Anaesthesia and analgesia for surgery in rabbits and rats: A comparison of the effects of different compounds

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ABSTRACT

Studies of anaesthesia and analgesia in rats and rabbit were undertaken with the aim to improve anesthesia techniques, for better animal welfare and research quality.

Induction of anaesthesia with the volatile halogenated agents isoflurane, sevoflurane and desflurane was compared in the New Zealand White rabbit. All agents caused struggling, breath holding and reflex bradycardia, desflurane having the least detrimental effects. Hypoxia was prevented by pre-oxygenation, and no cardiac arrhythmias were seen. Still, induction of anaesthesia in rabbits cannot be recommended with any of these agents.

Injection anaesthesia with ketamine (cyclohexamine)/medetomidine (alpha-2-adrenergic agonist) and the effect of adding the opioid butorphanol, were evaluated in the New Zealand White rabbit. Additionally, effects after subcutaneous and intramuscular administration of ketamine/medetomidine were compared. In a first study, a dose of 15/0.25 mg/kg of ketamine/medetomidine was found effective in producing surgical anaesthesia for 59 ± 18 min, but failed to produce surgical anaesthesia in some animals in a following study, possibly due to different stress levels at the time of induction. The anesthetic effects did not differ between the administration routes. Subcutaneous injection was easier to perform and seemed less painful. Pronounced hypoxia developed during anaesthesia (PaO$_2$ 4.8 ± 0.6 kPa, mean $\pm$ SD), indicating a need for oxygen supplementation. Blind tracheal intubation was easy to perform during anaesthesia. Addition of butorphanol increased duration of anaesthesia.

In Wistar rats, anaesthesia with ketamine/medetomidine, repeated six times with weekly intervals, and the effects of pre-medication with the opioid buprenorphine, were evaluated. Buprenorphine caused an increase in duration of anaesthesia as well as greater respiratory depression, and was associated with increased lethality. Repeated anaesthesia without buprenorphine was found safe and led to an increase in sleep times with successive anaesthetics.

The combination sufentanil (opioid)/medetomidine was also evaluated in Wistar rats. Anaesthesia was more efficiently produced after subcutaneous than after intraperitoneal administration, and a sc dose of 40/150 µg/kg of sufentanil/medetomidine produced surgical anaesthesia for 101 ± 49 min. Anaesthesia resulted in very low oxygen saturation levels (40 ± 20 %). Despite this, all animals recovered uneventfully. Oxygen supplementation is strongly recommended with this combination. Aanaesthesia was reversed within 7 min by administration of 0.2/0.5 mg/kg of butorphanol (mixed µ-opioid agonist/antagonist)/atipamezole (alpha-adrenergic antagonist).

Postoperative recovery and behavior were compared in Sprague-Dawley rats after abdominal surgery under isoflurane or ketamine/medetomidine anaesthesia, and the effect of perioperative treatment with the NSAID analgesic carprofen studied. Surprisingly, rats recovered body weight faster and showed less pain-related behavior when surgery was performed under isoflurane anaesthesia, and locomotion was also less reduced. The effects on body weight after surgery under ketamine/medetomidine anaesthesia were not just seen in the immediate postoperative period, but also for several days after. Perioperative treatment with carprofen reduced the detrimental effects in both isoflurane and ketamine/medetomidine anaesthetized animals. The

results show that the choice of anaesthesia may be just as important as the use of analgesic treatment for improved recovery and reduction of pain after surgery.

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CONTENTS

LIST OF ABBREVIATIONS

MAC	Mean alveolar concentration
IASP	International association for the study of pain
PaO_2	Partial pressure of oxygen in arterial blood
$PaCO_2$	Partial pressure of carbon dioxide in arterial blood
NSAID	Non-steroidal anti-inflammatory drug
CGRP	Calcitonin gene related peptide
ECG	Electrocardiogram
NMDA	N-methyl-D-aspartate
COX	Cyclo-oxygenase
PAG	Periaqueductal gray substance
NRM	Nucleus raphe magnus
MAP	Mean arterial blood pressure
Sc	Subcutaneous
Im	Intramuscular
Ip	Intraperitoneal
Iv	Intravenous
MAK	Makrolon
Bpm	Beats per minute
5-HT	5-hydroxytryptamine

1 INTRODUCTION

1.1 THE USE OF LABORATORY ANIMALS

Animal experiments are an important part of biomedical research with the aim to understand the nature of human disease and to develop effective and safe treatment. The use of laboratory animals has helped to improve health, prolong life and reduce suffering in man and also in domestic animals. With this use, however, ethical concerns regarding the welfare of laboratory animals arise. With the aim to improve health and reduce suffering in research animals, the field of laboratory animal science developed. One milestone in the history of experimental research ethics was the publication of "The Principles of Humane Experimental Technique" in 1959 by Russell and Burch, in which they introduce the three R's as guidance for animal experimentation: replacement of animal experiments and reduction of the number of animals used and refinement of procedures.

The number of animals used in medical research increased dramatically after World War II and continued to do so until the late 1970s (Baumans 2004). In the mid 1990s the downward trend was interrupted, when it became possible to genetically engineer mice (Fig 1). It has been estimated that 50-100 million vertebrate animals are used worldwide each year for scientific purposes (Orlans 1998), and of all species used, mice and rats account for approximately 80 %, while for example rabbits account for less than 1 % of all animals used.
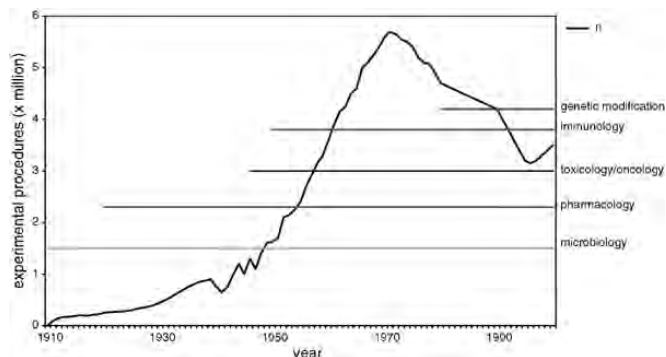
Fig 1. Development of animal use in the UK during the 20th century (number of experimental procedures per year). Reprinted by permission from Macmillan Publishers Ltd: Gene Therapy (Baumans 2004).

1.2 ANAESTHESIA

One important way of reducing animal suffering is the use of anaesthesia and analgesic treatment in conjunction with surgery and other painful procedures. Anaesthesia can be achieved with injection or inhalation of substances that induce a reversible state of unconsciousness. For scientific quality, anaesthetic techniques need to be reliable and safe, and the effects of the anaesthetic and analgesic compounds on the research animals must be well documented. This is especially important to consider when

experiments are performed and data collected under anaesthesia. Further, if animals undergo survival surgery, they need to recover quickly, and not suffer unnecessarily from pain. This is of importance for both animal welfare and scientific quality.

This thesis was performed to document some physiological and behavioural effects of different anaesthetics in rabbits and rats, with the aim to refine anaesthetic procedures, reduce suffering and improve scientific quality. The introduction will give a short overview over general aspects of anaesthesia, pain and analgesic treatment in animals.

1.2.1 Inhalation anaesthesia

Inhalation anaesthesia requires rather complex equipment for delivery of volatile agents, as well as effective ventilation of waste gases, but there are great advantages with its use, in the clinical as well as the research setting. The most prominent benefits are that the effect of anaesthesia is easy to control, and that animals recover fast and smoothly, especially if the newer volatile agents are used (Steffey 1996, Brunson 1997). Moreover, animals under inhalation anaesthesia benefit from the fact that oxygen is commonly used as the carrier gas, which improves tissue oxygenation during anaesthesia. Finally, most volatile agents undergo very little biotransformation; instead they are mostly exhaled in an unchanged form. They therefore typically interfere only to a small extent with liver function and metabolism of other drugs, which is especially important in pharmacology and toxicology research. Injection anaesthetics on the other hand often induce enzyme activity, which may cause cross-tolerance to other drugs.

1.2.1.1 Pharmacology

Among volatile agents commonly used in human and veterinary anaesthesiology are the aliphatic ethers isoflurane, sevoflurane and desflurane (Sakai *et al* 2005). For delivery, the volatile liquid is vaporized and mixed with a carrier gas. The vapour is absorbed in the lung alveoli into the blood and distributed to the central nervous system and other organs. The elimination of inhalation agents is primarily by expiration of the unchanged compounds. Isoflurane is metabolised to 0.2 %, sevoflurane to 2-3 % and desflurane to less than 0.1 %. During maintenance of anaesthesia, equilibrium is reached with the same constant partial pressure in the alveoli (P_A) as the brain. P_A is therefore used as an index of anaesthetic depth, and measured at the very end of expiration. The main property that determines the speed of induction and recovery is the blood:gas partition coefficient. Desflurane has the lowest blood:gas partition coefficient (0.42), and therefore the quickest induction and recovery, followed by sevoflurane (0.65) and isoflurane (1.4). The rapid effects are further accentuated in small animals, like rodents, in which equilibrium is reached much faster than in large animals (Brunson 1997). Mean alveolar concentration (MAC) is a measure of potency and equals the concentration at which 50 % of the anaesthetized subjects do not respond to a noxious stimulus. Thus MAC corresponds to the effective dose$_{50}$. In a single species the inter-individual variability in MAC for a given compound is generally small (Brunson 1997). Even between species the variability in MAC for a given agent is usually not large (Table 1). Anaesthetic potency is inversely related to MAC and direct related to the oil:gas partition coefficient. A very potent anaesthetic thus has a low MAC value and a high oil:gas partition coefficient.

Table 1: MAC values for humans, rabbits and rats in % (Steffey 1996)

	Human	Rabbit	Rat
Isoflurane	1.2	2.0	1.2-1.5
Sevoflurane	1.7-2.0	3.7	2.4-2.5
Desflurane	6.0-7.2	8.9	5.7-7.1

All common inhalation anaesthetics depress alveolar ventilation in a dose-related fashion, in humans and animals alike (Steffey 1996). Isoflurane, sevoflurane and desflurane also produce a mild decrease in myocardial contractility and increase the heart rate in dogs. All inhaled anaesthetics induce a dose-related depression of systemic arterial blood pressure, which is similar regardless of species studied (Steffey 1996). The arterial blood pressure declines mostly due to decreased cardiac output. Unlike halothane, the newer volatile agents lack effect to sensitize the heart to the arrhythmogenic effects of noradrenalin (Brunson 1997).

The mechanisms by which volatile agents cause anaesthesia are not fully understood (Campagna *et al* 2003). For a long time, anaesthetics were believed to act unitarily through perturbation of neuronal membrane lipids, the so called Meyer-Overton rule (Meyer 1899) Later work has indicated that halogenated agents may act by enhancing the function of inhibitory GABA$_A$ and glycine receptors and by inhibiting excitatory glutamate receptors, as well as by opening potassium channels (Solt & Forman 2007).

In relatively large animals like dogs and pigs intravenous induction usually forgoes endotracheal intubation and delivery of volatile anaesthetics. The reason is that mask induction with volatile anaesthetics is slower and associated with side effects like struggling and excitement (Mutoh *et al* 2001). Induction of anaesthesia with volatile agents may also be associated with upper-airway reflex responses such as apnoea, coughing, bronchoconstriction, laryngospasm and excessive mucus secretion. The degree of irritation from volatile anaesthetics varies with the agent used and its concentration. The newer volatile agent sevoflurane shows lower airway irritability compared with isoflurane, desflurane and halothane, and has therefore been recommended for mask induction in humans and dogs (Mutoh *et al* 2001, Sakai *et al* 2005).

Rabbits react violently to inhalation of halothane or isoflurane, and show extended periods of breath holding, which results in marked bradycardia (Flecknell *et al* 1996). In small research animals like rodents, induction with volatile agents is usually fast and smooth, and is easily achieved by placing the animal in a plastic chamber, to which the volatile agent is delivered (Brunson 1997). The first anaesthetic to be used in this fashion was ether, which is highly irritating to the respiratory tract, causing increased secretion in the respiratory tract and bronchoconstriction (Brunson 1997) as well as signs of distress (van Herck *et al* 2001). When more modern volatile agents (isoflurane, sevoflurane) are used for rodents, induction is usually without struggling or obvious signs of excitement (Brunson 1997).

The mucosa of the airways of animals and humans contain afferent sensory C-fibres that respond to a variety of irritant inhaled substances (Widdicombe & Lee 2001). The receptors for these reflexes are the free nerve endings, which lie in and

under the airway epithelium from nose to alveoli. They are polymodal by character and nociceptive and release calcitonin gene related peptide (CGRP) and substance P, which are important initiating mediators of neurogenic inflammation (Lundberg & Saria 1987). Activation of C-fibres leads to profound reflex responses, involving the autonomic nervous system and the respiratory and cardiovascular systems. In rabbits, the cardiovascular response includes bradycardia and reduction of cardiac output (Allison & Powis 1971), as well as dysrhythmias with depression of the ST complex of the ECG (Widdicombe & Lee 2001). The bradycardia caused by irritants like cigarette smoke or ammonia vapour in rabbits can be prevented by administration of atropine, which shows that this reflex is cholinergically (vagally) mediated.

Reflex apnoea most likely serves to protect the respiratory system from irritants, and is elicited by stimulation of receptors in the nasopharynx (Lindberg et al 1990). The afferents of this reflex are of trigeminal origin. The reflex is related to the diving reflex that is seen in aquatic animals, amphibians, and birds after contact with water, which helps the animals to stay under water for extended periods of time.

1.2.2 Injection anaesthesia

Injection anaesthetics can be administered intravenously, intramuscularly, subcutaneously or intraperitoneally. Administration by the intravenous route requires some skill and is not easy to accomplish in small rodents. The benefit of intravenous injection compared with intramuscular or intraperitoneal routes, is that the dose can be titrated until the right depth of anaesthesia has been reached, which reduces the risk of overdosing. By use of a quickly metabolised drug like propofol (a phenol derivative), the anaesthetic depth can be tightly controlled by adjustment of the infusion rate.

In rodents, it is common to use the intraperitoneal or subcutaneous routes when administering anaesthetic drugs, and for this the anaesthetic must not be metabolised too quickly, in order to accomplish a useful duration of anaesthesia. In comparison with inhalation anaesthesia, which is easy to control, the variation in duration and depth of anaesthesia is larger with injection anaesthesia administered in this manner. The variation is also larger in outbred rodent stocks (due to genetic variation) than in inbred strains, but there can also be large variations between different inbred strains (Flecknell 1997).

Another problem with injection anaesthesia induced by drugs that are slowly metabolised is the long duration of sleep that follows surgical anaesthesia. A long after-sleep is especially problematic in small animals like rodents, which have a high rate of general metabolism, and need to resume eating and drinking soon after anaesthesia. Due to their large body surface to body weight ratio, rodents are more prone to hypothermia, and without active warming the rate of drug biotransformation is reduced, and recovery prolonged even further. Fast recovery is also important in rabbits and guinea pigs, which have sensitive gastrointestinal tracts and can suffer from overgrowth of pathogenic bacteria as well as hepatic lipidosis if eating is interrupted for too long time. Rabbits under 3 kg develop metabolic acidosis and hypoglycaemia if food intake is interrupted for more than 12 h (Bonath *et al* 1982).

To overcome some of the difficulties with prolonged recovery, it is beneficial to use injectable anaesthetics which actions can be reversed by administration of antidotes.

1.2.2.1   Pharmacology

Few drugs have all the properties that are needed for anaesthesia and therefore, combinations of drugs are used to improve the quality of anaesthesia (Fish 1997). Little information is available on the biotransformation of anaesthetic drugs when used in combination. Very common are combinations including ketamine, which uses are on the increase for surgery in rodents (Richardson & Flecknell 2005). By addition of opiod drugs to ketamine combinations, the dose of anaesthetics may be reduced, side effects decreased and duration of anaesthesia prolonged (Tomizawa *et al* 1997, Ko *et al* 1997).

Ketamine is a phencyclidine derivative used since the 1970s (Craven 2007), and may be the most widely used injectable anaesthetic in animals (Fish 1997). Ketamine exerts its effects through an antagonistic action at N-methyl-D-aspartate (NMDA) receptors in the central nervous system (CNS). It produces a state of dissociative anaesthesia, in which the neuronal traffic between the thalamus and the cortex is interrupted (Alkire & Miller 2005). This state is characterized by unconsciousness, open eyes, sustained reflex movements and stiff muscles. Analgesia is potent enough to allow sole use for invasive procedures in many animal species, but to achieve improved conditions, such as good muscle relaxation and smooth recovery, ketamine is combined with a benzodiazepine or an alpha-2-adrengic agonist. Ketamine produces an increase in blood pressure, stroke volume and heart rate (Craven 2007).

The commercial preparations of ketamine are racemic mixtures of (S)- and (R)-ketamine. S-ketamine has four times the affinity for the NMDA receptor and also binds to μ- and κ-opioid receptors. Ketamine is metabolised in the liver by demethylation to the active metabolite norketamine (Ebert et al 1997), and subsequently by oxidation. Repeated dosing with ketamine in rats induces tolerance, so that sleep time is significantly and markedly reduced (Livingston & Waterman 1978).

Medetomidine was developed in the 1980s. It is a potent and highly specific alpha-2-adrenoceptor agonist with sedative, anxiolytic and analgesic properties (Sinclair 2003). Its effects may be reversed with a specific alpha-2-adrenoceptor antagonist like atipamezole.

Alpha-2-adrenoceptors are located throughout the CNS and periphery, and the subtypes (2A, 2B, 2C) differ in density and location between animal species. The state of awareness, arousal and vigilance is regulated by activity of the receptor subtype alpha-2A in the brainstem. The effects of medetomidine show marked species differences. In rats, but not in mice and rabbits, medetomidine causes heavy sedation with loss of the righting reflex (Fish 1997). Antinociceptive and sedative actions are inconsistent in guinea pigs and hamsters.

The adverse effects of medetomidine are mainly cardiovascular; bradycardia and associated arrhythmias, initial hypertension followed by hypotension, and reduced cardiac output. Side effects also include respiratory depression, increased blood glucose levels and diuresis (Fish 1997).

The mechanism behind apha-2-agonist-induced antinociception is not entirely understood, but both spinal and supraspinal sites of action are likely to be involved (Murell & Hellebrekers 2005). In the spinal cord, a high density of alpha-2-receptors is found in the substantia gelatinosa, and a part of them are located on primary afferent terminals. Possible ways of suppressing nociceptive signals are by inhibition of neurotransmitter release from primary afferents or by influencing descending modulatory systems from the brain stem. In the mouse all three receptor

subtypes are involved in the regulation of pain perception (Philipp *et al* 2002). In dogs and cats, analgesia is not present throughout the period of sedation, but lasts only for half the duration (Sinclair 2003).

Ketamine/medetomidine in combination has been used for anaesthesia in animals since the 1980s (Kommonen 1988). The combination is useful for producing surgical anaesthesia in many species including cat, dog, rat and rabbit (Verstegen *et al* 1989, Jalanka *et al* 1989, Nevalainen *et al* 1989). In mice and guinea pigs, the combination is only useful for immobilization (Arras *et al* 2001, Nevalainen 1989). Side effects include respiratory depression and bradycardia (Hellebrekers *et al* 1997, Dobromylskyj 1996, Verstegen *et al* 1989). In cats, blood pressure is initially increased and in rabbits it is decreased (Dobromylskyj 1996, Hellebrekers et al 1997).

1.3   PAIN IN ANIMALS

The definition of pain, as formulated by the International Association for the Study of Pain, states that pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (IASP 1979). Pain is a psychological state not to be confused with nociception, which is activity in nociceptive pathways from the periphery to the higher parts of the brain, where the activity ultimately may lead to the pain experience. In all vertebrates, including humans, reactions to injurious stimuli are generated by neural systems in the spinal cord and brain stem (Rose 2002). Responses like withdrawal of the stimulated body part, struggling, locomotion and vocalization are generated by the brainstem and spinal cord. Even associative learning is a response that may develop without cortical activation, and also occurs in invertebrate animals.

The pain experience on the other hand, is a brain process, more or less separate from the behavioural responses to nociceptive stimuli, and has at least three dimension: (1) a sensory-informational component that conveys the location, features and intensity of noxious stimulation, (2) an emotional dimension that constitutes the suffering and unpleasantness of the experience, and (3) a cognitive-evaluative component involving attention, previous experience and the perceived threat to the individual. These dimensions are processed in different parts of the cerebral cortex, including the parietal, frontal and prefrontal cortex (Rose 2002).

The processing of the emotional or affective component of the pain experience has been linked to certain brain regions (Johansen *et al* 2001). The evidence that the anterior cingulate cortex (ACC) is central for the affective component of pain includes reports after surgical ablation and using neuroimaging techniques in humans. In the rabbit and rat, neurons within the ACC that respond to noxious stimuli have large or whole-body receptive fields and bilateral nociceptor innervation which is consistent with a role in affective processing (Sikes & Vogt 1992, Yamamura *et al* 1996).

To examine the emotional part of the pain experience in animals, it is necessary to assess behavioural actions that are not preserved after decerebration, and thus prove involvement of the cerebral cortex (see below).

1.3.1   Pain assessment

In order to treat pain accurately, it needs to be assessed correctly. Because direct measurement of an animal's subjective experience is not possible, the use of objectively measurable parameters as a reflection of acute and chronic pain has been

investigated in companion, farm and laboratory animals (Viñuela-Fernandez *et al* 2007). These measures include physiological, biochemical and behavioural responses to nociceptive stimulation. However, most physiological and biochemical responses to nociception are non-specific and poorly related to scores of pain related behaviour. Pupil dilatation has however been shown to correlate to pain scores in dogs after orthopaedic surgery, but was not considered a practical means of pain assessment (Holton *et al* 1998).

Measurement of stress hormones can be useful in a certain context, even if they lack specificity for nociceptive stimulation and also show a "ceiling effect" (Viñuela-Fernandez *et al* 2007). Plasma cortisol/corticosterone levels are elevated after surgery in calves, lambs and mice, show correlation to behavioural changes, and are reduced by analgesic use (Kent et al 1993, Stafford et al 2002, Wright-Williams 2007).

The use of pain rating scales in animals has been adopted from human medicine (Viñuela-Fernandez *et al* 2007). An observer judges the animal's behaviour and scores it accordingly. The use of simple descriptive scales, like the visual analogue scale (VAS), has been shown to be reliable, valid and sensitive for postoperative pain scoring in humans (Coll *et al* 2004). However, when the same type of scale is used to assess postoperative pain in animals, by an observer, the reliability is unfortunately poor. Variation between observers is large and the correlation with analgesic treatment poor. The development of objective behavioural analysis has proven more reliable for postoperative pain assessment in farm and laboratory animals (Roughan and Flecknell 2003).

Not so long ago, analgesic treatment had a low priority in laboratory animal species, and it is still less common compared with companion animals. According to a study examining medical scientific publications from 2000-2002, only 19 % of laboratory rodents subjected to surgery received postoperative analgesic treatment (Richardson & Flecknell, 2005). Reasons why scientists are reluctant to use analgesic drugs may be fear of interference with scientific aims or difficulties in detecting pain. A problem with behavioural assessment of pain in prey species, is the lack of overt reaction to pain, which may be a part of their evolutionary strategy (Bateson, 1991).

Pain in farm animals has also traditionally been overlooked, partly because of lack of overt pain response (Viñuela-Fernandez *et al* 2007). Studies in lambs, calves and pigs after tail docking and castration, have however identified pain behaviours that are quantifiable, and either reduced (e.g. teat seeking) or increased (e.g. tail wagging) in frequency or duration, and respond to analgesic treatment (Molony *et al* 1995 and 2002, Hay *et al* 2003). Likewise, specific pain related behaviours have been identified after abdominal surgery in rats, and a validated pain-scoring scheme has been developed for practical use (Roughan & Flecknell 2003). A composite score of the frequencies of back arching, fall/stagger, writhe and poor gait was collected during 10 minutes and shown to correlate with the treatment with analgesics in a dose dependent fashion.

Our ability to determine the affective components of pain in animals is still very poor (Viñuela-Fernandez et al 2007). Many of the behaviours measured in animals are not measures of pain, but the simple outcome of nociceptive reflexes (Vierck 2005). For example, licking, guarding, vocalisation and orienting are all behaviours that are intact in decerebrated animals (by transection just above the brain stem), and thus constitute spinal-brain stem-spinal signalling, or so called supra-segmental reflexes.

Only learned, motivated responses are dependent on spinal-cerebral-spinal signalling, which is evident after decerebration. To measure such response, an operant escape model may be used, in which the animal needs to learn or remember that pushing on a lever or moving to a particular part of the apparatus would eliminate the aversive stimulus. Another recently described measurement of the affective component is the delayed vocalization after a noxious event (Viñuela-Fernandez et al 2007). Unlike immediate vocalization, that is medullary mediated, delayed vocalization is organized within the forebrain in rats (Nandigama and Borszcz 2003).

1.4 ANALGESIC TREATMENT OF EXPERIMENTAL ANIMALS

One of the aims of laboratory animal medicine is to alleviate pain and minimize suffering in research animals. Analgesic treatment in conjunction with surgery, aims not only to reduce pain, but also improve recovery and reduce morbidity and mortality. Surgery is associated with neuroendocrine, metabolic and immune alterations, resulting from tissue damage, anaesthesia and psychological stress (Shavit et al 2006). In rats, perioperative analgesic treatment with non-steroidal anti-inflammatory drugs (NSAIDs) or opioids has been shown to improve food intake and body weight gain after surgery, and decrease corticosterone levels. Pharmcodynamic effects of analgesic drugs vary between animals and humans and among animal species and even strains, but the greatest differences are in pharmacokinetics (Heavner 1997).

1.4.1 Non-steroidal anti-inflammatory drugs (NSAIDs)

All NSAIDs have very similar actions, and their main therapeutic effects are anti-inflammatory, analgesic and antipyretic (Rang et al 2008). The side effects are also similar for all NSAIDs and include gastric irritation, effects on renal blood flow and inhibition of platelet function. Most classical NSAIDs are carboxylic acids and inhibit the cyclo-oxygenase-enzymes (COX) 1 and 2. More modern NSAIDs are coxibs and mainly affect COX-2. Inhibition of COX leads to decreased production of prostaglandins and thromboxanes. COX-1 is a constitutive enzyme produced in most tissues whereas COX-2 is induced in inflammatory cells when they are activated. The anti-inflammatory action and probably the analgesic action are related to inhibition of COX-2, whereas many side effects are related to COX-1-inhibition. The use of at least some selective COX-2-inhibitors has, however, proved to increase the risk of cardiovascular events in humans, which has led to a setback of these drugs.

NSAIDs are effective against mild and moderate pain and especially pain arising from inflammation (Rang et al 2008) Peripherally, the decrease in prostaglandin synthesis leads to reduced sensitization of nociceptors to inflammatory mediators. Centrally, they are believed to have an action in spinal cord, where prostaglandins cause facilitation of nociceptive transmission.

In animals, NSAIDs generally show good bioavailability from oral, intramuscular and subcutaneous administration (Lees et al 2004). Marked species, and possibly breed and strain differences, are found in clearance and elimination half-life. The differences are most likely due to differences in hepatic clearance, as the high degree of binding to plasma protein leads to very limited ultra-filtration in the kidney and to relatively low concentrations in urine of parent drug. Details regarding efficacy

and safety of NSAIDs from a clinical perspective in laboratory animal species are scarce (Heavner 1997).

Among the NSAIDs licensed for use in animals that have been shown to have a good analgesic effect following surgery in many animal species is carprofen (Roughan and Flecknell 2004). Carprofen is a propionic acid derivative and commonly used in veterinary medicine. It is a weak inhibitor of COX enzymes and of lipoxygenas activity. Despite this, it is a good analgesic in both acute and chronic pain states and has excellent anti-inflammatory activity in laboratory animal models of inflammation. Carprofen is efficient for treating postoperative pain in dogs (Lascelles *et al* 1998), cats (Lascelles 2007) and rats (Roughan & Flecknell 2003). The volume of distribution is small and systemic clearance slow. Elimination is primarily by biotransformation. In dogs and rats carprofen is converted to glucoronide metabolites and mainly excreted in the faeces (Rubio *et al* 1980). Half-life is 8 h in the dog (McKellar *et al* 1994) and 20 h in the cat (Taylor *et al* 1996).

1.4.2 Opioids

The term opiod applies to any substance that produces morphine-like effects, which can be blocked by antagonists such as naloxone. Whether natural or synthetic, opioids produce their effects by binding to opioid receptors. They are classified as μ-, δ- and κ- receptors and are G-protein coupled. Knockout studies in mice have shown that the μ - receptor is the most important receptor for mediation of analgesia as well as side effects, of which respiratory depression is the most feared (Matthes *et al* 1996). Apart from centrally located μ-receptors, peripheral μ- and κ -receptors also mediate analgesia. κ –receptors are abundant in the dorsal horn of the spinal cord. Whereas μ- receptors mediate euphoria, κ –receptors are believed to mediate dysphoria in humans. Other side effects include constipation, nausea and bradycardia.

Opioids can be classified as pure agonists (e.g. morphine), partial agonists, mixed agonist-antagonists (e.g. buprenorphine) and antagonists (e.g. naloxone). Opioid agonists reduce neuronal excitability by hyperpolarisation and reduce transmitter release. However, opioids are also believed to increase the activity in certain neuronal pathways, by suppression of inhibitory neurons. Important areas include the periaqueductal gray (PAG), where descending pathways to the nucleus raphe magnus (NMR) arise. NRM is the origin of pain inhibitory pathways descending to the dorsal horn of the spinal cord. In the spinal cord, pain transmission is inhibited by an action on the dorsal horn neurons and possibly on the terminals of primary afferents. Peripheral local application of opioids can also induce analgesia (e.g. by local injection in the knee-joint). Apart from the antinociceptive effects, opioids also reduce the affective component of pain and have sedative properties. Opioids are drugs of abuse and cause, beside euphoria, dependence, tolerance and withdrawal symptoms.

Buprenorphine is a partial agonist on μ-receptors and has a long duration of action (6-12 h). It is a potent analgesic in many animal species (Roughan & Flecknell 2002). It is often used to provide postoperative pain relief, and will decrease the time to recovery after anaesthesia, if a full μ-agonist opioid was used as part of the anaesthetic regime. In rats and mice it is 25-40 times more potent than morphine when administered parenterally, and 6-10 times when given orally. The maximum antinociceptive effect is reached after one hour following subcutaneous administration in rats. The analgesic effect declines at doses in excess of 1 mg/kg in rats.

Butorphanol is a synthetic opioid and a partial agonist and mixed agonist-antagonist with affinity to all three opioid receptors (Commiskey *et al* 2005). It provides pain relief after surgery in cats and dogs (Al-Gizawiy & Rudé 2004). In rabbits, butorphanol has been used as an adjunct to ketamine/xylazine anaesthesia, in order to improve conditions for surgery (Marini *et al* 1992). Like buprenorphine, it may be used to reverse anaesthesia if a full μ-agonist was part of the anaesthetic regime.

Fentanyl and sufentanil are highly potent phenylpiperidine derivatives that are pure μ- and δ-agonists with a rapid onset and short duration of action. Their main use is to provide analgesia during surgery, thereby decreasing the doses of conjunct anaesthetics needed, and keeping the cardiovascular depression to a minimum. Respiratory depression may however require positive pressure ventilation (Wixson & Smiler 1997). Fentanyl has proven very useful for rodent and rabbit surgery in neuroleptanalgesic combinations (sedative + opioid), especially since recovery can be shortened by administration of a mixed opioid agonist/antagonist (Flecknell *et al* 1983, Flecknell & Mitchell 1984). Fentanyl has also been evaluated in combination with medetomidine, to achieve a fully reversible anaesthetic regimen, but the combination was shown to produce marked respiratory depression (Hu et al 1992).

2 AIMS

The overall aim was to establish safe and reliable anaesthesia for rabbits and rodents, and to examine how anaesthesia affects pain and recovery after surgery in rats.

Specific aims:

I.   To assess if anaesthesia can be induced with sevoflurane or desflurane in NZW rabbits without triggering a breath-holding response

II.   To compare the characteristics of anaesthesia induced with different dose combinations of ketamine/medetomidine in the NZW rabbit, to compare administration by the subcutaneous and intramuscular routes and to assess the effects of addition of butorphanol to this combination

III.   To evaluate the influence of buprenorphine on ketamine/medetomidine anaesthesia in Wistar rats, and assess the risk with using this combination to repeatedly anaesthetize rats

IV.   To assess the effects of sufentanil in combination with medetomidine administered by the intraperitoneal and subcutaneous routes, and its reversal with butorphanol and atipamezole in Wistar rats

V.   To evaluate postoperative pain and recovery in Sprague-Dawley rats after surgery under isoflurane or ketamine/medetomidine anaesthesia

3 MATERIALS AND METHODS

3.1 STUDIES IN NZW RABBITS (I-IV)

3.1.1 Animals

New Zealand White female rabbits were used. Animals were group housed in floor pens with dust-free shaving at a stock density of 6000 cm^2/animal, and fed commercial pelleted diet *ad libitum* and autoclaved hay. Room temperature was maintained at 19 ± 2°C and relative humidity at 50 %, with 18 air changes/hour. The rabbits were free from respiratory pathogens (Bordetella, Pasteurella spp, Myxomatosis, Rabbit Calicivirus Disease). Mean BW were between 2.2 ± 0.1 and 3.4 ± 0.3 kg. Group housing allows for social interaction and increased locomotion (running, hopping and rearing), but is only possible in female rabbits, due to fighting between males (Verga *et al* 2007).

3.1.2 Experimental design

Anaesthesia was repeated in a randomized block design, with 4 to 7 days between sessions. The animals were acclimatized to handling through daily weighing before the study.

3.1.3 Induction with inhalation anaesthesia (I-II)

Two studies were performed, one comparing isoflurane with sevoflurane and one comparing isoflurane with desflurane (Table 2).

Table 2: Inhalation anaesthesia studies in NZW rabbits

Study	N	Treatment
I	6	Slow isoflurane
		Slow sevoflurane
		Rapid sevoflurane
II	5	Slow isoflurane
		Rapid desflurane
		Slow desflurane

A small rubber mask attached to an unmodified Bain's circuit (a coaxial breathing system) was used for mask induction. A cannula at the apex of the mask was connected to a respiratory rate monitor, for counting respiratory rate. Isoflurane was delivered from an Isotech Mark III vaporizer in 100 % oxygen and at 4 l/min. Sevoflurane and desflurane were delivered from temperature and flow compensated vaporizers.

3.1.3.1 Induction of anaesthesia

After a settling phase of 5-10 min, baseline parameters were recorded. The animal was restrained manually and oxygen was delivered with the facemask for 2 min at 4 l/min. Following pre-oxygenation isoflurane was delivered at a rising concentration, with 0.5 % increments in vapour setting every 30 s, up to 5 %. Sevoflurane was delivered with 1 % increments every 30 s, up to 7 % (slow induction). Subsequently, all animals were

induced immediately with sevoflurane at 8 % (rapid induction). Desflurane was administered either by a rising concentration beginning at 2 % and stepwise increments of 2 % every 30 s up to 18 % (slow induction), or by immediate administration of 18 % (rapid induction). The anaesthetic concentration was increased or maintained at the maximum level until induction was complete, as judged by the loss of the righting and pedal withdrawal reflexes and a regular pattern of respiration.

Blood samples were collected and physiological parameters recorded at 2-min intervals. Periods of apnoea were noted and logged in the data acquisition system. In the first study anaesthesia was discontinued after induction was complete and in the second study anaesthesia was continued for 10 min. Once the anaesthetic vapour was switched off, and the rabbits had regained the righting reflex, they were placed in a heated incubator until fully recovered.

3.1.3.2 Cardiovascular monitoring

For electrocardiogram recording, electrodes were placed on the skin on the medial side of the upper forelegs and left hind leg, and a standard three lead system connected to a polygraph. A blood pressure module was used to record arterial blood pressure from a 22G cannula placed in the central ear artery. For blood gas analysis, a cannula was placed in the other ear. To minimize pain from cannulation, the ears were locally anaesthetized with EMLA cream, a mixture of lidocaine 2.5% and prilocaine 2.5%, for 45 min before cannulation.

All catheters and ECG leads were passed through a stockinet bandage applied around the shoulders. The blood pressure module and polygraph were interfaced to a microcomputer via an analogue to digital interface, which enabled continuous storage of blood pressure and ECG onto the computer hard disc.

3.1.3.3 Data processing and statistical analysis

Heart rate and blood pressure data were checked for arrhythmias and noted at the start of induction ($t = 0$), at 2-min intervals, and at the end of anaesthesia. PO_2 and PCO_2 values were noted at $t = 0$ and the maximum rise in pCO_2 ($t =$ high) and fall in pO_2 ($t =$ low) recorded.

The computer program SPSS was used for statistical evaluation. The Student's paired t-test for the comparison between isoflurane and sevoflurane. In the study comparing isoflurane, slow and rapid desflurane induction, general linear model (GLM) repeated measures were used to distinguish main effects of each technique, and one-way ANOVA for comparing mean calculations between groups, with Bonferroni post-hoc tests. A level of $p < 0.05$ was considered significant.

3.1.4 Injection anaesthesia with ketamine/medetomidine (III-IV)

From an initial dose finding study of intramuscularly administered ketamine/medetomidine, four dose combinations were selected for the study III (Table 3). From the results from study III, four dose combinations were chosen for study IV, which also evaluated the difference between subcutaneous (sc) and intramuscular (im) administration routes and the effect of adding butorphanol. The dose of butorphanol was selected based on a previous study of its analgesic effects in rabbits (Flecknell & Liles 1990).

Table 3: Doses of ketamine/medetomidine/butorphanol for anaesthesia in NZW rabbits

Study	N	Administration route	Ketamine (mg/kg)	Medetomidine (mg/kg)	Butorphanol (mg/kg)
III	5	Im	10	0.5	0
			15	0.25	0
			15	0.5	0
			25	0.25	0
IV	6	Im	15	0.25	0
		Sc	15	0.25	0
			15	0.25	0.4
			15	0.5	0

Before each injection the drugs were mixed in one syringe. The rabbits were prepared with a cannula in the ear artery for monitoring of blood pressure and removal of arterial blood gas for analysis. The ear was locally anaesthetized before cannulation like described under inhalation anaesthesia. ECG was measured like in the inhalation anaesthesia studies. Before induction of anaesthesia, recordings of heart rate, mean arterial blood pressure (MAP) and ECG were measured for 2 min and an arterial blood sample was obtained (t = 0). Heart rate, respiratory rate and body temperature were recorded at 5, 10, 15, 30, 45, 60, 75, 90 and 120 minutes. Depth of anaesthesia was evaluated at each time point by evaluation the righting response and the reaction to ear pinch and toe pinch.

An auto-regulated heat-pad with a rectal probe was used and set to 38 ° C to maintain body temperature. In study III, attempts were made to intubate the trachea in a blind fashion after loss of the righting reflex (tube size 3.0) and the tube was then immediately withdrawn. After 30 min of anaesthesia, 100 % oxygen was administered by facemask at a flow of 3.5 l/min for the remaining anaesthetic period in study III. Once the righting reflex was regained the rabbits were placed in a heated incubator until fully recovered.

Statistical analysis: In study III dose effects on physiological variables were examined using the SPSS GLM repeated measures procedure. This was also used to compare the duration of surgical anaesthesia and sleep time between groups. In study IV the effects of dose combinations and the route of administration were compared using repeated measures ANOVA, to establish overall group differences. Further univariate comparisons determined differences between routes of administration.

3.2   STUDIES IN RATS (V-VII)

3.2.1   Animals

In rats the effects of repeated anaesthesia with ketamine and medetomidine and the effect of pre-medication with buprenorphine were evaluated. Further, the effects of the anaesthetic combination sufentanil/medetomidine were evaluated, as well as the effect of administration route, and the reversal of anaesthesia with butorphanol and atipamezole.

For the study of repeated anaesthesia 22 male outbred Wistar rats aged 5-6 weeks were used (Table 4). For the sufentanil/medetomidine study, 23 male and female outbred Wistar rats aged 6-8 weeks were used (Table 5).

The effect of anaesthetic choice on the recovery of body weight and postoperative pain related behaviour was studied after splenectomy in rats. In this study, 56 male outbred Sprague-Dawley rats, aged 8-10 weeks were used. All rats originated form specific pathogen free breeding facilities, and the only evidence of pathogens was found by health monitoring in the animal facility where the Sprague-Dawley rats were housed (Helicobacter spp and *Clostridium piliforme*).

All rats were group-housed, 4-6 in plastic cages (floor area 1820 or 2128 cm^2) with sawdust or wooden chips. Food pellets and water was administered *ad libitum*. Room temperature was 20 ± 2 ºC, humidity 50 ± 10 % and the light cycle length varied between the studies (9-13h of light during the day time). Body weight was recorded before the study, by which the rats were accustomed to handling.

3.2.2   Repeated anaesthesia with ketamine/medetomidine (V)

3.2.2.1   Anaesthesia

Ketamine and medetomidine were mixed and diluted to give a suitable volume for intraperitoneal (ip) dosing. Anaesthesia was repeated 6 times at weekly intervals. In one group (n = 11), buprenorphine was administered sc one hour prior to anaesthesia induction (Table 4). After loss of the righting reflex, rats were placed on a heated pad, and covered to maintain a body temperature of 37.5 - 39 º C.

Table 4: Doses of ketamine/medetomidine/buprenorphine for repeated anaesthesia in Wistar rats

Study	N	Ketamine (mg/kg) ip	Medetomidine (mg/kg) ip	Buprenorphine (mg/kg) sc
V	11	60	0.4	0
	11	45	0.3	0.05

3.2.2.2   Recordings and statistics

Pedal withdrawal response, righting reflex and respiratory rate were assessed every 15 min until the righting reflex returned. Pedal withdrawal responses were assessed by pinching the hind foot metacarpal region, and were scored from 0 (no response) to 3 (strong withdrawal). For each group, means were calculated for pedal withdrawal score, respiratory rate, duration of surgical anaesthesia and total sleep time. Repeated-

measures ANOVA was used to compare data between successive anaesthesia days. On each day, one or more rats failed to develop surgical anaesthesia and therefore only days 1-4 could be incorporated in the repeated measures ANOVA. By calculation of means for each type of assessment over all anaesthesia days, one-way ANOVA could be used for comparing groups.

3.2.3 Sufentanil/medetomidine anaesthesia (VI)

3.2.3.1 Anaesthesia

Medetomidine was diluted and mixed with sufentanil and the combination administered by intraperitoneal or subcutaneous injection on up to four occasions. The treatment order was randomized for each group and equal numbers of females and males were used for each dose combination. Using rats of both sexes may cause a larger variation in response, and thereby necessitate a larger number of animals. Some anaesthetic drugs are metabolised more rapidly in male than female rats, due to higher concentrations of P450 liver isoenzymes (Ciccone & Holdcroft, 1999).

A minimum of one week was given between anaesthetics. After loss of the righting reflex, rats were placed on a heat pad, to maintain a body temperature of 36-38 º C. From an initial dose ranging study five dose combinations were selected.

Table 5: Doses and routes of administration of sufentanil/medetomidine in Wistar rats

Study	Route	N	Sufentanil (µg/kg)	Medetomidine (µg/kg)
VI	sc	6	40	150
		10	50	150
	ip	10	50	300
		6	60	300
		6	80	300

3.2.3.2 Recordings

Righting reflex, pedal withdrawal response, tail-pinch reflex, respiratory rate, heart rate and blood O_2 saturation were assessed every 15 min until the righting reflex returned. Tail pinch reflex was assessed by pinching the tip of the tail and scored like the pedal withdrawal reflex, from 0 to 3. Heart rate and O_2 saturation were measured by pulse oximetry with a sensor placed on one hind foot.

After completion of the main study, six animals were re-anaesthetized (sc 40/150 µg/kg sufentanil/medetomidine). After local lignocaine infiltration, the right carotid artery was cannulated with a 22 G catheter and blood gas tension was measured in 1-5 carotid arterial samples per animal. Simultaneously, pulse oximetry data was collected.

A further six animals were used to assess the efficacy of reversal of anaesthesia. Thirty minutes after induction of anaesthesia (sc 40/150 µg/kg sufentanil/medetomidine), atipamezole (0.5 mg/kg) and butorphanol (0.2 mg/kg) were given as a single subcutaneous injection. Reversal was assessed according to the latency of the ability to perform the righting reflex.

3.2.3.3 Data analysis

Calculations were made of latency until loss of righting reflex, pedal withdrawal and tail pinch reflexes. At 30 and 60 min post-injection mean respiratory rate, blood O_2 saturation, as well as tail pinch and pedal withdrawal scores, were collected for each dose combination. Means were compared between groups by one-way ANOVA.

3.2.4 Postoperative pain and recovery (VII)

3.2.4.1 Anaesthesia

Induction of anaesthesia was achieved by ip injection of a mixture of ketamine/medetomidine (75/0.5 mg/kg), or by inhalation of 5 % isoflurane in 100 % oxygen (flow rate 1 l/min) in a Plexiglas chamber. Rats were pre-treated with carprofen (5 mg/kg in a volume of 1 ml/kg) or saline (1 ml/kg) 90 minutes before induction of anaesthesia, and repeatedly for two days after surgery. After loss of righting reflex, animals were prepared for surgery by hair clipping and skin disinfection of the upper abdomen. The animals were placed on a heated surgical table, and additionally an infrared lamp was provided to maintain a body temperature of 37 ± 1 º C.

Isoflurane anaesthesia was maintained by a facemask, at a concentration of 3 % in oxygen, at a flow of 1 l/min. Rats anaesthetized with ketamine/medetomidine, received pure oxygen via the facemask (1 l/min). In animals that regained a positive pedal withdrawal reflex 20 min after ketamine/medetomidine injection, an additional 25 % of the original dose was administered ip. After completion of surgery, animals were placed in a heated chamber until they were fully awake. Rats that had received ketamine/medetomidine, were injected sc with atipamezole (1 mg/kg). Control groups included untreated rats, as well as rats undergoing isoflurane or ketamine/medetomidine anaesthesia without surgery. After recovery, rats were returned to their home cage.

3.2.4.2 Surgery

The spleen was removed after a midline incision in the upper abdominal wall. Two 4-0 vicryl ligatures were applied to the mesenteric vasculature and the abdominal wall and the skin were closed in two layers with 4-0 PDS sutures. The skin was sutured intracutaneously. The duration of surgery was approximately 30 min.

3.2.4.3 Evaluation of behaviour

Behaviour was recorded on six consecutive days, beginning on the day before surgery. For recording of pain related behaviour, rats were filmed one at a time with a video camera, for 10 min, in a Makrolon III cage with bedding. Immediately following, behaviours were automatically recorded with the LABORAS system, for one hour before, and one hour after the room lights were switched off. The LABORAS system is based on vibration and force signal analysis to determine both the behavior and the position of the animal (Van de Weerd *et al* 2001). For this**,** rats are singly kept in MAK III cages with food and water, and the duration and frequency of locomotion, rearing, grooming, eating and drinking, as well as maximum speed is automatically recorded and stored on a computer hard disc.

A trained observer scored pain related behaviour from the video recordings. The observer was unaware of which type of anaesthesia had been used and whether the

rats had received analgesic treatment. A validated pain scoring system developed by Roughan and Flecknell (2001) was used, in which the frequency of the following behaviours are counted: twitch, fall/stagger, back arch and abdominal stretch (writhing).

3.2.4.4 Statistical analysis

Body weight changes were compared between groups by one-way ANOVA and within groups by repeated measures ANOVA. Non-parametric analysis was used for behavioural measurements; within group analysis was performed with Friedman repeated measures ANOVA on ranks and between groups analysis was performed with Kruskal-Wallis ANOVA on ranks. A p-value < 0.05 was considered statistically significant.

4 RESULTS AND DISCUSSION

4.1 STUDIES IN NZW RABBITS (I-IV)

4.1.1 Induction with inhalation anaesthesia (I-II)

4.1.1.1 Baseline data

Induction of anaesthesia with sevoflurane and desflurane was evaluated in order to find a volatile agent that rabbits tolerate. After instrumentation, before induction of anaesthesia, rabbits were tachypnoeic and tachycardic (Table 6). Group-housed undisturbed resting rabbit have a mean respiratory rate of 59 ± 9 per minute (own data based on visual observation). Baseline heart rate in female mixed breed rabbits has been estimated to 147-161 bpm, and MAP to 78-82 mm Hg in a telemetry study (van den Buuse & Malpas 1997). Baseline respiratory rates differed significantly between groups in study II, between the slow desflurane group and the two other groups, respectively (Table 6). The baseline values in study II indicate that the rabbits on the whole were probably more stressed than in study I. There were no other major differences in baseline data between groups. Pre-oxygenation did not cause significant changes of baseline data.

Table 6: Baseline values of respiratory rate, heart rate and MAP in NZW rabbits

Study	N	Treatment	Respiratory rate/min (mean ± SD)	Heart rate/min (mean ± SD)	MAP (mean ± SD)
I	6	Slow isoflurane	188 ± 32	199 ± 41	80 ± 11
		Slow sevoflurane	227 ± 44	226 ± 68	71 ± 11
		Rapid sevoflurane	242 ± 52	184 ± 9	78 ± 8
II	5	Slow isoflurane	270 ± 47*	217 ± 13	63 ± 9
		Rapid desflurane	300 ± 40*	235 ± 24	67 ± 8
		Slow desflurane	349 ± 22	216 ± 11	76 ± 10

* $p < 0.01$, significantly different from slow desflurane

4.1.1.2 Effects of vapours on respiratory parameters and reactions during induction

After induction of anaesthesia with isoflurane, sevoflurane and rapid desflurane, all animals became apnoeic within the first 60 s of exposure. After a variable period of apnoea (30-190 s) a breath was taken, followed by further periods of apnoea. Time to onset of apnoea was longer and more variable during slow induction with desflurane (302 ± 296 s). Due to large interindividual variation within groups, the maximum recorded period of apnoea was not significantly different between groups, even though the mean value varied almost fourfold (Table 7). Apnoea eventually subsided and a normal respiratory pattern was re-established as the animals lost consciousness. Due to the fact that the rabbits had to be rigorously restrained, and due to their low tidal volume (20-30-ml), it would have been difficult to detect the apnoea without a respiratory monitor. It is likely that the apnoea along with the considerable stress caused by inhaling volatile anaesthetics, poses an increased anaesthetic risk. The

volatile anaesthetic halothane is known to sensitize the myocardium to catecholamines, which may induce severe ventricular arrhythmias (Hashimoto 2007). However, this sensitizing effect is specific for halothane, and not shared by the newer inhalant anaesthetics isoflurane, sevoflurane or desflurane (Eger 1992). Another risk factor during anaesthesia is ongoing respiratory infections, which can interfere with blood oxygenation (Lipman *et al* 1997). Before introduction of strict microbiological barrier housing, rabbits often had subclinical infections with respiratory agents (e.g. Pasteurella spp).

Table 7: Periods of apnoea during induction with volatile anaesthetics in NZW rabbits

Study	N	Treatment	Periods of apnoea in s (mean, range/SD)
I	6	Slow isoflurane	97 (30-180)
		Slow sevoflurane	85 (30-150)
		Rapid sevoflurane	95 (30-140)
II	5	Slow isoflurane	39 ± 22
		Rapid desflurane	72 ± 67
		Slow desflurane	25 ± 11

Due to these apnoeic periods, a gradual increase in inspired anaesthetic concentration was not achieved. Instead, when the rabbits finally took a breath, the vaporiser setting had been increased, and the inhaled concentration rather high. The irritating effects of volatile anaesthetics increase with higher concentrations (Mutoh *et al* 2001). Most animals excessively attempted to escape from the restraint upon exposure to isoflurane and sevoflurane. With desflurane, struggling was mild, especially with the slow induction technique (a mean of 2 escape attempts compared with 14 for isoflurane). Struggling suggests that inhalation of sevoflurane and isoflurane causes considerable distress, and it appears as if desflurane is a better choice regarding animal welfare.

These reactions in rabbits are different from those seen in man, in which single-breath induction with high concentrations of sevoflurane is well tolerated, while desflurane causes coughing, laryngospasm and breath holding in concentrations of 6-8 % (Young & Apfelbaum 1995). Hypothetically these species differences in reactions could be related to differences in the sensitivity of primary afferent nerve ending in the nasopharynx to various chemical compounds. A previous study showed that administration of the sedatives acepromazine (phenotiazine derivate) and medetomidine (alpha-2-adrenergic agonist) could prevent struggling, but not breath-holding, during induction (Flecknell & Liles 1996).

4.1.1.3 *Time to loss of reflexes*

Time to loss of righting reflex occurred significantly faster with rapid desflurane induction (Table 8). With slow desflurane induction, the time was much more variable. Time to loss of pedal withdrawal reflex, was significantly longer with slow desflurane induction than with rapid desflurane or isoflurane. The long induction time with slow desflurane administration makes this alternative very unpractical.

Mean time to loss of pedal withdrawal reflex with sevoflurane was not different from isoflurane. No differences were found between isoflurane and desflurane

groups for the time required to return of the righting reflex, once the vaporizer was switched off (approx 3 min).

Table 8: Time to loss of reflexes during induction with volatile anaesthetics in NZW rabbits

Study	N	Treatment	Time to loss of righting reflex in s (mean ± SD)	Time to loss of pedal withdrawal reflex in s (mean, range/SD)
I	6	Isoflurane	NA	240-420
		Sevoflurane	NA	350-420
		Sevoflurane 8 %	NA	240-360
II	5	Isoflurane	205 ± 48 §	390 ± 30*
		Rapid desflurane	139 ± 27*	230 ± 30 *
		Slow desflurane	337 ± 160	1150 ± 200 (mean ± SD)

§ p < 0.05 significantly different from rapid desflurane
* p < 0.05 significantly different from slow desflurane
NA: data not available

4.1.1.4 Effects on cardiovascular parameters

During apnoeic period, animals showed a significant reduction in heart rate, during isoflurane, sevoflurane and rapid desflurane induction (Table 9). The difference in the reduction of heart rates between the two studies may reflect a difference in stress levels. The heart rate decreased to a minimum within 2 min of rapid desflurane and isoflurane induction. During slow desflurane induction, heart rate increased slowly to a maximum of 279 ± 32 after 26 minutes.

Table 9: Changes in heart rate during induction with isoflurane, sevoflurane and desflurane in NZW rabbits (mean ± SD)

Study	N	Treatment	t=0	t=low
I	6	Isoflurane	199 ± 41	45 ± 11*
		Sevoflurane	226 ± 68	57 ± 32*
		Sevoflurane 8 %	184 ± 9	24 ± 4*
II	5	Isoflurane	217 ± 13	145 ± 69*
		Rapid desflurane	235 ± 24	185 ± 40*
		Slow desflurane	216 ± 11	216 ± 11

t=0: time before induction. t=low: lowest heart rate measured during induction
* p < 0.05 significantly different from t=0 in the same animals

Noxious vapours can elicit apnoea and bradycardia by the so-called nasopharyngeal reflex. This is activated by trigeminal afferents in the nasal mucosa (Nalivaiko et al

2003). The resulting cardiovascular pattern is similar to that seen with the related diving reflex. Vagally mediated bradycardia, which reduces cardiac oxygen consumption, and sympathetically mediated vasoconstriction, function to preserve oxygen to the heart and brain. Saturated formaldehyde vapour can be used to elicit the nasopharyngeal reflex in rabbits, which causes a reduction in heart rate of a similar magnitude as isoflurane (Nalivaiko *et al* 2003). Sedation of rabbits with acepromazine or medetomidine was shown to reduce the magnitude of bradycardia, even if breath holding was not prevented during induction with halothane (Flecknell & Liles 1996).

4.1.1.5 *Effects on blood gas values*

Pre-oxygenation increased baseline values of PaO_2 from 13 to 50-60 kPa. Within the first four minutes of induction (during apnoea), values fell significantly more during isoflurane induction than during rapid desflurane induction (Table 10). Slow desflurane induction only caused a small and gradual reduction at the end of induction. Blood $PaCO_2$ values increased significantly more during isoflurane induction than during rapid desflurane induction. Blood pH values did not differ between groups.

Table 10: Extreme values in arterial PaO_2 and $PaCO_2$ in kPa during induction with isoflurane, sevoflurane and desflurane in NZW rabbits (means ± SD)

Study	N	Treatment	PaO_2 at t=low	$PaCO_2$ at t=high
I	6	Isoflurane	24 ± 17	7.6 ± 0.4
		Sevoflurane	17 ± 10	7.6 ± 0.4
		Sevoflurane 8 %	12 ± 1	7.5 ± 0.9
II	5	Isoflurane	20 ± 17*	8.6 ± 1.0
		Rapid desflurane	44 ± 7	7.4 ± 1.0
		Slow desflurane	52 ± 1	5.0 ± 0.5

t=low: lowest PaO_2 measured during induction. t=high: highest $PaCO_2$ measured during induction. * $p < 0.05$ significantly different from rapid desflurane

Because oxygen had been pre-administered, hypoxia did not develop despite the long periods of apnoea. When PaO_2 levels decrease to levels < 8 kPa, tissue oxygenation is significantly impaired (Mason & Brown 1997). If oxygen had not been provided, it is possible that the apnoeic periods had been of a shorter duration, due to the hypoxic drive caused by low levels of oxygen. It is however not recommended to allow hypoxia to develop during induction of anaesthesia.

4.1.2 Injection anaesthesia with ketamine/medetomidine (III-IV)

The effects of ketamine/medetomidine anaesthesia were evaluated in NZW rabbits, including the route of administration and the addition of butorphanol.

4.1.2.1 *Time to loss of reflexes and duration of anaesthesia*

Induction was smooth and rabbits seemed to mind subcutaneous injection less than intramuscular injection. The time to loss of righting reflex did not differ between

injection routes, or change by addition of butorphanol. The time was 5 ± 3 min (mean $\pm$ SD) for the ketamine/medetomidine/butorphanol group.

Duration of surgical anaesthesia and sleep time were significantly increased when the im medetomidine dose was increased from 0.25 to 0.5 mg/ kg (Table 11). Increasing the dose of ketamine from 15 to 25 mg/kg also led to increased duration of surgical anaesthesia.

From study III it was concluded that earlier recommended doses in rabbits for combinations of ketamine (25 mg/kg) and medetomidine (0.5 mg/kg) (Becker 1996, Flecknell 1996) seem unnecessary high. In study IV however, the same dose combinations that worked well in study III, only induced surgical anaesthesia in half of the animals, and a statistical comparison was not considered meaningful. The reason for the differences in reaction between the two studies may be due to the stress level at the time of induction. Another possible source of variation in reaction to anaesthesia is different levels of hormones during the oestrus cycle, which have been shown to have subtle effects on anaesthetic responses (Lipman *et al* 1997). The duration of loss of ear pinch reflex was however significantly longer for the high-dose sc ketamine/medetomidine (mean $\pm$ SD: 75 ± 22 min), than the lower dose given either sc (41 ± 23 min) or im (44 ± 19 min). The duration was also significantly increased by the addition of butorphanol (78 ± 30 min).

Table 11: Duration of surgical anaesthesia and sleep time during ketamine/medetomidine/butorphanol anaesthesia in NZW rabbits (mean $\pm$ SD). Individual values are shown in groups where some of the rabbits never reached a level of surgical anaesthesia.

Study	N	Route	Ket/med/but (mg/kg)	Surgical anaesthesia (min)	Sleep time (min)
III	5	Im	10/0.5	28 ± 27 (n=3)	108 ± 12
			15/0.25	27 ± 28 *§	86 ± 22 *
			15/0.5	59 ± 18	111 ± 11
			25/0.25	57 ± 12	103 ± 23
IV	6	Im	15/0.25	30,30,30	86 ± 26
		Sc	15/0.25	10, 35, 50	79 ± 29
			15/0.25/0.4	45, 50, 70, 90	107 ± 11
			15/0.5	35, 45, 60	109 ± 19

* $p < 0.05$ significantly different from im15/0.5 mg/kg
§ $p < 0.05$ significantly different from im 25/0.25 mg/kg

The same dose combinations are probably not suitable for all rabbit strains. In a study by Nevalainen (1989) pigmented rabbits were not surgically anaesthetized even with 60/0.5 mg/kg of ketamine/medetomidine. In rats and mice, significant differences exist between albino and pigmented strains in the sensitivity to and metabolism of anaesthetic drugs (Creel 1980). Albino strains require lower doses than pigmented strains to achieve a similar effect from e.g. pentobarbital and sleep longer, probably due to lower levels of certain cytochrome P-450 isoforms.

4.1.2.2 Effect on respiratory and cardiovascular parameters

Respiratory rates dropped significantly more in NZW rabbits that had received butorphanol compared with those that did not (Table 12). This was however not reflected in the arterial PaO_2 or $PaCO_2$ levels.

Bradycardia developed during anaesthesia, but was not different between groups (Table 11). Hypotension also developed in all groups, and the lowest mean arterial blood pressure measured in any group was 57 ± 14 mm Hg (mean $\pm$ SD). It is recommended that blood pressure should be maintained over 60 mm Hg during anaesthesia for adequate tissue perfusion (Mason & Brown 1997).

Table 12: Lowest respiratory rates and heart rates after ketamine/medetomidine/butorphanol anaesthesia in NZW rabbits

Study	N	Route	Ket/med/but (mg/kg)	Respiratory rate per min at t=low (mean ± SD)	Heart rate per at t=low (mean ± SD)
III	5	Im	10/0.5	34 ± 6	153 ± 13
			15/0.25	38 ± 9	138 ± 14
			15/0.5	46 ± 13	141 ± 6
			25/0.25	49 ± 5	135 ± 18
IV	6	Im	15/0.25	49 ± 17§	157 ± 27
		Sc	15/0.25	49 ± 8 §	166 ± 24
			15/0.25/0.4	31 ± 13*	166 ± 17
			15/0.5	46 ± 17	152 ± 30

t=low: the lowest measured value during induction of anaesthesia
* $p < 0.05$, significantly different from sc 15/0.5 mg/ kg
§ $p < 0.05$, significantly different from sc 15/0.25/0.4 mg/kg

All animals were visibly cyanotic 10-15 minutes after induction, when hypoxia was most pronounced, but there were no significant differences between groups. The lowest arterial PaO_2 measured in any group was 4.8 ± 0.6 kPa (mean $\pm$ SD). PaO_2 levels < 8 kPa indicate significant impairment of tissue oxygenation and should lead to measures to improve oxygenation (Mason and Brown 1997). Supplementation of oxygen via mask is the easiest measure, but more efficient is to additionally mechanically ventilate the animals. However, ventilation requires that animals be intubated, and blind intubation was easy to perform in these rabbits, after induction of anaesthesia. The highest $PaCO_2$ levels in any group was 6.78 ± 0.8 kPa, and levels > 6 kPa indicate hypoventilation. Hypercapnia was most pronounced 15-60 min after induction. With mechanical ventilation it is possible to improve ventilation and restore $PaCO_2$ levels. The lowest pH in blood found in any of the groups was 7.35 ± 0.01 (mean $\pm$ SD).

4.2   STUDIES IN RATS (V-VII)

4.2.1   Repeated anaesthesia with ketamine/medetomidine

The effects of repeated ketamine/medetomidine anaesthesia six times, with weekly intervals, was evaluated in Wistar rats, and the effects of pre-medication with buprenorphine.

4.2.1.1   Duration of surgical anaesthesia and sleep time

Pre-medication with buprenorphine significantly increased duration and depth of anaesthesia. Surgical anaesthesia was increased from 104 ± 16 min (mean $\pm$ SD) to 146 ± 20 min. Depth of anaesthesia was measured by the pedal withdrawal score (Fig 2). Mean sleep time was also increased, from 157 ± 34 to 187 ± 22 vs. min (mean $\pm$ SD).
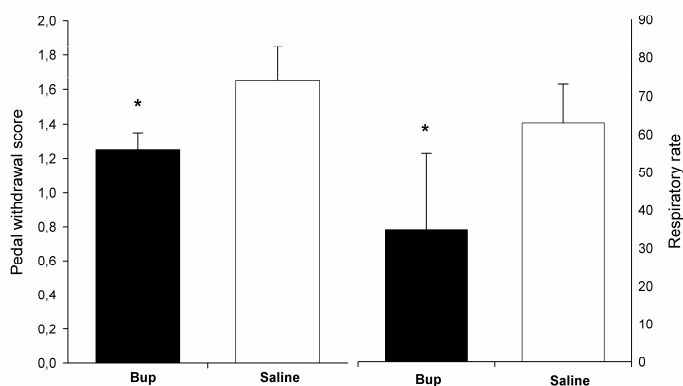

Fig 2: Study V: mean pedal withdrawal score and respiratory rate in Wistar rats anaesthetized with ketamine/medetomidine and pre-treated with buprenorphine or saline. Bup = buprenorphine pre-treated rats (0.05 mg/kg). * p < 0.05 significantly different from saline pre-treated rats.

Overall, with both groups included in the analysis, there were no significant effects of repeated anaesthesia on any variable. When the groups were independently examined, sleep time was significantly reduced between days 1 and 4 in the buprenorphine group (from 217 ± 44 to 168 ± 38 min). Duration of surgical anaesthesia was also reduced (from 178 ± 11 to 115 ± 36 min). Animals that did not receive buprenorphine, showed an increase in sleep times from day 1 to day 4 (from 123 ± 39 to 195 ± 31 min). Repeated anaesthesia with ketamine alone has been reported to progressively decrease anaesthetic effects in rats (Livingstone & Waterman 1978).

4.2.1.2   Effects on respiration

Pre-treatment with buprenorphine decreased respiratory rate (Fig 2). Two animals in the buprenorphine group died, after showing progressive reduction of respiratory rates. Attempts to use pulse oximetry were not successful, probably due to peripheral vasoconstriction produced by medetomidine. Otherwise, the anaesthetist might have

been alerted to impending problems and could have administered atipamezole in order to reverse the effects of medetomidine.

Since lower dose of ketamine and medetomidine than we used failed to consistently produce surgical anaesthesia following 0.05 mg/kg of buprenorphine (Roughan *et al* 1999), it seems that the margin of safety for this combination is rather low. Buprenorphine has a longer duration of action compared with butorphanol, which was our reason for choosing buprenorphine. Buprenorphine has however been shown to cause a greater degree of respiratory depression than butorphanol when used in conjunction with anaesthesia (Pircio *et al* 1976, Cowan *et al* 1977). In species such as the dog or pig, ketamine/medetomidine/butorphanol has been reported to provide safe and effective anaesthesia (Sakaguchi *et al* 1996, Tomizawa *et al* 1997), and the combination is frequently used in dogs and cats in small animal practice in the UK. However, our results show that repeated anaesthesia with ketamine/medetomidine alone seems to be safe.

4.2.2 Sufentanil/medetomidine anaesthesia (VI)

4.2.2.1 Time to induction and duration of anaesthesia

The aim was to evaluate the effects of anaesthesia, and of reversal with butorphanol/atipamezole, and to compare intraperitoneal with subcutaneous injection.

Subcutaneous administration proved more efficient than intraperitoneal injection. All rats in the sc dose groups (40/150 μg and 50/150 μg/kg sufentanil/medetomidine) lost the pedal withdrawal reflex, whereas after ip injection this was the case only in the highest dose group (80/300 μg/kg). In the two lower ip dose groups (50/300 and 60/300 μg/kg) only 4/8 and 4/5 rats lost the pedal withdrawal reflex. The righting reflex was lost within 7 min in all animals in the sc dose groups, whereas five rats that were dosed by the ip route never lost the righting reflex. For the remaining animals dosed by the ip route, it took up to 24 min to lose the reflex.

The pedal withdrawal reflex was lost within 15 min for all animals in the sc dose groups and 30 min in the ip dose groups. Another 10-15 min were needed for muscle relaxation to be maximized, which is consistent with reports of pharmacokinetics of medetomidine in the rat (Salonen 1989).

The duration of surgical anaesthesia was not different between the sc dose groups, whereas it was significantly longer in the highest ip dose group compared to the other two ip dose groups (Table 13).

The differences in effect after administration via different routes are probably due to a certain degree of first-pass hepatic metabolism after intraperitoneal injection. With intraperitoneal injection there is also the risk of injecting all or part of the solution in the intestines or retroperitoneal space (Steward *et al* 1968). With subcutaneous administration on the other hand, first pass liver metabolism is avoided and thereby a lower dose may be used to reach the equivalent plasma concentrations (Cerletti *et al* 1980). Subcutaneous administration is also technically easier for inexperienced persons, and seemingly less stressful to the animal.

Table 13: Duration of surgical anaesthesia in Wistar rats after injection with
sufentanil/medetomidine in different dose combinations sc and ip.

Study	Route	Dose sufentanil/medetomidine	Duration of surgical anaesthesia (min)
VI	Sc	40/150	101 ± 49
		50/150	124 ± 45
	Ip	50/300	13 ± 15 *
		60/300	21 ± 20*
		80/300	76 ± 23

* $p < 0.05$, significantly different from 80/300 mg/kg

4.2.2.2 *Effects on respiration*

All rats except those that received the lowest ip dose combination developed respiratory
depression (<50 % of the resting rate) within 30 min of drug administration. This
persisted for up to one hour. Some animals in both sc and ip dose groups were visibly
cyanotic. One rat in the sc 40/150 μg/kg dose group showed several periods of apnoea,
but recovered without the need of reversal with butorphanol/atipamezole.

Rats that received the highest ip dose combination (80/300 μg/kg) showed a
significantly greater reduction in O_2 saturation levels at 30 and 60 min compared with
the two lower ip dose combination groups. The lowest O_2 saturation level was at 30
min: 40 ± 26 % (mean $\pm$ SD), to be compared with 77 ± 12 % for the lowest ip dose
combination (50/300 μg/kg). Despite low O_2 saturation levels, all animals recovered
uneventfully. Low PaO_2 and high $PaCO_2$ levels were associated with low O_2 saturation
levels measured with pulse oximetry. Oxygen saturation levels below 80 % were
associated with PaO_2 levels of < 10kPa and those below 40 % with values of < 6 kPa.

4.2.2.3 *Reversal of anaesthesia*

After reversal of anaesthesia in six animals with sc butorphanol/atipamezole, the
righting reflex returned within 7 min in all animals. Recovery was abrupt and the
animals developed a "Straub tail" (opioid effect). Within 15 minutes, the animals
appeared fully recovered.

4.2.3 Postoperative pain and recovery (VII)

4.2.3.1 *Body weight change*

The study was undertaken to examine the influence of anaesthetic choice on recovery
and pain related behaviour after abdominal surgery in Sprague-Dawley rats.

Rats that underwent surgery under ketamine/medetomidine anaesthesia
without analgesia, lost more body weight during 6 h after surgery than untreated control
rats (-9 $\pm$ 7.1 vs. –0.9 $\pm$ 1.8 g). This was not the case in rats that underwent surgery
under isoflurane anaesthesia without analgesia, or in rats that received perioperative
analgesic treatment.

Four days after surgery, these rats had still gained less body weight
compared to unoperated controls (Fig 3). Rats in other groups were not different from
controls, although a tendency to impaired gain in body weight was seen in all rats that
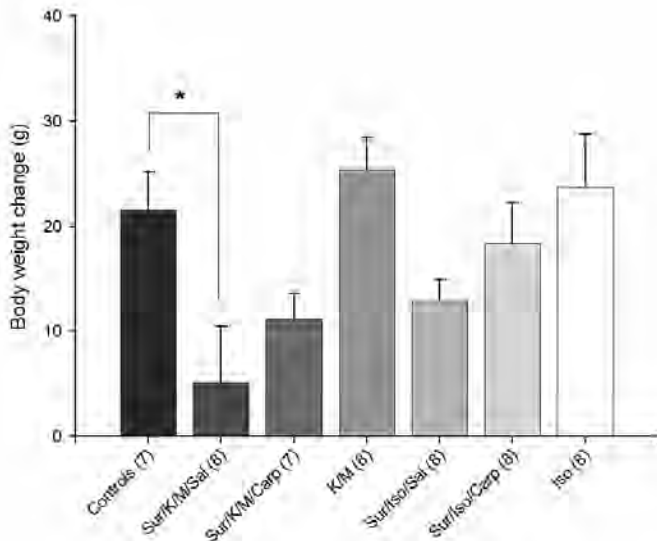underwent surgery.

Fig 3: Study VII: Mean body weight increase four days after abdominal surgery (Sur) in Sprague-Dawley rats during ketamine (K)/medetomidine (M) or isoflurane (Iso) anaesthesia. Rats were treated with carprofen (Carp) or saline (Sal) before and two days after surgery. Control groups include anaesthesia without surgery and untreated animals. * $p < 0.05$

4.2.3.2   Behaviour measured with LABORAS

Rats that underwent surgery under ketamine/medetomidine anaesthesia without analgesic treatment moved significantly less and at a slower speed eight hours after surgery, compared to un-operated controls (Fig 4). These differences were detected in the dark period. From the day after surgery, locomotion was not different from untreated animals in any of the operated groups.

Rats that were operated under isoflurane or ketamine/medetomidine anaesthesia without analgesic treatment also moved less eight hours after surgery, compared with baseline values (Fig 4). Locomotion was also reduced by ketamine/medetomidine anaesthesia alone, without surgery, compared to baseline values, and was still reduced on the next day (day 1). Isoflurane anaesthesia alone had no effect on locomotion.
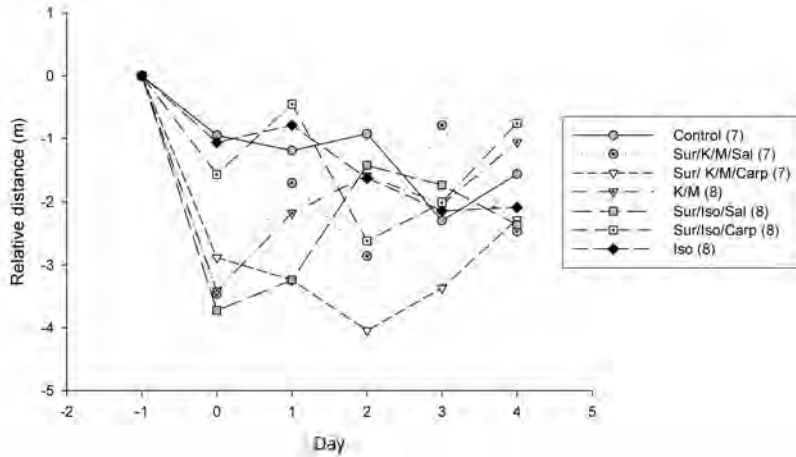
Fig 4: Study VII: Mean relative distance travelled 8 hours after abdominal surgery (Sur) under ketamine (K)/medetomidine (M) or isoflurane (Iso) anaesthesia, when measured in the dark. Sprague-Dawley rats were treated with carprofen (Carp) or saline (Sal) before and two days after surgery. Distance was measured with LABORAS during one hour and control groups include rats undergoing anaesthesia alone (no surgery) and untreated animals.

4.2.3.3 Pain related behaviour

Rats undergoing surgery with ketamine/medetomidine anaesthesia showed a higher median cumulative pain score compared with controls (Fig 5). A significantly higher pain score was seen on the day of surgery, compared with the day before, in groups undergoing surgery with ketamine/medetomidine anaesthesia, irrespective of carprofen administration (2/2/2.25 and 2/2/2.75 vs. 0/0/0). No increase in pain scores was seen after surgery performed under isoflurane anaesthesia. This was probably due to the fact that the first scoring was not performed until 6h after surgery (due to the effects of ketamine interfering with scoring during 6h after surgery). In a study performed in rats undergoing laparotomy under isoflurane anaesthesia, significant pain related behaviour could only be detected during the first 5 h after surgery (Roughan and Flecknell 2001).
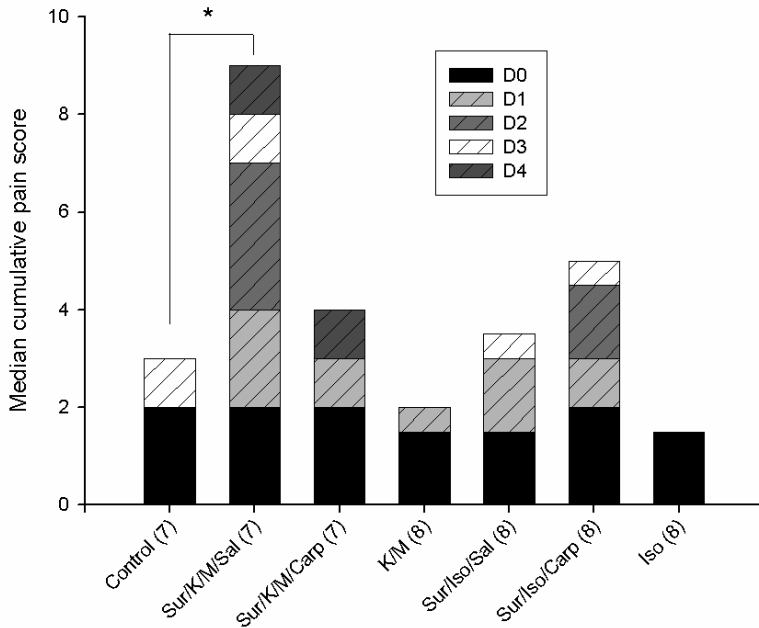
Fig 5: Study VII: Median total pain score at the day of surgery (D0) during four days after (D1-D4) abdominal surgery in Sprague-Dawley rats under ketamine (K)/medetomidine (M) or isoflurane (Iso) anaesthesia. Rats were treated with carprofen (Carp) or saline (Sal) before and two days after surgery. Control groups include animals undergoing anaesthesia alone and untreated rats. * $p < 0.05$

Surgery under isoflurane anaesthesia resulted in less signs of pain and less effect on body weight gain than surgery under ketamine/medetomidine anaesthesia. This was surprising, since ketamine and medetomidine are known to have antinociceptive actions via NMDA- and alpha-2-adrenergic receptors. The prediction was thus that these antinociceptive effects should have reduced centrally induced sensitization, and thereby the degree of postoperative pain, and that this form of anaesthesia would be the best choice to avoid postoperative pain. In a study of postoperative pain in cats, where pain scores were compared after abdominal surgery under either thiopentone/halothane anaesthesia or ketamine/medetomidine anaesthesia (Slingsby *et al* 1998), cats showed less pain related behaviour after ketamine/medetomidine anaesthesia.

Volatile agents like isoflurane however, also have specific antinociceptive effects that are separable from their hypnotic and amnesic properties. Isoflurane interacts with descending pain inhibitory pathways, acting on spinal α_{2A} adrenoceptors (Kingery *et al* 2002), and 5-HT$_{2A}$ receptors Zhang *et al* (Zhang *et al* 2003a), and also potentiates glycine transmission (Zhang *et al* 2003b). Volatile agents also open a type of potassium channels, which are thought to provide baseline regulation of membrane excitability (Franks and Honoré 2004). There may be differences between halothane and isoflurane in this respect, explaining the different outcomes in our rat study and the cat study referred to above (Slingsby *et al* 1998).

5 GENERAL DISCUSSION

5.1 INDUCTION WITH INHALATION ANAESTHESIA IN NZW RABBITS

The work in this thesis shows that NZW rabbits respond with struggling, reflex apnoea and bradycardia at induction with the halogenated volatile anaesthetics isoflurane, sevoflurane and desflurane. The struggling indicates that these agents are highly aversive to rabbits and cause distress, which is not consistent with good animal welfare. There is also a risk of injury to the animal from struggling. The major physiological effects that reflex apnoea leads to also poses a risk, and therefore induction with these agents is best avoided, and even contraindicated if animals are injured or in poor health. It is unfortunate that induction with volatile agents cannot be recommended for rabbits, since these agents show low toxicity and less effect on the cardiovascular system compared with many other anaesthetics (Brunson 1997). The ease of administration also favours mask induction with volatile agents. The search for a volatile induction agent that rabbits tolerate is therefore important.

Even though struggling and bradycardia can be reduced by pre-medication with sedatives, the benefits of using a single volatile agent for induction and maintenance would thereby be lost. The most important advantage with using inhalation anaesthesia is that concentrations of volatile anaesthetics can be measured continuously to ensure that all animals are at a similar anaesthetic depth (Brunson 1997), something that is important especially when experiments are performed during anaesthesia.

Breath holding seems to be more difficult to prevent than struggling. Sensory afferent fibres involved in the airway defence reflexes are of C-fibre type and their stimulation is known to elicit apnoea, coughing and bronchoconstriction in animals as well as humans (Mutoh *et al* 2002). Isoflurane and sevoflurane have been shown to stimulate capsaicin-sensitive C-fibres in the larynx in a dose-dependent manner (Mutoh *et al* 2001). The C-fibre induced activity may be inhibited by the use of local anaesthetics or opioids. Substance P is located in sensory afferent in tracheobronchial tissue and plays a role in bronchoconstriction (Nilsson *et al* 1977, Lundberg and Saria 1987). Morphine has been shown to inhibit substance P release from peripheral sensory nerve endings, and may thereby inhibit an inflammatory reaction (Brodin *et al* 1983). In humans, nebulized forms of opioids and lidocaine reduce airway reactivity in disease such as asthma and during bronchoscopy (Shirk 2006). Administration of nebulized drugs would be possible via facemask in rabbits before induction of inhalation anaesthesia. Another possibility to reduce breath holding may be by intravenous administration of a benzodiazepine, which has been shown to depress the sensitivity of upper airway reflexes in response to ammonia vapour in humans (Murphy *et al* 1994).

The reflex induced bradycardia is vagally mediated and can be triggered by noxious vapours, which activate trigeminal afferents in the nasal mucosa via the nasopharyngeal reflex (Nalivaiko *et al* 2003). The use of anticholinergic agents, like methylscopolamine, has been shown to inhibit the nasopharyngeal reflex elicited by inhalation of formaldehyde vapours in rabbits (Nalivaiko *et al* 2003). Glycopyrrolate, another anticholinergic agent, might also prove useful to prevent reflex bradycardia. Atropine would probably be less useful, due to its short duration of action in many rabbit strains, because of high levels of plasma atropinase (Olson *et al* 1994).

One radical way of circumventing the airway irritation induced by volatile anaesthetics would be by intravenous administration of the volatile fluid. The induction of anaesthesia is thereby accelerated, and airway side effects completely avoided. This can be achieved by mixing the anaesthetic in liquid form with a fat emulsion for intravenous use. Isoflurane has successfully been administered in mice and rats in this way (Eger & MacLeod 1995, Zhou *et al* 2006).

Finally, smooth induction could also be achieved by using a non-irritating inhalant anaesthetic agent. The noble gas xenon has many of the properties of an ideal anaesthetic agent, and is not irritating to the airways (Harris & Barnes 2008). It was first described to induce anaesthesia in mice in 1946 (Lawrence), and is licensed for use in humans. It causes a smooth induction and emergence from anaesthesia, has minimal effects on the cardiovascular system and is considered to be neuroprotective. So far, the relative high cost and limited availability has prevented a more widespread use.

5.2   INJECTION ANAESTHESIA IN NZW RABBITS

This thesis has shown that subcutaneous administration of ketamine/medetomidine in NZW rabbits is more efficient than intramuscular injection. Doses that produce surgical anaesthesia in most animals for 30-60 min were established, which is the time required for many surgical procedures. The inter-individual variation in duration and depth of anaesthesia can however be a problem in the research setting. One way of deepening the level of anaesthesia is to add a low concentration of isoflurane via facemask or tracheal tube. Since the combination of ketamine/medetomidine produces hypoxia to the extent that cyanosis is visible, provision of oxygen and even mechanical ventilation is warranted. When using this combination in young and healthy rabbits, the hypoxia that develops may not be life threatening, but the physiological effects must not be neglected. Even if rabbits seemingly recover well, the impact on the welfare of the animal as well as on the quality of research must be taken in account. When work is performed on sick rabbits, as models of disease, the risks when using this anaesthetic combination will be even greater.

Alternative anaesthetics for injection anaesthesia in rabbits are other combinations with ketamine, neuroleptanalgesic combinations (opioid with sedative) or combinations with propofol. Compared with these, ketamine/medetomidine has proven to more consistently produce surgical anaesthesia in rabbits or to cause less pronounced hypotension, but more pronounced respiratory depression (Hellebrekers *et al* 1997, Difilippo *et al* 2004, Henke *et al* 2005). An additional benefit of using ketamine/medetomidine is that the effect of medetomidine may be reversed pharmacologically, which decreases the time to recovery.

5.3   INJECTION ANAESTHESIA IN RATS

The combination of ketamine/medetomidine was found to be efficient and safe when administered six times in weekly intervals in Wistar rats. Surgical anaesthesia was consistently induced and sleep time increased with successive anaesthetic sessions. Medetomidine contains equal parts of two optical enantiomers, dexmedetomidine and levomedetomidine (Kuusela 2004). Levomedetomidine, the "inactive" medetomidine isomer, has been shown to prolong the hepatic metabolism of ketamine in studies with

human liver microsomes (Kharasch *et al* 1992). In rat liver microsomal incubations, both medetomidine enantiomers were able to inhibit the oxidative metabolism of several model substrates (Pelkonen *et al* 1991). This may explain why rats slept longer with repeated ketamine/medetomidine anaesthesia.

Pre-medication with buprenorphine increased duration and depth of anaesthesia but proved unsafe due to severe respiratory depression. In dogs, medetomidine has been shown to increase oxygen consumption of the myocardium, which adds to the decrease of oxygen saturation in medetomidine/ketamine anaesthesia (Vickery *et al* 1988). The reason for increase in oxygen consumption may be an increase in cardiac afterload and a decline of cardiac output, induced by bradycardia and vasoconstriction. The decrease in cardiac output may be up to 50 % (Sinclair 2003). Addition of buprenorphine will add to the decrease in heart rate, and possibly to the increase in oxygen consumption. Bradycardia may be counteracted by the administration of atropine, but even with intermittent administration of atropine during ketamine/medetomidine anaesthesia, the heart rate is lower than normal or under isoflurane anaesthesia, as has been shown in mice (Zuurbier *et al* 2002). However, it is possible that the safety margin of buprenorphine/ketamine/medetomidine anaesthesia may be increased by addition of atropine and oxygen.

A potential benefit of opioid administration is reduction of the anaesthetic dose needed to achieve surgical anaesthesia, and thereby reduction of dose-dependent side effects. Other benefits include reduced time to recovery and possibly a pre-emptive analgesic effect. Buprenorphine (0.03 mg/kg) has been shown to reduce the total dose of e.g. propofol needed for surgery in rats and to increase the level of awareness one hour after cessation of anaesthesia (Penderis & Franklin 2005). It has however also been shown to increase the mortality rate from 7 to 14 % when administered before methohexital anaesthesia for hypophysectomy in rats (Hansen et al 2002). The dose of buprenorphine used was however rather high (0.1 mg/ kg). The combination of ketamine/medetomidine/buprenorphine was shown to be safe for thoracic surgery in rabbits (Difilippo *et al* 2004), but the level of anaesthesia was insufficient for surgery and 0.75 % of isoflurane had to be added.

Instead of pre-medicating rats with buprenorphine before ketamine/medetomidine anaesthesia to increase anaesthetic depth or duration, one may consider using local anaesthesia or switch to inhalation anaesthesia, in which case opioid pre-medication is safe and reduces the concentration of the volatile agent needed for surgery (Criado *et al* 2000). An alternative and safe injection anaesthetic combination with good analgesic properties in rodents as well as rabbits is fluanisone/fentanyl/midazolam (Fleckell and Mitchell 1984, Green 1975).

The combination sufentanil and medetomidine was also efficient in producing surgical anaesthesia in rats, and subcutaneous administration was more efficient than intraperitoneal injection. Injection with butorphanol and atipamezole resulted in prompt reversal of anaesthesia. Surgical anaesthesia was associated with severe respiratory depression and rats were visibly cyanotic. Both sufentanil and medetomidine are known to cause respiratory depression and medetomidine alone was found to cause cyanosis in 33 % of dogs in one study (Monk et al 1988, Sinclair 2003). For cyanosis to be obvious, the oxygenated haemoglobin concentration has to reach levels lower than 50 g/L of blood. Cyanosis can develop when the blood is insufficiently oxygenated in the lungs, or when blood stagnates in the capillary beds. In the latter case, oxygen saturation levels may remain at normal values (Sinclair 2003).

Since the rats in our study showed respiratory depression of 50 % and oxygen saturation levels around 40 %, insufficient oxygenation is the most likely explanation to the cyanosis during sufentanil/medetomidine anaesthesia.

Despite respiratory depression, all rats recovered uneventfully. Due to severe respiratory depression, this anaesthetic combination can only be recommended for use in young and healthy rats, and only if oxygen is administered during anaesthesia. If the rats are intubated and mechanically ventilated as well, then the combination may be used for a wider population of animals.

5.4   ANAESTHESIA AND POSTOPERATIVE PAIN

Sprague-Dawley rats suffered from a greater weight-loss and showed more pain related behaviour after abdominal surgery under ketamine/medetomidine anaesthesia compared with surgery under isoflurane anaesthesia. This result was unexpected and not in line with a similar study performed in cats, comparing postoperative behaviour after surgery under thiopentone/halothane or ketamine/medetomidine anaesthesia (Slingsby *et al* 1998). Since both ketamine and medetomidine have specific actions with the possible capacity to reduce postoperative pain, it is surprising that rats showed more pain related behaviour than after surgery under isoflurane anaesthesia. It must however be kept in mind that volatile agents like isoflurane, also have specific antinociceptive effects that are separable from their hypnotic and amnesic properties.

Studies show that isoflurane produces significant attenuation of spinal sensitization and blocks nociceptive responses using the rat paw formalin test model (Abram & Yaksh 1993, Sanders *et al* 2005). The formalin model involves intense C-fiber stimulation leading to an increase in dorsal horn cell response to noxious stimulation, and is believed to share similarities with surgically induced sensitization (O'Connor & Abram 1995).

Despite the fact that rats did not show significant increase in pain related behaviour after surgery under isoflurane anaesthesia, this should not be used as a reason to omit perioperative analgesic treatment. Studies examining the more immediate postoperative period after surgery under isoflurane anaesthesia in rats, show changes in behaviour that are reduced by analgesic administration (Roughan & Flecknell 2000, 2001, 2003, 2004).

Whenever surgery is included in the development of animal models, it is important to allow enough time for animals to fully recover, before undertaking further experimental work, in order to minimize interference with collection of research data. By carefully planning the anaesthetic protocol and analgesic treatment, the animals are more likely to recover well and fast, which will benefit both animal welfare and scientific quality.

6 CONCLUSIONS

I. Anaesthesia cannot be induced with isoflurane, sevoflurane or desflurane in NZW rabbits without triggering a breath-holding response and severe bradycardia.

II. Ketamine/medetomidine anaesthesia in NZW rabbits is as effective after subcutaneous as after intramuscular administration. Dose combinations that induce surgical anaesthesia also cause hypoxia and hypercapnia. The addition of butorphanol prolongs the duration of anaesthesia.

III. Anaesthesia with ketamine/medetomidine can safely be repeated six times with weekly intervals in Wistar rats. Pre-medication with buprenorphine before ketamine/medetomidine anaesthesia in rats is however not safe, due to severe respiratory depression.

IV. Sufentanil/medetomidine anaesthesia is more efficient after subcutaneous than after intraperitoneal administration in Wistar rats, and is rapidly reversed with butorphanol and atipamezole. Severe hypoxia may develop during anaesthesia.

V. Sprague-Dawley rats lose less body weight, show less spontaneous pain related behaviour, and are more mobile after surgery performed under isoflurane than under ketamine/medetomidine anaesthesia. Perioperative treatment with carprofen reduces signs of pain and improves recovery.

7 SVENSK SAMMANFATTNING

Användning av försöksdjur har genererat kunskap som medfört ett minskat lidande och ökad livslängd hos människor och djur. Vid användning av försöksdjur är det vårt ansvar att minimera djurens obehag och lidande i möjligaste mån. Ett angeläget område där förbättringar är möjliga, är sövning och smärtlindring för kirurgiska ingrepp. Genom bättre metoder för anestesi och smärtlindring kan djurens lidande minskas, samtidigt som forskningens kvalitet ökar. Ökad kunskap om hur anestesin påverkar djuren är viktig, liksom att metoder för sövning är säkra och enkla att utföra. Arbetet syftar till att finna förbättrade anestesimetoder för kanin och råtta.

En fördel med medel för inhalationsanestesi är en ofta låg grad av metabolism i kroppen och därmed mindre påverkan på andra, tillförda substansers omsättning i kroppen. Detta har betydelse speciellt inom farmakologisk och toxikologisk forskning. Inhalationsanestesi kan induceras via mask, förutsatt att medlet som används inte är retande för andningsvägarna. Vid inandning av isofluran, ett vanligt anestesimedel, reagerar kaniner med flyktförsök och långa andningsuppehåll. Att hitta ett medel som kaniner tolererar vore önskvärt och i detta syfte studerades induktion med sevofluran och desfluran via mask på kaniner av rasen New Zeeland White. Kaninerna reagerade med flyktförsök och andningsuppehåll även vid induktion med dessa medel, dock var reaktionerna mindre med desfluran. Induktionen var snabbast med desfluran och reduktionen av hjärtfrekvensen, som reflexmässigt följer på andningsuppehållet, var lägre.

En bättre metod för induktion av anestesi på kanin är via injektion av anestesimedel. I detta syfte studerades effekten av olika doser av ketamin/medetomidin på New Zeeland White-kaniner. En jämförelse mellan intramuskulär och subkutan injektion visade att anslag och effekt av anestesin var likvärdig, samt att subkutan injektion var tekniskt enklare och till synes mindre smärtsam. Under anestesin utvecklas syrebrist, vilket kan avhjälpas genom syreadministrering via mask. Även förhöjda nivåer av koldioxid sågs, vilka endast kan motverkas genom intubering av luftstrupen och mekanisk ventilering. Intubering av luftstrupen utan visuell hjälp befanns vara lätt när djuren under sövning med ketamin/medetomidin. Initialt befanns en dos av 15/0,5 mg/kg ketamine/medetomidin medföra kirurgisk anestesi i ca 60 min, men i en påföljande studie visade sig dosen vara otillräcklig för en del av djuren, sannolikt beroende på en högre stressnivå hos dessa djur.

Effekter av upprepad sövning med ketamin/medetomidin undersöktes på Wistar-råttor, samt påverkan av premedicinering med buprenorfin. På andra djurslag medför användning av buprenorfin under anestesi förbättrade narkosförhållande för kirurgi, men på Wistar-råttor visade sig premedicinering med buprenorfin vara förenat med en större grad av andningsdepression. Den signifikant minskade andningsfrekvensen kan ha bidragit till att två råttor dog under anestesi. Sövning med ketamin och medetomin utan buprenorfin visade sig vara säker även vid upprepad användning, även om den tid som djuren sov ökade för varje narkostillfälle.

Effekter av anestesikombinationen sufentanil/medetomidin utvärderades på Wistar-råttor. Skillnader efter intraperitoneal och subkutan administrering undersöktes. Effekten inträdde snabbare och visade sig vara mer tillförlitlig efter subkutan administrering. Kombinationen orsakade emellertid uttalad syrebrist och

syretillförsel via mask rekommenderas. Med injektion av antidoterna butorfanol och atipamezol kunde effekterna av anestesin upphävas inom 7 min.

I en studie jämfördes postoperativ återhämtning och smärtrelaterat beteende hos råttor som varit sövda med isofluran eller ketamin/medetomidin under ett bukingrepp. Råttor som sövdes med ketamin/medetomidin minskad mer i vikt och uppvisade signifikant mer smärtrelaterat beteende jämfört med ooppererade råttor. Råttor som opererades under isoflurananestesi skiljde sig i detta avseende inte signifikant från ooppererade råttor, men uppvisade en minskad rörlighet jämfört med före operation. Behandling med det smärtstillande medlet karprofen lindrade de negativa effekterna på vikt och beteende. Resultaten visar att valet av anestesimedel kan vara lika viktigt som användning av smärtlindring för återhämtning och minskad smärta efter kirurgi.

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