated dogs were back to baseline or higher (Figure 4.6). In another study in conscious dogs receiving DEX 1, 3 or 10 μgkg^{-1} IV, Sabbe *et al*. (1994) reported no effect on the Et CO_2 and RR except with the highest dose, which induced a sudden decrease in Et CO_2 (non-significant) with a decrease (significant) in the RR soon after administration of the $alpha_2$-adrenoceptor agonist.

In the actual study, by time 10 min, the Et CO_2 values were higher than baseline and stayed elevated for the whole recording period. Respiratory depression after intravenous administration of MED or DEX has been described in animals (Nguyen *et al*., 1992; Sabbe *et al*., 1994; Bol *et al*., 1997; Lerche and Muir, 2004; Lerche and Muir, 2006) as well as in humans (Belleville *et al*., 1992). It often results in a decrease in the resting breathing rate and a decrease in the response to hypercapnoea resulting in an increase in $PaCO_2$ (Nguyen *et al*., 1992; Belleville *et al*., 1992; Sabbe *et al*., 1994; Bol *et al.*, 1997; Lerche and Muir, 2004; Lerche and Muir, 2006).

4.5 Conclusions

In this study, I have looked at the response to increasing doses of MED in the TCI PPF anaesthetised dogs. I determined the ED_{50} for the HR and the ABP. The different doses of MED were administered as an IV bolus over 1 min. Bolus injections result in high initial plasma concentration (Schnider *et al*., 1998). In humans, Ebert *et al*. (2000) reported that with an infusion scheme targeting a plasma concentration similar to the DEX plasma concentration obtained after a single IV bolus injection, the CV effects were less pronounced. They concluded that the difference resulted from that, during infusion without a bolus, the plasma concentration rises more slowly and allows DEX to equilibrate with the central nervous system. This results in an inhibition of the sympathetic system and therefore the perivascular effect action (vasoconstriction) is masked. This was also observed in other studies in humans (Talke *et al.*, 2003; Ickeringill *et al.*, 2004). It seems therefore that infusion of either MED or its active enantiomer DEX might be more appropriate if one wants to minimise or even eliminate CV effects while still producing analgesia and hypnotic sparing effect.

In this study, it has been confirmed that the CV effects such as bradycardia and hypertension resulting from administration of MED in TCI PPF anaesthetised dog were dose-dependent. The study also demonstrated that doses of MED below or equal to 0.1 μgkg^{-1} had no or minimum effects on the ABP and the HR, respectively. The CV effects appeared at lower doses in TCI PPF anaesthetised dogs than in conscious patients. The general anaesthetic agent may therefore amplify these CV effects, which may limit the use of MED as an analgesic in PPF anaesthetised dogs. However, this information added to pharmacokinetic data of MED in the dog enable further investigation of infusion schemes for MED exploiting the analgesic and anaesthetic agent sparing effects while minimising the CV consequences. The study also demonstrated that the respiratory effects of MED were minimal at low doses which confirmed the drug's potential suitability as an alternative to opioid analgesia.

CHAPTER 5:

PHARMACODYNAMIC AND PHARMACOKINETIC PROPERTIES OF MEDETOMIDINE AND DEXMEDETOMIDINE INFUSIONS IN DOGS ANAESTHETISED WITH PROPOFOL ADMINISTERED BY TCI.

5.1 Introduction

The inhibitory effect of medetomidine (MED) and its enantiomers on the metabolism of propofol (PPF) through the canine hepatic cytochrome P450's (CYPs) enzymatic system was demonstrated in chapter 3 in an *in vitro* study. However, from that investigation, no conclusion could be drawn regarding the possible negative impact of MED on the pharmacokinetics (PK) of PPF in the dog *in vivo*. Before undertaking a study to clarify this possible PK interaction between the alpha$_2$-adrernoceptor agonists and PPF *in vivo*, which might be expected to affect the performance of the target controlled infusion (TCI) system (developed in chapter 2), a dose response study in dogs anesthetised with TCI PPF was carried out. This indicated that cardiovascular (CV) effects resulting from administration of MED in TCI PPF anaesthetised dogs were dose-dependent (chapter 4). Doses of MED below or equal to 0.1 μgkg^{-1} injected over one min IV had no or minimum effects on the arterial blood pressure (ABP) and the heart rate (HR), respectively. This study also demonstrated that the observed CV effects appeared at lower doses in TCI PPF anaesthetised dogs than in conscious patients (Pypendop and Verstegen, 1998).

Building on the results of these previous studies, this latest study was designed to develop an infusion scheme for dexmedetomidine (DEX) and MED for co-infusion with PPF TCI and to study the PK and pharmacodynamic (PD) interactions between these agents and PPF in dogs. In parallel, an investigation was conducted to determine the plasma concentration (Cp_{50} and Cp_{95}) and the minimum infusion target (MIT_{50} and MIT_{95}) of PPF which would abolish the response to a supra-maximal noxious stimulus in 50 and 95%, respectively of PPF TCI anaesthetised dogs with and without co-infusion of DEX and MED.

5.1.1 Propofol and medetomidine/dexmedetomidine

In dogs (Vainio, 1991; Thurmon *et al.*, 1994; Bufalari *et al*., 1996) and less commonly in humans (Peden *et al.*, 2001), alpha$_2$-adrenoceptor agonists are administered as part of the premedication. Perioperative use of either MED or DEX by infusion has been reported during PPF anaesthesia in man (Aantaa *et al.*, 1997; Dutta *et al*., 2001; Larson and Talke, 2001) and in horses (Bettschart-Wolfensberger *et al.*, 2001b, 2005; Umar *et al.*, 2006, 2007).

Medetomidine and DEX infusions have been described in dogs during sevoflurane anaesthesia (Gomez-Villamandos *et al.*, 2008) and isoflurane anaesthesia (Pascoe *et al*., 2006), respectively. To the authors knowledge there appear to be no reported studies on the use of MED during PPF anaesthesia, though the use of DEX has been described (Lin *et al.*, 2008).

5.1.1.1 Pharmacokinetic studies

Two PK studies in dogs given a single injection of MED (80 and 40 μgkg^{-1} IV) produced the same value for the elimination half-life (57.6 min) (Salonen, 1989; Kuusela *et al*., 2000). In these two studies, total body clearance was 2 and 1.25 $Lkg^{-1}h^{-1}$ (33.3 and 20.8 $mlkg^{-1}min^{-1}$), respectively. For PPF, although a wide variation from 54 to 486 min, a majority of studies reported values around 70 min for the elimination half-life (Cockshott *et al.*, 1992; Nolan and Reid, 1993; Reid and Nolan, 1993; Zoran *et al.*, 1993; Hall *et al.*, 1994; Mandsager *et al.*, 1995; Reid and Nolan, 1996; Hughes and Nolan, 1999). In regard to the clearance, most of the authors recorded values around 40 (34–115) $mlkg^{-1}min^{-1}$ (Cockshott *et al.*, 1992; Nolan and Reid, 1993; Reid and Nolan, 1993; Zoran *et al.*, 1993; Hall *et al.*, 1994; Mandsager *et al.*, 1995; Reid and Nolan, 1996, Hughes and Nolan, 1999). For comparison, the values for the half-life elimination and the clearance used in the TCI developed in chapter 2 are 38 min and 54.6 $mlkg^{-1}min^{-1}$, respectively.

In the present study, a stepped infusion (chapter 1) scheme was developed to target specific MED blood concentrations using PK parameters provided by O. Vainio (personal communication, 2001) and derived from the study of Kuusela and colleagues (2000). Since the PK of DEX is similar to that of MED (Kuusela *et al.*, 2000), the same infusion scheme was used. The accuracy of a stepped infusion is dependent on the quality of the PK variables used. One objective of the present study was to assess the predictive performance of the stepped infusion scheme incorporating the selected PK variables, by comparing the MED or DEX concentrations predicted by computer simulation with the measured MED or DEX concentrations in venous blood samples taken at various time points during anaesthesia (Glass *et al.*, 1991; Coetzee *et al.*, 1995; Vuyk *et al.*, 1995; Short *et al.*, 1996; Glen, 1998; Oei-Lim *et al.*, 1998; Swinhoe *et al*., 1998; Varvel, 2002; Slepchenko *et al*., 2003; Li *et al.*, 2005; Ko *et al.*, 2007; White *et al*., 2008).

In vitro methods for drug interaction are relatively inexpensive, time and risk efficient studies (Venkatakrishnan *et al.*, 2003). Unfortunately they do not give an exact picture of the *in vivo* situation. Some methods have been described to allow extrapolation and/or prediction of clinical drug interaction on the basis of quantitative *in vitro* data on CYP inhibition. However, these methods are not well established or validated (Venkatakrishnan *et al.*, 2003) and *in vivo* studies are still needed. The effect of MED on PPF PK has been described *in vivo* in the dog (Hall *et al.*, 1994). In that study anaesthesia was induced and maintained with PPF in six dogs on two occasions with and without MED premedication. The authors concluded that the $alpha_2$-adrenoceptor agonist had no significant impact on the PK of PPF.

As in chapter 2, the evaluation of the predictive performance of the TCI system for PPF in the dog during MED/DEX co-infusion was carried out, according to the methodology described by Varvel (1992).

5.1.1.2 Pharmacodynamics

Several studies have examined the possible PD interactions between MED and PPF, and a more detailed description can be found in chapter 4. In summary, all the studies demonstrated a decrease in the amount of PPF necessary to induce as well as to maintain anaesthesia (in the studies where PPF was used for maintenance) (Vainio, 1991; Cullen and Reynoldson, 1993; Thurmon *et al.*, 1994; Bufalari *et al*., 1996). Induction with PPF resulted in an increase in ABP as well as in HR in all the studies as well as the one from Seliskar and colleagues (2007). In those studies where either atropine (Thurmon *et al.*, 1994; Bufalari *et al.*, 1996) was included in the premedication or where induction was not followed by PPF infusion (Cullen and Reynoldson, 1993), the ABP stayed higher than the baseline, while the HR decreased below baseline for some (Cullen and Reynoldson, 1993) or stayed above for others (Thurmon *et al*., 1994; Bufalari *et al*., 1996). Although Vainio (1991) reported a return towards baseline values for HR and ABP, the values stayed elevated in the study reported by Hellebrekers and Sap (1997).
The effect on the respiratory rate was variable with a decrease in some studies (Cullen and Reynoldson, 1993; Thurmon *et al.*, 1994; Bufalari *et al*., 1996) and no effect in others (Vainio, 1991, Seliskar *et al.*, 2007). Although Hellebrekers and Sap (1997) did not report the effect on the respiratory rate, they recorded the effect on the $PaCO_2$ and PaO_2. All 5 studies, but the one from Seliskar and colleagues (2007), showed an increase in $PaCO_2$, and two measured and reported a slight decrease in PaO_2 (Cullen and Reynoldson, 1993; Hellebrekers and Sap, 1997). Seliskar and colleagues (2007) recorded $PaCO_2$ and PaO_2 and did not observe any significant changes form baseline values.

The study reported by Thurmon *et al.* (1994) also considered effects on cardiac output (CO) and systemic vascular resistance (SVR) and reported that while the CO decreased and SVR increased after administration of MED (30 μgkg^{-1}) IM, no difference was observed during PPF infusion compared to baseline. The authors concluded that PPF alleviated MED-induced vasoconstriction.

Recently, Lin and colleagues (2008), in 10 PPF induced and anaesthetised mongrels, after an IV bolus of 1 μgkg^{-1}, started a 24 hour DEX infusion (1 $\mu gkg^{-1}h^{-1}$). Two hours after the start of the infusion, the anaesthesia was stopped. During the anaesthesia, compared with baseline they observed a significant decrease in HR, CO and cardiac index and a significant increase in central venous pressure as well as systolic and mean ABP and SVR. Respiratory rate, PaO_2 and $PaCO_2$ stayed around baseline over the 2 hour period.

5.1.1.3 Analgesia

5.1.1.3.1 Minimum medetomidine analgesic plasma concentration

The combination of MED (40 μgkg^{-1}, IM) and PPF anaesthesia (0.06 mgkg^{-1}min^{-1} after bolus of 2 mgkg^{-1}) provided acceptable operating conditions for elective ovario-hysterectomy in healthy bitches (Hellebrekers and Sap, 1997). Thurmon *et al.* (1994) used nerve stimulation (10 to 100 mV) through two subcutaneous 1 inch 18-gauge needles on either side of the tail (5th and 6th coccygeal vertebrae) and tail clamp to assess the degree of analgesia achieved with the combination of MED (30 μgkg $^{-1}$) and PPF bolus (2 mgkg^{-1}, IV) followed by PPF infusion (0.16 mgkg^{-1}min^{-1}, IV). No response to either stimulation voltage was observed during the 60 min of PPF anaesthesia. None of these studies measured MED blood concentrations, but it has been reported that the minimum blood concentration of MED necessary to provide analgesia in the conscious dog is between 2 and 9 ngml^{-1} (Kuusela *et al.*, 2000) while a review paper on MED reported that the minimum analgesic blood concentration in the dog was between 1 and 5 ngml^{-1} (Salonen, 1991). In a personal communication from Orion Pharma it was confirmed to the author from unpublished data that the minimum analgesic MED plasma concentration was about 1.7 ngml^{-1} and that the equivalent figure for DEX was 0.85 ngml^{-1} (Granholm, personal communication, 2001).

5.1.1.3.2 Determination of Minimum Infusion Target (MIT) for propofol

Minimum alveolar concentration or MAC corresponds to the minimum alveolar concentration of an inhalational anaesthetic agent that is required to abolish gross muscular movement in response to a supramaximal noxious stimulus in 50% of the population (Eger *et al.*, 1965). A similar approach has been used with the intravenous anaesthetic agents and the Cp_{50} and MIR_{50} have been defined as the plasma concentration and the minimum infusion rate of IV agent necessary to prevent a body movement to stimulus in 50% of the patients, respectively (Sear and Prys-Roberts, 1979; Flaishon *et al.*, 1997).

TCI systems allow the anaesthetist to maintain a constant target plasma concentration of an IV agent, thus allowing equilibration with the effect site resulting in maintenance of a steady-state effect site concentration. This provides better conditions for Cp_{50} determination and maintenance than constant rate infusion. Unfortunately, in-line analysis of PPF in blood is not yet available and so the anaesthetist cannot use the Cp_{50} information as a guideline to assess the level of anaesthesia. Howeve,r the introduction of a concept such as the minimum infusion target or MIT may be more clinically relevant for the anaesthetist using a TCI system to maintain anaesthesia as this system provides a prediction of the plasma concentration. Like Cp_{50} and MIR_{50}, the MIT_{50} would be

defined as follows: the minimum infusion target (predicted) necessary to abolish body movement to a painful stimulus in 50% of the population, while the MIT_{95} would be the target where 95% of the population would not respond to a similar stimulus. Compared with MIT where the computer will decrease the infusion rate exponentially over time to compensate for any accumulation of the agent infused, MIR does not take account of the PK of the agent used and infusion time and agent accumulation will have some influence on the real clinical value of this parameter.
To determine MIT, similar methods as described for the determination of MAC, MIR or CP_{50} could be used, such as response to skin incision, tail clamping and electrical stimulation (Eger *et al.*, 1965; Zbinden *et al.*, 1994; Andrews *et al.*, 1997).

5.1.2 Goals of the study

The objectives of this study were as follows

- To develop a co-infusion scheme for MED and DEX in dogs anaesthetised with PPF.
- To assess the PK parameters used to develop the MED and DEX stepped infusion in the TCI PPF anaesthetised dog.
- To determine the PK parameters of DEX in the TCI PPF anaesthetised dog.
- To assess the performance of the PPF TCI system in the dog during co-administration of MED or DEX.
- To assess the PD effect of MED and DEX infusion in TCI PPF anaesthetised dogs.
- To determine the effect of an infusion of MED or DEX on the minimum blood PPF target necessary to maintain anaesthesia during supra-maximal noxious stimulation.

5.2 Materials and Methods

5.2.1 Animals

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine of the University of Liege, Belgium. Six female, beagle dogs, aged between 5 and 8 years were included in the study. All dogs had full biochemical and haematological examinations before beginning of the study and were classified as ASA 1.

5.2.2 Development of a medetomidine/dexmedetomidine infusion schemes.

The following PK parameters for MED in the dog were obtained from O. Vainio (personal communication, 2001) and are derived from the study from Kuusela and colleagues (2000).

V1 = 470 $mlkg^{-1}$
K12 = 0.0954
K21 = 0.0438
K10 = 0.0489
K31 and k13 = 0

These parameters were used as inputs to a computer simulation using the program PK-SIM (Specialised Data Systems, Jenkintown, PA, USA) in order to determine stepped infusion schemes for MED and DEX, which would achieve predicted blood target concentrations of 1.7 $ngml^{-1}$ (MED and DEX) and 0.85 $ngml^{-1}$ (DEX). These infusion schemes were used in the experimental protocol described below (5.2.3).

5.2.3 Experimental design

The study was conducted over a 7 week period which consisted of 4 treatment weeks and 3 rest weeks. Medetomidine and DEX were provided by Orion Pharma (Turku, Finland).
There were four different treatments:

PS = PPF TCI with a co-infusion of saline
PM = PPF TCI with a co-infusion of MED (target concentration 1.7 $ngml^{-1}$)
PHD = PPF TCI with a co-infusion of high DEX (target concentration 1.7 $ngml^{-1}$)
PLD = PPF TCI with a co-infusion of low DEX (target concentration 0.85 $ngml^{-1}$)

Each dog was anaesthetised on 4 different occasions following a crossover design (Table 5.1)
During each test week 2 dogs were anaesthetised per day, on Monday, Tuesday and Wednesday, allowing a recovery period between treatments of 12 to 16 days for each dog.

The following table described the anaesthesia plan for each dog.

Week 1	Week 2	Week 3	Week 4
PS5	PS3	PHD3	PHD2
PHD4	PLD5	PM6	PLD4
PM2	PLD2	PS4	PM5
PLD6	PM4	PHD1	PHD6
PLD3	PLD1	PS2	PM1
PS1	PS6	PHD5	PM3

Table 5.1

Allocation of dogs (1-6) to 4 treatments on each of 4 treatment weeks

PS = Saline treatment; PM = medetomidine treatment; PHD = high dose dexmedetomidine 1.7 $ngml^{-1}$ treatment; PLD = low dose dexmedetomidine 0.85 $ngml^{-1}$ treatment; dogs 1 to 6.

5.2.4 Anaesthetic protocol

Before each treatment dogs were fasted for 12 h, but water was freely available up to 1 h prior to induction of anaesthesia. In addition, a routine pre-anaesthetic clinical examination of CV and respiratory systems and hydration status was carried out. Dogs were premedicated with acepromazine (ACP, Novartis Animal Health UK Ltd, Herts, UK) 0.03 $mgkg^{-1}$ and methadone (Martindale Pharmaceuticals, Essex, UK) 0.2 $mgkg^{-1}$ injected together intramuscularly 30 min prior to induction. Anaesthesia was induced with PPF, given through a preplaced cannula (Biovalve, Vygon, Cirencester, UK) inserted in the right cephalic vein, using the computer driven TCI system described in chapter 2, set to an initial induction target of 3 $\mu g\ ml^{-1}$. The end point of induction was considered to be when the animal assumed lateral recumbency and tolerated intubation of the trachea with no tongue movement or resistance. If the end point was not reached within 3 min, the target concentration was increased by 0.5 $\mu g\ ml^{-1}$ at 2 min intervals until induction was complete. After induction of anaesthesia, the dogs were placed in left lateral recumbency on an electric heating blanket and the induction target altered in increments of 0.5 μgml^{-1} as necessary to maintain a satisfactory depth of anaesthesia which was assessed subjectively on a clinical basis as described in chapter 2. The dogs breathed 100% oxygen throughout anaesthesia, delivered via a non-rebreathing circuit with an appropriate fresh gas flow rate.

The left pedal artery was cannulated and connected via a pressure transducer (Edwards Lifesciences Pressure Monitoring Set, Edwards Lifesciences LLC, Irvine, USA) to a Minimon 7132A amplifier and recorder (Kontron Instruments Ltd, England) for direct ABP monitoring. The

LiDCO^{TM} PLUS (LiDCO Ltd, Cambridge, UK) Flow Regulator (pump) and sensor for CO determination by lithium dilution was attached to the same arterial line using the LiDCO^{TM} Injectable Kit. Two additional cannulae were placed, one in the right jugular vein for blood sampling and the other in the left cephalic vein for the administration of Lactated Ringer's solution at 10 $mlkg^{-1}$ h^{-1} and for injection of the LiDCO^{TM} dye (lithium chloride) during CO determination. After instrumentation and calibration of the direct ABP and LiDCO monitoring systems, co-infusion of either saline (PS), MED (PM) or DEX (PHD and PLD) was started (Table 5.2).

The ECG (Kontron Micromon 7141, Kontron Instruments Ltd, England), pulse rate (PR), respiratory rate (RR), end-tidal carbon dioxide (Et CO_2) (Nellcor NPB 70; Nellcor Puritan Bennett Inc, CA, USA), oxygen saturation (SpO_2) (Nellcor N20; Nellcor Puritan Bennett Inc, CA, USA) and invasive ABP (Kontron Micromon 7141, Kontron Instruments Ltd, England) were monitored continuously. Data were recorded every 5 min throughout the period of anaesthesia. Rectal temperature was also recorded every 20-30 min to ensure normothermia. Cardiac output was measured using a lithium dilution technique (LiDCO^{TM} PLUS; LiDCO Ltd, Cambridge, UK) before the start of the co-infusion and 45 min later, at the time of the last change of the stepped infusion of MED or DEX. To measure the CO, a predetermined volume of lithium chloride (0.7-1 ml) was injected intravenously through the left cephalic vein and the lithium dilution curve was then measured by the LiDCO^{TM} computer system. Following the determination of the MIT to assess the analgesic effect of MED and DEX infusions, the co-infusion and PPF TCI were terminated. Thereafter time to extubation, sternal recumbency and standing were recorded for each animal. Time to extubation was recorded as the time from the end of the infusion to the first voluntary swallowing reflex; time to sternal corresponded to the time between the cessation of the infusions and the time when the dog adopted a sternal position; time to standing was the time between the cessation of the infusions and the time when the dog was able to stand without assistance.

In addition the following times were recorded for each dog: the instrumentation time which was the time between the start of the PPF infusion and the start of the co-infusion; the co-infusion time which was the time between the start of the co-infusion and the end of anaesthesia. For each individual, the amount of PPF infused during the instrumentation and co-infusion periods was recorded. These volumes, body weight and the duration of the various infusion periods were used to calculate the infusion rate of PPF during instrumentation, during the period of co-infusion and for the whole procedure. Similarly, the total amount of MED and DEX infused per kg body weight was recorded for each individual to derive the mean infusion rate of the co-infusion.

The following were noted for each individual: the number of unsuccessful attempts to intubate after 3 min and the number of increments of PPF needed to achieve intubation; the incidence and

duration of apnoea (absence of breathing for more than 30 sec); and the incidence and duration of involuntary movement, twitching or stiffness.

5.2.5 Determination of Minimum Infusion Target (MIT) for propofol

Fifteen min after the last step-down of the infusion of MED or DEX, or 60 min after the start of the saline infusion, electrodes were attached to 2 hypodermic needles (20G) placed subcutaneously at the level of the 4^{th} and 5^{th} coccygeal vertebrae. Using a nerve stimulator (Innervator 252, Fisher and Paykel Healthcare, Auckland, New Zealand), an electric stimulus (Tetanic twitch, 50Htz, 80mA) was applied for 5 sec maximum, or until purposeful movement was noted. If no movement was observed at the end of 5 sec, stimulation was stopped and the PPF blood target concentration was decreased by 0.4 $\mu g\ ml^{-1}$ and then maintained at that level for 10 min. The entire procedure was repeated after every 10 min equilibration period, until a response to tail stimulation was observed. If the animal moved in response to tail stimulation, the PPF blood target concentration was increased by 0.2 $\mu g\ ml^{-1}$ and after 5 min stabilisation, the tail was stimulated again. This was repeated until no purposeful movement was observed. The PPF blood target concentration was noted at each point and the mean value between the concentration at which the dog moved in response to the noxious stimulus and the concentration at which the dog stopped moving in reaction to the electrical stimulation was calculated to be the MIT for that individual.

Blood samples were taken for measured PPF each time the tail stimulus was applied, for comparison with the predicted values. During PPF MIT determination, blood samples for DEX or MED were taken when tail stimulation triggered a purposeful movement while PPF target blood concentration was being decreased, and when tail stimulation failed to trigger movement while the PPF blood target concentration was being increased. The mean of these 2 measured MED and DEX blood concentrations was taken to be the concentration at the time of MIT determination.

5.2.6 Blood sampling

Venous blood samples (1.5-2 ml) for PPF analysis were collected into fluoride oxalate tubes (Sarstedt Ltd, Leicester, UK). Samples were taken from all dogs before starting the co-infusion (time 0), at 10, 20 and 45 min after the start of the co-infusion and as described above during the MIT determination.

Venous blood samples (6 ml) for MED and DEX analysis were collected into serum separating tubes or SST (BD Vacutainer, Oxford, UK). Samples were taken at the end of each step of the co-infusion (5, 15, 30 and 45 min after the start of the co-infusion), just before the rate of infusion was

changed. Two samples were also taken during the MIT determination as described above. For the PHD treatment, one sample was also taken at 5, 10, 30, 60, 120, 300 and 420 min after the end of the co-infusion for the determination of the PK parameters of DEX.

5.2.7 Analyses

5.2.7.1 Propofol

After collection, the tubes were stored at -70ºC. At the end of week 2, 3 and 4, they were put into dry ice and transported to the Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow for analysis which was performed over the following 2 weeks by Dr Ana Monteiro. The methodology used was described in chapter 2.

5.2.7.2 Medetomidine and Dexmedetomidine

These analyses were performed by Orion Pharma Ltd (Turku, Finland). Samples were centrifuged after collection and the serum decanted into serum tubes (BD, Erembodegem-Aalst, Belgium) for storage at -70°C for the duration of the study. Thereafter they were packed in dry ice and transported to Orion Pharma Ltd (Turku, Finland) for analysis. Both DEX and LEV were determined in dog serum by liquid chromatography tandem mass spectrometry (LC-MS/MS). The analytes were extracted from serum samples with an organic solvent. The solvent was then evaporated to dryness and the residue redissolved in the LC mobile phase. Separation of the enantiomers was achieved in a chiral LC column and detection in an MS/MS system using atmospheric ionization and deuterated MED as the internal standard. The lower limit of quantification of the assay was 0.01 $ngml^{-1}$ for each enantiomer.
For MED, the blood concentrations were reported for each enantiomer DEX and LEV.
The accuracy was 97.8 ± 8.0% over the calibration range 0.010-25 $ngml^{-1}$.
The coefficient of variation was: 6.7% (0.025 $ngml^{-1}$); 5.4% (0.5 $ngml^{-1}$); 5.3% (20 $ngml^{-1}$).

5.2.8 Data Handling

As described in chapter 2, the target and predicted PPF concentrations were recorded at each sampling point and for each blood sample the percentage error (PE%) of the predicted concentration of PPF in the blood was calculated. For each animal the median prediction error (MDPE%) and the median absolute prediction error (MDAPE%) were calculated and from these values the treatment median MDPE% and MDAPE% were calculated to describe the overall performance of the TCI system (Varvel *et al.*, 1992; Coetzee *et al.*, 1995).

Using the computer simulation programme PK-SIM (Specialised Data Systems, Jenkintown, PA, USA), the predicted target concentrations for MED and DEX were determined and recorded at each sampling point. As for PPF, the PE% for each blood sample as well as the MDPE% and the MDAPE%, for each individual were calculated. Thereafter, the treatment median MDPE% and MDAPE% were calculated to describe the overall performance of the derived MED and DEX infusion schemes.

5.2.9 Determination of the PK parameters for dexmedetomidine

Data from the blood samples taken from the PHD treatment were fitted to a 2 compartment model using the software WinNonlin Professional 4.1 (Pharsight Corporation, Canada) to determine the PK of DEX. This software uses the Hartley's modification to the Gauss-Newton algorithm with the Levenberg –Marquardt modification.

The following parameters were calculated: V_1, K_{10}, K_{12} and K_{21}.

5.2.10 Statistical analysis

ANOVA for time and treatment effects was carried out for the systolic, mean and diastolic ABP, HR, Et CO_2, and RR using a General Linear model (Minitab 13) with Tukey 95% simultaneous confidence intervals.

The same model was used to look at the difference between the treatments for the infusion times (instrumentation, co-infusion and total); the amount of PPF infused (instrumentation, co-infusion and total); the CO; the PPF infusion rates (instrumentation, co-infusion and total); the different recovery times (extubation, sternal and standing times); the MDPE% and MDAPE% for PPF and DEX.

Differences were considered significant when $p < 0.05$.

Response to electrical stimulation (movement or not) was analysed using logistical equation (Origin 6.1; Microcal Software, Inc., Northampton, USA) to determine the PPF blood concentration (Cp) and the minimum infusion target (MIT) that prevented movement in 50% (Cp_{50} and MIT_{50}) and 95% (Cp_{95} and MIT_{95}) of the population.

$$Y = \frac{A1 - A2}{1 + (X / X_0)^p} + A2$$

Where x_0 is the centre

p is the power

A1 is the initial Y value

A2 is the final Y value

The Y value at X_0 is half way between the two limiting values A1 and A2:

$Y(X_0) = (A1 + A2)/2$

The y value corresponds to the probability of no body movement in response to the stimulus. For each PPF measured blood concentration (Cp_{50} and Cp_{95} determination) or predicted target blood concentration (MIT_{50} and MIT_{95} determination) a probability (y value) was calculated using the ratio between the number of dogs having still not reacted up to that specific concentration vs. the total number of dogs. The y (probability for no movement) and x values (blood concentration) were then used with the above logistical equation to determine a dose-response curve allowing the determination of Cp and MIT values.

A Kuskall Wallis test was used to compare the 4 treatments MDPE% and MDAPE% values for PPF, MED and DEX ($p \square 0.05$).

5.3 Results

5.3.1 Development of medetomidine and dexmedetomidine infusion schemes for co-infusion in dogs anaesthetised with propofol TCI

Using the computer simulation program PK-SIM, a range of different sequential infusion rates was evaluated and adjustments to infusion rate or duration were made until a scheme was devised which predicted the achievement of the desired blood concentrations of 1.7 $ngml^{-1}$ of MED (PM) and DEX (PHD) and 0.85 $ngml^{-1}$ of DEX (PLD). The resultant schemes selected for the study are illustrated in Figures 5.1 and 5.2. A summary of the infusion rates for each agent in the different treatments can be found in Table 5.2.

Time (min)	PS	PM	PLD	PHD
0	2 $mlkg^{-1}min^{-1}$	0.2 $\mu gkg^{-1}min^{-1}$	0.1 $\mu gkg^{-1}min^{-1}$	0.2 $\mu gkg^{-1}min^{-1}$
5	2 $mlkg^{-1}min^{-1}$	0.1 $\mu gkg^{-1}min^{-1}$	0.05 $\mu gkg^{-1}min^{-1}$	0.1 $\mu gkg^{-1}min^{-1}$
15	2 $mlkg^{-1}min^{-1}$	0.08 $\mu gkg^{-1}min^{-1}$	0.04 $\mu gkg^{-1}min^{-1}$	0.08 $\mu gkg^{-1}min^{-1}$
30	2 $mlkg^{-1}min^{-1}$	0.06 $\mu gkg^{-1}min^{-1}$	0.03 $\mu gkg^{-1}min^{-1}$	0.06 $\mu gkg^{-1}min^{-1}$
45	2 $mlkg^{-1}min^{-1}$	0.04 $\mu gkg^{-1}min^{-1}$	0.02 $\mu gkg^{-1}min^{-1}$	0.04 $\mu gkg^{-1}min^{-1}$

Table 5.2

Summary of the 5 steps of the co-infusion schemes for the PS (saline), PM (medetomidine target concentration of 1.7 $ngml^{-1}$), PLD (dexmedetomidine target concentration of 0.85 $ngml^{-1}$) and PHD (dexmedetomidine target concentration of 1.7 $ngml^{-1}$) treatments.

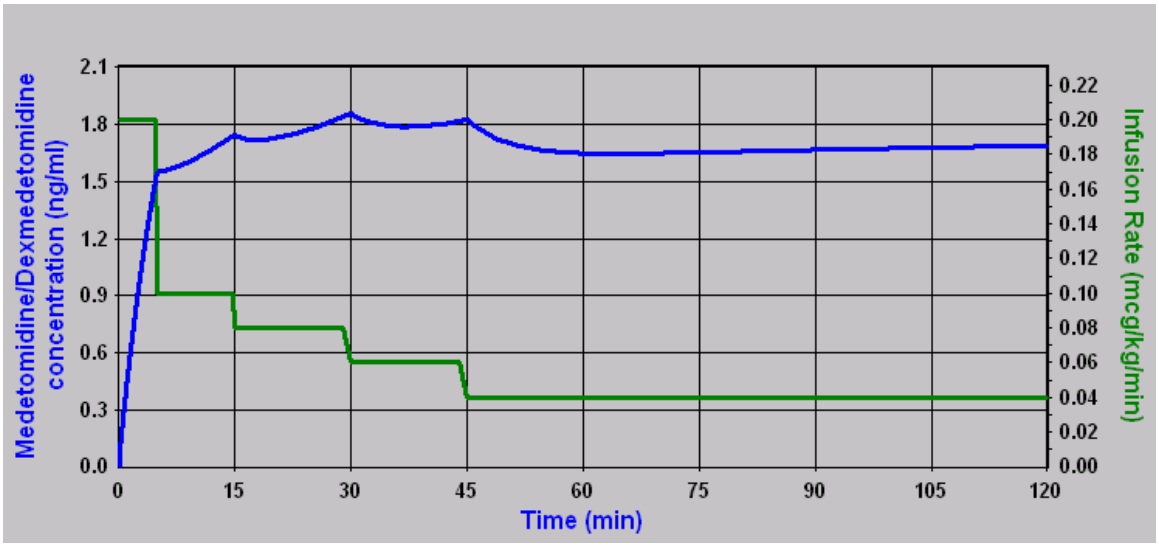


Figure 5.1

Five step infusion of medetomidine (MED) or dexmedetomidine (DEX) with a target blood concentration of 1.7 $ngml^{-1}$ (green line) and the predicted plasma concentration of MED or DEX (blue line), derived using the pharmacokinetic (PK) simulator PK-SIM and PK parameters of MED from O. Vainio (2001).

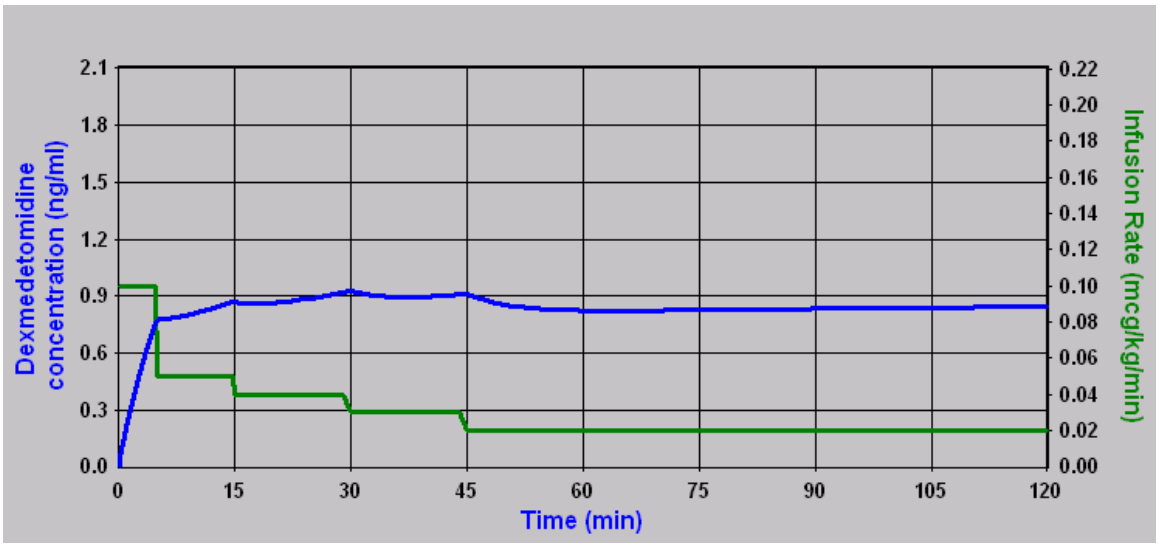


Figure 5.2

Five step infusion of dexmedetomidine (DEX) with a target blood concentration of 0.85 $ngml^{-1}$ (green line) and the predicted plasma concentration of DEX (blue line), derived using the pharmacokinetic (PK) simulator PK-SIM and PK parameters of MED from O. Vainio (2001).

5.3.2 Anaesthesia

Unless stated otherwise, mean values are quoted with their standard deviation in brackets.
Six female beagle dogs were included in this study. They were 7.3 (± 2.3) years old and weighed 16.1 (± 2.5) kg.

Instrumentation, co-infusion and total infusion times (min) are reported in Table 5.3. No significant difference between treatments was noted for these different times. For each treatment, the mean amount of PPF infused during instrumentation, co-infusion and for the whole procedure and the mean infusion rates of PPF are reported in Tables 5.4 and 5.5. There was no difference in infusion rate or in the amount of PPF infused between the treatments during the instrumentation time period, while a significant increase was observed in the PS treatment by comparison with the other 3 treatments during the co-infusion and total time periods.

The mean infusion rates of MED and DEX (PHD) were 0.071 (± 0.002) and 0.065 (± 0.003) $\mu gkg^{-1} min^{-1}$, respectively. These values correspond to a total amount per dog of MED and DEX of 5.36 and 5.85 μgkg^{-1}, respectively. The mean infusion rate of DEX in the PLD treatment was 0.036 (± 0.003) $\mu gkg^{-1}min^{-1}$ which is equivalent to a total amount of 2.84 μgkg^{-1} of DEX per dog.

Endotracheal intubation after 3 min was unsuccessful in 11 occasions. None of these 11 dogs needed more than one increment of PPF to achieve intubation and the PPF target concentration at this time was 3.5 μgml^{-1}. During the instrumentation period, localised muscle twitching was observed in 9 dogs (4 in treatment PS, 2 in treatment PM, 2 in treatment PLD and 1 in treatment PHD). Muscle twitching continued in the 4 dogs from treatment PS and 1 from t PM during the co-infusion. In the 4 remaining dogs, twitching stopped shortly after the start of the co-infusion.

Recovery times are reported for each treatment in Table 5.6. At extubation, there was a significant difference between treatments PS and PM. Although extubation time was similar in treatment PHD to that of treatment PM, the difference between treatments PS and PHD was not significant ($p = 0.084$). For the time to sternal position there were no significant differences between the treatments although the times to achieve sternal recumbency were markedly shorter in treatments PS and PLD compared with treatments PM and PHD. Dogs in the PS treatment stood up significantly sooner than in the PM and PHD treatments as did the dogs in the PLD treatment in comparison to these in the PHD treatment.

Treatments	Instrumentation (± SD)	Co-infusion (± SD)	Total (± SD)
PS	22 (2.1)	86.2 (17.3)	108.2 (17.1)
PM	26.5 (3)	75.5 (8.2)	102 (8.5)
PLD	23.3 (2.1)	79 (12.7)	102.3 (13.2)
PHD	26.5 (4)	90.8 (13.6)	117.3 (13.6)
The 4 treatments combined	24.4 (3.3)	83.5 (13.8)	107.9 (14)

Table 5.3

Mean (± SD) infusion times (min) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs separately and combined for the time periods instrumentation (time between the start of anaesthesia and the start of the co-infusion), co-infusion (time from the start of the co-infusion to the end of the procedure) and total (time for the whole procedure).

	PS (± SD)	PM (± SD)	PLD (± SD)	PHD (± SD)
Instrumentation	136 (25)	155 (29)	134 (15)	172 (29)
Co-infusion	463 (159)	190 (38)[a]	201 (33)[a]	192 (64)[a]
Total	600 (173)	345 (53)[a]	355 (41)[a]	364 (44)[a]

Table 5.4

Mean (± SD) amount (mg) of propofol infused during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs for the time periods instrumentation (time between the start of anaesthesia and the start of the co-infusion), co-infusion (time from the start of the co-infusion to the end of the procedure) and total (time for the whole procedure).

a = significant difference from treatment PS ($p \leq 0.0003$).

	PS (± SD)	PM (± SD)	PLD (± SD)	PHD (± SD)
Instrumentation	0.42 (0.04)	0.4 (0.04)	0.39 (0.08)	0.46 (0.15)
Co-infusion	0.36 (0.03)	0.17 (0.03)[a]	0.17 (0.02)[a]	0.14 (0.04)[a]
Total	0.37 (0.04)	0.23 (0.03)[a]	0.23 (0.03)[a]	0.21 (0.03)[a]

Table 5.5

Mean (±SD) infusion rate ($mgkg^{-1}min^{-1}$) of propofol during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs for the time period of instrumentation (time between the start of anaesthesia and the start of the co-infusion), co-infusion (time from the start of the co-infusion to the end of the procedure) and total (time for the whole procedure).

a = significant difference from treatment PS ($p < 0.0001$).

	PS (± SD)	PM (± SD)	PLD (± SD)	PHD (± SD)
Extubation	6.8 (6.7)	20.2 (10.1)[a]	12.5 (4.5)	18 (8.8)
Sternal	13.5 (10.9)	22.8 (8.2)	15 (4.3)	23.5 (9.3)
Standing	22.5 (11.8)	33.7 (7.8)[a]	23.3 (7.1)	36 (5)[b,c]

Table 5.6

Recovery times (min) for the 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs. Extubation time = time between end of anaesthesia and spontaneous swallowing; Sternal time = time between end of anaesthesia and the time when the dog adopts sternal position; Standing time = time between the end of anaesthesia and the time when the dog is able to stand without help.

a = significant difference from treatment PS ($p = 0.04$).

b = significant difference from treatment PS ($p = 0.013$).

c = significant difference from treatment PLD ($p = 0.02$).

5.3.3 Pharmacodynamic study

5.3.3.1 Heart Rate

The HR in each treatment is reported in Figure 5.3. There were no significant differences between the treatments before the start of the co-infusion. After the start of the co-infusion, decreased HR was evident for all three treatments and by 5 min the HR in treatments PM, PLD and PHD was significantly reduced in comparison to the rate prior to the co-infusion and to that of treatment PS. Thereafter, no significant differences were observed between treatments PM, PLD and PHD over time. In the PS treatment, no significant difference was observed over time.

5.3.3.2 Arterial Blood Pressure

The trends for the systolic and mean ABP for each treatment are reported in Figures 5.4 and 5.5.
There were no significant differences in systolic or mean ABP between the four treatments before the start of the co-infusion. After the start of the co-infusion, an increase in systolic ABP was evident in treatments PM, PLD and PHD and, from 5 to 45 min, the systolic ABP in treatments PM, PLD and PHD was significantly increased in comparison to the pressure prior to the co-infusion and to that of treatment PS. However, there were no significant differences between the three treatments. In the PS treatment, there were no significant differences in systolic ABP over time until the point of MIT determination. At that point, the systolic ABP was significantly increased from that recorded at 5, 15 and 30 min. At the time of MIT determination there was a significant difference in systolic ABP between treatments PS and PHD.
There was a significant increase in the mean ABP compared with baseline values in treatments PM, PLD and PHD at all time points and from treatment PS at 5, 15 and 30 min. At 45 min, mean ABP in treatments PM and PHD were significantly higher than treatment PS, but at the point of MIT determination there were no significant differences in mean ABP between treatments. At the times 5, 15 and 30 min, the mean ABP in the PLD treatment was significantly lower than that in the PHD treatment. At 5 min, there was a significant difference with the MIT determination point in the PLD treatment. There were no significant differences over time for the mean ABP in the PS treatment, although this did appear to rise at the point of MIT determination.

5.3.3.3 Cardiac output

Cardiac output measured before the start of the co-infusion was not significantly different between the treatments. However, at time 45 min, a significant decrease in the CO was observed in treatments PM, PLD and PHD with their baseline as well as with treatment PS (Figure 5.6).

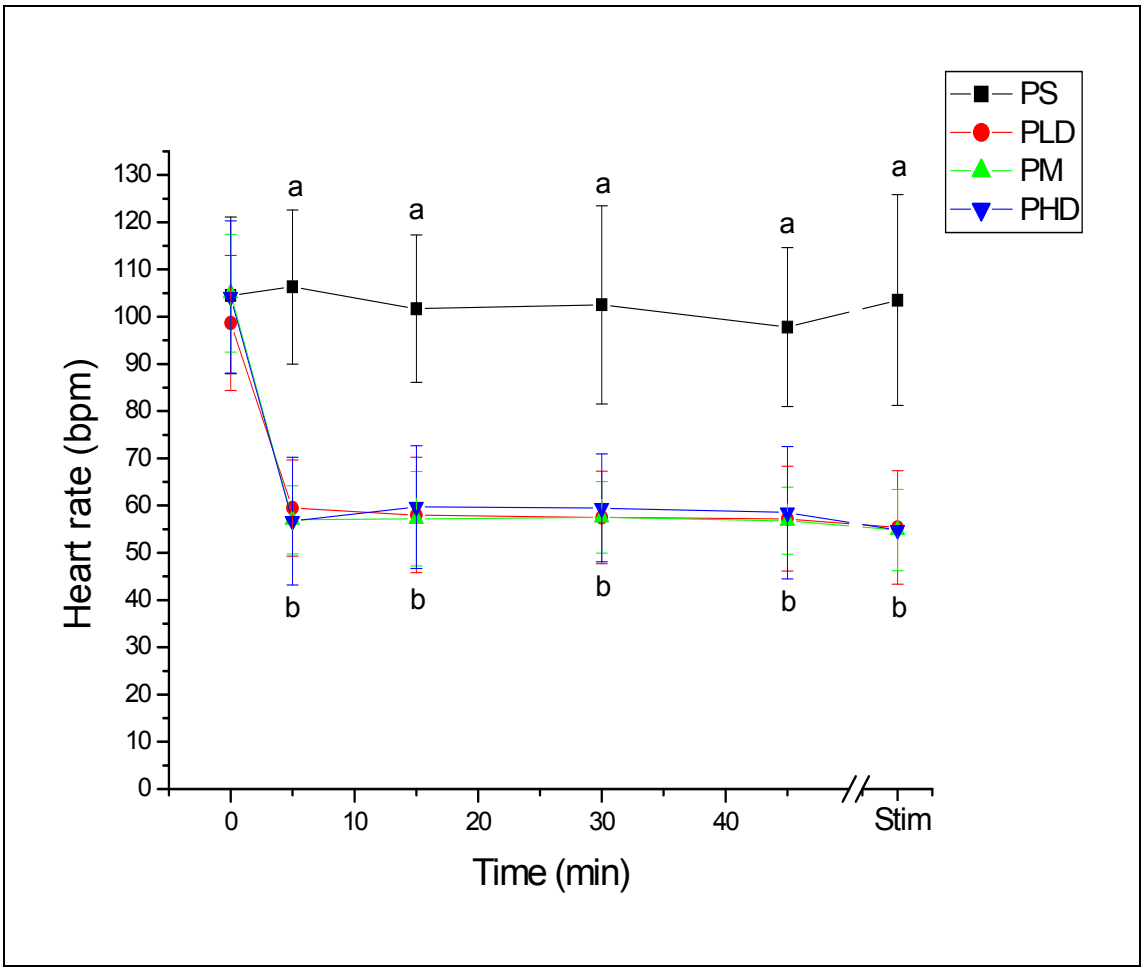


Figure 5.3

Mean (± SD) heart rate (BPM) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs from the start of the co-infusion (time 0) to the time of minimum propofol infusion target determination (Stim).

a = significant difference from treatments PM, PLD and PHD ($p < 0.0001$).

b = significant difference in treatments PLD, PM and PHD from baseline value (time 0) ($p < 0.0001$).

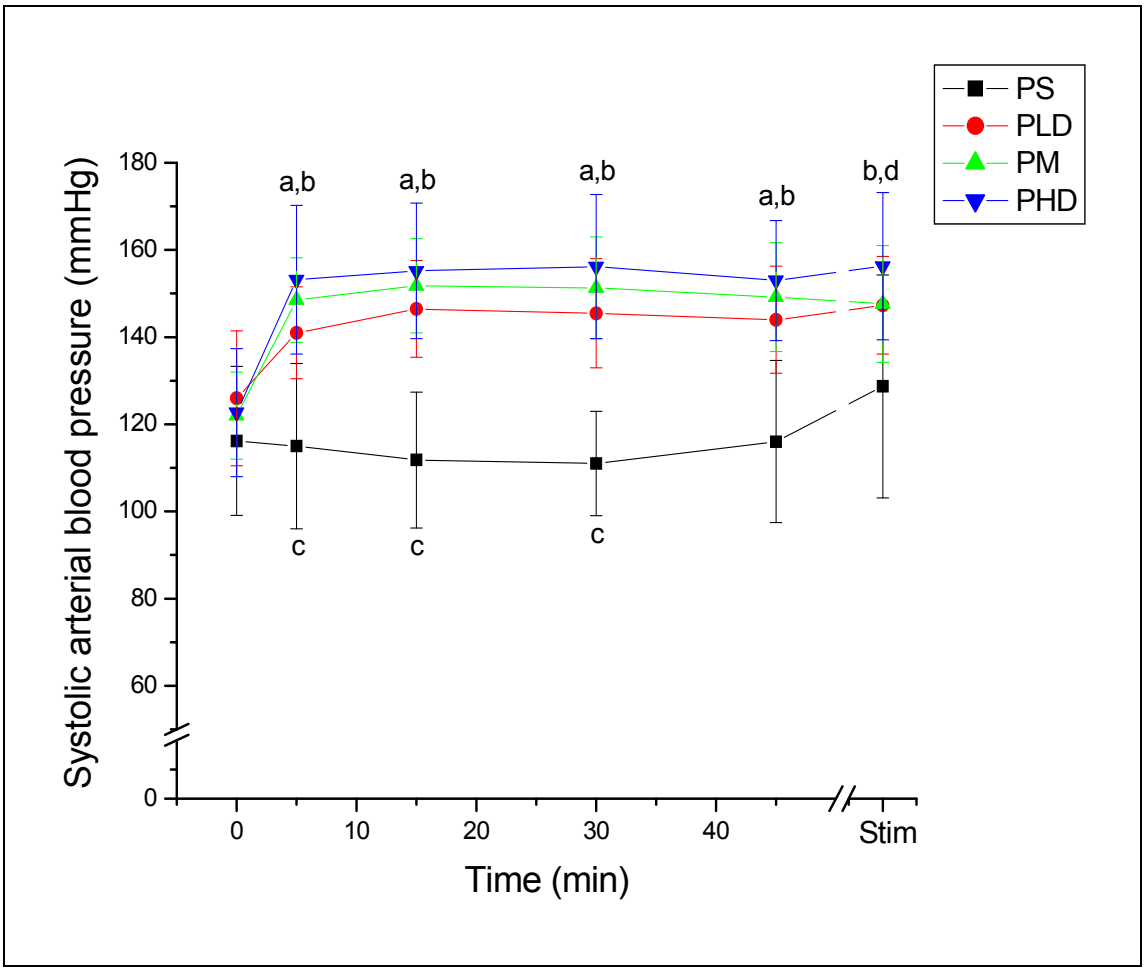


Figure 5.4

Mean (± SD) systolic arterial blood pressure (mmHg) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs from the start of the co-infusion (time 0) to the time of minimum propofol infusion target (MIT) determination (Stim).

a = significant difference in treatments PM, PLD and PHD from treatment PS ($p \leq 0.019$).

b = significant difference in treatments PM, PLD and PHD from baseline value (time 0) ($p < 0.00001$).

c = significant difference from MIT determination. For time points 5, 15 and 30 min, $p = 0.0365$, 0.0063 and 0.0039, respectively.

d = significant difference between treatment PS and treatment PHD ($p = 0.0196$).

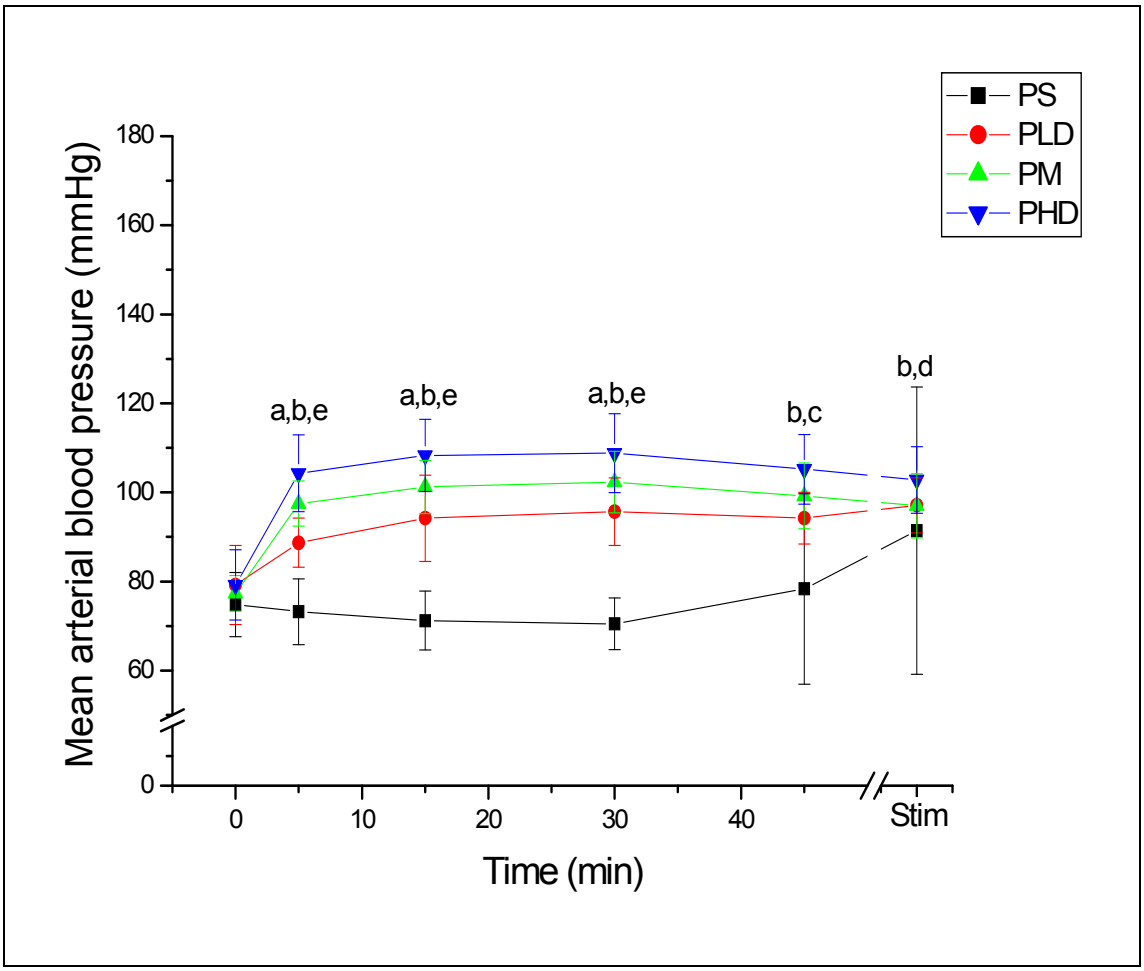


Figure 5.5

Mean (± SD) mean arterial blood pressure **(**mmHg**)** during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs from the start of the co-infusion (time 0) to the time of minimum propofol infusion target determination (Stim).

a = significant difference in treatments PM, PLD and PHD from treatment PS ($p \leq 0.038$).

b = significant difference in treatments PM, PLD and PHD from baseline value (time 0) ($p \leq 0.0181$).

c = significant difference (treatments PM and PHD) from treatment PS. P values for treatments PM and PHD = 0.0056 and 0.0022, respectively.

d = significant difference (treatment PLD) from 5 min time point ($p = 0.045$).

e = significant difference between treatments PLD and PHD ($p \leq 0.0063$).

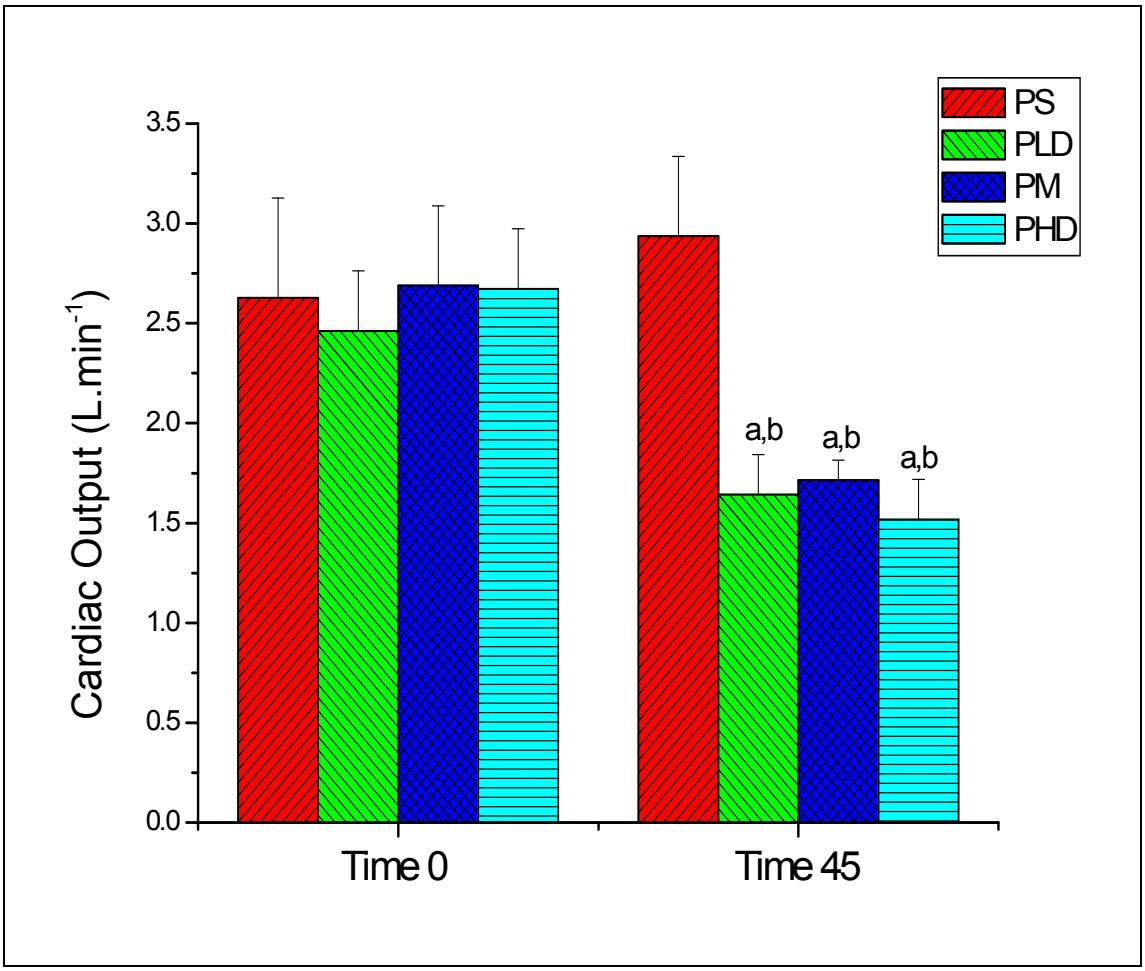


Figure 5.6

Mean (± SD) cardiac output ($Lmin^{-1}$) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs at time 0 (start of the co-infusion) and at time 45 (last step of the co-infusion).

a = significant difference from treatment PS ($p < 0.0001$).

b = significant difference from baseline value (time 0) ($p \leq 0.004$).

5.3.3.4 Respiratory variables

In treatment PHD there were significant differences from the baseline value for RR at all time points (Figure 5.7). Although there were no statistically significant differences in RR between the treatments at any time points, a significant difference was almost reached at the point of MIT determination between treatment PS and the other 3 treatments (p between 0.066 and 0.057). In treatment PS, RR at the 5 and 15 min time points was significantly different from that at the time of MIT determination.

Et CO_2 was not different from baseline values in any treatment. Except at the time of MIT determination when there was a significant difference between treatment PLD and treatments PS and PHD, there were no significant differences between treatments (Figure 5.8).

5.3.4 Pharmacokinetic results

5.3.4.1 Propofol

Before the start of the co-infusion (time 0), the predicted (Figure 5.9) and measured (Figure 5.10) PPF blood concentrations were the same between treatments. At time 45, the predicted and measured PPF blood concentrations for the control treatment (PS) were significantly higher from those in the other treatments. The predicted PPF blood concentration during MIT determination was also significantly higher in the PS treatment than in the other treatments as well as in the treatments PM and PLD when compared respectively with treatment PHD.

For the measured PPF blood concentrations during MIT determination, a significant difference was observed from treatment PS with the other treatments.
For the predicted PPF blood concentration, time 45 min was different from baseline in treatments PS, PM and PHD while at the time of MIT determination the difference was significant in all the treatments. For the measured PPF blood concentration, PLD showed a significant decrease from baseline at time of MIT determination while treatment PHD demonstrated a significant decrease from baseline at that time as well as time 45. In this last treatment, a significant decrease was also observed at time of MIT determination from time 45.

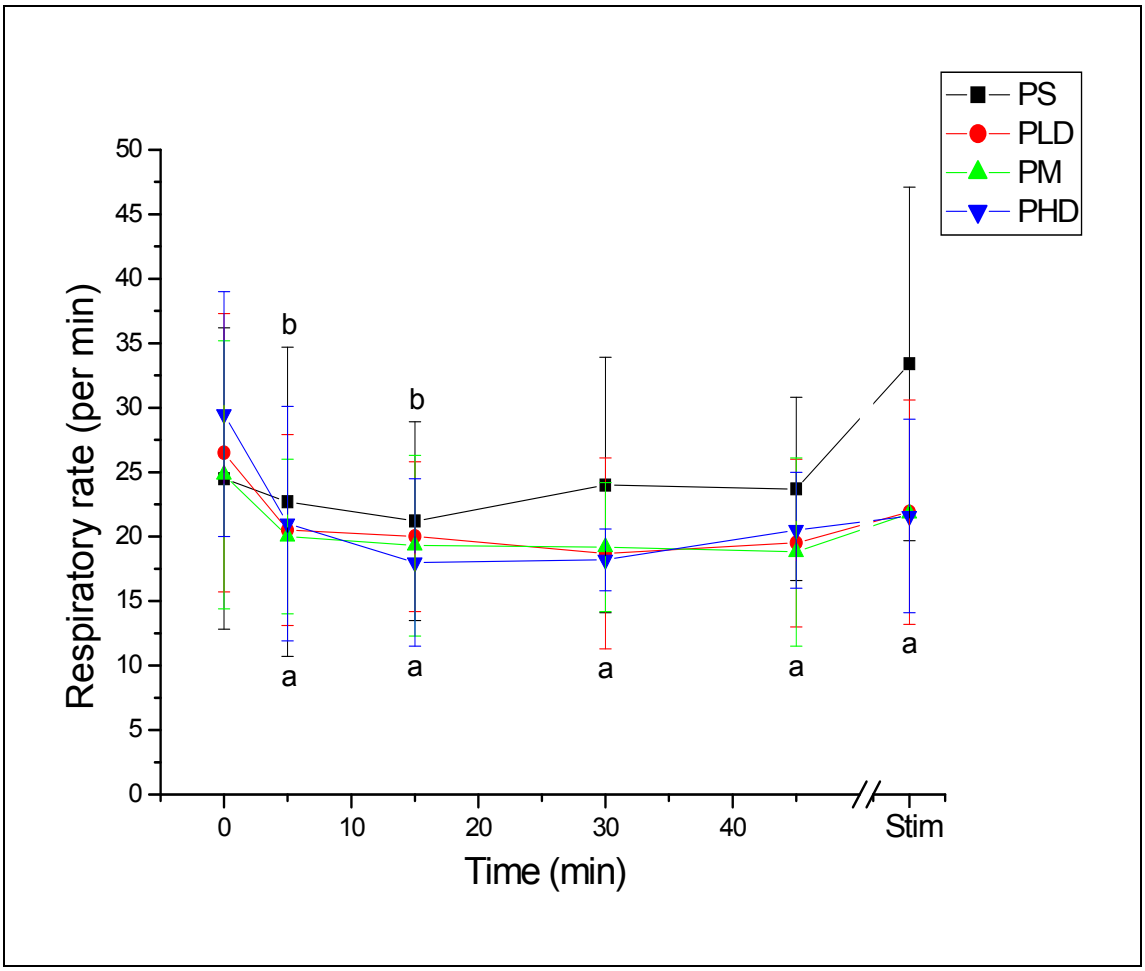


Figure 5.7

Mean (± SD) respiratory rate (Breath per min) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs from the start of the co-infusion (time 0) to the time of minimum propofol infusion target (MIT) determination (Stim).

a = significance difference (treatment PHD) ($p \leq 0.0454$) from baseline value (time 0).

b = significance difference (treatment PS) from MIT determination. P value at 5 and 15 min = 0.0296 and 0.0104, respectively.

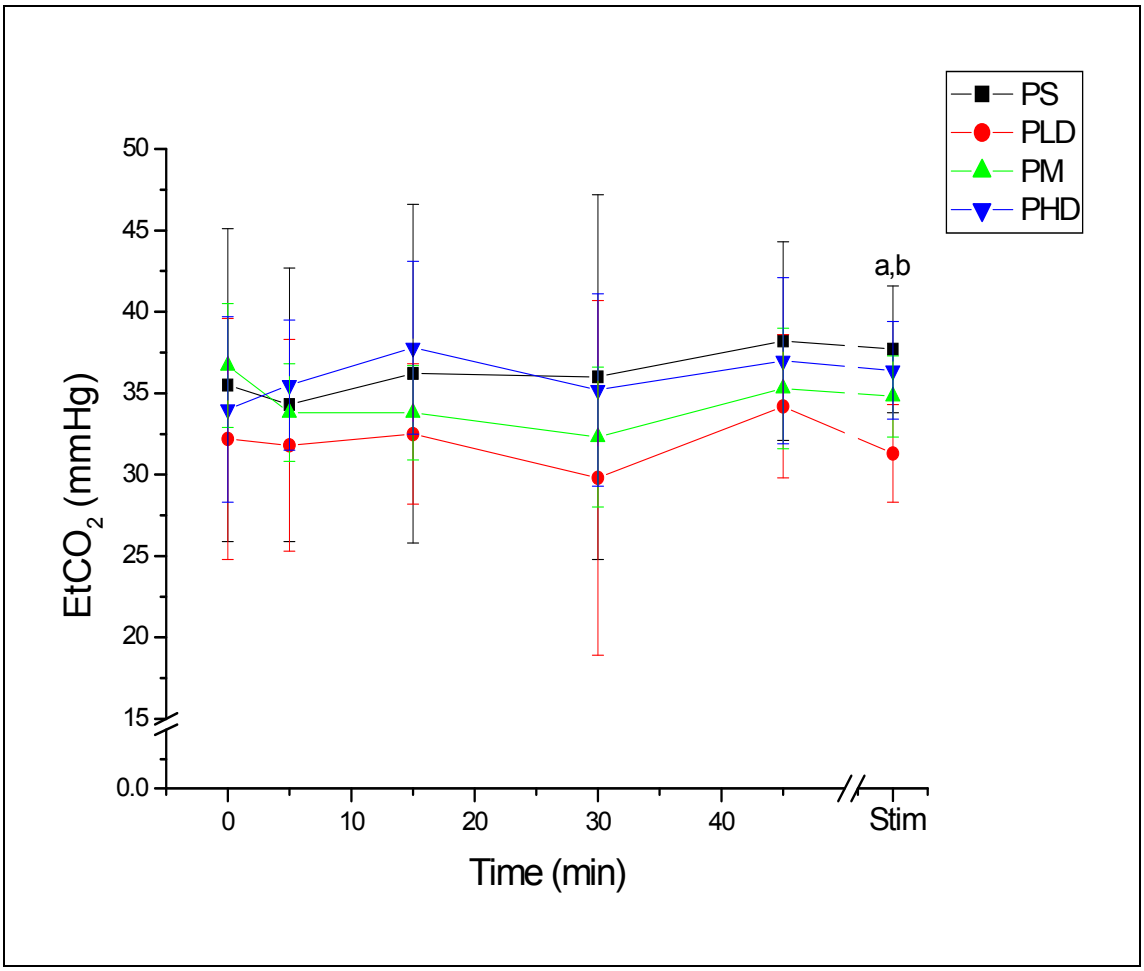


Figure 5.8

Mean (± SD) end tidal CO_2 ($EtCO_2$) (mmHg) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs from the start of the co-infusion (time 0) to time of minimum propofol infusion target determination (Stim).

a = significant difference between treatments PS and PLD (P = 0.0027).

b = significant difference between treatments PHD and PLD (p = 0.0149).

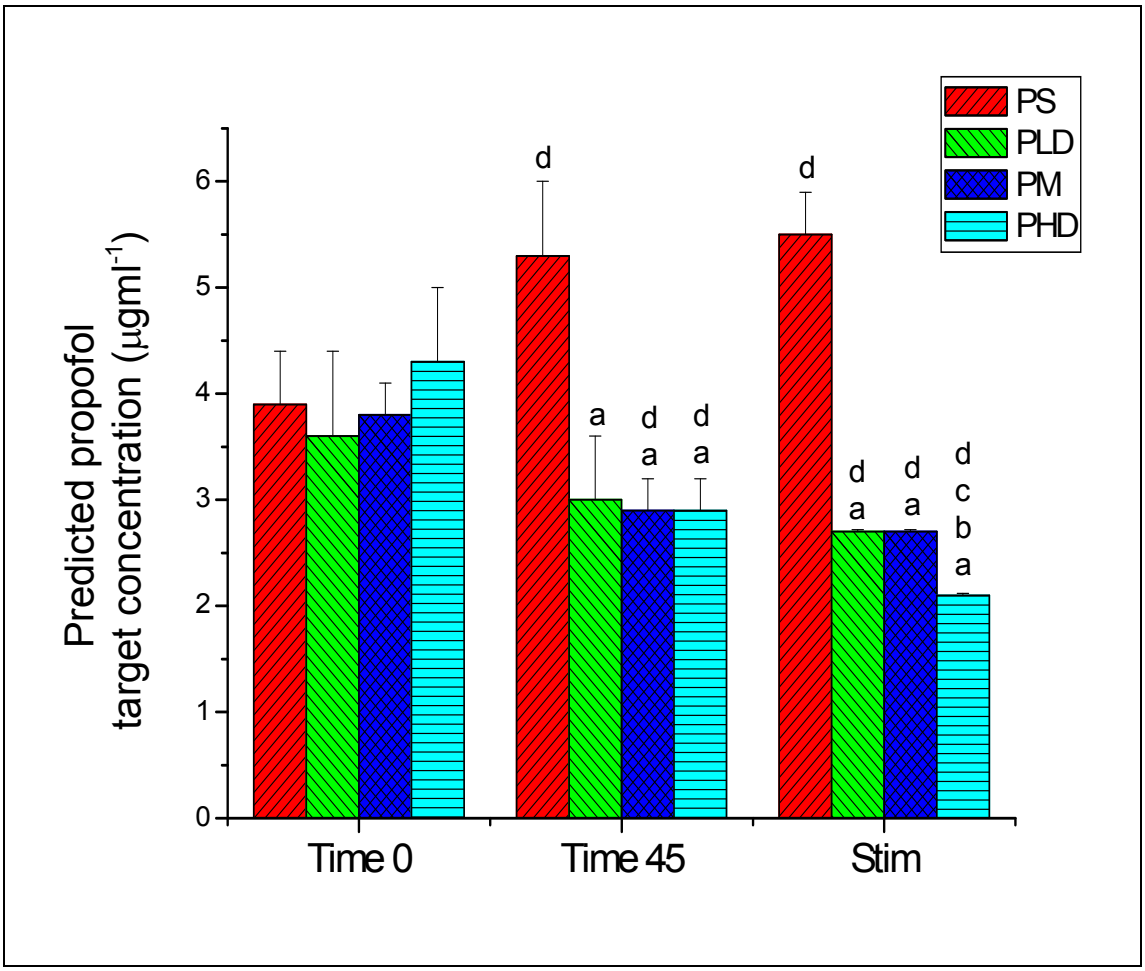


Figure 5.9

Mean (± SD) predicted propofol blood concentration (µgml^{-1}) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs at time 0 (before the start of the co-infusion), at time 45 (last step of the co-infusion) and at the time of minimum propofol infusion target determination (Stim).

a = significant difference from treatment PS ($p < 0.0001$).

b = significant difference from treatment PLD ($p = 0.0336$).

c = significant difference from treatment PM ($p = 0.0336$).

d = significant difference from baseline value (time 0) ($p \leq 0.0145$).

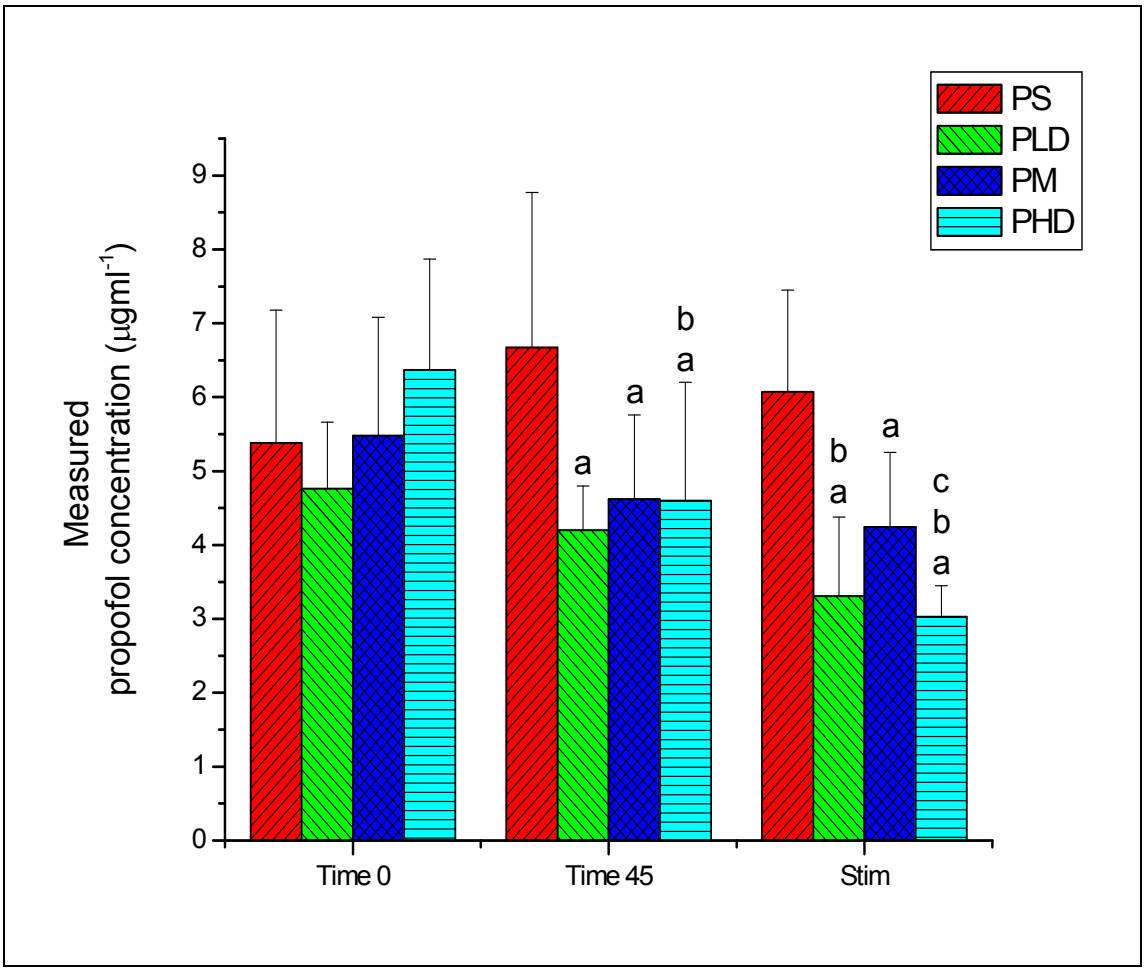


Figure 5.10

Mean (± SD) measured propofol blood concentration (μgml^{-1}) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs at time 0 (before the start of the co-infusion), at time 45 (last step of the co-infusion) and at the time of minimum propofol infusion target determination (Stim).

a = significant difference from treatment PS ($p \leq 0.027$).

b = significant difference from baseline value (time 0) ($p \leq 0.007$).

c = significant difference from time 45 value ($p = 0.0187$).

5.3.4.1.1 Assessment of the performance of the propofol TCI system

Figure 5.11 illustrates both measured and predicted PPF blood concentrations for each treatment at all blood sampling time points. In the 4 treatments the predicted values are lower than the measured value which demonstrates an underprediction of the TCI system. This is confirmed in Table 5.7 with a positive median MDPE% values present in all treatments. By comparison with treatments PM and PHD, the distance between the points in treatments PS and PLD seems to be smaller than in the 2 other treatments, which is confirmed in Table 5.7 by lower median MDAPE% values in these treatments.

Although no statistical difference was observed between the treatment groups for MDPE% and MDAPE% ($p \geq 0.227$), the figures in Table 5.7 suggest that the degree of underprediction was much greater in the presence of the alpha$_2$-adrenoceptor agonists, treatment PLD being the least affected.

5.3.4.1.2 Determination of the MIT for propofol

The minimum predicted and measured PPF concentrations required to abolish the response to a noxious stimulation are reported in Table 5.8. Both the minimum predicted and measured PPF blood concentrations in treatment PS were different from the other treatments. Treatment PHD showed significant differences with treatments PM and PLD for the predicted values.

Figure 5.12 represents the logistic regression used to determine the CP_{50} and CP_{95} for the measured PPF blood target concentration for the PS, PM, PLD and PHD treatments. Treatment PS needed a higher PPF blood concentration to prevent body movement to a stimulus than the other treatments. Treatment PHD had the best sparing effect on PPF, followed by treatment PLD then treatment PM. The CP_{50} and CP_{95} values for the measured PPF blood target concentration are reported Table 5.9 with the MIT_{50} and MIT_{95} values for comparison. In the 4 treatments, the plasma PPF concentrations were higher than the predicted concentrations resulting from an underprediction of the TCI system (see Figure 5.11 and Table 5.7).

For both parameters, Cp and MIT, treatment PS was less efficacious in abolishing body movement to electric stimulus that the 3 other treatments. In the DEX treated treatments, less PPF was required to provide a no response to a noxious stimulus in treatment PHD than in treatment PLD, but the difference was not significant. Of the PM, PLD and PHD treatments, although clinically, treatment PM was least effective in reducing the PPF concentrations required to abolish responses to a noxious stimulus, the difference was not significant.

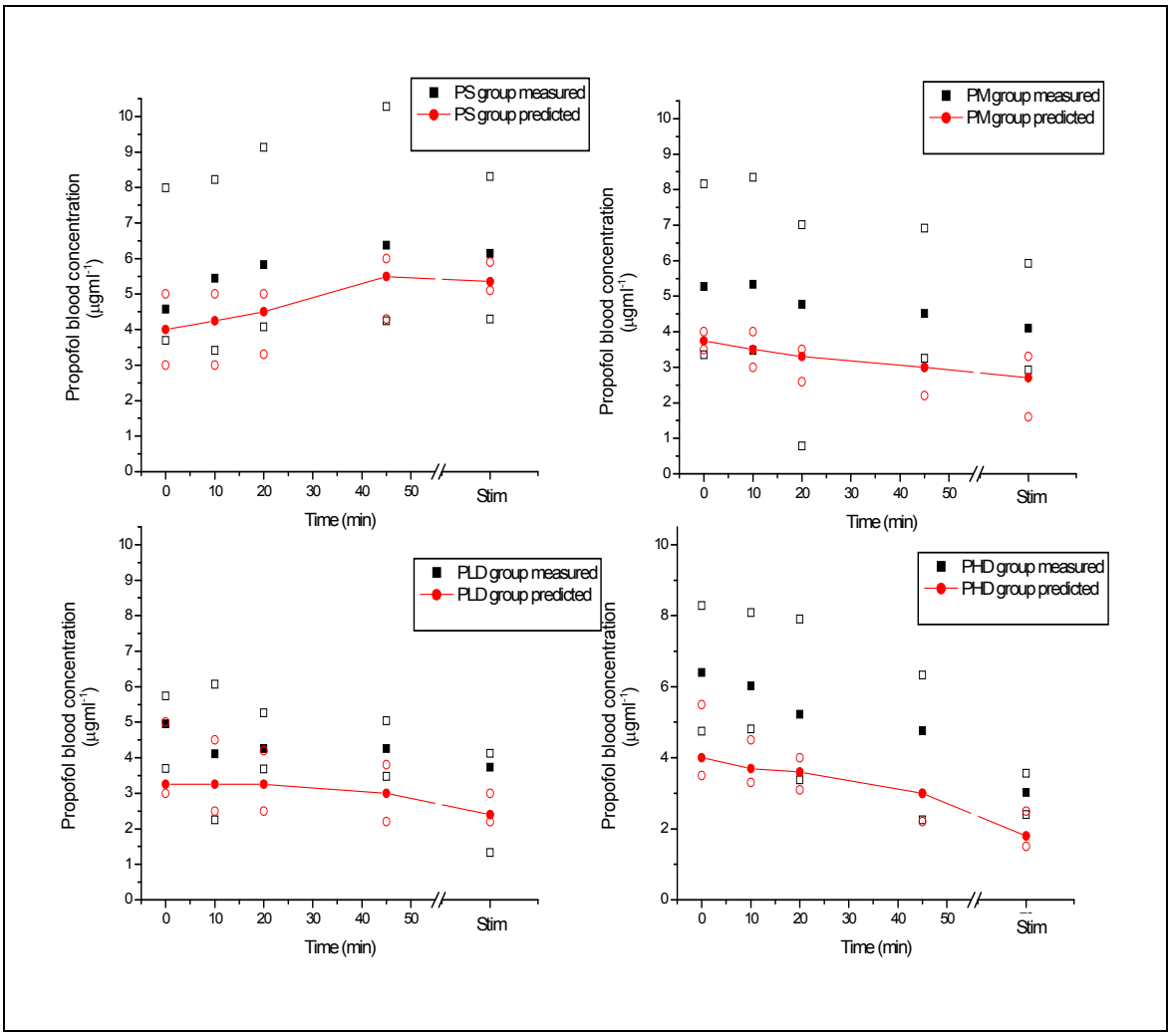


Figure 5.11

Comparison between measured and predicted propofol (PPF) blood concentrations (μgml^{-1}) during 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs at time 0 (before the start of the co-infusion); at 10 and 20 min (during the steps of the co-infusion); at time 45 (last step of the co-infusion); and at the time of minimum propofol infusion target (MIT) determination (Stim).

Each symbol, with the exception of those at the point of MIT determination, corresponds to the median (full symbol) and the range (open symbol) predicted (red circle) or measured (black square) blood PPF concentration for 6 dogs. At MIT determination, however, each symbol corresponds to the median (full symbol) and the range (open symbol) of the mean for 6 dogs, calculated between the PPF blood concentration at the time purposeful movement was observed while decreasing the PPF target blood concentration, and at the time when the movement was abolished while increasing the PPF target blood concentration.

	MDPE%				MDAPE%			
Dogs	**PS**	**PM**	**PLD**	**PHD**	**PS**	**PM**	**PLD**	**PHD**
1	18.50	61.07	23.59	35.25	18.50	61.07	23.59	35.25
2	19.39	74.38	55.83	98.79	19.39	74.38	55.83	98.79
3	-6.53	0.02	8.21	35.81	6.53	10.75	11.60	35.81
4	12.36	29.67	28.28	4.69	15.99	29.67	35.24	10.34
5	63.87	127.26	94.58	80.88	63.87	127.26	94.58	80.88
6	26.51	55.53	7.74	109.13	26.51	55.53	36.36	109.13
Median	18.85	58.30	25.94	58.35	18.85	58.30	35.80	58.35

Table 5.7

MDPE% (bias) and MDAPE% (accuracy) values for the propofol target controlled infusion system, for each individual during each treatment (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)).

a = significant difference from treatment PS ($p \leq 0.0412$).

	Predicted				Measured			
Dogs	**PS**	**PM**	**PLD**	**PHD**	**PS**	**PM**	**PLD**	**PMD**
1	5.2	2.9	3.2	2.5	5.4	4.6	4.1	3
2	5.6	1.8	2.7	1.8	6.1	2.9	3.6	3.4
3	5.1	2.9	2.5	1.8	4.3	3.9	2.9	2.4
4	5.9	3.3	2.9	2.5	6.2	4.3	3.9	2.8
5	5.5	2.7	2.4	2.2	8.3	5.9	4.1	3.6
6	5.85	2.5	2.4	1.7	6.8	3.8	1.3	3.1
Mean (± SD)	5.5 (0.44)	2.7[a] (0.02)	2.7[a] (0.02)	2.1[a,b] (0.02)	6.2 (1.35)	4.2[a] (1.06)	3.3[a] (1.04)	3[a] (0.42)

Table 5.8

The minimum predicted and measured propofol blood concentrations in $\mu g ml^{-1}$ required to abolish purposeful movement to electrical stimulation for each dog and during each treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)).

a = significant difference from treatment PS for the minimum predicted ($P \leq 0.001$) and measured propofol ($P \leq 0.033$) blood concentration.

b = significant difference from treatments PM ($p = 0.012$) and PLD ($p = 0.002$).

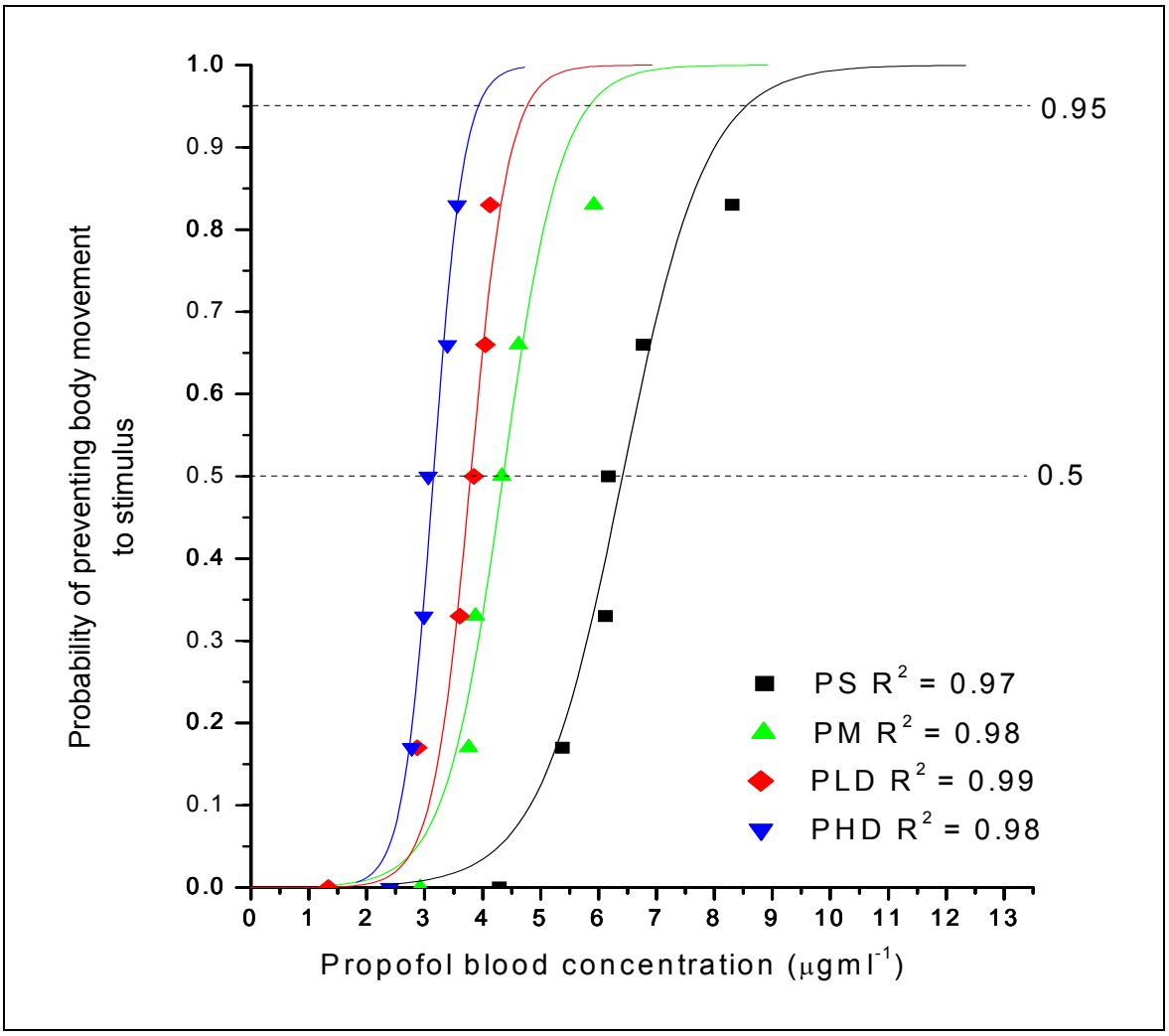


Figure 5.12

Logistic regression curves for the 4 treatments (Saline (PS), medetomidine (PM), low target dexmedetomidine (PLD) and high target dexmedetomidine (PHD)) in 6 dogs using the probability of preventing body movement to electric stimulus vs. measured propofol blood concentration (μgml^{-1}). Each dot represents the probability of a patient showing no response to a noxious stimulus at a given propofol blood concentration. The coefficient of correlation (R^2) is provided for each curve.

Treatments	PS	PM	PLD	PHD
Cp_{50} (μgml^{-1})	6.4	4.4	3.8	3.15
Cp_{95} (μgml^{-1})	8.6	5.8	4.8	3.9
MIT_{50} (μgml^{-1})	5.6	2.9	2.75	2.27
MIT_{95} (μgml^{-1})	6.35	3.65	3.4	3

Table 5.9

CP_{50-95} and MIT_{50-95} (measured and predicted blood propofol concentration where 50% or 95% of the population will not respond to a noxious stimulation) were determined using a logistic regression (see materials and methods). Treatments PM (medetomidine), PLD (low target dexmedetomidine) and PHD (high target dexmedetomidine) are more effective in reducing measured and predicted propofol blood concentration necessary to abolish response to a noxious stimulation than treatment PS (saline).

5.3.4.2 Medetomidine-Dexmedetomidine

Although the MDPE%, a measure of the bias, was very similar in the 3 treatments (p = 0.91), the PK parameters used to develop the infusions resulted in an overprediction (negative value) in treatments PLD and PHD and an underprediction (positive value) in treatment PM (Table 5.10). No statistical difference was found between the treatments for the MDAPE% (p = 0.91). Although more sampling times were available, MDPE% and MDAPE% for treatment PHD were only calculated from the start to the end of the co-infusion to allow a comparison with the other treatments. Figure 5.13 represents the levels of DEX and LEV measured in the PM treatment (MED blood concentration of 1.7 $ngml^{-1}$). The MED blood concentration reached at the time of the noxious stimulation was 1.52 (± 0.1) $ngml^{-1}$ with a DEX and LEV blood concentration of 0.92 (± 0.1) and 0.6 (± 0.1) $ngml^{-1}$, respectively.

5.3.4.2.1 Dexmedetomidine (PHD treatment) – Predicted vs. measured blood levels

Figure 5.14 represents the predicted *vs*. the actual DEX blood concentrations from the PHD treatment for the whole sampling period (from the start of the co-infusion up to 7 h after the end of the co-infusion). The PK parameters underpredicted the DEX blood concentration from time 10 to time 45 (last step of the co-infusion). Thereafter, during the last step and the wash out, there was an overprediction.

5.3.4.2.2 Determination of the PK parameters for dexmedetomidine (PHD treatment)

Table 5.11 shows the values of V1, K10, K12 and K21 for 6 dogs. The results for dog 4 are quite different from all the others with a V1 about 2-4 times higher than for the other dogs (V1 = 1123.56). These parameters, used with the PK-SIM software and the five step infusion scheme to simulate a DEX blood target concentration of 1.7 $ngml^{-1}$, resulted in a significant underprediction (Figure 5.15). Since a higher V1 in dog 4 may have increased the V1 for the whole treatment, resulting in a dilution effect and therefore an underprediction, it was decided to recalculate the PK parameters without dog number 4 and to redo the simulation. This resulted in a decrease of V_1 for the whole treatment from 514.6 (± 321.9) to 392.8 (± 105.5) $mlkg^{-1}$ and in a better prediction (Figure 5.16). Predicted blood concentrations of DEX (target blood concentration of 1.7 $ngml^{-1}$) using the new PK parameters in the dog, the 5-step infusion scheme used in the study for DEX and the PK-SIM software are reported Figure 5.15 (all the dogs) and Figure 5.16 (all the dogs minus dog 4).

	MDPE%			MDAPE%		
Dogs	**PM**	**PLD**	**PHD**	**PM**	**PLD**	**PHD**
1	11.18	-3.11	-15.08	11.18	10.58	15.08
2	16.09	2	4.02	16.09	15.19	8.53
3	-27.06	-29.56	-6.47	27.06	29.56	12.94
4	-12.91	-16.34	-27.09	12.91	19.12	27.09
5	19.63	68.33	49.56	21.31	68.33	49.56
6	-5.04	-2.80	13.27	12.66	8.54	13.27
Median	3.07	-2.95	-1.23	14.50	17.15	14.17

Table 5.10

MDPE% (bias) and MDAPE% (accuracy) values for medetomidine (treatment PM) and for dexmedetomidine (treatments PLD (0.85 μgml^{-1}) and PHD (1.7 μgml^{-1})), for each individual and for each treatment for the time period between the start and the end of the co-infusion (83.5 ± 13.8 min).

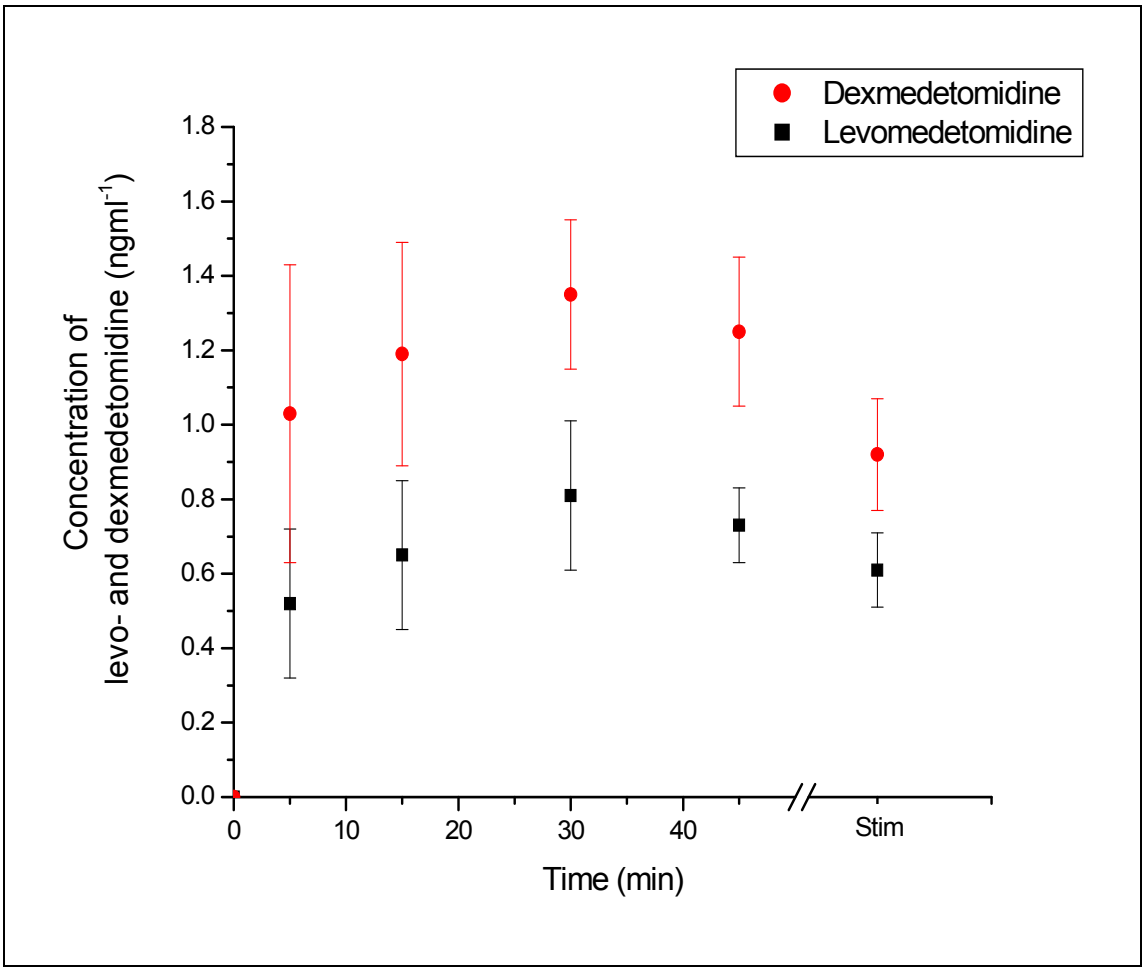


Figure 5.13

Measured plasma concentration of dexmedetomidine (DEX) and levomedetomidine (LEV) from the PM (medetomidine) treatment (medetomidine plasma concentration target of 1.7 $ngml^{-1}$) at time 0 (before the start of the co-infusion); at 5, 15 and 30 min (just before changing the rate of the co-infusion to the next step); at time 45 (last step of the co-infusion); and at the time of minimum propofol (PPF) infusion target (MIT) determination (Stim).

Each symbol, with the exception of those at the point of MIT determination, corresponds to the mean (± SD) measured blood DEX (red point) and LEV (black square) concentration for 6 dogs. At MIT determination, however, each symbol corresponds to the mean (± SD) for 6 dogs, calculated between the DEX and the LEV blood concentrations at the time purposeful movement was observed while decreasing the PPF target blood concentration, and at the time when the movement was abolished while increasing the PPF target blood concentration.

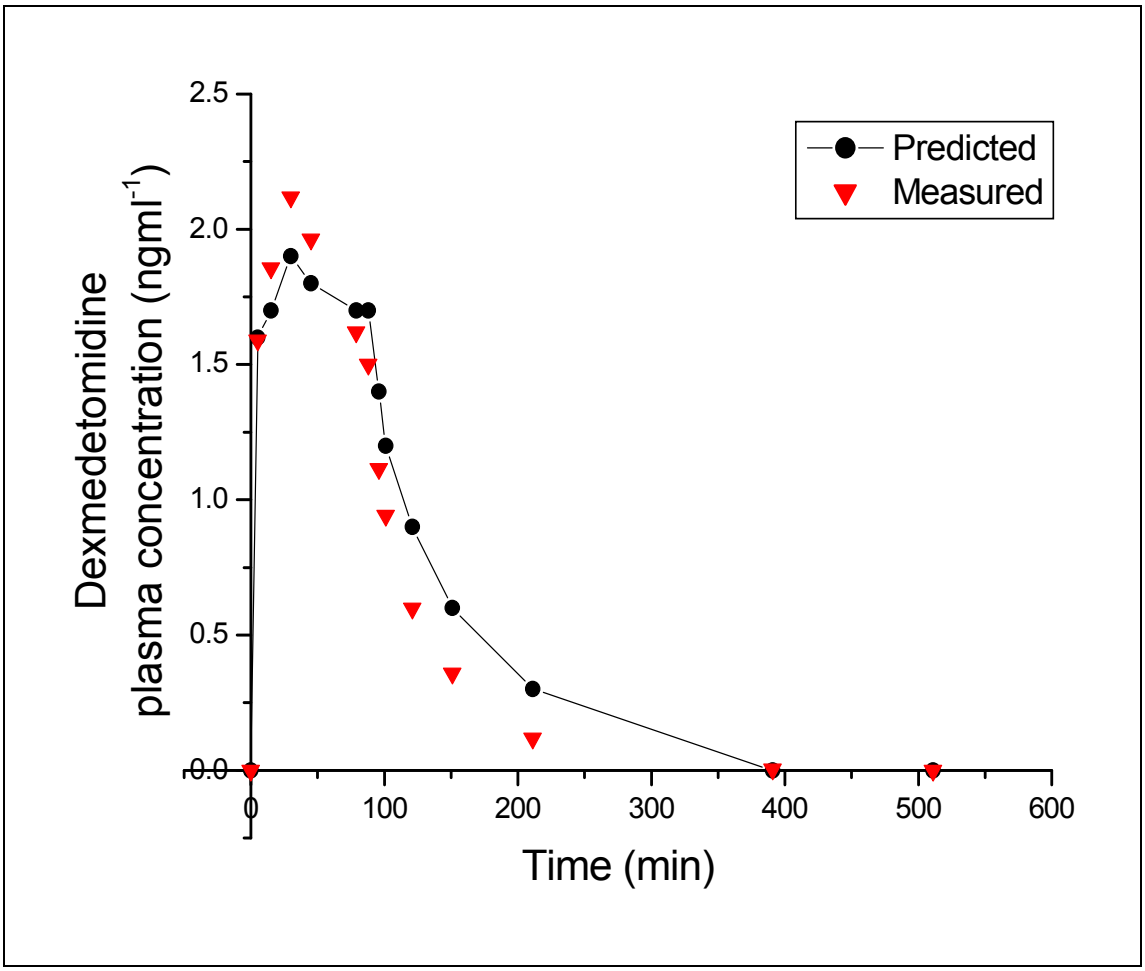


Figure 5.14

Predicted *vs*. measured plasma dexmedetomidine (DEX) concentration ($ngml^{-1}$) in treatment PHD (DEX plasma concentration target of $1.7\mu gml^{-1}$). Each point of the measured blood concentration curve is the mean value from the 6 dogs. The pharmacokinetic parameters used to stimulate the predicted concentration curve were taken from O. Vainio (2001).

	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	mean	St dev
V1	368.312	440.383	352.682	1123.56	260.070	542.608	514.603	312.899
K10	0.091	0.057	0.073	0.04	0.077	0.044	0.063	0.021
K12	0.237	0.068	0.744	0.01	0.046	0.041	0.191	0.283
K21	0.097	0.040	0.383	0.05	0.017	0.056	0.107	0.137

Table 5.11

New pharmacokinetic parameters derived for dexmedetomidine during the 5-step infusion up to 420 min after the end of the co-infusion in 6 target controlled infusion propofol anaesthetised dogs.

Treatment	MDPE %	MDAPE %
PHD (O.Vainio PK)	-10	19.47
PHD (new PK)	-1.25	16.25

Table 5.12

MDPE% (bias) and MDAPE% (accuracy) values for dexmedetomidine calculated from the time of the start of the co-infusion until 7 h after the end of the co-infusion. Comparison between the set of pharmacokinetic (PK) parameters from O. Vainio and the new PK parameters (without dog 4).

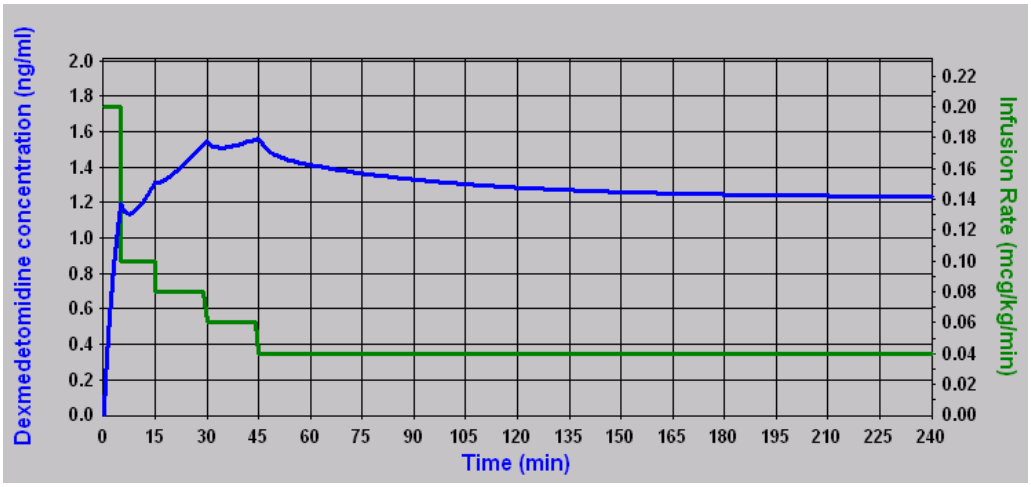


Figure 5.15

Predicted plasma concentration ($ngml^{-1}$) for dexmedetomidine (DEX) using the 5-step infusion scheme used in treatment PHD (DEX plasma concentration target of 1.7μgml^{-1}). The new pharmacokinetic parameters derived from the 6 dogs used in this study and achieved a concentration of approximately 1.3 $ngml^{-1}$ at steady-state.

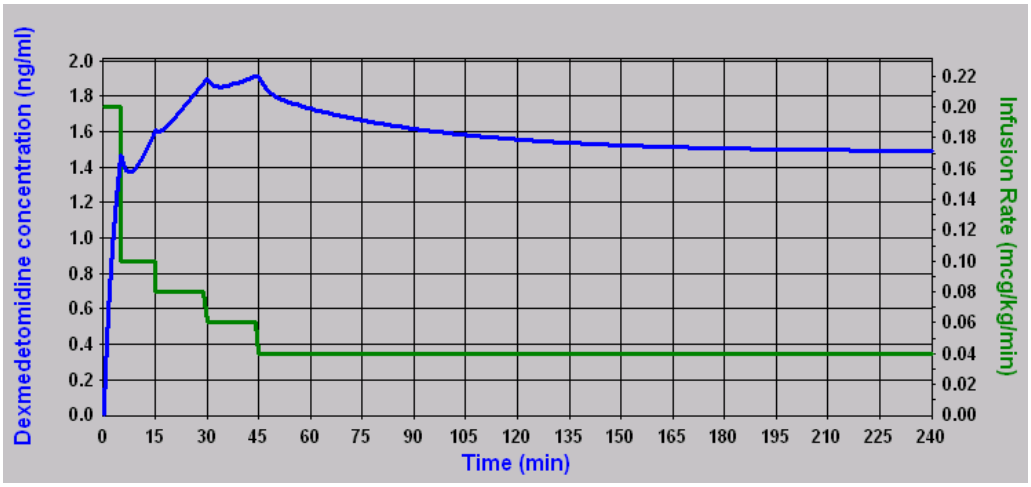


Figure 5.16

Predicted plasma concentration ($ngml^{-1}$) for dexmedetomidine (DEX) using the 5-step infusion scheme used in treatment PHD (DEX plasma concentration target of 1.7μgml^{-1}). The new pharmacokinetic parameters derived from the 6 dogs used in this study minus Dog 4, and achieved a concentration of approximately 1.5 $ngml^{-1}$ at steady-state.

The curves obtained in Figures 5.15 and 5.16 were thereafter compared with the measured DEX blood concentration obtained in this study and the better quality of the prediction using the new set of PK parameters was confirmed (Figure 5.17). A similar analysis was conducted comparing the new set of PK parameters (without Dog 4) with the set from O. Vainio. This resulted in very similar curves, with a better prediction in the wash-out period with the revised set of PK parameters (Figure 5.18). This apparent difference was confirmed where the 2 predictions (new set vs. O.Vainio PK parameters) were assessed against the actual DEX blood concentrations through determination of the MDPE% and MDAPE% (Table 5.12). Although the accuracy was very similar (16.25 and 19.47), the bias was almost abolished with the new set with a slight over-prediction for both sets of PK parameter.

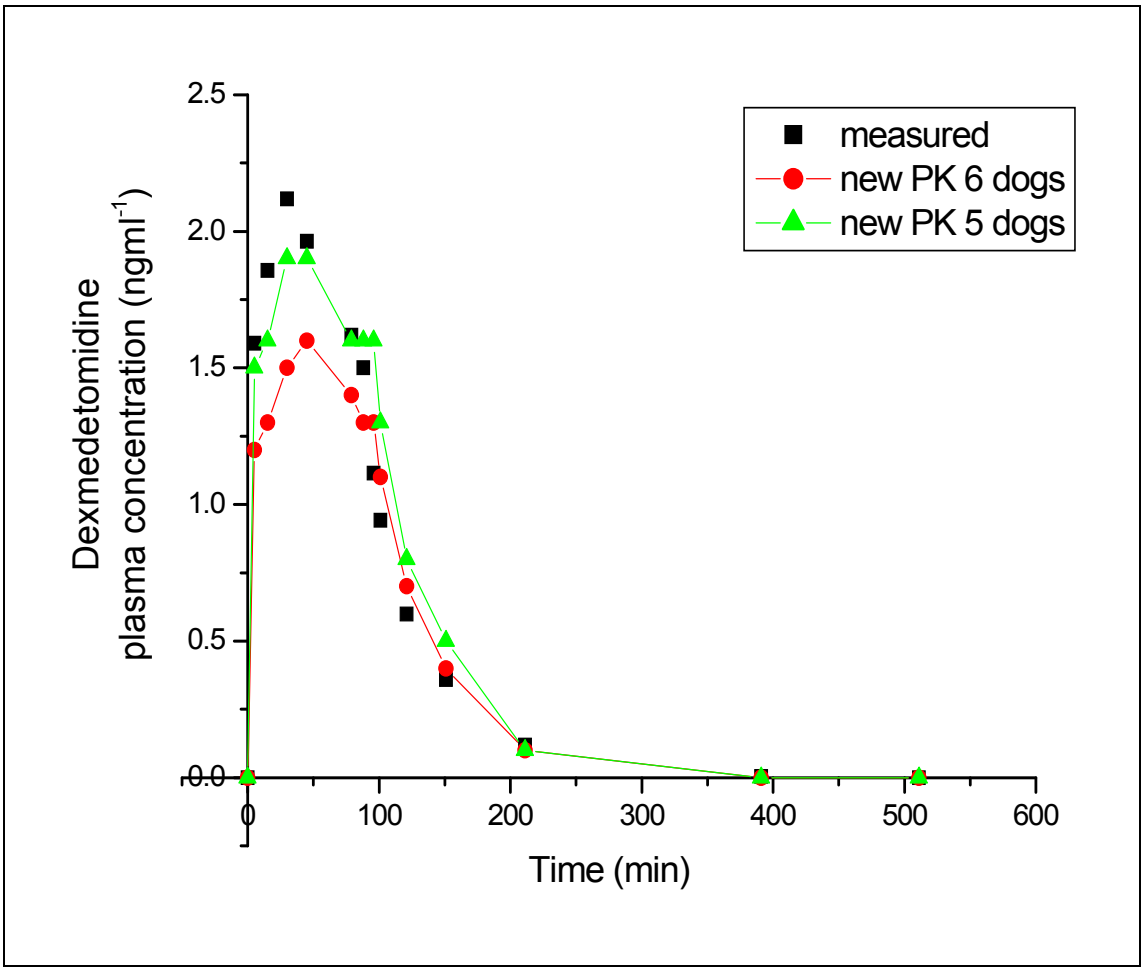


Figure 5.17

Comparison between the measured dexmedetomidine (DEX) concentration (black square) and the predicted DEX concentration ($ngml^{-1}$) during the 5-step infusion in dogs, using either the new pharmacokinetic (PK) parameters taken from all 6 dogs (red diamond) or the new PK parameters taken from the 6 dogs minus Dog 4 (green triangle).

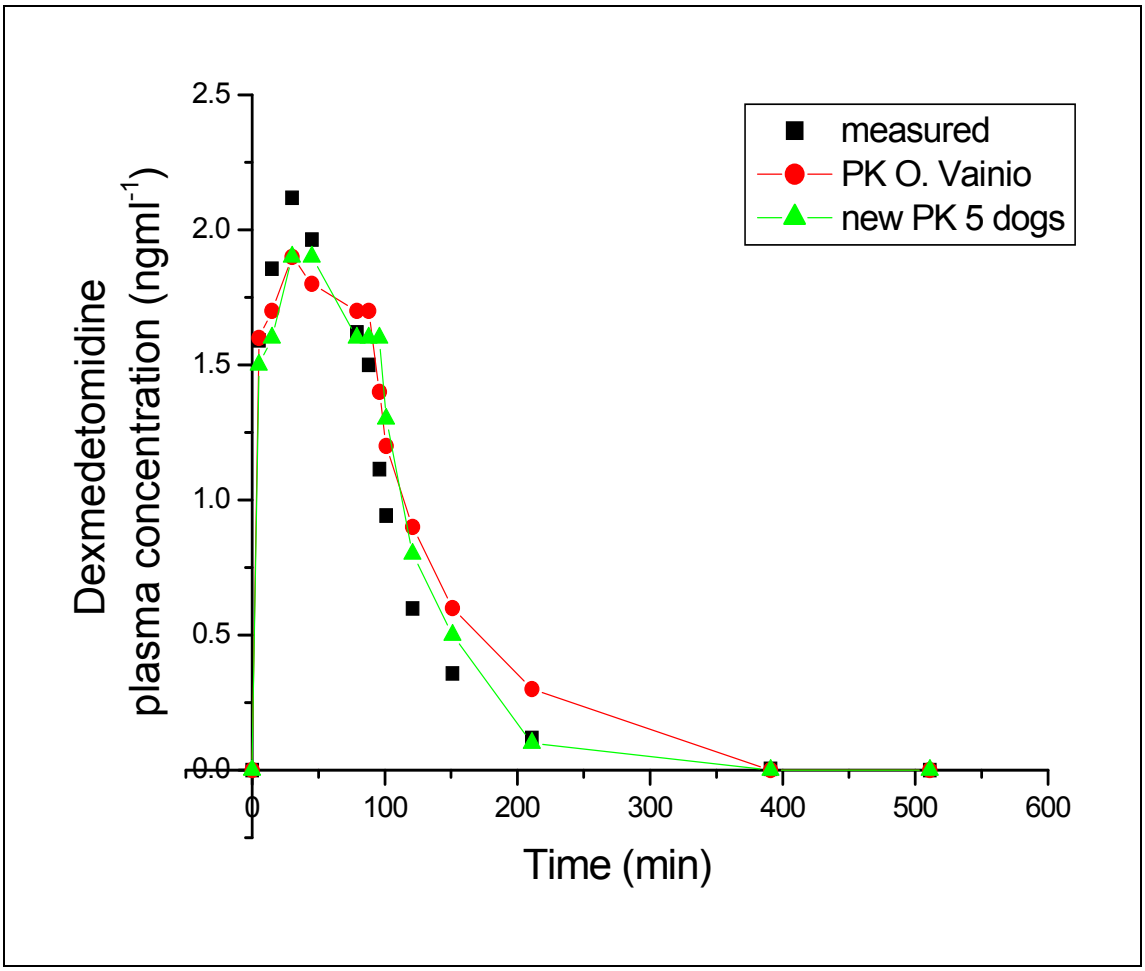


Figure 5.18

Comparison between the measured dexmedetomidine (DEX) concentration (black square) and the predicted DEX concentration ($ngml^{-1}$) during the 5-step infusion in 6 dogs, using either the pharmacokinetic (PK) parameters taken from O. Vainio (red diamond) or the new PK parameters taken from the 6 dogs minus Dog 4 (green triangle).

5.4 Discussion

This study investigated co-infusion of MED or DEX during TCI PPF anaesthesia in dogs. The influence of MED and DEX on the predictive performance of PPF administered by TCI (see chapter 2), was assessed and the predictive performance of an existing PK model for MED and DEX was also evaluated. Although the performance of the PK parameters (from O. Vainio) used to develop the infusion scheme in this study for MED and DEX was considered clinically acceptable, new PK parameters for DEX were calculated to see if any improvement could be achieved. The performance of the TCI system for PPF in the dog revealed a negative impact of the alpha$_2$-adrenoceptor agonists on the PK of PPF.

Even though it was judged clinically suitable, the combination of either MED or DEX infusion during PPF anaesthesia in the dog revealed important CV effects such as decrease in HR and CO with increase of the systolic and mean ABP. Low DEX plasma concentrations (PLD), while showing a similar effect on the HR and the CO as treatments PM and PHD, had less of an impact on the ABP while providing a better sparing effect on PPF requirement than the equivalent MED (PM) plasma concentration, with a lower negative impact on the predictive quality of the TCI system for PPF. These observations confirmed the more advantageous quality of the active enantiomer by comparison with the racemate.

5.4.1 Development of a medetomidine/dexmedetomidine infusion scheme

A DEX blood concentration target of 0.85 ngml^{-1} was chosen as it corresponds to the minimum analgesic blood concentration in the sedated dog (Granholm, personal communication from unpublished data, Orion, 2001) necessary to prevent response to thermally evoked skin twitch using a probe maintained at approximately 62.5 ± 0.5°C applied to shaved lumbar areas. As it is usually accepted that the activity of DEX is twice that of MED (Ansah *et al.*, 1998; Kuusela *et al.*, 2000, 2001a), the chosen MED blood concentration target was 1.7 ngml^{-1}. This study confirmed this observation as targeting a blood concentration of MED plasma concentration of 1.7 ngml^{-1} resulted in a measured plasma concentration of 0.92 (± 0.1) ngml^{-1} for DEX. This blood concentration (1.7 ngml^{-1}) is in the reported ranges of analgesic concentration (1-5 ngml^{-1}) for MED in the dog (Salonen, 1991). This target blood concentration was also used in a second DEX treatment (PHD) to allow a more precise study of the PK of the alpha$_2$-adrenoceptor agonist in the dog anaesthetised with PPF TCI. The minimum analgesic MED plasma concentration from Salonen (1991) is very different from that (9.5 ngml^{-1}) reported by Kuusela and colleagues (2000) in MED (40 µgkg^{-1} IV). However, it is difficult to compare the 2 studies as Salonen provided no information about the

method used in his study, while Kuusela *et al*. looked at withdrawal reflex in reaction to toe pinching.

With the aim of minimising the CV effects and keeping the alpha$_2$-adrenoceptor agonist blood concentration steady, a stepped infusion was developed (Smith and White, 1998a). The choice of number of steps and therefore the infusion rates was critical.

In a dose response study in conscious beagles, Pypendop and Verstegen (1998) showed that the CV effects of MED were dose-dependent. This was also observed in TCI PPF anaesthetised dogs in chapter 4 of this work. It has been shown in humans that a blood concentration of 0.9 ngml^{-1} of DEX can be achieved after a bolus of 1 μgkg^{-1} IV, but this results in an increase in ABP and a decrease in HR (Ebert *et al*., 2000). When the same target blood concentration was reached over 5 min using a TCI like system, the observed CV effects were markedly attenuated (Ebert *et al*., 2000). This illustrated that not only is the blood concentration important, but the speed of injection is also. Therefore, in theory, the choice of a small number of steps for this study might be expected to result in higher initial infusion rates and therefore more CV effects while the choice of more steps should result in less CV effect. However, the main disadvantage of a high number of steps is the time taken to reach the chosen blood concentration target.

To compromise between speed of infusion and time to reach the MED and DEX blood target concentrations, a 5-step infusion was arbitrarily chosen using 0.2 μgkg^{-1}min^{-1} as the infusion rate for the first step for MED. This rate was chosen as it corresponds to an infusion rate described in chapter 4 of this work, where the effect on the HR was only 50% of the maximum while the effect on ABP was minimal. The following four steps were modelled using the PK-SIM and the MED PK parameters from O. Vainio as they are reported to be similar to those from DEX (Kuusela *et al.*, 2000).

5.4.2 Anaesthesia

A PPF target of 3 μg ml^{-1} was used for induction as it was observed in chapter 2 to be a suitable blood target concentration to allow endotracheal intubation within 3 min in dogs premedicated with acepromazine and an opioid. In chapter 4, the same blood target concentration allowed endotracheal intubation in 100% of dogs 3 min after the start of the TCI system. In a more recent study, using similar premedication, Musk and colleagues (2005) reported that a PPF blood concentration target of 3 μgml^{-1} allowed tracheal intubation in 3 min in only 80% of the cases, compared with 54% (13/24) in this study. They also concluded that a target of 3.5 μgml^{-1} was more appropriate as it allowed endotracheal intubation in 100% of the patients with few side effects such

as post induction apnoea. Eleven patients in this study required an increase in target from 3 to 3.5 μg ml^{-1} after 3 min. All were then intubated in the next 1 min.

With an induction target of 3 μgml^{-1}, none of the patients from this study suffered from post-induction apnoea. In chapter 4 and in the Musk study (2005), using the same premedication as in this study the same induction target induced post induction apnoea in 22.5% and 30% of the dogs, respectively. As in the previous chapter, this frequency of apnoea is very similar to that described in the literature (Morgan and Legge, 1989; Cullen and Reynoldson, 1993; Smith *et al*., 1993; Nolan *et al.*, 1993; Muir and Gadawski, 1998; Quandt *et al*., 1998; Lerche *et al.*, 2000).

Signs of pain at injection, vomiting during recovery, excitation, paddling, muscle twitching, and opisthotonos are some of the side effects previously described with the use of PPF in the dog (Davies, 1991; Smith *et al*., 1993; Smedile *et al.*, 1996). In this study some muscle twitching was observed in 9 dogs. These signs disappeared soon after the start of the co-infusion in 4 dogs (1 from PM, 2 from PLD and 1 from PHD). In 1 dog (PM), the twitching continued, but was very much attenuated. Although the origin of these movements is not yet understood, the quality of the sedation and the addition of other drugs such as diazepam seem to have some beneficial effects (Short and Bufalari, 1999). This is consistent with the results reported here as the addition of MED or DEX stopped (4/9) and attenuated (1/9) the signs. In the last 4 dogs, the start of the co-infusion had no effect. These dogs belonged to the placebo (PS) treatment and confirm the positive effect of the addition of sedative and or muscle relaxant agents such as diazepam or, as in our study, an $alpha_2$-adrenoceptor agonist.

In this study, before the start of the noxious stimulation, the co-infusion of DEX or MED allowed a decrease of the infusion rate of PPF necessary to maintain the same depth of anaesthesia by about 50%. The infusion rates of PPF observed in this study (PM, PLD and PHD = 172, 173 and 142 μg$kg^{-1}min^{-1}$, respectively) for that time period, are very similar to the rates used in other studies in dogs after premedication with MED (150-165 μg$kg^{-1}min^{-1}$) (Vainio, 1991; Thurmon *et al*., 1994) or DEX (200 μg$kg^{-1}min^{-1}$) (Kuusela *et al.*, 2003). However, in those studies the dose of $alpha_2$-adrenoceptor agonist used was 40 and 30 μgkg^{-1} for MED and 10 μgkg^{-1} for DEX given IM in the premedication *versus* 5.64 (PM and PHD) and 2.82 μgkg^{-1} (PLD) only in this study.

Although clinical differences were observed for extubation time when comparing PS and PLD with PM and PHD, it was only significant between PS and PM ($p < 0.05$). When compared with the studies of Vainio (1991), Thurmon *et al.* (1994) and Kuusela *et al.* (2003), the times for extubation in this study (PM, PLD and PHD = 20.2, 12.5 and 18 min, respectively) were much shorter than 29.3 min (Thurmon *et al*., 1994) or similar at about 20 min (Vainio, 1991; Kuusela *et al*., 2003). The mean time to standing was reported to be 88.2 and 100 min for Thurmon *et al.* (1994) and

Kuusela *et al.* (2003), respectively, while in this study it was 23.3, 33.7 and 36 min for the PLD, PM and PHD treatments, respectively. When comparing these previous two studies with the present work, the PPF infusion rates were very similar, as well as the breed of dogs, although the anaesthesia time was longer in this study (107.9 *vs*. 60 min). Therefore, the difference in the recovery times between those two studies and this investigation is more likely to result from the total dose of MED or DEX used: 5.64 μgkg^{-1} (PM and PHD) and 2.82 μgkg^{-1} (PLD) *versus* 10 (Kuusela *et al.*, 2003) and 30 μgkg^{-1} (Thurmon *et al.*, 1994) of DEX and MED, respectively.

For the sternal and standing times, the clinical difference between PS and PLD was minor compared to the two other treatments. Although the target of DEX in the PLD treatment was supposedly equivalent to the MED target (PM), there was a clinical difference for the sternal and standing times between these two treatments. The observed difference could have resulted from the fact that the two targets were not equivalent in effect. Another possible reason for the delayed recovery in the PM treatment was an excess of PPF. It is notable that although the prediction for PPF was the same between the two treatments at the end of the treatments, the measured concentration was 27% higher in the PM treatment (Table 5.8) despite the delivery of a similar infusion rate of PPF in these two treatments (Table 5.4).

It has been shown in humans (Takizawa *et al.*, 2004b*,* 2005c; Takizawa *et al.*, 2006), in sheep (Upton *et al*., 1999; Myburg *et al.*, 2001) and in pigs (Kurita *et al*., 2002) that CO and PPF blood concentrations during constant rate infusion (CRI) or TCI are inversely related. Therefore a difference in CO between the PLD and PM treatments could have resulted in a difference in PPF concentrations and therefore in the recovery times. However, this cannot be confirmed as CO was not measured at later time points. Hepatic cytochrome P450 dependent metabolism of PPF is more important in dogs than in humans (Court *et al*., 1999). Medetomidine and its enantiomers are imidazole derivatives and are potential CYP inhibitors (Kharasch *et al.*, 1991; Pelkonen *et al.*, 1991; Kharasch *et al.*, 1992; Rodrigues and Roberts, 1997; Lennquist *et al.*, 2008). As the negative effect of these agents on the hepatic CYP dependent metabolism of PPF has been demonstrated in dog and in rat tissues in chapter 3, a further possible explanation for the higher concentration of PPF in the PM treatment and therefore the delayed recovery could be that MED, as it contains both DEX and LEV, inhibited PPF metabolism to a greater extent than clinically equipotent doses of DEX.

5.4.3 Pharmacodynamics

Bradycardia and an increase in ABP are common side effects of MED (Savola, 1989; Vainio and Palmu, 1989). The infusion rate of the first step (0.2 μgkg^{-1} min^{-1}) was chosen as it corresponded to the ED_{50} for the HR with little effects on the ABP (see chapter 4). In this study, after 5 min of this

infusion rate, the HR decreased by about 50% and the systolic ABP increased by about 30%. Although the infusion rate was similar to the study in chapter 4, the infusion time was longer in the actual one (5 vs. 1 min) and therefore the total dose of MED administered (1 vs. 0.2 μgkg^{-1}). Using the computer program PK-sim and the MED PK parameters from O. Vainio, the MED plasma concentration reached at 2 min (time of the ED_{50} determination in chapter 4) in this study and in chapter 4 can be determined and corresponded to 0.75 vs. 0.37 $ngml^{-1}$, respectively, while after 5 min, these values were 1.55 and 0.25 $ngml^{-1}$, respectively (appendix 35 and 36). In this study, the MED blood concentration was twice and 6 times higher at 2 and 5 min, respectively than that of the study in chapter 4. The dose and blood concentration dependent CV effect of DEX and MED have already been demonstrated in humans (Ebert *et al.*, 2000) and in dogs (Pypendop and Verstegen, 1998) and might explain the difference in results between the 2 studies regarding the HR and the ABP. The bradycardia observed here was accompanied by an increase in ABP, which is therefore believed to result more from the peripherally induced vasoconstriction than being centrally mediated (Hall and Clarke, 1991b; Pypendop and Verstegen, 1998).

In conscious humans, a dose response study using a TCI-like system showed that blood concentrations between 0.7 and 1.2 $ngml^{-1}$ of DEX slightly decreased HR and ABP while concentrations higher than 1.9 $ngml^{-1}$ increased ABP and SVR while decreasing HR (Ebert *et al.*, 2000). Still in humans, an IV bolus of 1 μgkg^{-1} of DEX resulted in a decrease in HR and increase in ABP, while the blood concentration reached a maximum of only 0.9 $ngml^{-1}$ (Bloor *et al.*, 1992). In this study, the concentration of DEX varied between 0.85 (PLD) and 1.7 $ngml^{-1}$ (PHD), which is very similar to the concentrations used in the 2 studies in man described above.

An infusion of DEX of 0.2 – 0.4 $\mu gkg^{-1} h^{-1}$ (0.003 – 0.006 $\mu gkg^{-1}min^{-1}$) in humans in intensive care units provided analgesia and sedation, accompanied by slight decreases in HR and ABP (Ickeringill *et al.*, 2004). These infusion rates are far smaller than the one used in this study (0.04–0.2 $\mu gkg^{-1} min^{-1}$), but were done in conscious patients. In a study in anaesthetised humans, DEX blood concentrations between 0.075 and 0.6 $ngml^{-1}$ showed similar CV effects to those reported in this study in dogs (Talke *et al.*, 2003). As discussed in chapter 4, general anaesthesia, by reducing the sympathetic tone decreases or eliminates the centrally mediated sympatholytic effect of the $alpha_2$-adrenoceptor agonists, in favour of their peripherally mediated vasopressor effect (Flacke *et al.*, 1990; Flacke *et al.*, 1993; Talke *et al.*, 1999; Talke *et al.*, 2003) resulting in the CV changes observed in this study. In their study in dog anaesthetised with PPF and a co-infusion of DEX (1 $\mu gkg^{-1} h^{-1}$), Lin and colleagues (2008) stopped the anaesthesia after 2 hours while carrying on a DEX CRI for another 22 hours. During that period, the DEX plasma concentration was between 0.35 and 0.45 $ngml^{-1}$. Even though, as in this study, they observed an increase in ABP and a decrease in HR during the anaesthesia, they did not measure the ABP during the following 22 hours

and although the HR stayed low, no conclusion can be taken regarding the possible emergence of a centrally mediated sympatholytic effect with PPF disappearance and the regain of consciousness.

During noxious stimulation the HR and the systolic and mean ABP did not change from the values recorded before stimulation in the PLD, PM and PHD treatments, while a significant increase was observed in the PS treatment. This apparent blunting of the sympathetic response to noxious stimulation and haemodynamic stability with alpha$_2$-adrenoceptor agonists has been previously reported in humans (Flacke *et al*., 1987; Aho *et al.*, 1992b; Scheinin *et al*., 1992; Talke *et al.*, 2000b), and is used to advantage in humans undergoing CV surgery where stable CV parameters are of importance (Mukhtar *et al.*, 2006).

In dogs and in man, a dose dependent decrease in CO is a common feature of the use of an alpha$_2$-adrenoceptor agonist (Vickery *et al.*, 1988; Bloor *et al.*, 1992; Flacke *et al*., 1993; Pypendop and Verstegen, 1998; Ebert *et al*., 2000). The effect appears to reach a maximum with doses of MED of about 5 μgkg^{-1} when given IV in conscious dogs (Pypendop and Verstegen, 1998). Using the PK-Sim software and the PK parameters from O.Vainio for MED, this bolus dose ($5\mu gkg^{-1}$) corresponds to a blood concentration of 10.6 $ngml^{-1}$. In this study, a maximum effect on CO might already have been reached with a DEX blood concentration of only 0.85 $ngml^{-1}$ (MED blood concentration of 1.7 $ngml^{-1}$), as no difference in CO was observed between that treatment and the PHD treatment. In a study in halothane anaesthetised dogs receiving 1, 3 or 10 μgkg^{-1} of MED IV over 15 min (corresponding to 0.067, 0.2 and 0.67 $\mu gkg^{-1}min^{-1}$, respectively), a maximum effect on the CO measured 10 min after the infusion was already reach with the 0.2 $\mu gkg^{-1}min^{-1}$ dose (Vickery and Maze, 19889). As above, using the PK software PK-Sim and the PK parameters from O. Vainio, it can be concluded that the MED plasma concentration in that study at the time of CO determination corresponded to 1.3 $ngml^{-1}$ (appendix 37). In chapter 4 it has been hypothesised that most of the differences in the CV effects of MED observed between PPF anesthetised and conscious dogs resulted from the lack of centrally mediated sympatholytic effect from the alpha$_2$-adrenoceptor agonist and the predominance of peripherally mediated vasoconstriction in anaesthetised patient. Therefore we can speculate that similarly, the effect on the CO observed in this study have been enhanced by the lack of sympathetic tone in anesthetised dogs.

In this study, lithium dilution (LiDCO) was used to measure the CO. This recent method has been validated in humans (Linton *et al*., 1997), horses (Linton *et al.*, 2000) and dogs (Mason *et al*., 2001). Compared to thermodilution, the LiDCO system is as accurate if not better (Kurita *et al.*, 1997). This method was also chosen for this study as it only requires the placement of a catheter in a superficial vein as well as in a superficial artery instead of a Swan-Ganz catheter in the pulmonary artery for the thermodilution technique. Placement of such a catheter is not without risk and requires access to fluoroscopy or pressure-wave analysis (Mason *et al.*, 2001). Although the

manufacturer of the LiDCO system advises the use of a central venous site for the injection of the lithium, Mason *et al*. (2002) showed that a peripheral site (cephalic vein) is totally acceptable.

5.4.4 Pharmacokinetics

5.4.4.1 Propofol

5.4.4.1.1 Assessment of the performance of the propofol TCI system

In chapter 2 of this work, the performance of the TCI system for PPF was assessed in greyhounds and in mixed-breed dogs. The parameters used to programme the TCI system in the dog were considered clinically acceptable in both treatments studied (greyhound and mixed-breed). In this study, it was observed that these parameters for PPF were equally acceptable in beagle dogs, with figures for bias and inaccuracy in the control treatment similar to those found in other breeds reported in chapter 2. On the other hand, the predictive performance of the PPF TCI system was impaired during the co-administration of MED or DEX, with the greatest degree of underprediction occurring in the PM and PHD treatments.

The performance of a TCI system will deteriorate if the distribution or the clearance of the drug being delivered differs from the parameters of the average model used to programme the system. Drug-drug PK interactions are well recognized (Benet *et al.*, 1996) and occur when one agent alters the distribution or clearance of the other (Vuyk 1998). When co-infusing either MED or its active enantiomer DEX, the performance and therefore the predictability of the TCI system for PPF in the dog decreased and became clinically unacceptable in the PM and PHD treatments, with MDPE% and MDAPE% values of 58.3 and 58.35 respectively for both agents. Not only did the accuracy decrease, but also the bias started to become very positive (underprediction of the TCI system). In the two treatments, the co-infusion of the alpha$_2$-adrenoceptor agonists resulted in a marked decrease in CO. As it has been discussed previously, the PPF concentration is influenced by changes in CO (Upton *et al.*, 1999; Myburg *et al.*, 2001; Kurita *et al.*, 2002; Takizawa. *et al.*, 2004, 2005c; Takizawa *et al.*, 2006). Therefore, any infused drug such as the alpha$_2$-adrenoceptor agonists which have an effect on the CO has the potential to modify the performance of the TCI system for PPF in the dog, as has been proposed for remifentanil in humans (Ludbrook and Upton, 2003).

Two studies in humans anaesthetised with 1% sevoflurane and a constant target of PPF (2 μg ml^{-1}) showed that the PPF concentration decreased while the CO increased after either infusion of dopamine (Takizawa *et al.*, 2005c) or injection of atropine (Takizawa *et al.*, 2006), resulting in an over-prediction of the TCI system. In this study, the co-infusion of either MED or DEX resulted in

a decrease in CO. This decrease could have resulted in an increase in PPF blood concentration and in the underprediction observed. However, as there was no difference in CO between the PM, PHD and PLD treatments measured at 45 min, and the PLD treatment performed much better than the two other treatments with a MDPE% and MDAPE% of 25.94 and 35.80, respectively, additional factors appear to be involved. The CYP inhibitory effects of MED and its enantiomers (Kharasch *et al.*, 1991; Pelkonen *et al.*, 1991; Kharasch *et al.*, 1992; Rodrigues and Roberts, 1997; Lennquist *et al.*, 2008), might explain the difference in the performance of the TCI system as it did for the difference in recovery times (see last paragraph of 5.4.2). The better performance of the TCI system in the PLD treatment compared with the two others may be a consequence of the smaller amount of alpha$_2$-adrenoceptor agonist in the blood of the dogs of that treatment, resulting in less CYP inhibition of metabolism.

5.4.4.1.2 Determination of the MIT for propofol

Since 1963 in a study in dogs by Merkel and Eger, MAC has been used in human as well as in veterinary anaesthesia as the most objective indicator of inhalant anaesthetics potency (Koblin *et al.*, 1981). In a similar way, anaesthetists have used the Cp_{50} to compare the intravenous agents' potency (Kenny and Stutcliffe, 1997). As the MAC and the Cp_{50} correspond to the effective drug concentration (ED_{50}) for the inhalants and the IV agents, respectively, they allow a more accurate comparison between their relative and adverse effects by providing a better appreciation of equipotent doses (Kenny and Stutcliffe, 1997). Although real time PPF blood concentration measurement might be possible in the near future (Hornuss *et al.*, 2007; Takita *et al.*, 2007) it is not currently possible and therefore the Cp_{50} value is of limited practical application in clinical anaesthesia (Flaishon *et al.*, 1997). The MIR, developed to be of a more practical interest for the clinician, is unfortunately also of little value in the clinical environment, as it depends on the PK of the agent and ignores drug accumulation (time dependent measure) making the maintenance of a steady-state difficult (Flaishon *et al.*, 1997). Although this is correct for most drugs, one can argue that the MIR concept is a very reliable concept for agents with little to no apparent cumulative properties such as PPF (Smith and White, 1998b) or, better, remifentanil (Lozito *et al.*, 1994).

Computer controlled infusion systems such as the TCI system for PPF are ideal for Cp_{50} determination as they allow the anaesthetist to maintain a steady-state effect site concentration by targeting specific PPF blood concentrations (Smith and White, 1994; Andrews *et al.*, 1997). As the TCI system provides the anaesthetist with the predicted PPF blood concentration, it seemed that the MIT_{50} would be of a more practical interest in the clinical environment and was therefore also determined in this study. Although CP_{50} values, like MAC, are relatively constant in a given population, it is not the same for the MIT_{50} values, which are derived from predictions of drug plasma concentration and are PK dependent. As a result, the MIT_{50} values determined in this study

will only be of interest and relevance to clinicians using a TCI system programmed with the same PPF PK parameters as in this study.

It is usually accepted that the anaesthetic dosage required to maintain anaesthesia during moderate surgical procedures is estimated to be 95% of the effective dose or ED_{95} (Sear, 1992). In this study, the Cp_{95} was determined in the 4 treatments of dogs (Cp_{95} for treatments PS, PM, PLD and PHD were 8.6, 5.8, 4.8 and 3.9 μgml^{-1}, respectively) and can therefore be considered as the PPF blood concentrations required to maintain anaesthesia in dogs undergoing moderate surgical procedure. It can be speculated that more traumatic surgeries will require higher PPF and/or $alpha_2$-adrenoceptor agonist blood concentration and/or the addition of other analgesic agents such as opioids. These results compare very well with human studies where to maintain anaesthesia and prevent reaction to skin incision in 95% of patients, targets of 4 to 27.4 μgml^{-1} PPF have been reported, with this variation being dependent on whether or not an analgesic adjuvant was used (Smith *et al.*, 1994; Andrews *et al*., 1997; Kazama *et al.*, 1997; Handa-Tsuitsui and Kodaka, 2007).
For the volatile anaesthetics, 1.2-1.4 MAC is used as an approximation of the ED_{95} or MAC_{95} (Steffey, 1999). In this study, the same comment could be made as the Cp_{95} in the 4 treatments was equal to 1.2-1.4 times the corresponding Cp_{50}. In a study in horses where MIR_{50} for PPF was determined after premedication with xylazine by stimulating the oral mucosa with an electric current (Oku *et al.*, 2005), 1.2-1.4 MIR_{50} corresponded to the MIR_{95} calculated using a linear regression. This MIR_{95} obtained, by analogy with MAC, was considered by the authors as adequate for basic infusion rate of PPF required for moderate surgical operation in TIVA of horses. In a similar manner, it is proposed that in this study, a target of 1.2-1.4 MIT_{50} (corresponding to the MIT_{95}) would be adequate in providing anaesthesia in the PPF TCI anesthetised dog undergoing a surgical procedure of moderate or lower intensity.

In humans, skin incision or electrical currents are used as the noxious stimulation for the determination of the MAC or Cp_{50} value for an inhalant or an IV agent, respectively, (Quasha *et al.*, 1980, Smith *et al.*, 1994; Zbinden *et al.*, 1994; Andrews *et al.*, 1997; Kazama *et al.*, 1997; Stuart *et al.*, 2000). In the dog, in addition to these stimulations, the tail clamp is also an accepted method (Eger *et al.*, 1965; Quasha *et al.*, 1980; Weitz *et al.*, 1991; Branson *et al.*, 1993; Thurmon *et al.*, 1994; Grimm *et al.*, 2000; Valverde *et al.*, 2003).

In a study in man defining the anaesthetic depth in the isoflurane-anesthetised patient using multiple noxious simulations (Zbinden *et al.*, 1994), it was shown that different stimuli resulted in different MAC values determination. They found that the MAC value determined using a supramaximal tetanic stimulation of the muscles of the forearms (50 Hz, 50 mAmp for 10 sec) was lower than MAC determined with a skin incision. They also concluded that the disadvantage of the skin incision was that it could not be repeated in contrast to electrical stimulations. In animals, the

comparison between electrical stimulation and skin incision gave different results in a comparative study in dogs and in rabbits (Valverde *et al.*, 2003). In both species MAC values for halothane (dog) and for isoflurane (rabbit) were lower with the skin incision than with tail clamping or electrical stimulation (ulnar and tibial area for the forelimb and hind limb respectively; 50 volt, 50 Hz for 10 msec), which were equipotent. This observation and the advantage of the repeatability resulted in the choice in this study of the tetanic twitch 50Htz and 80mA applied for 10 sec on the tail at the level of the 5^{th} and 6^{h} coccygeal vertebrae as the supramaximal noxious stimulation for the determination of the PPF Cp_{50}.

In a recent study on isoflurane anesthetised dogs, Pascoe *et al.* (2006) observed the MAC reduction effect of two DEX infusion rates, 0.5 and 3 $\mu gkg^{-1} h^{-1}$. The decrease of the MAC value was 18 and 59%, with DEX blood concentrations of 0.198 and 1.903 $ngml^{-1}$. In this study, DEX blood concentrations of 0.85 and 1.7 $ngml^{-1}$ decreased the PPF Cp_{50} by 41 and 51%, respectively (from 6.4 to 3.8 and 3.15 μgml^{-1}, respectively). In humans, Aanta *et al*. (1997) observed very similar results with a MAC reduction of isoflurane by about 35% and 50% with DEX target plasma concentration of 0.3 and 0.6 $ngml^{-1}$, respectively.

In 2 studies in dogs receiving IV boluses (Kuusela *et al.*, 2000 and 2001b) of DEX and MED, the authors observed that, regarding analgesia, DEX was slightly more potent than equivalent doses of MED. This result corroborates that observed in this study, where the Cp_{50} and Cp_{95} values in treatment PLD were lower than in treatment PM, (3.8 and 4.4, vs. 4.8 and 5.8, respectively). It is possible that LEV might interact with DEX in some manner causing antagonism or competition at the same receptor site or that LEV might have an action on $alpha_1$-adrenoceptors (Kuusela *et al.*, 2000).

5.4.4.2 Medetomidine/Dexmedetomidine

The parameters provided by Outi Vainio and used to design an infusion scheme for MED were adequate as the blood target concentrations and the actual blood concentrations were very close. This is confirmed by the clinically acceptable MDPE% and the MDAPE% values (3.07 and 14.5, respectively) obtained. Moreover these parameters were equally acceptable for the active enantiomer as the MDPE% and MDAPE% values for the PLD (-2.95 and 17.5, respectively) and PHD (-1.23 and 14.17%, respectively) treatments were also clinically acceptable. This confirmed the result of a study in dogs which demonstrated that MED and its active enantiomer had very similar pharmacokinetics, LEV had a faster clearance (Kuusela *et al*., 2000). Although the present study was not designed to look at the PK of LEV, Figure 5.13 confirmed that LEV has a different PK from DEX. The difference between the 2 enantiomers might have been the result of a higher clearance and/or a bigger volume of distribution for LEV as its plasma concentration was lower

than DEX in the PMD treatment. In their study Kuusela and colleagues did not look at the PK of DEX or LEV when administered simultaneously (MED) and concluded that the difference in PK observed between these 2 agents could be caused by a divergent metabolic pathway and by the fact that DEX could reduce its own elimination rate via its haemodynamic effects. In the actual study, as DEX and LEV were infused simultaneously (MED), the haemodynamic of DEX influenced both DEX and LEV PK and, therefore, the difference in PK might have resulted mainly from a different metabolic pathway. The results from this study show that PPF had little effect on the pharmacokinetics of MED or DEX.

The study undertaken was also designed to calculate the PK parameters of DEX in the PPF anaesthetised dog. A simulation of the 5-steps infusion with these new PK parameters for DEX (Table 5.10) using the PK-SIM software showed an important underprediction (Figure 5.16). When looking at the data used to calculate the DEX parameters, dog 4 showed a very large volume of distribution when compared with the other dogs. When repeating the same simulation but with the PK parameters of 5 dogs (dog 4 being removed) instead of 6, the prediction obtained was much closer to the actual DEX blood concentrations (Figure 5.16). The new PK parameters produced similar accuracy for the system as those from O. Vainio, but induced less bias (Table 5.18).

5.5 Conclusion

Previous studies have shown advantages of using DEX instead of the racemate in the dog (Kuusela *et al*., 2000, 2001a, 2001b). In this study the use of DEX 0.85 $ngml^{-1}$ had a similar PPF sparing effect to the equivalent MED blood concentration of 1.7 $ngml^{-1}$, but with less effects on the ABP, as well as on the performance of the TCI for PPF in the dog. Thus DEX is more advantageous than MED given by infusion in PPF anaesthetised dogs.

The study also confirmed the validity of the PK of MED from the previous study (O. Vainio, personal communication, 2001). The study redefined specific PK parameters for DEX, although the MED PK parameters could also be used. The study indicated that DEX blood concentrations as low as 0.85 $ngml^{-1}$ decreased the measured PPF blood concentrations necessary to maintain anaesthesia during noxious stimulation by about 41%. However, although this study supported the suitability of the co-infusion of DEX during PPF anaesthesia in the dog, and the analgesic/sedative effects of DEX were present at the lowest blood concentrations, the CV effects were marked. Further studies are therefore necessary to establish if a lower blood concentration of DEX will provide analgesia while preserving the CV system. It was also observed that the PPF sparing effect resulting from co-infusion of MED or DEX was not accompanied by any benefit in terms of shortening of recovery times.

CHAPTER 6:

GENERAL DISCUSSION AND CONCLUSIONS

In humans, a target controlled infusion (TCI) system developed firstly for propofol (PPF) revolutionised TIVA at the end of the eighties, by making it as simple to administer and as rapid to titrate as inhalational anaesthesia. This system enabled the anaesthetist to predict and maintain any desired target drug plasma concentration. This project sought to investigate the development of such a system for PPF in the dog, which would be suitable for use in general practice. Propofol is a poor reflex suppressor, and agents such as opioids are commonly used to provide analgesia. Since respiratory depression and apnoea are common side-effects of these agents, their use is challenging to veterinarians in practice. This study looked at the development of a co-infusion scheme using PPF and the alpha$_2$-adrenoceptor agonist medetomidine (MED) and its active enantiomer dexmedetomidine (DEX). This class of drug is well known for its analgesic, muscle relaxant and anaesthetic agent sparing properties in many domestic species. In the dog few respiratory effects have been reported although effects on the CV system can be significant.

The main findings of the study were as follows

- The PPF TCI system performed well in a group of mixed-breed dogs (10) and greyhounds (6) (chapter 2) as well as in a group of beagles (6) (chapter 5).
- An induction target concentration of 3 μgml^{-1} appeared to be optimal in dogs premedicated with acepromazine and methadone or pethidine, causing little CV and respiratory depression (chapters 2, 4 and 5).
- Ideal maintenance target concentrations varied between 2 and 6 μgml^{-1}, depending on the premedication, the level of the noxious stimulation, and the co-infusion of an analgesic agent such as MED or DEX (chapters 2 and 5)
- MED and its enantiomers are cytochrome P450 inhibitors and were found to impair the *In vitro* metabolism of PPF in rat and canine hepatic microsomes in a dose-dependent manner (chapter 3).
- The *In vitro* inhibitory effects of MED and DEX were evident at concentrations higher than the minimum effective analgesic plasma concentrations described in the dog for these two agents.
- The CV effects of MED in the PPF TCI anaesthetised dogs were dose dependent and occurred at lower doses than in the awake patient (chapter 4). Bradycardia and an increase in arterial blood pressure were the predominant effects. Significant hypotension was not observed, even after IV doses of MED as low as 0.001μgkg^{-1} (chapter 4).
- The ED_{50} and ED_{95} for MED in the PPF TCI anesthetised dog were 0.187 and 3.1 μgkg^{-1} respectively for the HR and 2.05 and 18.1 μgkg^{-1} respectively for the systolic ABP, while MED doses below or equal to 0.1 μgkg^{-1} had no or minimal effects on the ABP and the HR, respectively (chapter 4).
- MED and DEX have very similar PK parameters in PPF TCI anesthetised dogs (chapter 5).

- DEX infusion impaired the performance of the TCI system in a concentration dependent manner. This impairment of the PPF TCI system was less with DEX infusion than with an equivalent MED infusion, confirming the clinical advantage of DEX vs. MED (chapter 5).
- A plasma concentration of 0.85 $ngml^{-1}$ of DEX had less effect on the ABP than an equivalent MED plasma concentration (1.7 $ngml^{-1}$), while the effects on the HR and the CO were similar (chapter 5).
- CP_{50-95} and MIT_{50-95} (measured and predicted blood PPF concentrations where 50% or 95% of the dog population would not respond to a noxious stimulation) were clinically lower during DEX infusion targeting a plasma concentration of 0.85 $ngml^{-1}$ than during infusion of MED targeting an equivalent plasma concentration (1.7 $ngml^{-1}$) (chapter 5).
- The infusion of DEX to a target plasma concentration of 0.85 $ngml^{-1}$ during TCI PPF anaesthesia in dogs had little effect on the respiratory rate and ventilation, while decreasing the HR (± 50%) and increasing ABP (± 15%) (chapter 5).
- DEX infusion appeared to be more advantageous than an equivalent MED infusion in terms of PPF sparing effect and PPF TCI system performance.

Although the pharmacokinetics (PK) for anaesthetic drugs and for PPF in particular have been shown to vary between breeds (Sams *et al.,* 1985; Court *et al.,* 1999), the TCI system developed in this study was clinically acceptable in greyhounds, beagle dogs and in a group of mixed breed dogs. While the PK parameters used to programme the TCI system might represent a wide range of the canine population, PK variation exists between patients and groups of patients in the same population and between different populations and so the system might not perform well in some individual dogs. It has been shown that as long as the actual plasma concentrations are still in the therapeutic window, the difference with the predicted values and therefore the lack of performance of the system is likely to be of little consequence (Li *et al.,* 2005), and in this event, the TCI system is still a valuable tool in terms of TIVA, providing the ability to titrate to effect, to maintain stable plasma concentration and to make changes to the plasma concentrations and therefore anaesthetic depth, easily (Schuttler and Ihmsen, 2000; Li *et al.,* 2005). The number of dogs in the studies assessing the performance of the TCI system (chapter 2 and 5) was small, and therefore some caution is needed interpreting the results. More studies with higher number of dogs might be necessary to confirm these results, however the data were encouraging.

Although the studies assessing the performance of the TCI system used dogs aged from 2 to 10 years old, the study was not designed to consider elderly patients. In humans, different studies have used TCI systems programmed with general PK parameters. Although the authors agree that as long as the anaesthetist titrates to effect and that the PK model incorporates age as a covariate, the performance of the system will be clinically acceptable, they advised that specific PK parameters for the elderly should be incorporated into the system (Ouattara *et al.,* 2003; Passot *et al.,* 2005; Xu

et al., 2005, White *et al.,* 2008). Reid and Nolan (1996) showed that, in geriatric dogs (which they defined as > 8 years old) PK parameters for PPF, such as the volume of distribution and the elimination half-life, were not different from young dogs, while the clearance was a bit longer (Reid and Nolan 1996). Although the TCI system devised here might still perform adequately in the elderly, more studies are necessary to confirm this. Similarly, neonates and puppies were not considered while programming this TCI system. In humans, the PPF PK parameters of the adult were replaced with specific PK parameters for children (Absalom *et al.,* 2003). Therefore, further studies in dogs would have to be undertaken to assess the PK parameters for PPF in puppies and thereafter to define new PK parameters as appropriate for use in the TCI system.

With knowledge of the PK parameters of DEX in the dog (chapter 5) and the technology of the TCI system, more studies could be undertaken to look at the analgesic and/or sedative DEX concentrations with minimal CV effects. These results could be used thereafter to optimise the PPF-DEX PK/PD relationship.

A negative effect on the performance of the TCI system resulted from the co-infusion of MED or DEX with PPF. The predicted PPF blood concentrations were lower than the measured concentrations (under-prediction). While a possible PK interaction resulting from a direct inhibitory effect of the alpha$_2$-adrenoceptor agonists on the hepatic CYPs was highlighted (chapter 3), the study reported another possible origin: the effect of these drugs (alpha$_2$-adrenoceptor agonists) on sympathetic drive, altering cardiac output (CO) and hepatic blood flow. This has already been illustrated in humans co-infused with PPF and remifentanil (Wietasch *et al.,* 2006). In man, studies looking at the effect of opioids such as remifentanil or alfentanil have shown from 17 up to 60% underprediction from the computerised infusion system (TCI or similar) when compared with measured PPF plasma concentrations (Pavlin *et al.,* 1996; Crankshaw *et al.,* 2002; Hoymork *et al.,* 2003; Mertens *et al.,* 2004; Wietasch *et al.,* 2006). Although the performance of the PPF TCI is affected, these PK changes are believed to be insignificant compared to inter-individual pharmacodynamic variability, and by consequence to be of little effect on the clinical performance of the TCI system. Therefore, it is considered that such interactions will not interfere with the quality of anaesthesia, as the clinician is continuously titrating to effect (Vuyk 1998; Li *et al.,* 2005). Although this was confirmed in chapter 5 in beagles, where anaesthesia was easy to maintain even though the predictions of the TCI system were erroneous in the treatment groups (co-infusion of MED and DEX), the small number of dogs used in that study does not allow firm conclusions to be made. Further studies are therefore necessary to study these interactions and maybe to define a new set of PPF PK parameters for use with DEX infusion.

When considering drug-drug relationships, PK interactions are often minimally variable compared to PD interactions. Inter-individual PK variability of a single agent is in the order of 70-80%, while

inter-individual PD variability frequently ranges between 300-400% (Vuyk 1998; Lichtenbelt *et al.,* 2004). More studies are necessary to establish a better knowledge and understanding of the PD interaction of PPF and DEX and to develop optimal PPF-DEX infusion regimens using PK/PD modelling. In humans, PK/PD modelling of PPF and different opioids has been used to maximize the use of both agents (Vuyck 2001). PK/PD modelling determines the various optimal PPF-opioid concentrations associated with intra-operative adequacy of anaesthesia and the most rapid return to consciousness thereafter. The optimal PPF concentration changes with the opioid used and the length of infusion (Vuyck 2001; Lichtenbelt *et al.,* 2004). Similar studies could be done with DEX and PPF using either isobolographic analyses or response surface modelling techniques in order to maximize and make the interaction more beneficial for the anaesthetist and for the patient (i.e. optimising speed of induction of anaesthesia; increasing haemodynamic stability at induction and during maintenance of anaesthesia; decreasing time to awakening; optimising spontaneous respiration, minimising the level of post-operative pain) (Vuyck 2001; Minto and Vuyck 2003; Lichtenbelt *et al.,* 2004).

Co-infusion of DEX and PPF appeared to provide excellent analgesia with little respiratory depression, however, CV effects were observed. The study was not designed to determine an infusion scheme for DEX low enough to maintain analgesia with minimal CV effects. On the other hand the study determined the minimum PPF target blood concentration necessary to maintain anaesthesia during a supra-maximal noxious stimulation and the decrease of this blood concentration by up to 35.5% by DEX blood concentration as low as 0.85 $ngml^{-1}$.

The TCI system for PPF in the dog was developed in the context of anaesthesia; however there is more to TCI than anaesthesia. The TCI system can be used to provide sedation as well as analgesia. By replacing the PK parameters with those for DEX or for an opioid such as remifentanil, fentanyl or even morphine, a powerful and precise analgesic tool would be made available for use pre, peri and postoperatively or in the ICU (Milne and Kenny, 1998a). In fact, any IV agent that can be administered by infusion may benefit from the TCI mode of administration: intravenous antibacterials, antiarrhythmics, chemotherapeutic agents and inotropes to cite a few (Glen, 2003).

The performance of a TCI system will only be as good as the PK parameters used to programme it. While incorporating PK parameters derived from studies conducted in groups of individuals into the system is reasonable, work in humans with alfentanil has shown that the gain compared to the use of population PK parameters was minimal (Maitre *et al.,* 1987). Egan (2003) in a review on target-controlled drug delivery confirmed that programming a TCI system with an individual's own PK does not markedly improve the performance of the TCI. Currently, studies are being carried out into 'in-line' monitoring devices, which would allow real time measurement of blood PPF concentrations (Hornuss *et al.,* 2007; Takita *et al.,* 2007). This would allow the anaesthetist to

know the exact PPF plasma concentration in the patient. However, this would not change the predictive performance of the machine, unless the system "learns" from the actual blood concentration in that specific patient, and adapts the PK accordingly. One could argue that this would not really change the quality of the anaesthesia, as the clinician would still titrate and change the target to a desired clinical effect.

Knowing the drug blood concentration is not critical. What is important is the effect site concentration. In humans, an effect site PK parameter, or ke0, has been incorporated into TCI systems to reflect the passage of the drug from the blood to the effect site (Wakeling *et al.,* 1999). In 2000, Struys and colleagues compared a TCI system targeting plasma concentration vs. a TCI system targeting the effect site concentration. They concluded that targeting the effect site compartment resulted in less variability and greater predictability in the time to loss of consciousness. They also observed that when targeting the effect site, the onset of drug effect was quicker and without adverse haemodynamic consequences. For TCI PPF in the dog, the ke0 (the plasma effect site equilibration rate constant) value for anaesthesia could be determined and incorporated into the TCI system. This would allow the anaesthetist to know if the effect site concentration has been achieved, how long it would take to reach the effect, and also how long it would take for the patient to recover. The Ke0 can be determined during an integrated PK/PD study (Minto *et al.,* 2003). Unfortunately, with this method, the Ke0 value will be specific for this set of PK parameters and will result in poor predictions of the time course of drug effect if one wanted to use it with a different set of PK parameters (Gentry *et al.,* 1994; Wakeling *et al.,* 1999). To be able to link the Ke0 from an integrated PK/PD study to PK parameters determined in another study, Minto and colleagues (2003) introduced the time of maximum effect site concentration (t_{peak}) (Minto *et al.,* 2003). Knowing the t_{peak} for a specific agent, the investigator can calculate the value of Ke0 that accurately predicts t_{peak} when using the set of parameters of interest. With simulations for thiopentone, remifentanil and PPF, Minto and colleagues showed that the Ke0 determined through the t_{peak} method better approximated the time course of drug effect than the simple transfer of a Ke0 value from one set of PK parameters to another.

Although the knowledge of the Ke0 will allow the anaesthetist better control of anaesthesia by being able to predict the time course of effect of the drug used, like any other PK parameter calculated from a population, there will still be some inter-individual variability and the anaesthetist will still use his/her clinical expertise to titrate to effect.

Better than predicting the effect site concentration, is measuring the effect, namely the depth of anaesthesia. Most of the work regarding monitoring the depth of anaesthesia in man has focussed on electroencephalographic (EEG) assessment. There are 2 approaches: BIS (Bispectral Index) and AEP (Auditory Evoked Potential) monitoring, which are being used with increased frequency in

humans (Kreuer *et al.,* 2003; Johansen, 2006; Plourde, 2006), and with some success in dogs (Pypendop *et al.,* 1999; Greene *et al.,* 2002, 2003; Carrasco-Jimenez *et al.,* 2004; Joubert, 2004; Murell *et al.,,* 2004). In humans, the combination of the TCI system for PPF and these monitors of the depth of anaesthesia results in an anaesthesia technique called 'closed loop anaesthesia' or CLAN (Kenny and Mantzaridis, 1999). With this technique, the BIS or AEP system measures the level of consciousness in the patient and titrates the drug dose to individual requirements through the TCI system (Morley et *al*., 2000; Absalom *et al.,* 2002; Struys *et al.,* 2005a; Liu *et al.,* 2006). The advantages of the CLAN technique are: continuous responsive control of anaesthesia which is considered to improve the quality of care; dose delivered corresponding to individual requirements, which decreases the problems of inter-individual PK and PD differences; removal of the risk of under- or overdosing and removal of observer bias (Milne and Kenny, 1998a; Morley *et al.,* 2000; Absalom *et al.,* 2002; Struys *et al.,* 2005a).

In parallel to the improvements in technology with regard to PK/PD modelling and anaesthesia monitoring, progress has been made in developing the 'ideal' IV agent. Several drug companies are developing new agents, but others are revisiting "old agents" and making them more TIVA friendly (Sneyd, 2004). These attempts at "revamping" are of various kinds: isolation of active enantiomers such as for MED and DEX, ketamine and s-ketamine, methadone and l-methadone; replacement of an "unsuitable" excipient e.g. cyclodextrine replacing Cremophor EL for solubilising alphaxolone (Alfaxan CD), or replacing the intralipid solution used to emulsify PPF; transforming an agent into a prodrug to make it more water soluble e.g. PPF phosphate. Detailed information regarding these developments have been reported in recent years (Fechner *et al.,* 2003; Calvo *et al.,* 2004; Sneyd, 2004; Gibiansky *et al.,* 2005; Struys *et al.*, 2005b; Morey *et al.,* 2006; Kim *et al.,* 2007).

Finally, this work shows how the use of a TCI system facilitates TIVA by allowing better control of the delivery of the anaesthetic agent. Another advantage of the system is in research where it allows the maintenance of steady plasma concentrations to study PD interactions of drugs (Smith and White 1994; Andrews *et al.,* 1997; Milne and Kenny 1999; Glen 2003). In humans, research in this area has increased with the advent of TCI (Van den Nieuwenhuyzen *et al.,* 2000, Glen 2003). Hopefully these advantages will be more widely appreciated in the veterinary setting and TCI systems will start being used not only for research, but also in practice to improve anaesthesia and critical care for our patients, as well as in cancer therapy and antimicrobial therapy to improve treatment outcomes (Glen 2003).

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APPENDICES

Appendix 1: Measured (cm) vs. Predicted (Ccalc) propofol concentration (μgml^{-1}) and PE% in a group of 10 propofol TCI anaesthetised mixed breed dogs undergoing dental surgery.

Dog 1	Time (min.)	Cm (μgml^{-1})	Ccalc (μgml^{-1})	PE%
	2.00	0.28	8.00	-96.50
	5.00	5.63	8.00	-29.63
	30.50	4.87	7.00	-30.43
	33.00	4.58	7.00	-34.57
Dog 2	5.00	16.31	11.20	45.63
	7.00	9.59	8.60	11.51
	10.00	5.44	6.50	-16.31
	30.00	7.01	5.00	40.20
	35.00	4.47	4.30	3.95
	38.00	3.47	3.50	-0.86
	43.00	2.78	2.40	15.83
	55.00	2.50	1.60	56.25
Dog 3	5.00	5.59	8.80	-36.48
	10.00	5.29	6.00	-11.83
	15.00	6.49	6.00	8.17
	23.00	3.26	4.30	-24.19
	27.00	3.76	4.00	-6.00
	30.00	2.47	3.20	-22.81
	33.00	2.24	2.70	-17.04
	38.00	0.78	1.90	-58.95
Dog 4	2.00	5.04	6.00	-16.00
	5.00	5.89	6.00	-1.83
	7.00	5.38	6.00	-10.33
	10.00	3.05	5.00	-39.00
	17.00	3.63	3.00	21.00
	20.00	2.28	3.00	-24.00
	33.00	1.10	2.60	-57.69
	36.00	1.48	2.10	-29.52
	45.00	0.82	1.20	-31.67
	47.00	0.89	1.10	-19.09

Dog 5	Time (min.)	Cm (μgml^{-1})	Ccalc (μgml^{-1})	PE%
	2.50	1.97	3.00	-34.33
	4.00	4.41	4.00	10.25
	7.00	4.76	4.00	19.00
	12.00	4.22	4.00	5.50
	22.00	4.66	4.00	16.50
	40.00	4.19	3.60	16.39
	50.00	5.15	4.90	5.10
	55.00	5.12	4.70	8.94
	62.00	1.88	3.30	-43.03
	67.00	1.50	2.40	-37.50
	77.00	0.84	1.50	-44.00
Dog 6	1.00	10.00	9.00	11.11
	3.00	5.77	1.80	220.56
	6.00	4.80	2.60	84.62
	8.00	4.70	2.80	67.86
	15.00	8.41	3.50	140.29
	18.00	6.14	3.80	61.58
	20.00	6.62	3.90	69.74
	40.00	6.06	4.00	51.50
	53.00	4.43	3.40	30.29
	56.00	2.99	2.70	10.74
	58.00	2.62	2.50	4.80
	63.00	2.29	1.90	20.53
	70.00	1.90	1.50	26.67
	71.00	1.88	1.30	44.62
Dog 7	5.00	3.88	2.50	55.20
	8.00	2.51	2.90	-13.45
	10.00	2.57	3.20	-19.69
	13.00	5.03	3.50	43.71
	16.00	4.58	3.90	17.44
	18.00	4.17	3.90	6.92
	37.00	3.67	4.00	-8.25
	51.00	2.51	3.50	-28.29
	54.00	1.58	2.60	-39.23
	56.00	1.37	2.20	-37.73
	60.00	1.26	1.70	-25.88
	65.00	1.05	1.50	-30.00
Dog 8	5.00	5.44	3.00	81.33
	8.00	5.39	3.60	49.72
	10.00	5.43	3.80	42.89
	13.00	5.42	3.90	38.97
	16.00	5.14	4.00	28.50
	18.00	4.21	4.00	5.25
	37.00	2.91	3.30	-11.82
	51.00	2.31	2.60	-11.15
	54.00	3.05	2.20	38.64
	56.00	35.47	1.60	2116.88

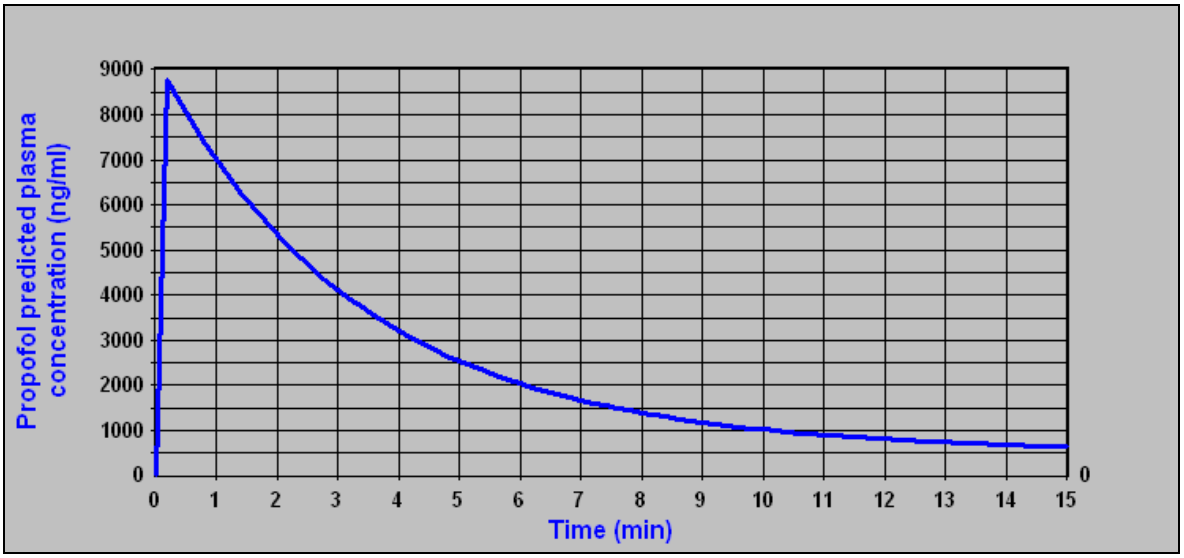
Dog 9	Time (min.)	Cm (μgml^{-1})	Ccalc (μgml^{-1})	PE%
	2.00	5.47	3.00	82.33
	5.00	5.06	3.00	68.67
	7.00	4.32	3.00	44.00
	9.00	5.41	3.00	80.33
	30.00	6.14	3.00	104.67
	35.00	1.67	3.00	-44.33
	38.00	2.21	2.50	-11.60
	40.00	2.05	2.10	-2.38
	42.00	0.44	1.90	-76.84
	44.00	1.18	1.70	-30.59
	50.00	0.47	1.20	-60.83
Dog 10	6.00	3.10	3.00	3.33
	9.00	3.26	3.50	-6.86
	12.00	3.58	4.00	-10.50
	15.00	3.82	4.00	-4.50
	18.00	4.12	4.00	3.00
	38.00	4.83	4.00	20.75
	41.00	4.78	3.80	25.79
	43.00	3.64	3.10	17.42
	46.00	3.33	2.60	28.08
	49.00	3.24	2.10	54.29
	54.00	2.64	1.70	55.29

Appendix 2: Measured (Cm) vs. Predicted (Ccalc) propofol concentration (μgml^{-1}) and PE% in a group of 6 propofol TCI anaesthetised greyhound dogs undergoing dental surgery.

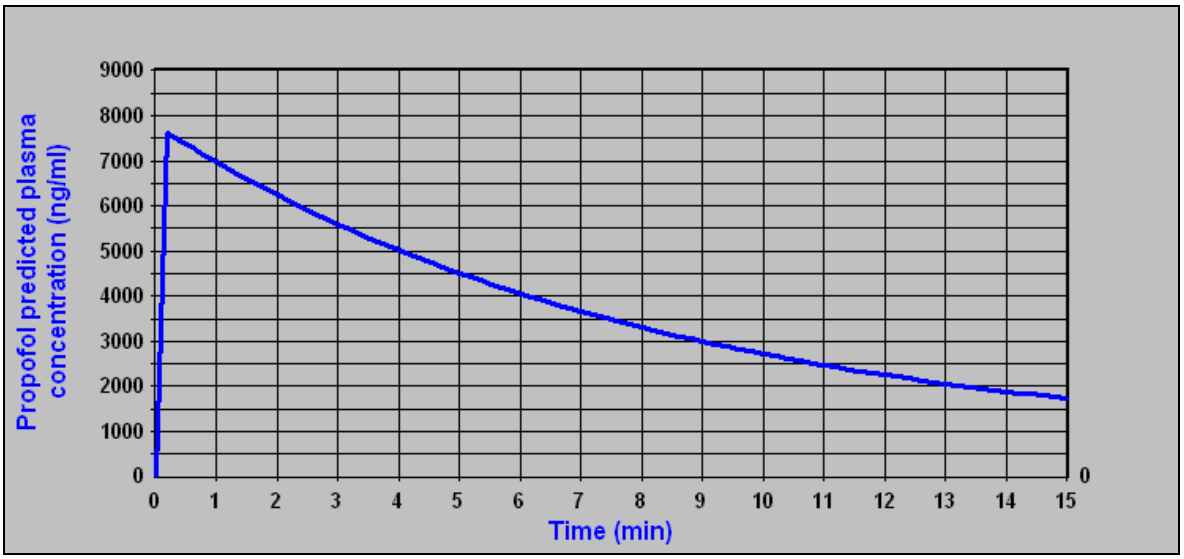
Grey 1	Time (min.)	Cm (μgml^{-1})	Ccalc (μgml^{-1})	PE%
	4.00	1.83	2.50	-26.80
	6.00	0.56	2.50	-77.60
	9.00	2.87	2.50	14.80
	11.00	1.96	3.00	-34.67
	14.00	1.63	3.00	-45.67
	17.00	3.22	3.50	-8.00
	20.00	2.88	3.50	-17.71
	22.00	2.60	3.50	-25.71
	25.00	2.71	3.50	-22.57
	35.00	2.72	3.50	-22.29
	44.00	1.53	3.50	-56.29
	49.00	1.46	2.40	-39.17
	51.00	0.36	2.00	-82.00
	52.00	1.28	1.90	-32.63
Grey 2	2.00	1.86	2.50	-25.60
	4.00	1.88	2.50	-24.80
	9.00	1.88	2.50	-24.80
	22.00	2.65	2.50	6.00
	24.00	2.95	3.00	-1.67
	26.00	1.83	3.00	-39.00
	29.00	2.49	3.00	-17.00
	31.00	1.83	3.00	-39.00
	52.00	1.88	3.00	-37.33
	70.00	2.11	3.00	-29.67
	72.00	2.47	3.00	-17.67
	75.00	1.76	2.10	-16.19
	77.00	1.78	1.90	-6.32
	81.00	1.47	1.50	-2.00
Grey 3	4.00	2.19	3.50	-37.43
	6.00	2.63	3.50	-24.86
	9.00	2.22	3.50	-36.57
	11.00	2.63	3.50	-24.86
	24.00	2.76	3.50	-21.14
	44.00	2.17	3.50	-38.00
	64.00	2.62	3.50	-25.14
	75.00	2.39	3.50	-31.71
	77.00	1.51	2.80	-46.07
	80.00	1.11	2.50	-55.60
	92.00	1.03	1.30	-20.77

Grey 4	Time (min.)	Cm (µgml^{-1})	Ccalc (µgml^{-1})	PE%
	3.00	2.13	3.00	-29.00
	6.00	7.91	3.50	126.00
	8.00	7.44	4.00	86.00
	10.00	5.48	4.00	37.00
	12.00	6.24	4.00	56.00
	15.00	6.49	4.00	62.25
	25.00	6.75	4.00	68.75
	30.00	5.74	3.50	64.00
	32.00	6.13	3.50	75.14
	37.00	5.62	3.50	60.57
	39.00	4.65	3.10	50.00
	42.00	2.23	2.70	-17.41
	44.00	3.30	2.30	43.48
	52.00	2.30	1.40	64.29
	88.00	1.43	0.50	186.00
Grey 5	7.00	3.35	3.50	-4.29
	9.00	3.6	3.50	2.86
	11.00	3.69	3.50	5.43
	16.00	2.97	3.50	-15.14
	26.00	3.08	3.50	-12.00
	36.00	3.37	3.50	-3.71
	54.00	1.13	1.30	-13.08
	57.00	1.24	1.10	12.73
	82.00	0.88	0.60	46.67
Grey 6	3.00	3.03	3.00	1.00
	5.00	3.14	3.00	4.67
	7.00	3.59	3.00	19.67
	10.00	3.93	3.00	31.00
	22.00	4.29	3.00	43.00
	29.00	4.12	3.00	37.33
	31.00	2.63	2.50	5.20
	34.00	3.21	2.50	28.40
	36.00	3.37	2.50	34.80
	38.00	2.74	2.20	24.55
	40.00	1.31	1.80	-27.22
	42.00	1.81	1.60	13.13
	50.00	0.68	1.00	32.00

Appendix 3: Predicted propofol plasma concentration after 2 $mgkg^{-1}$ IV injection bolus using human pharmacokinetic parameters (Marsh, Br J Anaesth 67:41, 1991) and the PK-SIM simulator software (PK-SIM; Specialised Data Systems, Jenkintown, PA, USA).



Appendix 4: Predicted propofol plasma concentration after 5.95 $mgkg^{-1}$ IV injection bolus using canine pharmacokinetic parameters (see chapter 2) and the PK-SIM simulator software (PK-SIM; Specialised Data Systems, Jenkintown, PA, USA).



Appendix 5: Time study: Propofol (10, 7.5, 5 and 2.5 μgml^{-1}) degradation by hepatic cytochrome P450 over time (min.) in 6 rats.

	Times (min)	0	5	10	15	30	45
Propofol							
10 μgml^{-1}	Rat 1	9.66	8.95	8.31	8.13	6.68	5.56
	Rat 2	9.75	9.08	8.59	7.77	6.27	5.07
	Rat 3	9.59	9.15	8.26	8.49	6.91	5.71
	Rat 4	9.83	9.05	8.72	8.58	7.71	6.57
	Rat 5	11.86	9.64	8.59	8.46	7.17	6.02
	Rat 6	10.37	10.10	9.23	9.27	7.59	6.53
	Mean (±SD)	10.18 (0.9)	9.33 (0.4)	8.62 (0.3)	8.45 (0.5)	7.05 (0.5)	5.91 (0.6)
7.5 μgml^{-1}	Rat 1	6.99	5.96	5.22	4.54	3.54	2.79
	Rat 2	6.87	6.27	5.24	4.59	3.55	2.58
	Rat 3	7.00	6.34	5.93	5.46	4.00	3.03
	Rat 4	7.23	6.58	6.32	5.61	4.86	3.58
	Rat 5	6.94	6.33	5.81	5.31	4.22	3.44
	Rat 6	7.12	6.55	5.88	5.64	4.22	3.16
	Mean (±SD)	7.02 (0.1)	6.34 (0.2)	5.73 (0.4)	5.19 (0.5)	4.06 (0.5)	3.10 (0.4)
5 μgml^{-1}	Rat 1	5.69	4.60	4.09	4.13	3.58	3.01
	Rat 2	5.28	4.81	4.73	4.52	3.55	3.12
	Rat 3	5.55	4.85	4.65	4.51	3.83	3.42
	Rat 4	5.19	4.72	4.61	4.33	3.83	3.48
	Rat 5	5.07	4.35	4.29	4.23	3.65	3.38
	Rat 6	5.65	5.17	4.76	4.65	3.81	3.26
	Mean (±SD)	5.40 (0.3)	4.75 (0.3)	4.52 (0.3)	4.39 (0.2)	3.71 (0.1)	3.28 (0.2)
2.5 μgml^{-1}	Rat 1	2.25	2.01	1.62	1.40	0,89	0.53
	Rat 2	2.42	2.05	1.72	1.54	1.00	0.58
	Rat 3	2.61	2.25	2.01	1.69	1.17	0.82
	Rat 4	1.96	1.84	1.74	1.52	1.11	0.78
	Rat 5	2.13	1.96	1.81	1.71	1.14	0.77
	Rat 6	2.33	2.15	1.85	1.51	0.98	0.59
	Mean (±SD)	2.28 (0.2)	2.04 (0.1)	1.79 (0.1)	1.56 (0.1)	1.05 (0.1)	0.68 (0.1)

Appendix 6: Time study: Propofol (10, 7.5, 5 and 2.5 μgml^{-1}) degradation by hepatic cytochrome P450 over time (min.) in 6 dogs.

	Times (min)	0	5	10	15	30	45
Propofol							
10 μgml^{-1}	Dog 1	10.17	9.32	8.66	8.09	6.09	4.78
	Dog 2	10.49	9.54	9.28	8.80	7.16	6.72
	Dog 3	10.52	9.84	9.24	8.75	7.94	7.14
	Dog 4	9.47	8.29	8.15	7.67	7.12	6.47
	Dog 5	10.84	9.69	8.10	7.97	6.85	5.60
	Dog 6	11.23	8.98	8.68	7.99	6.52	4.42
	Mean (±SD)	10.45 (0.6)	9.28 (0.6)	8.69 (0.5)	8.21 (0.5)	6.95 (0.6)	5.85 (1.1)
7.5 μgml^{-1}	Dog 1	7.57	6.18	5.02	4.53	2.60	1.52
	Dog 2	7.09	6.50	5.93	5.34	3.67	2.67
	Dog 3	7.23	6.23	5.71	5.53	4.51	3.37
	Dog 4	7.19	6.80	6.44	6.28	5.71	4.64
	Dog 5	6.91	6.14	5.67	4.54	3.18	2.08
	Dog 6	6.72	5.91	5.26	4.51	2.95	2.20
	Mean (±SD)	7.12 (0.3)	6.29 (0.3)	5.67 (0.5)	5.12 (0.7)	3.77 (1.2)	2.75 (1.1)
5 μgml^{-1}	Dog 1	5.36	4.36	3.89	3.58	2.56	1.91
	Dog 2	6.50	5.28	4.95	4.45	3.77	3.04
	Dog 3	5.14	4.75	4.19	4.01	3.60	2.88
	Dog 4	5.35	5.09	4.74	4.46	3.76	3.25
	Dog 5	5.14	4.48	3.99	3.51	2.77	1.43
	Dog 6	4.88	4.21	3.83	3.75	2.74	2.07
	Mean (±SD)	5.40 (0.6)	4.70 (0.4)	4.26 (0.5)	3.96 (0.4)	3.20 (0.6)	2.43 (0.7)
2.5 μgml^{-1}	Dog 1	2.29	1.45	0.83	0.53	0.13	0.01
	Dog 2	2.70	1.89	1.42	1.00	0.38	0.10
	Dog 3	2.42	1.74	1.26	1.09	0.43	0.17
	Dog 4	2.5	1.81	1.49	1.21	0.65	0.34
	Dog 5	2.26	1.55	1.01	0.73	0.15	0.02
	Dog 6	2.26	1.47	1.07	0.75	0.20	0.05
	Mean (±SD)	2.41 (0.2)	1.65 (0.2)	1.18 (0.3)	0.89 (0.3)	0.32 (0.2)	0.11 (0.1)

Appendix 7: Propofol (2.5 μgml^{-1}) degradation by rat hepatic cytochrome P450 after an incubation time of 15 min.; inhibitory effect of different doses of medetomidine (MED) ($ngml^{-1}$).

MED	0	0.0007	0.00237	0.007	0.0237	0.07	0.237	0.7	2.37
Rat 3	1.31	1.38	1.38	1.46	1.60	1.65	2.07	2.21	2.20
Rat 4	1.53	1.73	1.62	1.71	2.03	2.18	2.45	2.54	2.42
Rat 5	1.20	1.37	1.60	1.41	1.41	1.44	1.94	2.17	2.26
Rat 6	1.01	1.26	1.11	1.19	1.40	1.90	2.26	2.45	2.60
Rat 7	1.56	1.61	1.53	1.67	1.98	2.21	2.40	2.48	2.50
Rat 8	1.56	1.68	1.66	1.83	2.09	2.25	2.46	2.68	2.68
Mean (±SD)	**1.36 (0.22)**	**1.51 (0.19)**	**1.48 (0.21)**	**1.55 (0.23)**	**1.75 (0.31)**	**1.94 (0.33)**	**2.26 (0.21)**	**2.42 (0.20)**	**2.47 (0.23)**

Appendix 8: Propofol (2.5 μgml^{-1}) degradation by rat hepatic cytochrome P450 after an incubation time of 15 min.; inhibitory effect of different doses of dexmedetomidine (DEX) (μgml^{-1}).

DEX	0	0.0007	0.00237	0.007	0.0237	0.07	0.237	0.7	2.37
Rat 3	1.35	1.39	1.33	1.29	1.51	1.90	2.21	2.36	2.34
Rat 4	1.42	1.53	1.42	1.52	1.75	2.05	2.33	2.57	2.66
Rat 5	1.30	1.61	1.58	1.64	1.67	1.87	2.22	2.40	2.54
Rat 6	1.00	1.14	1.03	1.19	1.26	1.54	2.49	2.40	2.59
Rat 7	1.38	1.49	1.35	1.47	1.51	1.46	2.25	2.69	2.79
Rat 8	1.52	1.48	1.56	1.58	1.58	1.65	2.31	2.46	2.59
Mean (±SD)	**1.33 (0.17)**	**1.44 (0.16)**	**1.38 (0.20)**	**1.45 (0.17)**	**1.55 (0.16)**	**1.75 (0.22)**	**2.30 (0.14)**	**2.48 (0.14)**	**2.58 (0.17)**

Appendix 9: Propofol (2.5 μgml^{-1}) degradation by rat hepatic cytochrome P450 after an incubation time of 15 min.; inhibitory effect of different doses of levomedetomidine (LEV) (μgml^{-1}).

LEV	0	0.0007	0.00237	0.007	0.0237	0.07	0.237	0.7	2.37
Rat 4	1.48	1.67	1.53	1.70	1.98	2.29	2.33	2.76	2.64
Rat 5	1.69	1.85	1.75	1.89	2.17	2.33	2.41	2.42	2.52
Rat 6	1.11	1.32	1.19	1.23	1.62	1.97	2.10	2.41	2.42
Rat 7	1.22	1.56	1.48	1.49	1.84	2.12	2.38	2.50	2.53
Rat 8	1.52	1.59	1.71	1.86	1.84	2.13	2.40	2.71	2.61
Rat 9	1.34	1.44	1.50	1.63	1.61	1.85	2.30	2.36	2.37
Mean (±SD)	**1.39 (0.20)**	**1.57 (0.18)**	**1.53 (0.21)**	**1.63 (0.24)**	**1.84 (0.20)**	**2.12 (0.18)**	**2.32 (0.12)**	**2.53 (0.18)**	**2.52 (0.14)**

Appendix 10: Propofol (2.5 μgml^{-1}) degradation by canine hepatic cytochrome P450 after an incubation time of 15 min.; inhibitory effect of different doses of medetomidine (MED) (μgml^{-1}).

MED	0	0.000236	0.0007	0.00236	0.007	0.0236	0.07	0.236	0.7
Dog 1	0.93	0.84	0.83	0.81	0.92	1.21	1.62	1.96	1.93
Dog 2	0.52	0.50	0.51	0.54	0.64	0.91	1.72	2.10	2.04
Dog 3	0.65	0.64	0.55	0.69	0.77	1.19	1.93	2.22	2.24
Dog 4	0.97	0.99	0.99	1.08	1.13	1.64	1.95	2.01	2.03
Dog 5	0.35	0.36	0.39	0.38	0.44	0.72	1.58	2.16	2.23
Dog 6	0.34	0.41	0.39	0.39	0.45	0.70	1.28	1.74	1.81
Mean (±SD)	**0.63 (0.27)**	**0.62 (0.25)**	**0.61 (0.25)**	**0.65 (0.27)**	**0.72 (0.27)**	**1.06 (0.36)**	**1.68 (0.25)**	**2.03 (0.17)**	**2.05 (0.17)**

Appendix 11: Propofol (2.5 μgml^{-1}) degradation by canine hepatic cytochrome P450 after an incubation time of 15 min.; inhibitory effect of different doses of dexmedetomidine (DEX) (μgml^{-1}).

DEX	0	0.000236	0.0007	0.00236	0.007	0.0.236	0.07	0.236	0.7	2.36
Dog 1	0.23	0.26	0.24	0.29	0.25	0.45	0.81	1.91	2.25	2.31
Dog 2	0.58	0.58	0.57	0.56	0.68	0.86	1.33	1.74	1.82	1.87
Dog 3	0.26	0.31	0.25	0.28	0.32	0.48	0.91	1;81	1.86	2.03
Dog 4	0.21	0.19	0.19	0.19	0.22	0.36	0.74	1.63	1.85	1.91
Dog 5	0.15	0.12	0.13	0.15	0.15	0.29	0.66	1.79	2.06	2.25
Dog 6	0.29	0.30	0.28	0.31	0.34	0.48	0.9	2.04	2.16	2.25
Mean (±SD)	**0.28 (0.15)**	**0.29 (0.16)**	**0.28 (0.15)**	**0.30 (0.14)**	**0.33 (0.19)**	**0.49 (0.20)**	**0.89 (0.24)**	**1.82 (0.14)**	**2.00 (0.18)**	**2.10 (0.19)**

Appendix 12: Propofol (2.5 μgml^{-1}) degradation by canine hepatic cytochrome P450 after an incubation time of 15 min.; inhibitory effect of different doses of levomedetomidine (LEV) (μgml^{-1}).

LEV	0	0.000236	0.0007	0.00236	0.007	0.0236	0.07	0.236	0.7	2.36
Dog 1	0.22	0.24	0.27	0.25	0.27	0.59	1.87	2.14	2.24	2.13
Dog 4	0.81	0.77	0.86	0.91	1.12	1.91	2.07	2.12	2.18	2.17
Dog 5	0.27	0.30	0.30	0.35	0.44	1.04	2.04	2.29	2.16	2.15
Dog 6	0.27	0.22	0.26	0.30	0.43	0.97	2.00	1.92	2.08	2.08
Dog 7	0.17	0.19	0.20	0.25	0.30	0.55	1.77	2.16	2.10	2.02
Dog 8	0.32	0.32	0.38	0.41	0.56	0.89	2.08	2.15	2.21	2.28
Mean (±SD)	**0.34 (0.23)**	**0.34 (0.22)**	**0.38 (0.24)**	**0.41 (0.25)**	**0.52 (0.31)**	**0.99 (0.49)**	**1.97 (0.13)**	**2.13 (0.12)**	**2.16 (0.06**	**2.14 (0.09**

Appendix 13: Age and weight in 8 groups (1 to 8) of 10 TCI propofol anaesthetised dogs given saline (Control Group) or MED (0.01, 0.03, 0.1, 0.3, 1, 3 and 10 μgkg^{-1}, respectively).

Control group

Dog	Age (months)	Weight (kg)	Gender	Breed
1	9	33	NM	Chow Chow
2	26	25	NF	Boxer
3	48	39.5	NM	Labrador
4	81	44	NM	Rottweiler
5	37	18	NF	Cocker Spaniel
6	9	31	NM	Retriever cross
7	56	17	NF	Jack Russell Terrier
8	72	32	NF	Huntaway Collie
9	82	45	M	Flat Coated Retriever
10	18	41	M	Bernese Mountain Dog

Group 0.01 μgkg^{-1}

Dog	Age (months)	Weight (kg)	Gender	Breed
1	9	29	M	Golden Retriever
2	45	33	F	Weimaraner
3	72	39	M	Labrador Retriever
4	8	10.75	F	Corgi
5	72	29	M	Old English Sheep Dog
6	60	27.5	FN	German Shorthaired Pointer
7	8	14	M	Lakeland Terrier
8	10	29	M	Golden Retriever
9	7	27.5	NM	Chow Chow
10	7	30	M	Golden Retriever

Group 0.03 μgkg^{-1}

Dog	Age (months)	Weight (kg)	Gender	Breed
1	10	34	F	Rottweiler
2	9	23	F	Golden Retriever
3	22	36	NF	Labrador Retriever
4	36	21.5	MN	Spaniel cross
5	8	16	FN	Labrador Retriever
6	72	27	F	Weimaraner
7	9	33	NM	Labrador Retriever
8	60	21	M	Bearded Collie
9	35	32.5	M	Greyhound
10	61	16	M	Welsh Springer Spaniel

Group 0.1μgkg^{-1}

Dog	Age (months)	Weight (kg)	Gender	Breed
1	6	25	F	Labrador Retriever
2	12	29	M	Labrador Retriever
3	96	30	NM	Lurcher cross
4	11	24	F	German Shepherd
5	71	12	M	Cavalier King Charles Spaniel
6	67	21	M	Setter cross
7	40	26	NF	Labrador Retriever
8	96	11	NM	West Highland White Terrier
9	17	29	F	Labrador Retriever
10	84	34	M	Rough Collie

Group 0.3 μgkg^{-1}

Dog	Age (months)	Weight (kg)	Gender	Breed
1	10	35	M	Rottweiler
2	9	28	M	German Shepherd
3	13	39	M	Rottweiler
4	51	35	NM	Boxer
5	54	27	NF	Rough Collie
6	60	40	M	Doberman
7	73	28	F	Weimaraner
8	99	39	NM	German Shepherd
9	23	29	NF	Labrador Retriever
10	7	9	F	Dachshund

Group 1 μgkg^{-1}

Dog	Age (months)	Weight (kg)	Gender	Breed
1	96	37	M	Labrador Retriever
2	70	7.5	NF	Tibetan Spaniel
3	61	38	F	Bernese Mountain Dog
4	84	15.5	NF	Spaniel cross
5	60	7.5	NF	Tibetan Spaniel
6	12	25	F	Dalmatian
7	86	30	NM	Retriever cross
8	26	39	NM	Labrador Retriever
9	60	22	M	Staffordshire Bull Terrier
10	60	31	NF	Golden Retriever

Group 3 μgkg^{-1}

Dog	Age (months)	Weight (kg)	Gender	Breed
1	88	35	M	Rough Collie
2	14	28	M	Labrador Retriever
3	62	38.5	M	Weimaraner
4	16	33.5	NF	Labrador Retriever
5	44	29	NF	Poodle
6	12	18.5	NM	Cavalier King Charles Spaniel
7	96	7	M	Miniature Longhaired Dachshund
8	61	32	M	Weimaraner
9	61	28	NF	Boxer
10	72	17	M	Cavalier King Charles Spaniel

Group 10 μgkg^{-1}

Dog	Age (months)	Weight (kg)	Gender	Breed
1	69	26	NM	Irish Setter
2	7	19	M	Border Collie
3	14	18	F	Sharpei
4	36	27	M	Labrador Retriever
5	95	19	M	Border Collie
6	78	22	F	Rough Collie
7	49	44	NF	Bullmastiff
8	103	35	NM	Cross Breed
9	28	17.5	NF	Border Collie
10	10	31	NF	Labrador Retriever

Appendix 14: Heart rate (beats per min.) in 10 propofol TCI anesthetised dogs from 10 min. before up to 20 min. after the injection of either saline (control group) or medetomidine (groups 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 $\mu g ml^{-1}$).

Control group										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	125	96	96	80	140	60	100	129	71	63
-5	128	92	84	80	132	64	104	116	76	70
0	115	92	84	80	130	60	104	120	80	70
2	116	96	80	74	136	60	104	124	76	64
5	112	92	80	72	132	60	108	120	84	64
10	112	88	84	72	135	56	104	112	80	63
15	96	84	76	75	128	64	104	116	76	66
20	120	84	80	72	125	70	100	108	76	63
Group 0.01 $\mu g ml^{-1}$										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	68	80	122	82	81	76	136	78	119	88
-5	70	72	120	75	81	75	138	72	119	84
0	68	68	113	72	80	71	138	79	120	92
2	72	65	116	77	75	71	136	77	122	96
5	68	64	117	75	79	75	138	84	111	84
10	72	64	105	77	79	78	135	76	113	92
15	72	60	104	75	81	84	127	76	108	88
20	70	66	107	75	83	78	138	78	120	88
Group 0.03 $\mu g ml^{-1}$										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	84	78	88	112	98	121	92	65	60	85
-5	82	76	76	96	98	125	89	61	54	84
0	80	77	82	96	95	119	89	58	54	84
2	64	74	68	100	92	113	78	54	54	80
5	75	76	70	96	99	117	75	54	54	80
10	80	80	72	96	99	113	88	60	54	80
15	80	72	68	100	97	114	86	60	56	84
20	72	73	72	100	96	115	87	60	54	78
Group 0.1 $\mu g ml^{-1}$										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	86	93	88	68	157	51	75	118	83	160
-5	90	93	84	69	146	62	73	122	77	140
0	84	91	89	65	141	58	79	107	75	140
2	60	75	84	58	111	60	55	79	49	104
5	72	80	90	68	120	57	60	91	64	104
10	76	90	84	70	124	64	62	91	68	104
15	84	90	80	77	125	59	64	85	72	100
20	76	91	84	69	127	59	64	85	72	104

Group 0.3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	79	88	96	117	60	84	68	115	60	117
-5	78	85	100	120	56	92	71	111	63	115
0	68	82	92	133	60	97	74	107	60	111
2	51	68	80	80	45	92	41	76	48	95
5	69	85	92	98	47	96	43	83	49	92
10	66	84	92	106	53	94	48	92	55	92
15	72	90	92	112	55	100	48	88	54	92
20	64	93	100	111	55	100	50	89	57	92
Group 1 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	82	72	72	73	72	84	112	80	92	103
-5	84	80	68	72	72	81	96	80	78	113
0	87	72	63	73	80	98	96	84	84	113
2	66	44	44	51	44	73	72	52	60	60
5	75	68	60	57	64	77	76	64	68	75
10	78	68	62	62	68	76	80	76	68	84
15	86	80	59	65	64	66	80	68	64	84
20	91	80	60	68	60	68	84	76	68	88
Group 3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	92	86	120	72	130	104	72	108	72	108
-5	89	100	120	78	120	88	68	116	64	108
0	89	92	120	78	124	84	68	112	64	100
2	40	50	48	52	72	60	40	72	42	56
5	70	60	100	52	84	68	52	72	48	56
10	76	60	92	58	84	80	60	76	56	80
15	88	68	92	60	84	100	64	80	62	88
20	72	72	92	52	84	88	60	88	92	65
Group 10 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog 8	dog 9	dog10
-10	104	72	84	60	80	78	104	80	106	100
-5	92	72	80	54	88	80	93	88	100	93
0	96	72	80	60	84	76	96	95	92	96
2	80	40	40	58	38	52	52	60	36	36
5	80	56	90	44	48	76	72	120	92	52
10	80	72	84	52	52	80	92	100	72	64
15	84	71	90	48	60	80	80	100	60	68
20	87	64	88	52	52	76	81	100	64	84

Appendix 15: Systolic arterial blood pressure (mmHg) in 10 propofol TCI anesthetised dogs from 10 min. before up to 20 min. after the injection of either saline (control group) or medetomidine (groups 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 $\mu g ml^{-1}$).

Control group										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	93	126	103	98	98	102	104	89	121	109
-5	94	122	100	101	94	98	113	80	117	111
0	96	126	99	104	100	104	116	83	100	103
2	93	124	96	109	96	102	110	94	114	116
5	97	122	100	105	92	96	113	85	111	107
10	102	128	104	97	103	99	109	89	107	106
15	99	123	96	96	87	110	108	83	113	112
20	102	122	98	100	90	122	110	79	103	108
Group 0.01 $\mu g ml^{-1}$										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	98	103	131	110	110	115	158	105	117	91
-5	100	92	132	107	115	111	147	104	112	97
0	99	102	132	103	118	121	144	108	119	96
2	101	97	136	107	120	114	141	107	121	97
5	106	100	137	116	124	110	141	107	118	97
10	104	100	128	115	141	108	138	116	121	95
15	106	84	137	115	130	118	133	105	119	96
20	103	97	137	115	140	112	146	117	111	101
Group 0.03 $\mu g ml^{-1}$										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	106	103	96	127	142	151	103	111	112	97
-5	104	118	96	127	146	148	99	109	114	100
0	100	118	92	122	146	155	108	120	106	98
2	108	117	100	119	158	159	113	118	113	102
5	98	113	89	124	160	162	110	111	105	101
10	98	117	92	129	163	165	112	111	103	105
15	95	109	94	121	160	159	108	78	107	96
20	95	116	88	127	169	166	109	95	105	103
Group 0.1 $\mu g ml^{-1}$										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	110	105	97	91	103	98	112	114	103	121
-5	109	97	99	92	99	103	117	120	90	116
0	106	99	100	90	104	105	114	116	89	119
2	109	103	106	94	108	104	116	110	nr	119
5	109	101	103	90	99	94	114	110	101	119
10	109	99	108	94	98	97	110	108	90	119
15	109	103	105	94	104	80	113	116	90	114
20	109	106	109	93	97	100	105	108	87	118

Group 0.3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	109	89	105	112	123	94	140	116	87	100
-5	103	104	92	113	126	91	142	112	90	117
0	95	100	96	116	124	99	140	121	83	110
2	113	122	113	127	131	101	132	127	95	116
5	111	115	109	116	137	100	134	141	90	97
10	109	101	101	117	130	100	133	129	83	90
15	109	104	101	118	122	101	151	123	84	104
20	102	98	109	113	127	108	143	125	81	103
Group 1 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	96	111	118	94	160	92	103	138	110	105
-5	95	107	112	89	150	87	97	132	106	113
0	96	104	112	88	158	83	96	132	108	107
2	113	120	128	112	150	112	135	148	120	118
5	106	122	118	116	150	108	118	132	112	111
10	100	114	112	101	142	102	110	136	108	110
15	99	109	112	90	134	98	93	130	100	103
20	92	103	110	94	138	97	100	130	106	109
Group 3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	90	88	112	132	106	131	118	138	122	100
-5	92	86	116	128	108	131	108	148	118	98
0	90	86	118	128	108	128	118	146	122	99
2	134	106	180	178	144	148	148	198	121	nr
5	135	104	178	168	162	151	146	178	133	135
10	130	98	158	154	142	156	134	170	132	130
15	132	90	152	153	142	158	128	164	137	119
20	126	88	156	150	140	156	124	158	127	119
Group 10 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	106	103	96	127	142	151	103	111	112	97
-5	104	118	96	127	146	148	99	109	114	100
0	100	118	92	122	146	155	108	120	106	98
2	108	117	100	119	158	159	113	118	113	102
5	98	113	89	124	160	162	110	111	105	101
10	98	117	92	129	163	165	112	111	103	105
15	95	109	94	121	160	159	108	78	107	96
20	95	116	88	127	169	166	109	95	105	103

nr = not recorded

Appendix 16: Respiratory rate (breaths per min.) in 10 propofol TCI anesthetised dogs from 10 min. before up to 20 min. after the injection of either saline (control group) or medetomidine (groups 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 μgml^{-1}).

Control group										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	6	4	0	10	30	20	20	10	0	6
-5	10	5	0	12	45	20	20	10	0	10
0	8	5	0	8	40	25	19	10	0	8
2	7	5	0	10	45	25	21	11	0	7
5	9	5	0	8	45	25	20	11	13	9
10	8	6	0	8	40	20	22	12	12	8
15	7	9	0	8	39	23	20	15	12	7
20	6	10	0	12	36	22	18	13	12	6
Group 0.01 μgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	20	10	8	23	11	12	0	10	12	19
-5	20	10	8	21	5	14	0	10	15	21
0	17	12	10	18	10	14	0	15	15	20
2	17	15	8	20	11	13	0	12	15	18
5	17	12	8	26	8	5	14	20	20	20
10	17	15	8	22	10	7	15	20	12	20
15	15	15	8	23	12	14	15	20	15	21
20	70	66	107	75	83	78	138	78	120	88
Group 0.03 μgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	20	22	20	7	50	8	15	10	0	15
-5	20	13	23	3	52	11	20	12	0	20
0	20	14	30	3	48	12	20	13	0	15
2	20	16	13	3	52	11	20	11	0	12
5	20	13	22	3	50	12	20	9	0	15
10	20	15	22	6	51	12	20	11	0	15
15	20	15	22	3	49	12	20	11	0	15
20	20	13	20	6	48	13	20	11	0	15
Group 0.1 μgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	8	38	3	0	12	0	10	5	16	12
-5	15	42	0	0	23	0	10	9	20	12
0	10	38	0	0	25	0	10	8	16	10
2	10	40	0	0	25	0	10	15	12	10
5	2	37	0	0	25	0	12	11	12	10
10	20	40	6	0	27	0	15	14	15	10
15	10	41	10	0	40	0	20	14	10	10
20	20	39	5	0	56	0	15	10	8	10

Group 0.3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	12	9	8	0	0	0	12	15	2	20
-5	10	6	6	0	0	0	11	15	8	20
0	9	6	6	0	0	0	11	15	0	15
2	8	6	10	0	0	0	10	12	0	15
5	8	6	7	9	0	0	10	8	6	20
10	9	6	8	10	0	0	12	10	7	15
15	10	6	8	10	0	9	15	12	6	18
20	9	6	12	10	0	5	15	6	0	20
Group 1 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	0	0	0	16	0	7	0	20	7	60
-5	0	0	0	15	0	6	0	22	7	50
0	0	0	0	15	0	10	0	24	7	0
2	0	0	0	4	0	7	0	20	7	0
5	0	0	0	7	0	9	0	14	7	0
10	0	0	0	7	0	8	0	15	7	0
15	0	0	0	10	0	11	0	12	7	22
20	0	0	0	12	0	8	0	12	4	30
Group 3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	30	0	10	30	30	15	5	40	27	0
-5	46	0	11	34	24	18	6	33	28	0
0	58	0	12	37	26	19	7	40	29	0
2	30	0	15	36	24	23	0	15	23	0
5	15	0	10	30	19	0	7	18	20	0
10	14	0	10	0	13	0	8	25	11	0
15	15	0	9	0	8	12	11	23	13	0
20	19	0	10	20	13	21	15	20	22	0
Group 10 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	0	15	10	30	18	14	8	32	6	13
-5	0	16	12	20	20	18	8	32	8	18
0	0	18	12	30	27	12	8	34	8	19
2	0	11	3	0	12	0	0	15	0	21
5	0	6	6	0	8	0	0	14	0	0
10	0	5	0	0	5	0	0	18	0	0
15	0	0	0	0	0	0	0	19	0	0
20	0	0	0	0	0	0	0	18	0	0

Appendix 17: $EtCO_2$ (mmHg) in 10 TCI propofol anesthetised dogs from 10 min. before up to 20 min. after the injection of either saline (control group) or medetomidine (groups 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 μgml^{-1}).

Control group										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	43	33	50	46	30	36	30	45	nr	43
-5	43	32	41	43	35	38	31	43	nr	43
0	44	32	44	41	32	35	32	42	nr	40
2	42	32	39	42	36	34	31	41	nr	41
5	44	33	43	44	32	32	31	41	nr	40
10	43	33	45	44	35	35	30	42	nr	38
15	44	30	41	42	32	35	31	39	nr	38
20	45	35	41	40	32	34	32	41	nr	37
Group 0.01 μgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	41	nr	44	37	44	41	nr	49	38	39
-5	40	nr	42	35	46	40	nr	50	39	34
0	40	nr	44	34	43	45	nr	44	38	36
2	39	nr	44	34	42	47	nr	43	37	37
5	40	nr	44	32	44	44	nr	41	38	38
10	42	nr	43	32	43	47	nr	41	38	35
15	41	nr	42	32	43	51	nr	42	38	35
20	40	nr	45	32	44	44	nr	41	38	37
Group 0.03 μgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	38	38	nr	44	34	38	40	53	41	nr
-5	38	37	nr	45	36	35	37	44	41	nr
0	38	38	nr	44	37	32	38	56	41	nr
2	38	38	nr	45	35	33	38	51	42	nr
5	37	38	nr	45	36	34	38	53	42	nr
10	37	38	nr	43	35	32	38	49	45	nr
15	37	37	nr	44	32	32	38	47	45	nr
20	38	39	nr	41	33	32	38	47	40	nr
Group 0.1 μgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	52	42	41	nr	48	50	42	38	nr	nr
-5	51	38	45	nr	44	50	44	40	nr	nr
0	53	37	47	nr	45	49	43	37	nr	nr
2	53	38	45	nr	46	50	38	31	nr	nr
5	55	38	42	nr	46	50	42	35	nr	nr
10	48	34	41	nr	45	50	41	33	nr	nr
15	51	38	40	nr	44	50	39	35	nr	nr
20	47	38	43	nr	40	53	38	34	nr	nr

Group 0.3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	38	33	43	nr	nr	45	nr	nr	47	nr
-5	37	32	44	nr	nr	44	nr	nr	52	nr
0	38	34	45	nr	nr	46	nr	nr	52	nr
2	35	37	44	nr	nr	45	nr	nr	54	nr
5	35	37	46	nr	nr	43	nr	nr	44	nr
10	34	34	44	nr	nr	50	nr	nr	50	nr
15	32	32	40	nr	nr	49	nr	nr	52	nr
20	31	32	39	nr	nr	49	nr	nr	50	nr
Group 1 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	nr	30	40	34	30	41	nr	53	40	nr
-5	nr	32	32	32	26	41	nr	53	38	nr
0	nr	32	35	32	27	41	nr	47	35	nr
2	nr	34	30	11	23	45	nr	47	36	nr
5	nr	34	36	38	27	45	nr	52	35	nr
10	nr	35	39	38	30	47	nr	60	40	nr
15	nr	33	33	32	28	47	nr	58	47	nr
20	nr	28	35	32	27	46	nr	56	45	nr
Group 3 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	44	32	47	38	29	38	46	33	35	38
-5	38	29	47	35	25	37	45	33	35	41
0	38	35	41	31	26	37	47	33	36	40
2	34	27	26	26	23	23	30	24	32	36
5	47	29	39	35	22	37	45	29	35	43
10	52	37	50	38	26	42	52	36	47	44
15	51	36	52	41	32	50	53	36	51	47
20	47	33	52	49	27	44	45	35	49	39
Group 10 µgml^{-1}										
Time (min)	dog1	dog2	dog3	dog4	dog5	dog6	dog7	dog8	dog9	dog10
-10	35	38	41	38	47	37	44	49	50	38
-5	34	38	33	42	45	38	44	42	46	38
0	35	38	35	38	42	35	44	43	47	35
2	38	41	25	23	28	39	39	41	49	26
5	33	50	30	24	50	45	35	48	50	29
10	35	56	50	22	58	49	47	53	50	35
15	33	47	50	53	45	40	48	50	50	30
20	37	55	41	51	48	38	48	62	48	32

nr = not recorded

Appendix 18: Anaesthesia times (min.) before and after the start of a co-infusion of saline (group PS), medetomidine plasma target concentration of 1.7 $ngml^{-1}$ (group PM) and dexmedetomidine plasma target concentration of 0.85 (group PLD) and 1.7 $ngml^{-1}$ (group PHD) in 6 propofol TCI anaesthetised beagles.

		Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Group PS							
	Before	20	21	25	20	25	21
	After	85	77	85	73	77	120
Group PM							
	Before	30	29	27	22	27	24
	After	68	86	70	71	72	86
Group PLD							
	Before	25	23	25	20	25	22
	After	68	89	100	74	70	73
Group PHD							
	Before	24	29	24	33	27	22
	After	77	103	104	72	91	98

Appendix 19: Extubation (Ext), Sternal (Ster) and standing (Stan) times (min.) in 4 groups (PS, PM, PLD and PHD) of 6 propofol TCI anaesthetised beagles.

		Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Group PS							
	Ext	5	20	3	7	2	4
	Ster	5	21	11	32	6	6
	Stan	20	34	16	40	14	11
Group PM							
	Ext	13	15	33	32	19	9
	Ster	17	20	34	32	20	14
	Stan	35	35	38	42	33	19
Group PLD							
	Ext	15	19	9	15	10	7
	Ster	15	19	12	18	18	8
	Stan	30	27	16	29	25	13
Group PHD							
	Ext	5	31	19	12	20	21
	Ster	18	37	32	12	21	21
	Stan	34	45	36	30	37	34

Appendix 20: Amount of propofol (mg) used before and after the start of a co-infusion of saline (PS group), medetomidine plasma target concentration of 1.7 $ngml^{-1}$ (group PM) and dexmedetomidine plasma target concentration of 0.85 (group PLD) and 1.7 $ngml^{-1}$ (group PHD) in 6 propofol TCI anaesthetised beagles.

		Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Group PS							
	Before	120	145	110.7	115	170	157
	After	479	429	305.3	378	422	766
Group PM							
	Before	203	167.5	128	126.4	147	155
	After	200	163.5	136	194.6	199	247
Group PLD							
	Before	160	124.4	142.4	120	124	130.7
	After	230	248.6	199.6	186	158	186.3
Group PHD							
	Before	127	181	194	207.7	157	165
	After	264	220	91	143.3	203	232

Appendix 21: Cardiac output (CO) ($l.min^{-1}$) before the start and after the change of the last step of a co-infusion of saline (group PS), medetomidine plasma target concentration of 1.7 $ngml^{-1}$ (group PM) and dexmedetomidine plasma target concentration of 0.85 (group PLD) and 1.7 $ngml^{-1}$ (group PHD) in 6 propofol TCI anaesthetised beagles.

		Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Group PS							
	Before	2.48	2.33	2.7	2.14	2.68	3.44
	After	3.38	2.79	2.93	2.21	3.06	3.25
Group PM							
	Before	3.1	nr	2.45	2.39	2.33	3.17
	After	1.65	nr	1.56	1.66	1.75	1.95
Group PLD							
	Before	2.49	2.19	2.74	2.09	2.43	2.83
	After	1.74	1.94	1.5	1.38	1.6	1.7
Group PHD							
	Before	2.52	2.24	3.09	2.68	2.49	3.02
	After	1.67	1.49	1.26	1.35	1.6	1.74

nr = not recorded

Appendix 22: Heart rate (beats per min.), breathing rate (breaths per min.), systolic and mean arterial blood pressure (mmHg), $EtCO_2$ (mmHg) and propofol target concentration (μgml^{-1}) in 6 TCI propofol anaesthetised beagles with a co-infusion of saline (group PS) recorded at the time of the start of the co-infusion (time 0), at times 5, 15, 30 and 45 min., and during the MIT determination (Stim + and Stim -).

Dog 1						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	120	38	136	84	17	3
5	121	35	138	82	18	3
15	115	32	132	82	16	3.3
30	135	40	127	80	15	3.5
45	114	30	150	121	29	5.7
Stim +	105	38	176	153	30	4.9
Stim -	176	74	174	157	33	5.3
Dog 2						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	112	22	101	69	44	4
5	105	15	99	69	35	4
15	109	16	100	69	37	4.5
30	109	25	99	70	38	4.5
45	108	30	97	68	42	5.5
Stim +	116	31	102	73	38	5.1
Stim -	112	25	108	74	38	5.7
Dog 3						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	80	38	97	66	35	4
5	81	35	96	65	36	4
15	77	30	97	67	38	4.5
30	75	30	103	69	35	4.5
45	73	30	108	70	37	4.5
Stim +	79	35	109	70	37	4.5
Stim -	88	35	115	86	34	5.7
Dog 4						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	92	25	116	71	40	4
5	100	30	108	66	42	5
15	90	18	104	63	44	5
30	88	20	104	62	47	5.5
45	83	20	106	62	46	5.5
Stim +	83	20	107	62	47	5.5
Stim -	80	25	119	70	38	6.3

Dog 5						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	122	13	137	81	41	4.5
5	127	9	139	80	39	5
15	117	15	131	74	44	5
30	112	16	125	70	44	5.3
45	112	16	122	74	41	6
Stim +	100	21	133	85	38	5.2
Stim -	116	60	138	91	43	5.4
Dog 6						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	101	11	110	78	36	4
5	104	12	110	77	36	4
15	102	16	107	72	38	4.3
30	96	13	108	72	37	4.3
45	97	16	113	75	34	4.3
Stim +	97	17	113	75	38	4.3
Stim -	90	20	150	101	38	7.5

Appendix 23: Heart rate (beats per min.), breathing rate (breaths per min.), systolic and mean arterial blood pressure (mmHg), $EtCO_2$ (mmHg) and propofol target concentration (μgml^{-1}) in 6 TCI propofol anaesthetised beagles with a co-infusion of medetomidine target plasma concentration of 1.7 $ngml^{-1}$ (group PM) recorded at the time of the start of the co-infusion (time 0), at times 5, 15, 30 and 45 min., and during the MIT determination (Stim + and Stim -).

Dog 1						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	113	18	116	77	37	4
5	60	20	157	102	32	3.5
15	67	16	162	107	32	3.3
30	68	20	165	111	32	3
45	65	17	165	110	37	3
Stim +	66	28	164	108	32	2.6
Stim -	62	27	166	109	31	2.8
Dog 2						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	103	19	126	78	42	3.5
5	51	15	150	103	35	3
15	46	12	147	102	30	3
30	47	14	150	107	27	2.4
45	46	13	148	102	29	2.2
Stim +	43	7	141	94	37	1.4
Stim -	45	9	141	96	37	1.8
Dog 3						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	90	35	107	72	38	4
5	49	30	148	98	38	3.8
15	45	29	155	107	37	3.5
30	52	28	154	106	37	3.1
45	51	27	147	101	37	3
Stim +	48	25	146	99	36	2.6
Stim -	46	24	145	98	36	2.8
Dog 4						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	91	41	137	78	31	4
5	65	24	161	98	30	4
15	65	24	163	103	32	3.5
30	61	20	162	101	30	3
45	62	29	162	100	37	3
Stim +	65	25	163	99	37	3
Stim -	65	33	162	102	35	3.6

Dog 5						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	120	18	123	75	38	3.5
5	52	16	138	89	36	3.5
15	55	23	150	92	37	3.3
30	55	15	143	93	38	3
45	58	15	141	89	39	3
Stim +	56	20	139	88	36	2.6
Stim -	55	27	142	90	37	2.8
Dog 6						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	113	18	123	84	34	3.5
5	65	15	137	95	32	3.3
15	65	12	134	96	35	3.3
30	62	18	134	96	30	3
45	59	12	132	93	33	3
Stim +	54	20	130	92	32	2.2
Stim -	52	16	132	89	31	2.4

Appendix 24: Heart rate (beats per min.), breathing rate (breaths per min.), systolic and mean arterial blood pressure (mmHg), $EtCO_2$ (mmHg) and propofol target concentration (μgml^{-1}) in 6 TCI propofol anaesthetised beagles with a co-infusion of dexmedetomidine target plasma concentration of 0.85 $ngml^{-1}$ (group PLD) recorded at the time of the start of the co-infusion (time 0), at times 5, 15, 30 and 45 min., and during the MIT determination (Stim + and Stim -).

Dog 1						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	119	18	147	82	26	3.5
5	76	19	160	94	22	3.5
15	76	21	162	102	31	3.5
30	75	20	162	101	31	3.3
45	77	23	159	98	30	3.3
Stim +	74	20	158	99	29	2.9
Stim -	75	30	158	100	30	3.1
Dog 2						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	108	25	106	68	36	3
5	64	15	132	89	36	3
15	66	14	142	93	27	3
30	61	15	141	94	24	3
45	61	12	138	93	29	3
Stim +	60	11	139	90	32	2.2
Stim -	55	12	148	96	32	2.8
Dog 3						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	89	47	114	89	36	5
5	51	34	137	91	32	4.5
15	41	28	150	99	31	4.2
30	47	17	149	100	11	4
45	45	21	149	98	37	3.8
Stim +	37	24	150	97	17	2.2
Stim -	38	20	159	108	36	2.4
Dog 4						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	80	20	127	78	41	4
5	54	21	146	94	41	4
15	51	20	155	105	40	4
30	54	27	156	105	41	3.5
45	51	23	155	101	40	3
Stim +	51	25	157	102	38	2.6
Stim -	51	30	159	107	27	2.8

Dog 5						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	92	29	122	70	33	3
5	49	21	133	83	32	3
15	54	24	136	86	34	2.7
30	51	26	136	87	37	2.5
45	53	27	136	87	37	2.5
Stim +	50	32	138	88	32	2.1
Stim -	59	35	138	88	32	2.3
Dog 6						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	104	20	140	88	21	3
5	63	13	138	81	28	3
15	60	13	134	80	32	2.5
30	57	7	129	87	35	2.2
45	56	11	127	88	32	2.2
Stim +	60	6	133	97	40	1.8
Stim -	55	18	130	93	31	2.6

Appendix 25: Heart rate (beats per min.), breathing rate (breaths per min.), systolic and mean arterial blood pressure (mmHg), $EtCO_2$ (mmHg) and propofol target concentration (μgml^{-1}) in 6 TCI propofol anaesthetised beagles with a co-infusion of dexmedetomidine target plasma concentration of 1.7 $ngml^{-1}$ (PHD) recorded at the time of the start of the co-infusion (time 0), at times 5, 15, 30 and 45 min., and during the MIT determination (Stim + and Stim -).

Dog 1						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	126	32	127	80	33	4
5	80	16	163	110	36	3.8
15	78	13	164	111	39	3.8
30	78	20	166	112	40	3.2
45	77	25	163	112	35	3
Stim +	73	20	167	109	35	2.2
Stim -	70	20	168	111	35	2.4
Dog 2						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	97	26	98	67	24	4
5	54	15	156	109	29	3.5
15	57	18	157	113	29	3.3
30	57	20	161	115	37	3
45	58	21	158	114	36	3
Stim +	53	15	150	102	34	1.4
Stim -	58	13	151	103	35	1.8
Dog 3						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	84	40	142	90	37	4.5
5	44	35	173	113	35	4.3
15	39	22	173	118	37	3.7
30	44	20	173	120	32	3.3
45	38	25	166	110	36	3
Stim +	40	32	172	105	32	1.4
Stim -	45	30	178	110	33	1.8
Dog 4						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	91	40	130	82	35	5.5
5	55	30	162	107	41	5.5
15	57	29	163	111	42	4
30	54	18	164	109	25	3
45	48	20	158	101	30	2.2
Stim +	55	26	164	104	39	2.2
Stim -	51	32	169	113	35	2.8

Dog 5						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	111	22	117	74	41	3.5
5	44	17	136	93	38	3.5
15	59	14	144	100	44	3.3
30	59	17	145	101	41	3
45	62	19	143	99	45	3
Stim +	52	25	148	97	38	1.8
Stim -	55	20	151	99	41	2.2
Dog 6						
Time	**HR**	**RR**	**SP**	**MP**	**EtCO2**	**Target**
0	116	17	122	82	34	4
5	63	13	129	94	34	3.7
15	68	12	130	97	36	3.5
30	65	14	128	96	36	3.3
45	68	13	130	95	40	3
Stim +	54	15	128	90	37	1.4
Stim -	52	11	129	91	43	1.6

Appendix 26: Measured (cm) vs. Predicted (Ccalc) propofol concentration (µgml^{-1}) and PE% values in 6 TCI propofol anaesthetised beagles with a co-infusion of saline (group PS), at times 0 (start of the co-infusion), 10, 20 and 45 min. and at the time of MIT determination (Stim+ and Stim-).

Dog 1	Time (min.)	Cm (µgml^{-1})	Ccalc (µgml^{-1})	PE%
	0.00	4.27	3.00	42.33
	10.00	3.41	3.00	13.67
	20.00	4.07	3.30	23.33
	45.00	7.35	5.70	28.95
	Stim+	5.34	4.90	8.98
	Stim-	5.42	5.30	2.26
Dog 2				
	0.00	7.99	3.50	128.29
	10.00	5.46	4.50	21.33
	20.00	6.29	4.50	39.78
	45.00	6.46	5.50	17.45
	Stim+	5.68	5.10	11.37
	Stim-	6.56	5.70	15.09
Dog 3				
	0.00	3.70	4.00	-7.50
	10.00	3.79	4.00	-5.25
	20.00	4.64	4.50	3.11
	45.00	4.25	4.50	-5.56
	Stim+	3.68	4.50	-18.22
	Stim-	4.90	5.70	-14.04
Dog 4				
	0.00	4.08	**5.00**	-18.40
	10.00	6.13	**5.00**	22.60
	20.00	5.89	**5.00**	17.80
	45.00	6.28	**5.50**	14.18
	Stim+	6.08	**5.50**	10.55
	Stim-	6.25	**6.30**	-0.79
Dog 5				
	0.00	7.35	4.50	63.33
	10.00	8.22	5.00	64.40
	20.00	9.13	5.00	82.60
	45.00	10.28	6.00	71.33
	Stim+	8.35	5.20	60.58
	Stim-	8.27	5.40	53.15
Dog 6				
	0.00	4.89	4.00	22.25
	10.00	5.41	4.00	35.25
	20.00	5.74	4.30	33.49
	45.00	5.40	4.30	25.58
	Stim+	5.48	4.30	27.44
	Stim-	8.05	7.50	7.33

Appendix 27: Measured (cm) vs. Predicted (Ccalc) propofol concentration (µgml^{-1}) and PE% values in 6 TCI propofol anaesthetised beagles with a co-infusion of medetomidine target plasma concentration of 1.7 ngml^{-1} (PM group), at time 0 (start of the co-infusion), 10, 20 and 45 min. and at the time of MIT determination (Stim+ and Stim-).

Dog 1	Time (min.)	Cm (µgml^{-1})	Ccalc (µgml^{-1})	PE%
	0.00	5.82	4.00	45.50
	10.00	5.50	3.50	57.14
	20.00	4.89	3.30	48.18
	45.00	5.14	3.00	71.33
	Stim+	4.29	2.60	65.00
	Stim-	4.95	2.80	76.79
Dog 2				
	0.00	6.17	3.50	76.21
	10.00	5.57	3.00	85.70
	20.00	0.78	2.60	-70.04
	45.00	3.35	2.20	52.38
	Stim+	2.73	1.40	95.21
	Stim-	3.11	1.80	72.55
Dog 3				
	0.00	3.35	4.00	-16.25
	10.00	3.48	3.80	-8.42
	20.00	3.21	3.50	-8.29
	45.00	3.25	3.00	8.33
	Stim+	2.94	2.60	13.08
	Stim-	4.81	2.80	71.79
Dog 4				
	0.00	4.68	4.00	17.00
	10.00	5.18	4.00	29.50
	20.00	4.64	3.50	32.57
	45.00	3.89	3.00	29.67
	Stim+	3.89	3.00	29.67
	Stim-	4.76	3.60	32.22
Dog 5				
	0.00	8.16	3.5	133.14
	10.00	8.35	3.5	138.57
	20.00	7.01	3.3	112.42
	45.00	6.92	3	130.67
	Stim+	5.82	2.60	123.85
	Stim-	6.02	2.80	115.00
Dog 6				
	0.00	4.72	3.5	34.86
	10.00	5.12	3.3	55.15
	20.00	5.04	3.3	52.73
	45.00	5.19	3	73.00
	Stim+	3.43	2.20	55.91
	Stim-	4.09	2.40	70.42

Appendix 28: Measured (cm) vs. Predicted (Ccalc) propofol concentration (µgml^{-1}) and PE% values in 6 TCI propofol anaesthetised beagles with a co-infusion of dexmedetomidine target plasma concentration of 0.85 ngml^{-1} (group PLD), at time 0 (start of the co-infusion), 10, 20 and 45 min. and at the time of MIT determination (Stim+ and Stim-).

Dog 1	Time (min.)	Cm (µgml^{-1})	Ccalc (µgml^{-1})	PE%
	0.00	3.69	3.50	5.43
	10.00	3.86	3.50	10.29
	20.00	4.25	3.50	21.43
	45.00	4.15	3.30	25.76
	Stim+	3.79	2.90	30.69
	Stim-	4.47	3.10	44.19
Dog 2		5.74		
	0.00	4.84	3.00	91.33
	10.00	5.11	3.00	61.33
	20.00	4.51	3.00	70.33
	45.00	3.12	3.00	50.33
	Stim+	4.09	2.20	41.82
	Stim-	5.74	2.80	46.07
Dog 3				
	0.00	4.59	5.00	-8.20
	10.00	4.35	4.50	-3.33
	20.00	4.26	4.20	1.43
	45.00	4.37	3.80	15.00
	Stim+	2.67	2.20	21.36
	Stim-	3.06	2.40	27.50
Dog 4				
	0.00	5.45	4.00	36.25
	10.00	2.48	4.00	-38.00
	20.00	4.09	4.00	2.25
	45.00	3.67	3.00	22.33
	Stim+	3.49	2.60	34.23
	Stim-	4.21	2.80	50.36
Dog 5				
	0.00	5.32	3.00	77.33
	10.00	6.07	3.00	102.33
	20.00	5.26	2.70	94.81
	45.00	5.04	2.50	101.60
	Stim+	3.62	2.10	72.38
	Stim-	4.47	2.30	94.35
Dog 6				
	0.00	3.77	3.00	25.54
	10.00	2.25	2.50	-10.06
	20.00	3.68	2.50	47.17
	45.00	3.48	2.20	58.06
	Stim+	1.50	1.80	-16.64
	Stim-	1.17	2.60	-55.01

Appendix 29: Measured (cm) vs. Predicted (Ccalc) propofol concentration (µgml^{-1}) and PE% values in 6 TCI propofol anaesthetised beagles with a co-infusion of dexmedetomidine target plasma concentration of 1.7 ngml^{-1} (group PHD), at time 0 (start of the co-infusion), 10, 20 and 45 min. and at the time of MIT determination (Stim+ and Stim-).

Dog 1	Time (min.)	Cm (µgml^{-1})	Ccalc (µgml^{-1})	PE%
	0.00	6.37	4.00	59.25
	10.00	4.90	3.80	28.95
	20.00	5.27	3.80	38.68
	45.00	4.23	3.00	41.00
	Stim+	2.90	2.20	31.82
	Stim-	3.07	2.40	27.92
Dog 2				
	0.00	7.65	4.00	91.25
	10.00	7.17	3.50	104.86
	20.00	6.36	3.30	92.73
	45.00	5.29	3.00	76.33
	Stim+	2.90	1.40	107.14
	Stim-	3.88	1.80	115.56
Dog 3				
	0.00	4.75	4.50	5.56
	10.00	4.89	3.70	32.16
	20.00	5.16	3.70	39.46
	45.00	3.35	3.00	11.67
	Stim+	2.04	1.40	45.71
	Stim-	2.75	1.80	52.78
Dog 4				
	0.00	4.75	5.50	-13.70
	10.00	4.81	4.50	6.99
	20.00	4.57	4.00	14.17
	45.00	2.25	2.20	2.39
	Stim+	2.25	2.20	2.27
	Stim-	3.30	2.80	17.97
Dog 5				
	0.00	6.42	3.50	83.43
	10.00	7.14	3.30	116.36
	20.00	3.38	3.10	9.03
	45.00	6.12	3.00	104.00
	Stim+	3.21	1.80	78.33
	Stim-	3.90	2.20	77.27
Dog 6				
	0.00	8.29	4.00	107.25
	10.00	8.09	3.70	118.65
	20.00	7.90	3.50	125.71
	45.00	6.33	3.00	111.00
	Stim+	2.85	1.40	103.57
	Stim-	3.27	1.60	104.38

Appendix 30: Measured (cm) vs. Predicted (Ccalc) dexmedetomidine target plasma concentration of 0.85 $ngml^{-1}$ (group PLD) in 6 TCI propofol anaesthetised beagles at times 0 (start of the co-infusion), 5, 10, 15, 30 and 45 min. and at the time of MIT determination (Stim+ and Stim-).

		Cm ($ngml^{-1}$)					
Time (min.)	**Ccalc. ($ngml^{-1}$)**	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
5	0.80	0.38	0.59	0.27	0.72	1.17	0.63
15	0.90	0.76	0.94	0.55	1.09	1.42	0.87
30	0.90	0.88	1.00	0.74	0.76	1.66	1.12
45	0.90	0.86	1.12	0.67	0.71	1.59	0.93
Stim+	0.80	1.18	0.65	0.55	0.60	1.42	0.69
Stim-	0.80	0.84	0.80	0.57	0.66	1.28	0.78

Appendix 31: Measured (cm) vs. Predicted (Ccalc) medetomidine target plasma concentration of 1.7 $ngml^{-1}$ (group PM) in 6 TCI propofol anaesthetised beagles at times 0 (start of the co-infusion), 5, 10, 15, 30 and 45 min. and at the time of MIT determination (Stim+ and Stim-).

		Cm ($ngml^{-1}$)					
Time (min.)	**Ccalc. ($ngml^{-1}$)**	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
5	1.60	1.74	1.636	0.83	1.09	2.60	1.40
15	1.70	1.93	2.024	1.13	1.59	2.46	1.92
30	1.90	2.21	2.592	1.63	1.90	2.38	2.22
45	1.80	2.25	2.455	1.65	1.73	2.05	1.72
Stim+	1.70	1.72	1.599	1.23	1.37	1.40	1.61
Stim-	1.70	1.77	1.923	1.25	0.00	1.51	1.42

Appendix 32: Measured (cm) vs. Predicted (Ccalc) dexmedetomidine target plasma concentration of 1.7 $ngml^{-1}$ (group PHD) in 6 TCI propofol anaesthetised beagles at times 0 (start of the co-infusion), 5, 10, 15, 30 and 45 min., at the time of MIT determination (Stim+ and Stim-) and at times 5, 20, 60, 120, 300 and 420 min. after the end of anaesthesia.

		Cm ($ngml^{-1}$)					
Time (min.)	**Ccalc. ($ngml^{-1}$)**	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
5	1.60	1.43	1.76	1.20	0.74	2.91	1.51
15	1.70	1.39	1.78	1.49	1.38	3.02	2.08
30	1.90	1.64	2.30	2.01	1.34	3.15	2.27
45	1.80	1.64	1.86	2.21	1.49	2.40	2.18
Stim+	1.70	1.42	1.58	1.69	1.28	1.93	1.82
Stim-	1.7	1.34	1.53	1.47	1.11	1.78	1.77
0	1.4	0.905	1.19	1.26	0.834	1.09	1.41
5	1.2	0.797	0.966	1.09	0.689	0.998	1.11
20	0.9	0.556	0.632	0.658	0.417	0.641	0.691
60	0.6	0.331	0.498	0.343	0.121	0.516	0.341
120	0.3	0.135	0.218	0.042	0.023	0.188	0.11
300	nd	nd	0.015	nd	nd	0.014	nd
420	nd	nd	nd	nd	nd	nd	nd

nd = not detected

Appendix 33: PE% values in 6 TCI propofol anaesthetised beagles from treatments PLD, PM and PHD at times 0 (start of the co-infusion) 5, 10, 15, 30 and 45min., and at the time of MIT determination (Stim+ and Stim-). For the PHD treatment, the PE% values at times 0, 5, 20, 60, 120, 300 and 420 min. after the end of anaesthesia are also provided.

	Low Dexmedetomidine (0.85 ngml^{-1})					
Time (min.)	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
5	-52.50	-25.75	-65.88	-9.75	46.25	-21.13
15	-15.78	4.00	-39.00	21.11	57.78	-3.22
30	-1.78	11.00	-18.11	-15.56	84.44	24.44
45	-4.44	24.44	-25.44	-21.33	76.67	3.33
Stim+	47.50	-19.38	-30.88	-24.50	77.50	-13.75
Stim-	5.37	0.00	-28.25	-17.13	60.00	-2.38
	Medetomidine (1.7 ngml^{-1})					
5	9.00	2.25	-48.25	-32.19	62.50	-1231
15	13.35	19.06	-33.76	-6.65	44.41	13.00
30	16.42	36.42	-14.32	-0.26	25.21	17.05
45	25.17	36.39	-8.22	-4.11	14.06	-4.61
Stim+	0.88	-5.94	-27.71	-19.18	-17.41	-5.47
Stim-	3.88	13.12	-26.41	-100.00	-11.06	-16.47
	High Dexmedetomidine (1.7 ngml^{-1})					
5	-10.63	10.00	-25.00	-54.06	81.88	-5.63
15	-18.24	4.71	-12.35	-18.82	77.65	22.35
30	-13.68	21.05	5.79	-29.47	65.79	19.47
45	-8.89	3.33	22.78	-17.22	33.33	21.11
Stim+	-16.47	-7.06	-0.59	-24.71	13.53	7.06
Stim-	-21.18	-10.00	-13.53	-34.71	4.71	4.12
Stop infusion						
5	-35.36	-15.00	-10.00	-40.43	-22.14	0.71
10	-33.58	-19.50	-9.17	-42.58	-16.83	-7.50
30	-38.22	-29.78	-26.89	-53.67	-28.78	-23.22
60	-44.83	-17.00	-42.83	-79.83	-14.00	-43.17
120	-55.00	-27.33	-86.00	-92.33	-37.33	-63.33
300	nd	nd	nd	nd	nd	nd
420	nd	nd	nd	nd	nd	nd

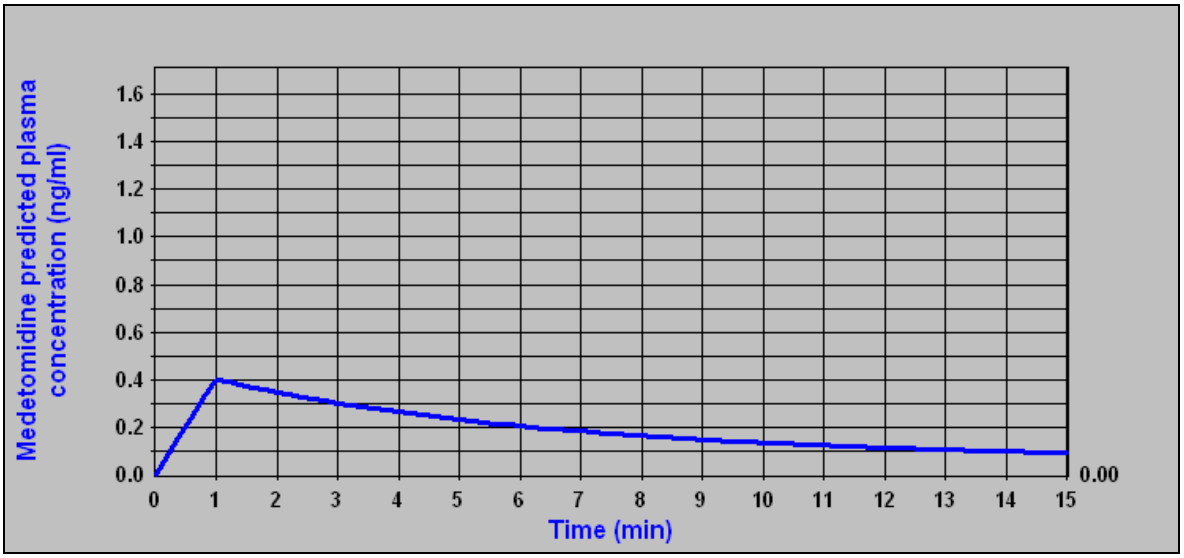
nd = not determined

Appendix 34: PE% values in 6 TCI propofol anaesthetised beagles from treatments PHD calculated with the new PK parameters (5 dogs). at times 0 (start of the co-infusion) 5, 10, 15, 30 and 45min., at the time of MIT determination (Stim+ and Stim-) and at times 0, 5, 20, 60, 120, 300 and 420 min. after the end of anaesthesia.

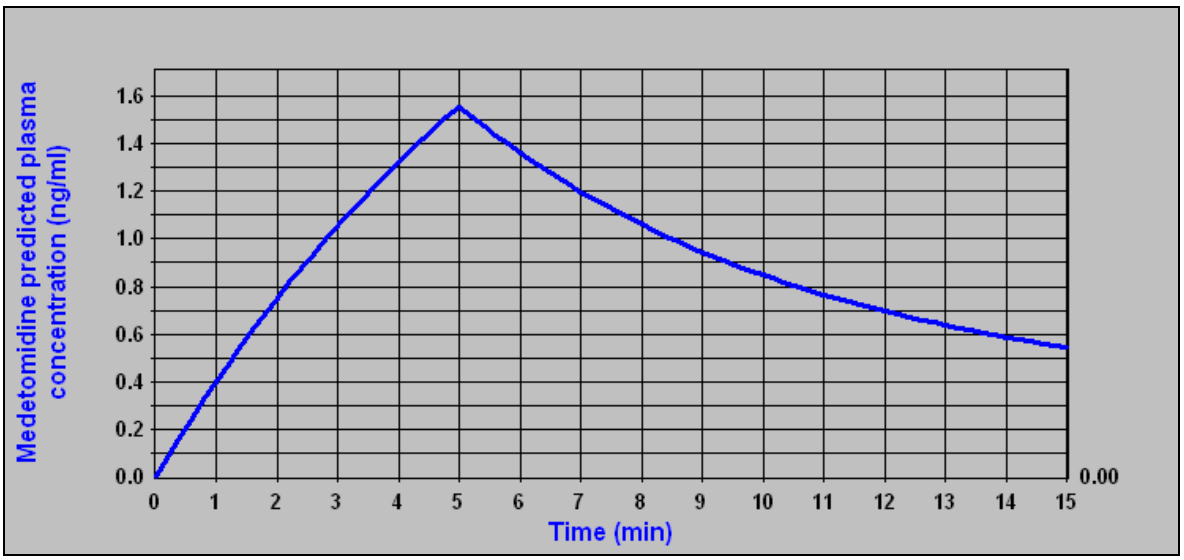
High Dexmedetomidine (1.7 ngml^{-1}) – New PK parameters (5 dogs)							
Time (min.)	**Ccalc (ngml^{-1})**	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
5	1.00	43.00	76.00	20.00	-26.50	191.00	51.00
15	1.60	-13.13	11.25	-6.88	-13.75	88.75	30.00
30	2.00	-18.00	15.00	0.50	-33.00	57.50	13.50
45	2.10	-21.90	-11.43	5.24	-29.05	14.29	3.81
Stim+	1.80	-21.11	-12.22	-6.11	-28.89	7.22	1.11
Stim-	1.7	-21.18	-10.00	-13.53	-34.71	4.71	4.12
Stop infusion							
5	1.5	-39.67	-20.67	-16.00	-44.40	-27.33	-6.00
10	1.3	-38.69	-25.69	-16.15	-47.00	-23.23	-14.62
30	0.8	-30.50	-21.00	-17.75	-47.88	-19.88	-13.63
60	0.4	-17.25	24.50	-14.25	-69.75	29.00	-14.75
120	0.1	35.00	118.00	-58.00	-77.00	88.00	10.00
300	0	nd	nd	nd	nd	nd	nd
420	0	nd	nd	nd	nd	nd	nd

nd = not determined

Appendix 35: Predicted medetomidine plasma concentration after a 0.2 $\mu gkg^{-1}min^{-1}$ IV injection over 1 min using canine pharmacokinetic parameters (O. Vainio, personal communication, 2001) and the PK-SIM simulator software (PK-SIM; Specialised Data Systems, Jenkintown, PA, USA).



Appendix 36: Predicted medetomidine plasma concentration after a 0.2 $\mu gkg^{-1}min^{-1}$ IV injection over 5 min using canine pharmacokinetic parameters (O. Vainio, personal communication, 2001) and the PK-SIM simulator software (PK-SIM; Specialised Data Systems, Jenkintown, PA, USA).



Appendix 37: Predicted medetomidine plasma concentration after a 0.2 $\mu gkg^{-1}min^{-1}$ IV injection over 15 min using canine pharmacokinetic parameters (O. Vainio, personal communication, 2001) and the PK-SIM simulator software (PK-SIM; Specialised Data Systems, Jenkintown, PA, USA). Predicted medetomidine plasma concentration 10 min after the end of infusion = 1.3 $ngml^{-1}$.