Abstract

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Pigs are commonly used in biomedical research, often subjected to complicated and invasive surgical procedures. The knowledge of appropriate analgesia and anaesthesia in pigs however is limited. Therefore, the general aim of the present thesis was to establish and evaluate opioid analgesia suitable for abdominal surgery in growing pigs.

Isoflurane minimal alveolar concentration (MAC) was determined in growing pigs using claw pinching. Thereafter, each pig was randomly studied thrice to determine the MAC values in the following treatments: induction of anaesthesia with medetomidine and tiletamine/zolazepam given intramuscularly (MTZ); MTZ followed by epidural morphine (MTZ/M); and MTZ followed by intramuscular buprenorphine (MTZ/B). Pigs were subjected to abdominal surgery during isoflurane anaesthesia and physiological and behavioural effects of MTZ/M and MTZ/B compared to MTZ were evaluated. Transdermal fentanyl was applied and the effects were evaluated for 60 h in conscious pigs and in pigs treated with MTZ/M. Opioid serum concentrations were monitored up to 72 h after drug administration. Behaviour was analysed utilizing videotape recordings of pigs' activity level before and after surgery.

Induction of anaesthesia with MTZ reduced the isoflurane MAC in pigs by 68%. Additional epidural morphine and systemic buprenorphine decreased MTZ isoflurane MAC by 33% and 50%, respectively. Pigs treated with epidural morphine or systemic buprenorphine prior to abdominal surgery attained surgical anaesthetic depth with reduced isoflurane requirement. Induction of anaesthesia with MTZ improved arterial blood pressure and oxygenation compared to isoflurane induction. Epidural morphine did not influence the cardiorespiratory functions during anaesthesia but systemic buprenorphine affected the respiratory response in spontaneously breathing pigs. The postoperative activity level after epidural morphine was lower but the pigs gained weight and the feed intake was similar compared to before surgery. Combining epidural morphine and transdermal fentanyl resulted in initial return to regular activity levels and weight gain after surgery. Twelve hours after surgery these pigs showed decreased activity but still gained weight. Transdermal fentanyl alone in conscious pigs did not cause inactivity or sedation but resulted in inter-individual variations in fentanyl serum concentrations. Systemic buprenorphine caused unpredictable activity levels with postoperative decrease in weight and feed consumption.

The analgesic properties of MTZ contributed to a substantial reduction in concentration of isoflurane required for maintenance of inhalation anaesthesia. Additional preoperative opioid analgesia further reduced the requirements of isoflurane needed to maintain an adequate anaesthetic depth. The opioids evaluated resulted in different behaviour postoperatively. Pigs treated with epidural morphine with or without transdermal fentanyl had good appetite and gained weight after abdominal surgery indicating improved postoperative recovery.

Keywords: swine, anaesthesia, analgesia, epidural morphine, buprenorphine, transdermal fentanyl, minimal alveolar concentration, isoflurane, pain assessment, behaviour

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Contents

Abbreviations

EP	Epidural injection
ETiso	End-tidal isoflurane concentration
FIO_2	Fraction of inspired oxygen
HR	Heart rate
IM	Intramuscular injection
mABP	Mean arterial blood pressure
MAC	Minimal alveolar concentration
MTZ	Medetomidine and tiletamine/zolazepam
MTZ/B	Medetomidine and tiletamine/zolazepam with buprenorphine
MTZ/M	Medetomidine and tiletamine/zolazepam with morphine
O_2-sat Hb	Oxygen saturation of haemoglobin
$PaCO_2$	Arterial carbon dioxide partial pressure
PAO_2	Alveolar oxygen partial pressure
PaO_2	Arterial oxygen partial pressure
$P(A\text{-}a)O_2$	Alveolar – arterial oxygen partial pressure difference
PIO_2	Partial pressure of inspired oxygen
RR	Respiratory rate
TV	Tidal volume
VE	Expired minute ventilation

Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I: Malavasi, L.M., Jensen-Waern, M., Augustsson, H. & Nyman, N. 2005. Changes in minimal alveolar concentration of isoflurane after treatment with medetomidine and tiletamine/zolazepam, epidural morphine and systemic buprenorphine in pigs. (Manuscript).

II: Malavasi, L.M., Jensen-Waern, M., Jacobson, M., Rydén, A., Öhagen, P. & Nyman, G. 2005. Effects of epidural morphine on end-tidal isoflurane concentration and physiological parameters in pigs undergoing abdominal surgery: a clinical study. *Veterinary Anaesthesia and Analgesia (accepted)*.

III: Malavasi, L.M., Nyman, G., Augustsson, H., Jacobson, M. & Jensen-Waern, M. 2005. Effects of epidural morphine and transdermal fentanyl analgesia on physiology and behaviour after abdominal surgery in pigs. *Laboratory Animals (accepted).*

IV: Malavasi, L.M., Jensen-Waern, M., Augustsson, H., Lindberg, J.E. & Nyman, G. 2005. Effects of preoperative epidural morphine and intramuscular buprenorphine in pigs subjected to abdominal surgery: a pilot study. (Manuscript).

V: Malavasi, L.M., Augustsson, H., Jensen-Waern, M. & Nyman, G. 2005. The effect of transdermal delivery of fentanyl on activity in growing pigs. *Acta Veterinaria Scandinavica (in press).*

Introduction

Background

The veterinary surgical procedures in growing pigs performed under field conditions are limited to minor operations such as hernias and castrations. The use of analgesia and anaesthesia in the swine industry must thus meet these requirements. In contrast, when pigs are used in biomedical research the animal can be involved in complicated and invasive surgical procedures. These circumstances require advanced analgesia and anaesthesia. Unfortunately, the knowledge of appropriate analgesic and anaesthetic protocols designed for pigs is restricted. The aim of this thesis is to some extent to fill this gap.

Animals and humans present many similarities in anatomical and physiological pathways (Morton & Griffiths, 1985). Pigs, in particular, provide researchers with an excellent animal model for human conditions (Almond, 1996) and sometimes pigs are submitted to extensive experimental procedures such as hemorrhagic shock (Jernigan, Croce & Fabian, 2004) and organ transplantation experiments (Guarrera *et al.,* 2005). The importance of pigs as an animal model in biomedical research is illustrated by the trend that pigs are replacing dogs and nonhumans primates (Almond, 1996). According to Swedish Animal Welfare Agency statistics for 2003, 2047 pigs were used as laboratory animals compared to 734 dogs and 56 old-world apes (http://.djurskyddsmyndigheten.se.;27-May-2005). Therefore, from an animal welfare point of view, investigations of analgesia and anaesthesia in porcine models are of great importance.

Anaesthetic and analgesic drugs used in biomedical research have well known effects in human medicine. The physiological effects of these drugs are then generally extrapolated to the pigs used as laboratory animal model. It is known that swine respond in a unique way to many anaesthetics. For instance, pigs are relatively more resistant to the effects of opioids and alpha-$_2$-agonists compared to cattle (Moon & Smith, 1996). In addition to the animal welfare issue, most of the animal experiments in biomedicine are acute experiments where the animals are euthanized under general anaesthesia and do not recover from it. Consequently, there is a lack of knowledge about the behavioural effects of the anaesthetics and analgesics during the postoperative period. Therefore, the present thesis is a contribution to the limited literature evaluating analgesia in pigs used as a laboratory animal model.

Assessment of clinical pain in pigs

Pain in mammalian animals is considered universal and it is protective in nature and is part of the fight and flight mechanism (Sanford *et al*., 1986; Short, 1987). It is generally accepted that animals perceive and react to pain in a similar manner to humans (Short, 1999). Therefore, the clinical assessment and evaluation of the intensity of pain is based on signs exhibited by the animal and, based on personal experience, the interpretation of those signs.

The pain process can be summarized in three stages: transmission of the signal provoked by the noxious stimulus to the brain (nociception); perception of an unpleasant experience; and the behavioural (or cognitive) response to pain (Fig. 1)

(Livingston & Chambers, 2001). However, in pigs the species-specific pain-related response is not entirely known.

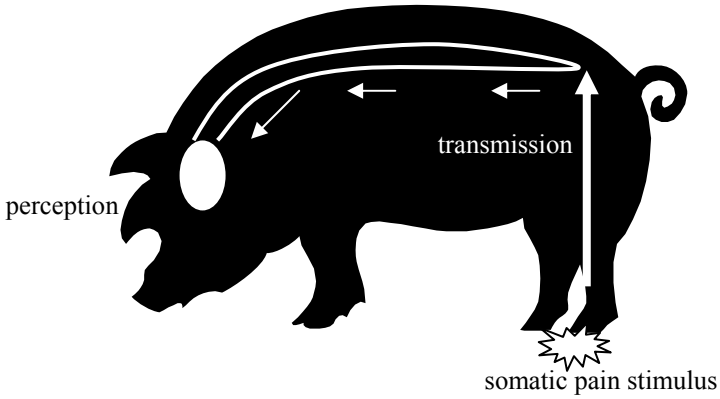

Fig. 1. Pain signal from peripheral to central nervous system.

Extensive efforts have been made to define subjective and objective methods to determine the site and extension of the pain in animals. Pain alters physiological and behavioural parameters both of which should be integrated for a clinical pain assessment.

Pain influences the autonomic nervous system. Heart rate, blood pressure, respiration rate and body temperature frequently increased together with reduced blood supply to the body's extremities (Morton & Griffiths, 1985; Sanford *et al.,* 1986; Short, 1999). Also, the respiratory rate and pattern can change in response to pain stimuli but these alterations are partly related to the site of pain (Short, 1999). For instance, when an animal is subjected to a thoracotomy the respiration is shallow and with high frequency. Pain can also result in endocrinal changes and release of hormones from the pituitary, thyroid, parathyroid and adrenal glands (Wood *et al.,* 1991; Short, 1999). Although autonomic reflexes and hormonal responses are commonly used in animal pain assessment both are considered unreliable indicators of pain (Table 1). The autonomic reflexes may induce alterations caused by stress-related endocrinal activity, drugs or external physical effects (e.g. ambient temperature). Many of the hormones in the body are released in a pulsatile manner and the circulating level varies during a short period of time (Dobromylskyj *et al.,* 2001; Livingston & Chambers, 2001).

Pain may also induce behavioural changes for example in gait, posture and even in the activity level. Usually, pigs squeal and attempt to escape from handling; they may be unwilling to move and may hide in the bedding materials. Additionally, pigs demonstrate inactivity and recumbency, aggressiveness, depression, head pressing and stereotypic chewing (Sanford *et al.,* 1986; Short, 1999; Harvey-Clark, Gilespie & Riggs, 2000; Dobromylskyj *et al.,* 2001). It has been suggested that pigs with abdominal pain would remain lying on the floor with spread hind limbs and with abdominal musculature contracted (Dobromylskyj *et al.*, 2001). In our experience, pig's exhibit hunched back posture in response to abdominal pain. Pain also generates undesirable physiological changes that result in further behavioural responses. For instance,

animals can display decreased feed and water consumption in response to postoperative pain (Dennis & Melzack 1983; Flecknell, 1999; Short, 1999; Flecknell, 2001). An adequate energy supply is important factors for the wound-healing process (Short, 1999). Therefore, a reduced intake of food and water postoperative can result in slower return to homeostasis. Pigs are prone to vocalize in response to pain. However, vocalization can be a non-reliable sign of pain because pigs vocalize when they are simply restrained. Thus, studies have been performed in an attempt to distinguish the different calls by range and frequency of these sounds (Short, 1999; Taylor & Weary, 2000).

Despite these endeavours to understand animal response to pain, animal pain assessment is difficult. Researchers such as Short (1999), Reyes *et al.* (2002) and Molony, Kent & McKendrick (2002) have tried to assess pain in animals by visual analogue scores (VAS) and numerical rating scale (NRS) using both physiological and behavioural parameters. The reliability of these scales is low and, consequently, to achieve the necessary skills to use them takes time, especially as human beings are still responsible for the interpretation of pain in the animal.

Table 1. *Signs of pain in animals according to Short (1999)*

System	Signs
Neurological	Twitching, tremors, convulsion, paralysis, dilated pupils, hyperesthesia, reflexes sluggish, absent or exaggerated areas of numbness
Cardiovascular	Changes in heart rate, cardiac dysrhythmias, vascular resistance, blood pressure, blood flow, changes in cardiac output
Respiratory	Changes in respiratory rate, minute volume, oxygen saturation, blood gases and pH
Musculoskeletal	Lameness, unsteady gait, muscle flaccidity, rigidity, reluctance to move, muscle twitching, tetanus, atrophy
Digestive	Body weight loss or poor growth, faeces altered in volume, colour, amount or consistency, vomiting, jaundice, bleeding
Urinary	Urine retention, decrease in volume, change in specific gravity
Endocrine	Hyperactivity, sluggishness, depression

Antinociceptive testing

Many animals including pigs have been used in antinociceptive tests. During these tests the animal is submitted to some type of nociceptive pain which can be somatic or visceral (Table 2). The stimulation of the afferent neural fibres A-δ and C that are involved in these types of pain can be evoked by injury, disease or inflammation (Coda & Bonica, 2001). The classification of somatic and visceral pain is based on the origin of the stimulus. Pain arising from visceral organs is denominated visceral pain, whereas that arising from skin, muscle, joint capsules and bones is denominated somatic pain (Molony & Kent, 1997;

http://jcaho.org/news+room/health+care+issues/pain_mono_npc.pdf; 9-Aug-2005).

Table 2. *Examples and characteristics of somatic and visceral pain (*http://jcaho.org/news+room/health+care+issues/pain_mono_npc.pdf; 9-Aug-2005*)*

	Superficial somatic pain	**Deep somatic pain**	**Visceral pain**
Nociceptor location	Skin, subcutaneous tissue and mucous membranes	Muscles, tendons, joints, fasciae and bones	Visceral organs*
Potential stimuli	External mechanical, chemical or thermal events Dermatological disorders	Overuse strain, mechanical injury, cramping, ischemia, inflammation	Organ distension, muscle spasm, traction, ischemia, inflammation
Localization	Well localized	Localized or diffuse and radiating	Poor localized

* Visceral organs include the heart, lungs, gastrointestinal tract, pancreas, liver, gallbladder, kidneys, and bladder.

Somatic pain and visceral pain differ in the manner in which their nociceptive stimulation is processed until the unpleasant experience is perceived. This difference contributes to determining the most suitable analgesia to remove this sensation. Therefore, it is important to know which nociceptive pain that has been evoked in the test and whether the pain relief is adequate. These antinociceptive tests are used to determine minimal alveolar concentrations or cardiopulmonary effects of anaesthetics. Also, they are used to evaluate analgesic effects of drugs.

Analgesia in pigs

During porcine experimentation, it is common practice to administer an analgesic drug pre-emptively, i.e. prior to surgery. The objective of the procedure is to reduce the afferent nerve impulses involved in the nociceptive process. This initial analgesic treatment can be sufficient to achieve adequate analgesia, depending on the drug chosen and the degree of surgical trauma (Moon & Smith, 1996; Smith, Ehler & Swindle, 1997; Dobromylskyj *et al.,* 2001). However, few drugs are currently approved for use in pigs that are destined for the food market. This scarcity is due to the lack of information about the minimal residual limit (MRL) and appropriate withdrawal time of most of these drugs (Moon & Smith, 1996). Thus, caution should be taken when the analgesic protocol is to be used in future field procedures.

In pigs, most analgesic drugs possess a short half-life, and therefore their uses as postoperative analgesic have some limitations (Smith, Ehler & Swindle, 1997). Nevertheless, administration of analgesic drugs in pigs reduces the degree of central hypersensitivity. For postoperative pain relief a new concept has been applied, the multi-modal pain therapy. It consists of the administration of different classes of analgesic drugs to the animal, where each drug acts on different parts of the pain process. Multi-modal therapy can also diminish some of the problems associated with the different times to onset of action of the several drugs involved

(Flecknell, 1999; Dobromylskyj *et al.,* 2001). Another important aspect of postoperative analgesia in pigs is that injections are often stressful, and may prolong the total recovery time (Smith, Ehler & Swindle, 1997). Alternatively, methods of drug administration associated with minimal stress such as transdermal patches and oral preparations could be utilized more frequently (Moon & Smith 1996; Dobromylskyj *et al.,* 2001).

Opioids

Opioid drugs are used for pain relief in both humans and animals. Common opioid analgesics used in veterinary practice are morphine, fentanyl, buprenorphine and butorphanol (Swindle, 1994; Rang, Dale & Ritter, 1996; Smith, Ehler & http://criver.com/techdocs/anesth.html.; 15- Sep-2004; Branson & Gross, 2001; Nolan, 2001).

Morphine sulphate

Morphine sulphate is a full agonist and relatively water-soluble and poorly lipid-soluble compared to other opioids and because of the hydrophilic –OH group in its structure (Fig. 2). When administered intravenously the poor lipid solubility limits the passage of morphine from plasma to tissues, especially to the central nervous system (Glare & Walsh, 1991). When administered intravenously to pigs, morphine produces not only analgesia but also respiratory depression (Steffey *et al.,* 1994). Systemic morphine is also reported to produce other side effects, such as decreased gastrointestinal motility, nausea and vomiting, pupillary constriction, bradycardia, euphoria and histamine release in many species e.g. dogs, cats and horses (Branson & Gross, 2001; Nolan, 2001). To avoid many of the systemic physiological side-effects, morphine can be administered epidurally. The morphine then acts locally on all opioid receptors located in the spinal cord, enhancing the analgesic effect (Rang, Dale & Ritter, 1996; Nolan, 2001; http://www.esraeurope.org/abstracts/abstracts99/puig.htm.; 30-May-2002). In addition, because morphine has low lipid solubility, it remains for a longer period in the cerebrospinal fluid than in the plasma, prolonging the analgesic effect for 13 - 33 h in pigs (Ummenhofer *et al.,* 2000). In many species, such as dogs, cats, horses and cattle, this analgesic effect does not produce major motor impairment, in contrast to epidural administration of other drugs such as lidocaine (Nolan, 2001; Branson & Gross, 2001). Opioids given epidurally are expected to have the onset time of action 20 - 60 min after administration, but the nociceptive input is not totally abolished (Ummenhofer *et al.,* 2000). Additionally, it has been reported that epidural morphine reduces the minimal alveolar concentration of inhalation anaesthetics such as halothane in dogs (Valverde, Dyson & McDonell, 1989).

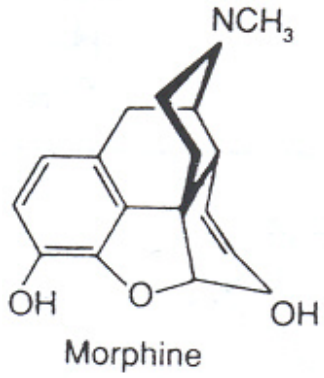

Fig 2. Structures of morphine sulphate (Rang, Dale & Ritter, 1996).

Buprenorphine hydrochloride

Buprenorphine hydrochloride is a partial agonist and a morphine-like opioid drug (Fig. 3) that has a relatively long analgesic action and minimal adverse effects in pigs compared to other opioids (Hermansen, Pedersen & Olesen, 1986). This drug is a partial agonist with affinity for the OP3 receptor (μ receptor) but only with partial activity. Due to its high lipophilic property, buprenorphine slowly associates to and dissociates from the opioid receptors compared to morphine (Cowan, Doxey & Harry, 1977; Sadée, Rosenbaum & Herz, 1982; Branson & Gross, 2001). In pigs, buprenorphine administered intramuscularly has an onset of action of approximately 30 - 60 min (Hermansen, Pedersen & Olesen, 1986) and the full analgesic effect has been reported to last 7 - 24 h when given in a high dose (Harvey-Clark, Gilespie & Riggs, 2000).

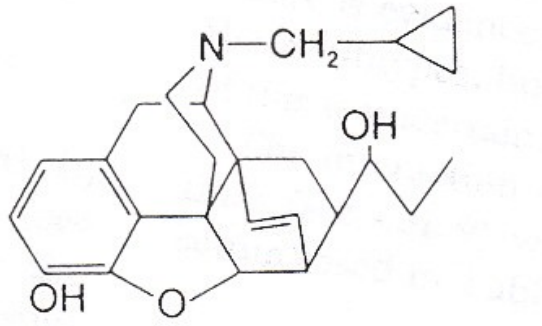

Fig. 3. Structures of buprenorphine hydrochloride (Rang, Dale & Ritter, 1996).

Fentanyl citrate

Fentanyl citrate is a full agonist and a synthetic opioid (Fig. 4) at least 100 times more potent and lipid soluble than morphine and with affinity on the OP3 receptor (see Branson & Gross, 2001). The high liposolubility contributes to the rapid onset (2 - 5 min) of fentanyl and the short duration of action ranging from 5 to 20 min (Branson & Gross, 2001; Robertson & Taylor, 2004). Also, fentanyl is often the analgesic drug of choice during general anaesthesia because it can be given as

intermittent intravenous boluses or by infusion (Nolan, 2001; Robertson & Taylor, 2004). The side-effects of fentanyl are reported to be respiratory depression and bradycardia (Nolan, 2001). Recently, fentanyl has been selected over other opioids for use in a transdermal delivery system for both pre-emptive and postoperative analgesia in humans (Bowdle, 1998; France *et al.,* 1998), dogs (Rang, Dale & Ritter, 1996; Pettifer and Hosgood, 2004), cats (Egger *et al.,* 2003), pigs (Szeit, Riggs & Harvey-Clark, 1996; Harvey-Clark, Gilespie & Riggs, 2000), goats (Carroll *et al.,* 1999) and horses (Thomasy *et al.,* 2004). Transdermal delivery of fentanyl in pigs has been suggested to provide continuous and systemic delivery of fentanyl of up to three days per patch application (Harvey-Clark, Gilespie & Riggs, 2000; Wilkinson *et al.*, 2001; Thomasy *et al.*, 2004).

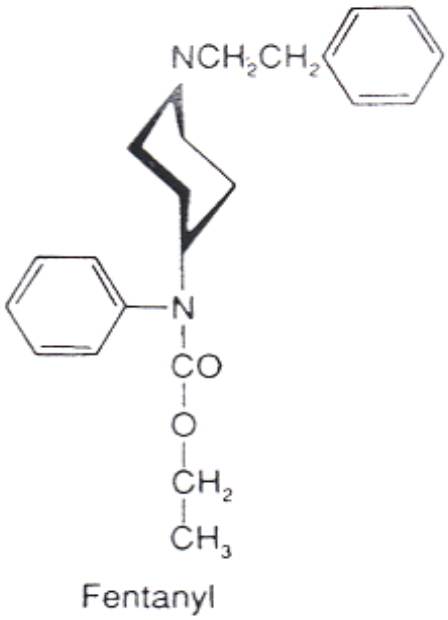

Fig. 4. Structures of fentanyl citrate (Rang, Dale & Ritter, 1996).

Non-steroidal anti-inflammatory drugs (NSAIDs)

The non-steroidal anti-inflammatory drugs are a group of analgesics that include organic acids, carboxylic acids e.g. aspirin, carprofen, flunixin and enolic acids e.g. phenylbutazone, meloxicam (Nolan, 2001). The analgesia produced by these drugs is related to the potent inhibitory effect on prostaglandin. Consequently their side-effects are known to be gastrointestinal, hepatic and renal toxicity (Waterman-Pearson, 2001). In addition to the analgesia, the NSAIDs are reported to have anti-inflammatory and antipyretic properties (http://criver.com/techdocs/anesth.html.; 15- Sep-2004; Smith, Ehler & Swindle, 1997; Boothe, 2001; Nolan, 2001). When the objective of the research is to evaluate inflammation or infection processes the researchers involved are reluctant to use NSAIDs. The pigs used in the present thesis were also involved in a swine dysentery project. Therefore, the NSAIDs were avoided as analgesic drugs.

Miscellaneous drugs

In veterinary medicine, there are other drugs that are primarily sedatives or anaesthetics but that also have analgesic properties. Alpha$_2$-adrenoceptor agonists (e.g. medetomidine), and N-metyl-D-Aspartate receptor antagonist (e.g. tiletamine, ketamine) can contribute to the reduction of pain. These classes of drugs are frequently used to induce anaesthesia (Golden *et al.,* 1998; Slingsby & Waterman-Pearson, 2000; Jang *et al.,* 2004). Volatile and gaseous agents such as methoxyflurane and nitrous oxide are shown to have some analgesic properties (Waterman-Pearson, 2001). Local anaesthetics e.g. lidocaine produces complete local analgesia by blocking all sensory input from a specific area (Hall & Clark, 1991; Ngo *et al.,* 1997; Valverde *et al.*, 2004).

Aims of the study

The general aim of this thesis was to establish and evaluate opioid analgesia suitable for abdominal surgery in growing pigs. The pigs were included in a project on experimental swine dysentery and NSAIDs were thus not an option.

The specific aims were:

- To evaluate and compare the effects of medetomidine and tiletamine/zolazepam, epidural morphine and systemic buprenorphine on isoflurane minimal alveolar concentration and physiological parameters (Study **I**).

- To study the analgesic and cardiorespiratory effects of pre-operative epidural morphine and systemic buprenorphine during intestinal cannula insertion and isoflurane anaesthesia in pigs induced with medetomidine and tiletamine/zolazepam (Study **II-IV**).

- To study postoperative physiological and behavioural effects of epidural morphine, systemic buprenorphine and transdermal fentanyl patches (Study **III, IV** and **V**).

Material and methods

Pigs

The experimental protocol was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

A total of 58 crossbreed pigs (Swedish Landrace x Yorkshire) were purchased from a conventional gilt-producing herd. On arrival, the pigs were seven weeks old and clinically healthy, and there were an equal number of males and females. During Study **II, III, IV** and **V**, the pigs were housed in individual pens and for Study I pigs were housed in two large pens. All pens had a solid concrete floor and straw as bedding, and all pigs were kept within sight and sound of one another with a light regime of 8 h light/16 h dark. The pigs were fed twice daily with a commercial finisher diet and had free access to water. Only pigs included in Study **IV** were kept on a restricted diet with a total of one kilogram of commercial finisher diet. All pigs were allowed 2 - 5 weeks to acclimatize to the stabling and staff before start of the studies. When the experiments started the animals weighed 23 ± 4 kg.

All pigs were randomly chosen for a cross-over test in each study. However, due to technical problems data were collected from 52 of the 58 pigs. The behavioural analysis in Study **III** used 10 of the 14 pigs used during Study **II**. In Study **I**, each pig was treated thrice with at least one week of "wash-out" period in between. In Study **V**, each pig was treated twice with one week of interval in between (Table 3).

Table 3. *Summary of data for the pigs used in the studies*

Study	Treatment group	No. of pigs
I	Isoflurane Medetomidine+tiletamine/zolazepam Medetomidine+tiletamine/zolazepam+morphine Medetomidine+tiletamine/zolazepam+buprenorphine	11
II	Morphine+fentanyl Saline (control)	14
III	Morphine+fentanyl Saline (control)	10
IV	Morphine Buprenorphine	9
V	Fentanyl Anaesthesia+fentanyl	8

Anaesthesia and instrumentation

Before anaesthesia all pigs were fasted for 12 h but had free access to water. Anaesthesia was induced in each pig with either medetomidine (0.05 mg kg^{-1}; Domitor®vet 1 mg mL^{-1}; Orion, Espoo, Finland) and tiletamine-zolazepam (2.5 mg kg^{-1} each; Zoletil forte vet; Virbac, Carros, France), intramuscularly or isoflurane

(IsoFlo™ vet; Orion Pharma Animal Health; Sollentuna, Sweden) delivered via a face mask at 5% as described in detail in Study **I**. After tracheal intubation, anaesthesia was maintained with isoflurane in oxygen and air (inspired oxygen 50%; vaporizer Isotec 5; Datex-Ohmeda, Helsinki, Finland) using a small animal anaesthetic circle system. In Study **I**, ventilation was controlled to maintain the end-tidal CO_2 between 5.5 – 6.0 kPa. All pigs were placed in ventral recumbency during instrumentation and an experienced animal nurse, unaware of the treatment, continuously checked the end-tidal concentration of isoflurane (**II, III and IV**). This concentration was adjusted according to the animal's reflexes (i.e. leg movements) and the hemodynamic response to surgery (e.g. increased heart rate and arterial blood pressure).

A 22-gauge catheter (BD Venflon; Helsingborg, Sweden) was placed in one ear artery, for blood sampling and monitoring, and another placed in the opposite ear vein, for intravenous fluid. Electrolyte solution (Rehydrex - glucose 25 mg mL^{-1}, Fresenius Kabi AB, Uppsala, Sweden) was administered at dosage of 5 mL $kg^{-1}h^{-1}$.

Analgesia

In addition to the drugs used to induce anaesthesia, the pigs were randomly chosen to receive epidural morphine, intramuscular buprenorphine or epidural morphine and a fentanyl patch.

Epidural morphine was given at a dosage of 0.1 - 0.12 mg kg^{-1} (Morfin epidural 2 mg mL^{-1}; Pharmacia & Upjohn, Stockholm, Sweden) diluted in saline (**I, II** and **III**). The epidural injection was administered according to the technique recommended by Strande (1968). This procedure was carried out in the anaesthetized animal in ventral recumbency at least 5 min before the pig was positioned in lateral recumbency for the surgery. For maximal distribution of the drug into the spinal channel, morphine was diluted with saline to a final dosage of 1 ml of solution for pigs with a vertebral length of up to 40 cm. Then, an additional 1.5 ml of saline was added for every additional 10 cm of vertebral length (Fig. 5) (Strande, 1968). The final volume was delivered slowly over 1 - 2 min.

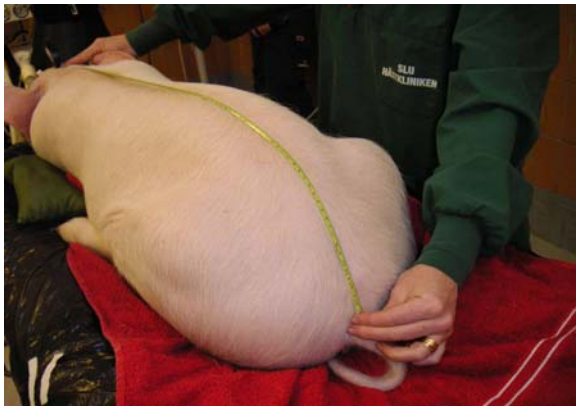

Fig. 5. Pig placed in ventral recumbency. Measuring its vertebral column with a common measuring tape to define what final volume should be administered by epidural route.

Intramuscular buprenorphine was administered at a dosage of 0.1 mg kg^{-1} (Temgesic® 0.3 mg mL^{-1}; Schering-Plough, Brussels, Belgium) immediately after onset of general anaesthesia (**I** and **IV**). A repeated injection was given 12 h after the first administration.

The fentanyl patch (50 µg h^{-1}; Durogesic, Janssen-Cilag AB, Sollentuna, Sweden) was applied for 60 h per pig (**III** and **V**). The patch was attached to the skin behind the ear. This skin area was shaved with care to avoid abrading the skin but it was not washed prior to attachment of the patch. The attachment procedure was in accordance with the manufacture's recommendations for humans. In order to protect the patches and prevent their being rubbed off, a piece of canvas was sutured onto the skin and checked at least twice daily.

Monitoring of physiological parameters

Clinical parameters were monitored continuously and recorded every 10 min throughout the anaesthesia and surgery. These included respiratory rate (RR), tidal volume (TV), expired minute ventilation (VE), inspired oxygen fraction (FIO_2), end-tidal carbon dioxide concentration ($ETCO_2$), end-tidal isoflurane concentration (ETiso) (Capnomac Ultima; Datex-Ohmeda, Helsinki, Finland), oxygen saturation of haemoglobin (O_2-sat Hb), heart rate (HR) (Cardiocap II; Datex, Helsinki, Finland) and invasive mean arterial blood pressure (mABP), measured from an auricular catheter (Sirecust 730; Siemens, Germany).

Blood samples were collected from an auricular artery after epidural administration of morphine or saline and again immediately after the surgical procedure, for analysis of arterial carbon dioxide partial pressure ($PaCO_2$), arterial oxygen partial pressure (PaO_2) and pH (ABL™ 5; Radiometer Medical A/S, Copenhagen, Denmark). Rectal temperature was measured with a digital thermometer at the time of blood sampling.

In Study **I**, according to Lentner (1990) the following calculations were made from blood gas data. Alveolar oxygen partial pressure:
(PAO_2 [kPa]) = (PIO_2 [kPa]) – $PaCO_2$/0.8 (respiratory exchange ratio), where PIO_2 is partial pressure of inspired O_2.

Abdominal Surgery

In Study **II, III** and **IV** the intestinal surgery was performed under aseptic conditions and by the same surgeons. The pigs were positioned in left lateral recumbency. The skin over the surgical area and over the area for the epidural analgesia was shaved, washed with surgical soap and disinfected with antiseptic solution.

According to the method described by van Leeuwen *et al.* (1991), a 10-cm incision was made in the skin, 4 cm below the transverse processes and 2 cm behind and parallel to the last rib. The subcutaneous layer, the muscular layers and the peritoneum was opened. The caecum was located, the ileocaecal ligament transsected, and the nearby vessels ligated. An intestinal clamp was applied across the corpus opposite to the papilla ilealis and a purse-string suture placed below the

intestinal clamp. Consecutively, the caecum was transsected between the intestinal clamp and the purse-string suture, and ingesta were evacuated.

The inner part of the cannula was inserted into the remaining part of the caecum, and a second purse-string suture, below the first one, was applied to tighten and secure this part of the cannula. A further incision was made through the skin 3 cm caudal to the first laparotomy incision, and the muscular layers were dissected bluntly in the direction of the fibres. Through this second incision, the barrel of the inner part of the cannula, packed with gauze, was exteriorized. Before attaching the second part of the cannula, the peritoneum and the muscular layers from the first incision were closed with continuous sutures. Finally, the skin was closed with single sutures. To complete the surgery, the outer part of the cannula was attached to the cannula barrel, which had been exposed through the skin, then a plug was inserted and two nylon straps secured both parts (van Leeuwen *et al.,* 1991).

MAC determination

The MAC isoflurane was evaluated by the recording of responses (i.e. response versus no response) to noxious stimuli created by application of a digital calliper square (Digimatic calliper; Mitutoyo Corporation; Kanagawa, Japan).

The calliper square was applied to a claw, just below the coronary band, and an interdigital space from the hind limbs of the pigs. To provoke noxious stimuli the calliper square was closed tightly to decrease the total thickness by 20% of the claw and 55% of the interdigital space. The noxious stimulus was applied to a different claw or interdigital space after 20 min of equilibration. The claw and interdigital space were stimulated for 60 sec or until a purposeful movement occurred. Purposeful movement was defined as movement of the legs or head.

Based on earlier observations of the requirement of isoflurane concentration during surgery the stepwise increase or decrease in isoflurane concentration was dependent on the treatment. During the baseline isoflurane MAC study ETiso was increased by 0.1% if a purposeful movement was observed. Otherwise, in the absence of purposeful movements the ETiso was decreased by 0.1%. When pigs received treatment MTZ, MTZ/M or MTZ/B the stepwise increase or decrease of ETiso was 0.2%. After observing the ETiso that permitted a positive response during treatment MTZ, MTZ/M and MTZ/B isoflurane concentration was increased by 0.1% to determine the lowest concentration preventing the response. The ETiso value midway between these determinations was recorded as the isoflurane MAC for each pig.

Biochemical analyses

Cortisol and β-endorphin measurements

Blood samples (5 mL) were collected by repeated needle puncture of the external jugular vein. The tubes contained EDTA, EDTA and Trasylol, or no additives, depending on the different measurements desired. Blood samples were taken and analyzed for cortisol and β-endorphin at five times: before endotracheal

intubation, immediately after surgery, and 24, 48 and 72 h postoperatively. The postoperative samples were collected daily at 15:00 h.

Serum was separated by centrifugation at +4ºC within 10 min after blood sampling, and stored at -70ºC until analyzed. Cortisol was assayed by solid-phase radioimmunoassay (Coat-A-Count, DPC, Los Angeles, USA), according to the recommendations of the manufacturer. The intra-assay coefficients of variation from three control samples were 3.8% (36 nmol L^{-1}), 9% (77 nmol L^{-1}) and 4% (635 nmol L^{-1}). The corresponding interassay coefficients of variation were 0, 0 and 8.5%, respectively.

The plasma concentration of β-endorphin was assayed with a human radioimmunoassay kit (EURIA-β-ENDORPHIN; Euro-diagnostica AB, Malmö, Sweden). This method was validated for pigs. For this assay, blood was collected in tubes containing EDTA and Trasylol. The sample was immediately cooled in an ice bath, and plasma was separated by centrifugation at +4ºC. The samples were stored at -20ºC prior to analysis, and for adequate results the samples were re-centrifuged for 10 min at a velocity of 4000 x G. Beta-endorphin was extracted using sep-pak C18 (360 mg) cartridges (Waters Assoc., Milford, M.A., USA). The extracts were analyzed by a competitive radioimmunoassay using antibodies against synthetic human β-endorphin, and counted by a multigamma counter.

Morphine and Buprenorphine measurements

Blood samples (4 mL) were collected from the external jugular vein into vacutainer tubes without additives at five different times: 30 min after the first drug application and then 1 h, 1 h 30 min, 12 h and 24 h. In the conscious animal, blood sampling was performed while the pig was restrained with a nose twitch. Plasma was separated by centrifugation at +4ºC within 10 min after blood sampling, and stored at -70ºC until analyzed. The analysis of plasma samples was modified accordingly to the technique of liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS/MS) as described by Murphy & Huestis (2005). The internal standards [2H4]-buprenorphine (7.2 ng) and [2H3]-morphine (7.2 ng) were added to every plasma sample (1.0 mL). After addition of 5.0 mL dichloromethane/2-butanol and 1.0 mL of saturated sodium carbonate buffer (pH 9.3) the mixture was extracted for 20 min. After centrifugation the organic phase was transferred to a new glass tube and evaporated to dryness in a stream of nitrogen at 50^{o}C. The residue in each vial was reconstituted in 50 µL of 20mM ammonium acetate, 0.01% formic acid. The reconstituted samples were quantified with LC-ESI-MS/MS. LC/MS/MS analyses were performed using a Surveyor LC system interfaced to a Finnigan TSQ Quantum Ultra (Thermo Electron Corporation, San José, CA, USA) mass spectrometer. The mass spectrometer was run in selected reaction monitoring mode and the limit of quantification was 0.001 ng mL^{-1} and 0.05 ng mL^{-1} for buprenorphine and morphine, respectively. The standard curve was linear in the range 0.001 to 110 ng mL^{-1} for buprenorphine and 0.05 to 245 ng mL^{-1} for morphine. The coefficients of variance at the 1 ng mL^{-1} level were 7% (n=5) and 5% (n=5) for buprenorphine and morphine, respectively. The samples were analyzed in a single determination.

Fentanyl measurement

Blood samples (4 mL) were collected from the external jugular vein into vacutainer tubes without additives, in Study **III**, before endotracheal intubation and 24, 48 and 72 h postoperatively. In Study **V**, samples were collected at six different time points: 1 h after application of the fentanyl patch and then 6, 12, 24, 48 and 72 h. The animals were restrained with a nose twitch during the blood sampling procedure. Serum was separated by centrifugation at + 4ºC within 10 min after blood sampling, and stored at -70ºC until analysed at the University Hospital of Linköping. The serum fentanyl concentration was measured by gas chromatography with mass-selective detection as described by Szeit, Riggs & Harvey-Clark (1996). Fentanyl was extracted from 1 mL of serum by using 2-octanol, and deuterium-labelled fentanyl was used as internal standard (Fentanyl-D5). This method was calibrated to detect fentanyl concentrations above 0.05 ng mL^{-1}.

Ethogram

The protocol for the behavioural analysis was determined from experience gained in a previous pilot study involving four pigs of the same age, breed and weight. An ethogram, which is a list of activities that the pig was expected to perform, was established. The activities were grouped in two major categories, inactive and active behaviours (Table 4 and 5, respectively). Inactive behaviour related to activities that indicated mental or physiological impairment, whereas active behaviour considered activities that includes both mental and physical activity. In Study **V**, the activities "interacting with blanket", "interacting with straw", "running" and "jumping" were included in the ethogram. During night time, it was not possible to distinguish which activities were performed by the pig while it was under the heating lamp (**III-IV**). Therefore an additional inactive behaviour "staying under a heat lamp" was included.

To minimize human disturbance on the pig behaviour, animal care taking, feeding and videotape changing were executed daily at same time (8:00 h and 15:00 h).

Table 4. *Expected inactive activities of pigs included in the studies*

Activity	**Definition**
Lying down quietly	Pig in recumbency on the floor with no legs or head movements.
Lying down agitatedly	Pig in recumbency on the floor with frequent movement of legs and/or head.
Sitting position	Pig with lower extremity of the body in contact with the ground and supporting most of its weight.
Staying under heating lamp	Pig positioned under heating lamp; other activities not clearly visible.

Table 5. *Expected active activities of pigs included in the studies*

Activity	Definition
Tentative to stand up	Rolling the body with legs and head tossing without success.
Standing up without balance	Pig on four legs with support of solid object (e.g. walls) and/or with presence of legs spread posture.
Standing up with balance	Pig on four legs with normal posture.
Scooting	Pig dragging posterior legs and rear end across the floor.
Walking	Pig on four legs and in motion.
Running	Pig on four legs and in fast motion around the pen.
Jumping	Pig bouncing up in the air whiles two or four legs do not touch the ground.
Interacting with blanket	Pig dragging, biting or rolling with the blanket.
Interacting with straw	Pig dragging straw with the mouth or feet or rolling itself on the straw.
Rooting	Pig digging the ground with the snout or nose, together with standing or walking activity.
Eating	Opening and closing the mouth containing food or straw several times
Drinking	Sucking the water nipple.

Behavioural recordings

Two black and white video cameras with a wide-angle lens (Computar CE IP66) were positioned approximately one metre in front of each pen. Behaviour was recorded with a time-lapse video cassette recorder (Panasonic, AG-TL350) and a video multiplexer (Panasonic, WJ-FS409) (Fig. 6). The picture-sampling interval was 0.18 second, and the time code in hours/minutes/seconds was recorded on the tape.

Fig. 6. Video cassette recorder and video multiplexer used for the recording of pig's behaviour.

To evaluate the efficiency of the analgesic drugs used in these studies the individual pig activity level was considered as control values for the behavioural analysis (**III, IV** and **V**). Thus, each pig was videotape recorded for 24 h at least one week before treatments. Behavioural recordings also covered the period of 60 h (**IV**) after each treatment, beginning when the animal was left alone in its pen after completion of the treatment. In Study **III and V**, the pigs received a fentanyl patch that is reported to provide analgesia at least 72 h (Harvey-Clark, Gilespie & Riggs, 2000). Therefore, the post-treatment recordings in these studies included the 72-h period.

A researcher, blinded to the treatments, watched all videotapes and manually recorded the behaviour of the pigs. The recordings collected before and after treatments were sampled with instantaneous sampling method. This method of behaviour sampling involves observing the animal at regular and predetermined times and then accounting for whether each of the range of activities is being shown at that instant. However, rare and short-lasting activities might be missed altogether (Fraser & Broom, 1990). The interval of predetermined times chosen in these studies was 10 min. Based on previous evaluation, this interval was found sufficient for behavioural analysis in pigs compared to sampling intervals of 5 and 15 min.

Behavioural effects after castration are reported to be the greatest during the first 8 h in 7-week-old pigs (McGlone & Hellman, 1988). Hence, we decided that the behaviour of pigs subjected to major abdominal surgery should be closely analysed during the first 12 h immediately after surgery. When using the instantaneous sampling method some short-lasting behaviour could be missed; hence, the videotape recordings for the first 12 h postoperatively were registered continuously. We estimated the frequency and latency of each activity during this earlier postoperative period. If an active behaviour occurred simultaneously with an inactive behaviour, such as "sitting" and "rooting" activities, it was considered active.

Clinical examination

The general condition of pigs and signs of pain-related behaviour (e.g. hunched back) were examined at least three times a day by a veterinarian experienced in swine medicine (**III, IV** and **V**). This physical examination included also RR, HR, appetite and estimates of faecal colour and consistency.

Weight and feed consumption

All pigs were weighed in a wooden box on an electronic scale immediately before surgery. Pigs were then weighed once daily during the two consecutive postoperative days in Study **III, IV** and **V**.

Daily feed consumption was measured at each feeding time in Study **IV**, including the day before surgery and the two consecutive days. Each pig was given 500 g of dry feed humified with approximately 100 mL of water at each feeding time. Feed intake was then calculated as the amount of feed left from the previous feeding time.

Statistical analyses

For Study **I**, differences between treatments when isoflurane MAC was determined were tested using General Linear Model and Tukey's method for further comparison. Changes in physiological parameters measured in Study **II-IV** were analyzed using repeated measurement ANOVA for each treatment groups. For Study **II**, differences in data between treatment groups were analyzed at specific points using a Mann Whitney U test. For Study **IV**, values from blood gas analysis were tested with two-sample *t*-test.

Behavioural data recorded at pre-treatment period were used as control data. The activity/inactivity levels and each specific activity were analyzed by repeated measurement ANOVA. Data from behaviour analysis are presented as individual values in Study **IV**. The latency of the first observation of each different behaviour was analyzed with the Kruskal-Wallis non-parametric test (**III**). Weight and feed consumption were calculated as the difference between pre-treatment value and the values obtained post-treatment and analyzed with repeated measurement ANOVA. Serum concentrations were analyzed by a General Linear Model. All results were analyzed with statistical software (Minitab, Inc., State College, PA, USA; and SAS Institute Inc.; Cary, NC, USA) and the results are given as mean ± SD. The level of statistical significance was set at $p < 0.05$.

Results and discussion

During general anaesthesia

Induction with medetomidine and tiletamine/zolazepam

Induction of anaesthesia with the combination of medetomidine and tiletamine/zolazepam (MTZ) in pigs has been used at our department for many years (Henrikson, Jensen-Waern & Nyman, 1995). This combination is easy to apply since the volume of the mixture is small enough for single intramuscular injection. Also, sedation produced by this combination is relatively fast (within 10 – 15 min) and smooth. In Study **I**, medetomidine together with tiletamine/zolazepam was found to reduce the isoflurane MAC by 68% (average ETiso 0.6%) compared to pigs induced and maintained with isoflurane as the sole anaesthetic agent (average ETiso 1.9%). This reduction was seen in pigs experiencing somatic noxious stimuli evoked by claw pinching. In Study **II**, pigs treated in a similar manner but submitted to surgery including both somatic and visceral pain required 0.9 ± 0.2% isoflurane. When pigs are induced with MTZ the isoflurane requirement is lower than the ETiso measured in pigs with isoflurane as the sole anaesthetic drug. Medetomidine (0.03 mg kg^{-1}) alone has been reported to reduce the isoflurane MAC by 47% in dogs (Ewing *et al*., 1993). Tiletamine/zolazepam has also been described to reduce the isoflurane MAC by 29% (0.55 mg kg^{-1}) and 77% (8.8 mg kg^{-1}) in goats (Doherty *et al*., 2002). Combining medetomidine and tiletamine as induction drugs was expected to have a sparing effect on the volatile anaesthetic agent since these drugs have analgesic properties.

In Study **I**, after induction with MTZ the mean arterial blood pressure was higher and the alveolar – arterial oxygen partial pressure difference was lower compared to the face mask induction with isoflurane. The mABP during isoflurane anaesthesia in the MTZ treated pigs was 73 ± 12 mm Hg, which is comparable to 70 mm Hg reported in pigs after 1 h of anaesthesia with tiletamine/zolazepam and xylazine (Henrikson, Jensen-Waern & Nyman, 1995). The isoflurane face mask-treated pigs showed a mABP of 51 ± 12 mm Hg. The higher mABP in the pigs treated with MTZ might be related to the reduced requirement of isoflurane to maintain a surgical depth of anaesthesia. Consequently, the peripheral vasodilatation induced by isoflurane is minimized (Egger *et al.,* 2003; Pettifer & Hosgood, 2004). There is also a possibility that an α_2-agonist such as medetomidine could affect the blood pressure. The α_2-agonist medetomidine produces a dose dependent increase in arterial blood pressure due to vasoconstriction (Sinclair, 2003). The $P(A\text{-}a)O_2$ calculated for the MTZ treated pigs was 5.2 ± 2.1 kPa and for the isoflurane-induced pigs 11.2 ± 5.3 kPa. Thus, the pigs treated with MTZ had a better gas exchange compared to pigs treated with isoflurane by face mask. Volatile anaesthetics are well known to impair the pulmonary gas exchange and respiratory mechanism (Hedenstierna, 1995), which can result in decreased arterial oxygenation (Eisenkraft, 1990; Kleinsasser *et a*l., 2001). Whether the impaired gas exchange is related to alterations in pulmonary circulation was not studied in the present investigation.

Induction with additional opioid analgesia

Systemic buprenorphine

In Study **IV**, preoperative administration of buprenorphine (0.1mg kg^{-1}), in addition to MTZ, resulted in serum concentration between 11.2 - 18.5 ng mL^{-1} one hour after the intramuscular injection. A light plane of anaesthesia with short episode of leg movement was seen during surgery in two out of four pigs. In addition, it was found that systemic buprenorphine at this high dose affected mean arterial blood pressure, respiratory rate and $PaCO_2$ during the abdominal surgery. The mABP and $PaCO_2$ were increased 60 min after the drug application. At this time point, the respiratory rate was lower compared to pigs treated with epidural morphine. In Study **IV**, an apnoea episode was observed in one pig. Two other pigs showed a transient respiratory depression 10 min after the intramuscular injection (Fig. 7). In other animal species, buprenorphine was suggested to produce fewer respiratory side-effects compared to other opioids, for example in rats (Liles & Flecknell, 1992; Roughan & Flecknell, 2004) and sheep (Nolan, Livingston & Waterman, 1987). According to Dahan *et al.* (2005) a dose of 0.1 mg kg^{-1} of buprenorphine did not produce respiratory effects in rats. The recommended doses of buprenorphine in pigs range from 0.005 to 0.1 mg kg^{-1} (Rodriguez, Cooper, & Risdahl, 2001). It has been shown that 0.1 mg kg^{-1} provides sufficient and long-lasting analgesia in pigs subjected to noxious stimuli such as hot plate, needle prick and cannulation of ear vein (Hermansen, Pedersen, & Olesen, 1986). However, based on the few pigs studied in this work, it seems that this dose of buprenorphine is associated with respiratory depression in spontaneously breathing pigs during inhalation anaesthesia.

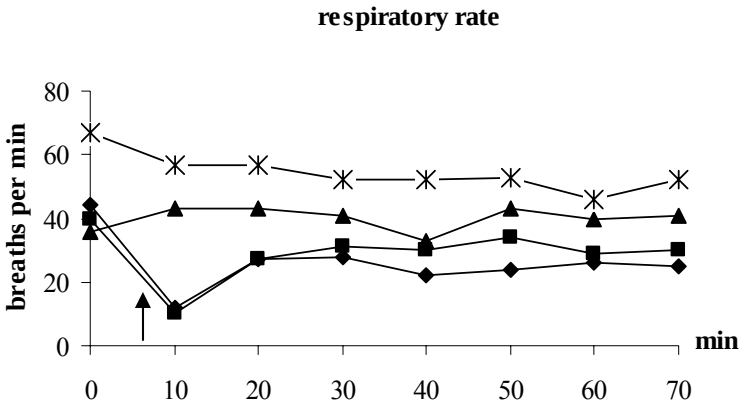

Fig. 7. Individual respiratory rate (breath per min) measured throughout the abdominal surgery in pigs (n=4) treated with intramuscular buprenorphine (Study **IV**). Apnoea episode is indicated with an arrow.

In Study **I**, the pigs were not subjected to abdominal surgery. The only effect of the systemic buprenorphine was an increase in mABP during the isoflurane anaesthesia. All pigs were mechanically ventilated and thus both respiratory rate and minute ventilation were controlled. Therefore, the higher blood pressure was probably related to the lower isoflurane concentration (average 0.3%) required to avoid the pain sensation evoked in this study (i.e. somatic pain). In contrast, in Study **IV**, pigs were subjected to a major abdominal surgery, which evokes both somatic and visceral pain. Hence, the isoflurane concentration measured during surgery was 0.5 ± 0.1%.

Epidural morphine

In Study **IV**, one pig out of five exhibited a light plane of anaesthesia with a short episode of leg movement during abdominal surgery. In this study, the administration of epidural morphine resulted in serum concentration ranging between 23.6 – 141.1 ng mL^{-1} after 1 h from injection. When pigs were subjected to only somatic pain (Study **I**), mean arterial blood pressure was higher 30 min after the epidural injection compared to pigs treated with MTZ. This higher mABP was probably related to the isoflurane sparing effect of the opioid in the pigs. In Study **I**, the isoflurane requirement was further reduced by 30% from ETiso 0.6% to 0.4% when additional opioid analgesia was added to the MTZ induction. Also, preoperative epidural morphine given immediately after MTZ induction reduced the requirement of isoflurane by 33% during caecum intestinal cannulation compared to pigs treated with epidural saline (**II**). Thus, the reduction of isoflurane concentration again minimized the cardiovascular effects of isoflurane.

Influence of the type of pain stimulus on the isoflurane requirement

This thesis included antinociceptive testing that evoked both somatic and visceral pains. In Study **I,** the claw pinching evoked somatic pain whereas in Study **II-IV** the abdominal surgery evoked somatic and visceral pain. The different types of pain were found to influence the isoflurane requirement during isoflurane general anaesthesia (Fig. 8). It was seen that an average 0.26% increase in the isoflurane concentration was required to abolish somatic and visceral pain compared when only somatic pain is present. This emphasizes the importance of which type of nociceptive pain is included in the study and what influences it might have in the results.

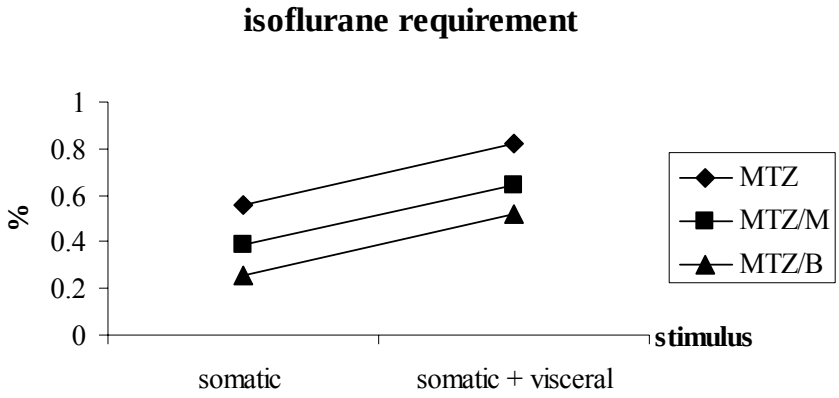

Fig. 8. Mean end-tidal isoflurane concentration (%) after induction by medetomidine and tiletamine/zolazepam given intramuscularly (MTZ), medetomidine and tiletamine/zolazepam followed by epidural morphine (MTZ/M), and medetomidine and tiletamine/zolazepam followed by intramuscular buprenorphine (MTZ/B), during two different antinociceptive tests.

Pig behaviour

Before opioid analgesia

From the video tape recordings of 29 pigs it was seen that the activity level during 24 h prior to the treatments was 30 ± 9% (Fig. 9).

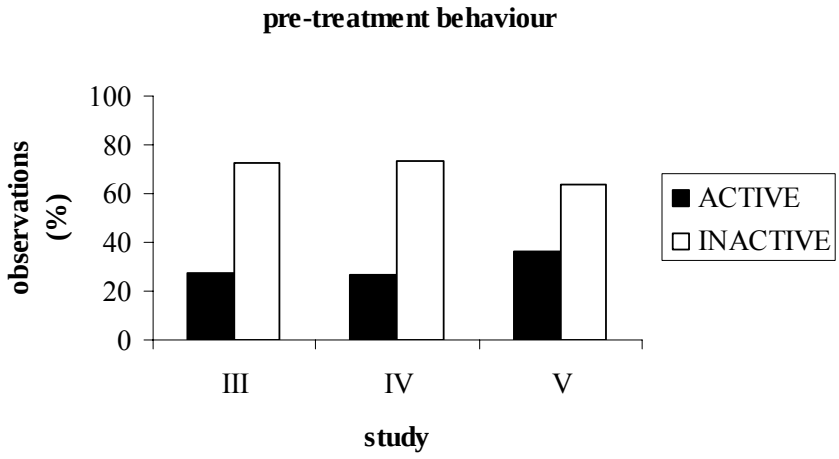

Fig. 9. Mean percentage numbers of observations of active and inactive behaviours in pigs from Study **III** (n=10), **IV** (n=9) and **V** (n=10) during 24 h prior to the treatments.

The activities mostly seen during this period was "lying down quietly" (67 ± 9%), "rooting" (18 ± 10%) and "eating" (7 ± 4%) (Fig. 10). In Study **IV**, the pigs

were kept on restricted diet but that did not influence the "eating" activity. During Study **V**, the pigs displayed more percentage of "rooting", which might be related to the fact that this study was conducted during winter time. Consequently, the active behaviours would help maintain a higher body temperature.

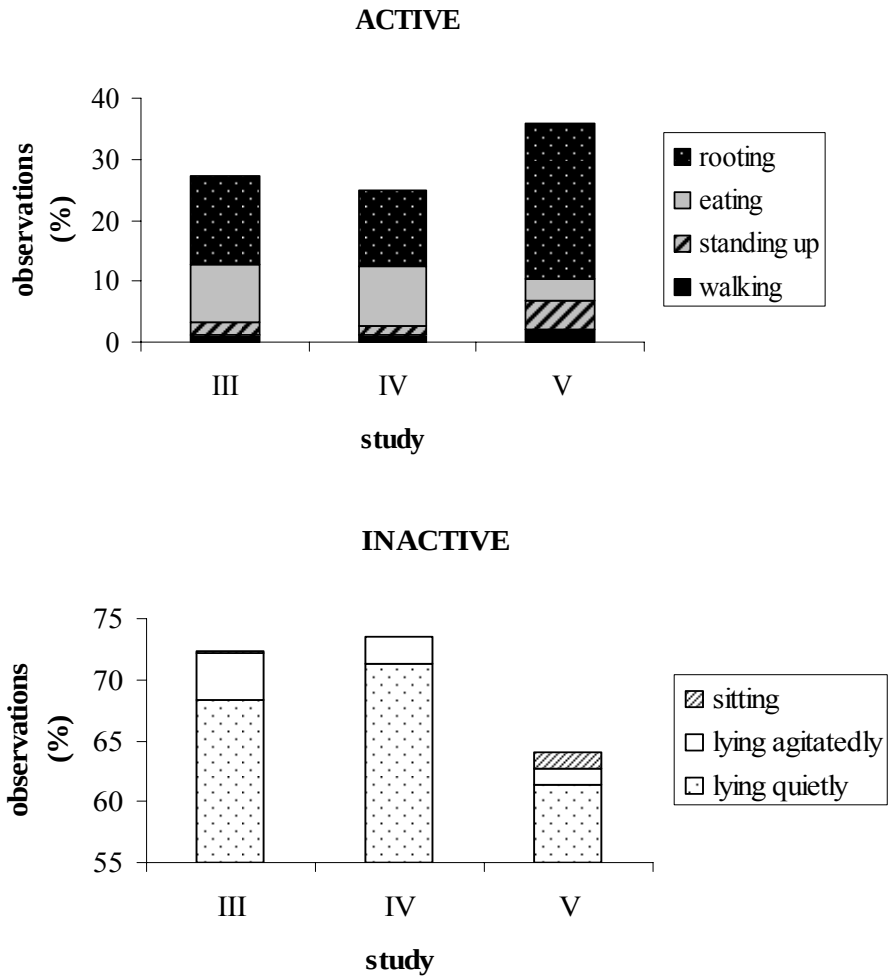

Fig. 10. Percentage number of observations of selected activities, in pigs from Study **III** (n=10), **IV** (n=9) and **V** (n=10), during 24 hours prior to the treatments.

Induction with medetomidine and tiletamine/zolazepam

Although MTZ improved cardiovascular function and pulmonary gas exchange it was not sufficient for postoperative analgesia in pigs. In Study **III**, no obvious pain-related behaviour was observed in pigs subjected to caecum intestinal cannulation through direct observation by the experienced veterinarian. The absence of pain-related behaviour does not guarantee that the pigs were not experiencing pain. However, based on the videotape recordings, these pigs showed lower activity level (6 ± 3%) compared to their preoperative values (25 ±

9%). Also, the pigs which did not received additional opioid analgesia decreased in weight during the next two consecutive days postoperatively. The weight and feed measurements were important indicators of animal welfare in this study. Conventional growing pigs have a high feed-conversion rate and ability to gain weight. Thus, poor physiological conditions will be reflected in a low daily weight gain (Wallgren, 1994).

Induction with additional opioid analgesia

Systemic buprenorphine

In Study **IV**, the dosage of buprenorphine used caused unpredictable postoperative behavioural effects in the four pigs included (pig no. 3, 6, 7 and 9). During the first 12 h after injection, pigs 7 and 9 had increased activity of 16% and 15%, compared to pre-treatment values (Fig. 11). The other two pigs (pig 3 and 6) showed a decrease in activity of 29% and 21%, respectively. The increase of spontaneous locomotory activity caused by buprenorphine has earlier been reported in rats (Cowan, Doxey & Harry, 1977; Liles & Flecknell, 1991; Roughan & Flecknell, 2004), mice (Cowan, Doxey & Harry, 1977), sheep (Nolan, Livingston & Waterman, 1987) and pigs (Hermansen, Pedersen & Olesen, 1986; Harvey-Clark, Gilespie & Riggs, 2000). In our pigs the change in activity was seen together with loss in weight, lower feed consumption (Fig. 13) and buprenorphine serum concentrations that can indicate that pigs might have needed additional analgesia. Harvey-Clark, Gilespie & Riggs (2000) have stated that all pigs treated with intramuscular buprenorphine (0.1 mg kg^{-1}) after thoracotomy required supplemental dose of buprenorphine (0.1 mg kg^{-1}) 5 – 8 h after the initial dose. Even rats treated with buprenorphine after abdominal surgery needed additional analgesia (Roughan & Flecknell, 2004).

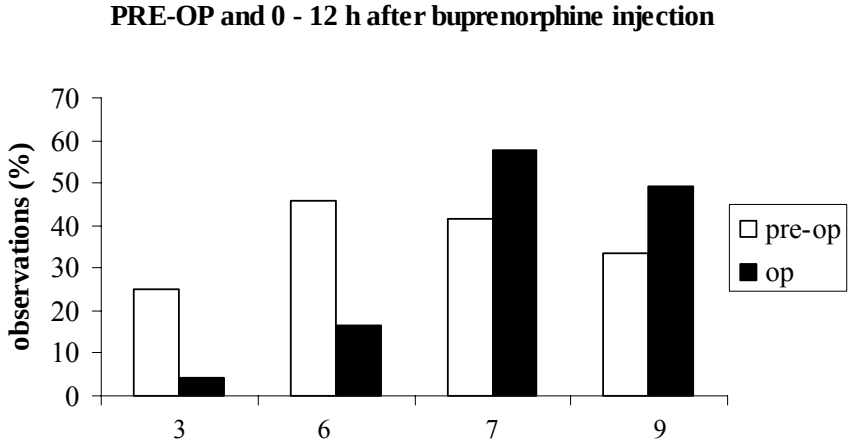

Fig. 11. Percentage numbers of observations of active behaviour in pig no. 3, 6, 7 and 9, treated with intramuscular buprenorphine, during the pre-operative day and the first 12 h after surgery (Study **IV**).

Epidural morphine

In Study **III**, five pigs treated with epidural morphine and fentanyl patch displayed similar activity level (33 ± 10%) in the early postoperative period compared to the pre-treatment values (30 ± 10%). In contrast, in Study **IV**, five pigs treated with only epidural morphine showed a lower activity level of 6 ± 3% compared to their pre-treatment values (35 ± 13%). Considering all pigs treated with epidural morphine from Study **III** (n=5) and **IV** (n=5), we observed that seven out of the ten pigs had a low activity level ranging between 4 – 14 % (Fig. 12). From these ten pigs, two of them showed a high activity level of 43% and 47%, respectively. Also, one pig out of the ten treated with epidural morphine exhibited an activity level greater than 50%. Therefore, epidural morphine with or without fentanyl patch resulted in unpredictable behaviour.

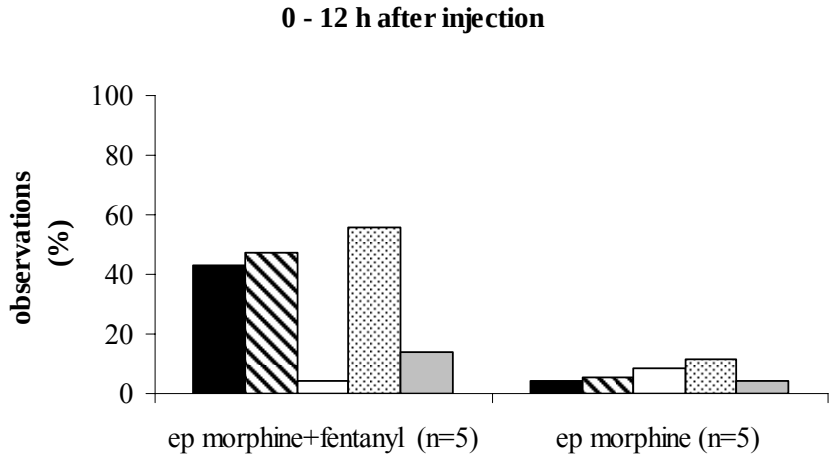

Fig. 12. Individual percentage numbers of observations of active behaviour in pigs, treated with epidural morphine with and without fentanyl patch, during the first 12 h after surgery.

During the first 12 h postoperative, it was seen that seven out of the ten pigs in Study **III-V** treated with morphine performed behaviours such as "eating" and "rooting" while they were "sitting". In Study **III**, this locomotor dysfunction in pigs receiving epidural saline was also observed. In these situations, the active behaviour was registered in priority to the inactive behaviour. In human medicine there are some reports of numbness and weakness of one or both legs after receiving epidural injection (Littrell, 1991). Additionally, the high volume solution used in the present study might have affected the motor function. The technique chosen was earlier proved to result in standardized penetration of a solution in the epidural space in young pigs. Using epidural radio-opaque contrast, Strande (1968) was able to recommend this technique for surgeries on the abdominal cavity or caudal regions of the body. However, Strande's study did not include a behaviour aspect and the effects on locomotion have until now not been reported. Despite his recommendation, the motor impairment seen in our pigs might affect the postoperative behaviour.

The next 12 – 36 h after abdominal surgery, all pigs treated with epidural morphine both from Study **III-IV** showed lower activity level (16 ± 5% and 11 ± 3%, respectively) compared to their preoperative data (30 ± 9% and 36 ± 13%, respectively). A possible factor that can result in low activity is when pain causes an animal to become immobile (Dennis & Melzack, 1983; Flecknell, 1999). However, when analyzing weight measurements in these studies, the morphine treated pigs gained more weight compared to pigs treated with epidural saline or buprenorphine (Fig. 13). In Study **IV**, although the pigs were kept on a restricted diet they weighed more than pigs treated with saline or buprenorphine. The weight gains in study **III-IV** were at least similar to that of conventional herds (Wallgren, 1994).

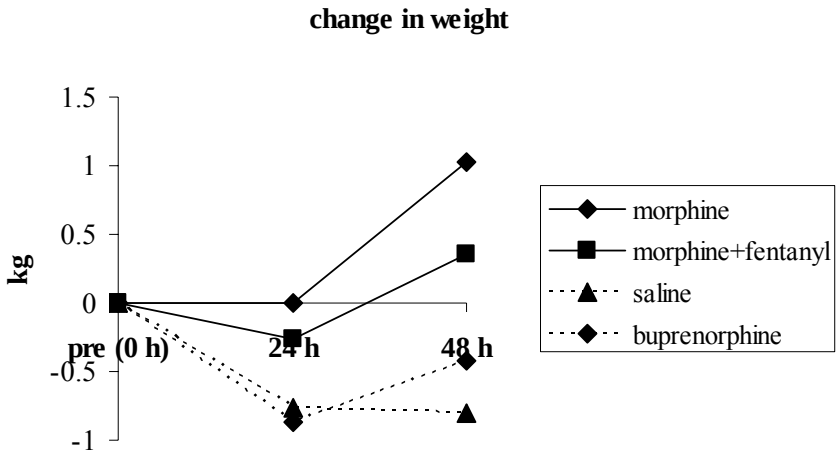

Fig. 13. Mean change in weight (kg) compared to data measured before surgery (pre) in pigs treated with epidural morphine (n=5), epidural morphine and fentanyl patch (n=5), epidural saline (n=5) or intramuscular buprenorphine (n=4) during the two next postoperative days.

Epidural morphine and transdermal fentanyl

It is suggested that when fentanyl patch is used as the only analgesic drug, it should be placed on to the skin of an animal at least 12 h prior to any surgical procedure (Robertson & Taylor, 2004). In general, the onset time until a steady state of plasma concentration is achieved after 6 - 12 h in cats and 18 - 24 h in dogs (Riviere & Papich, 2001). In Study **III**, the fentanyl patch was applied immediately after surgery. The epidural morphine given prior to the surgery was expected to give sufficient analgesia at least for 12 h and until the effect of fentanyl was present. Fentanyl patch (50 μg h^{-1}) applied to pigs subjected to caecum intestinal cannulation resulted in serum fentanyl concentrations ranging between 0.15 – 0.75 ng mL^{-1} after 24 h from application. However, these pigs had a low activity level during 12 – 36 h after surgery compared to their pre-operative values (Fig. 14).

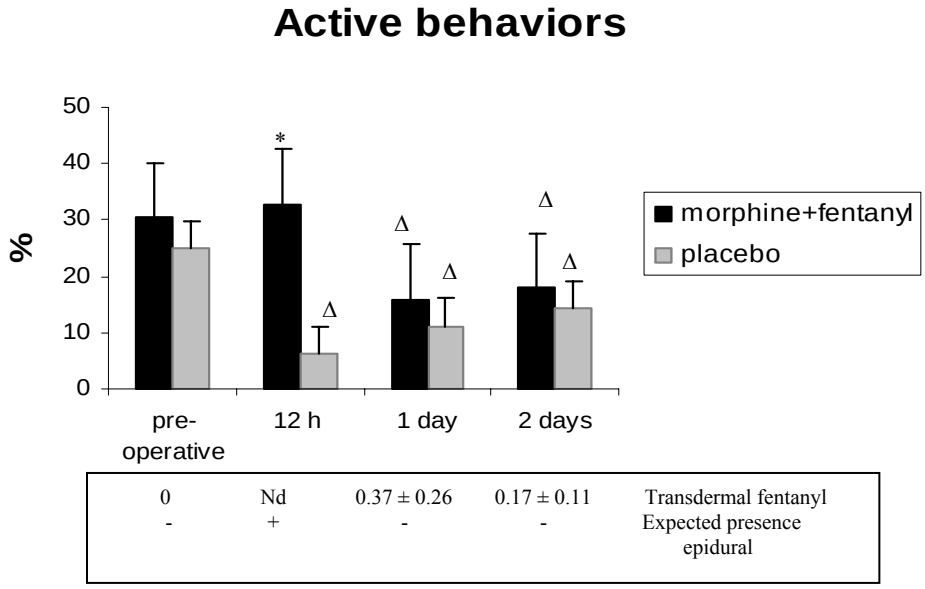

Fig. 14. Percentage (mean ± SD) numbers of observations of active behaviours in pigs, treated with epidural morphine and transdermal fentanyl or regular patch; preoperatively, during the first 12 hours after surgery, 1 day and 2 days postoperatively. Mean serum fentanyl concentrations ± SD (ng mL^{-1}) are given. Serum morphine concentrations were not measured. However, the expected effect of morphine is indicated by presence (+) or absence (-) during the experimental period. (*) Significant differences compared to placebo group ($p < 0.05$). (Δ) Significant difference compared to data from preoperative day ($p < 0.05$). (Nd) Not determined. (Study **III**).

During these first and second postoperative days, it was expected that only fentanyl would be the analgesic agent in action. Whether this change in behaviour was related to opioid-induced sedation or to insufficient pain relief was then investigated in Study **V**. The treatments in this study consisted in applying a fentanyl patch for 60 h immediately after 30 min of isoflurane anaesthesia or in awaken pigs. In Study **V** pigs, which were not subjected to any surgery, had serum fentanyl concentrations ranging between 0.01 – 0.6 ng mL^{-1} 24 h after application. The serum fentanyl concentrations measured differed significantly between individuals and between the treatments but no effects on behavioural could be seen (Fig. 15). This unpredictability of fentanyl absorption might explain the somewhat irregular behavioural effect seen in pigs treated with both morphine and fentanyl patch after surgery in Study **III**.

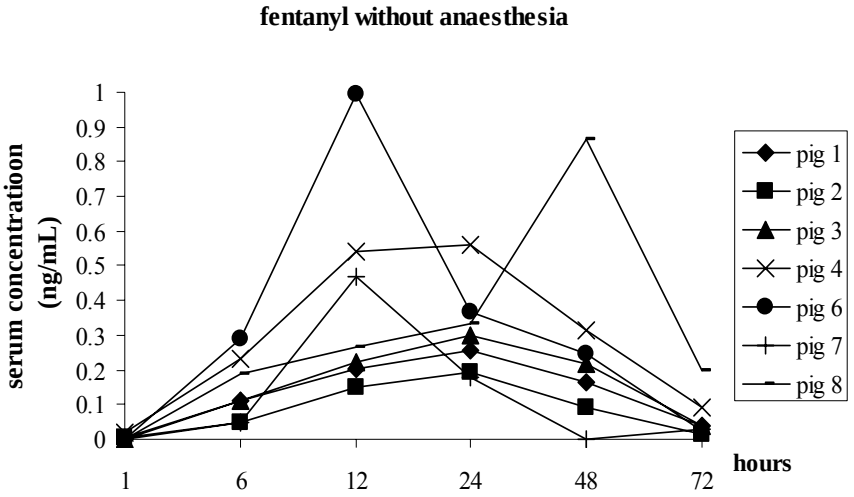

Fig. 15. Individual serum fentanyl concentrations (ng/mL) in pigs treated with transdermal fentanyl (patch 50 μg^{-1} h^{-1}) without isoflurane anaesthesia. Values obtained 1, 6, 12, 24, 48 and 72 hours after the patch application are shown (Study **V**).

Conclusions

- Induction of anaesthesia with medetomidine (0.05 mg kg^{-1}) and tiletamine/zolazepam (2.5 mg kg^{-1}, each) reduced the isoflurane MAC in pigs by 68%. Epidural morphine (0.1 mg kg^{-1}) and systemic buprenorphine (0.1 mg kg^{-1}) given in addition to the drugs used for induction of anaesthesia decreased the isoflurane MAC further by 33% and 50%, respectively. Induction of anaesthesia with medetomidine and tiletamine/zolazepam enhanced arterial blood pressure and arterial oxygenation compared to isoflurane induction.

- Pigs treated with epidural morphine or systemic buprenorphine prior to abdominal surgery attained anaesthetic depth with reduction in requirement of isoflurane. During anaesthesia, epidural morphine did not produce hemodynamic or respiratory changes but systemic buprenorphine affected the respiratory response in spontaneously breathing pigs.

- Transdermal fentanyl (50 μg h^{-1}) did not cause inactivity in awaken pigs. Preoperative epidural morphine decreased the activity in growing pigs during 60 h after abdominal surgery. Notable was that the combination of epidural morphine and transdermal fentanyl resulted in regular activity levels during the first 12 h postoperatively. Systemic buprenorphine caused either decreased or increased activity levels during the first 12 h after administration. The inter-individual variations measured in serum

fentanyl concentration indicate that drug absorption from transdermal patches is unpredictable and sometimes deficient. Epidural morphine and transdermal fentanyl resulted in weight gain after treatment and abdominal surgery. Weight and feed consumption decreased after systemic buprenorphine.

Recommendations

Medetomidine and tiletamine/zolazepam can be used in growing pigs for induction of anaesthesia. Additional preoperative epidural morphine indicated improved postoperative recovery with weight gain and unaltered feed intake. Due to the unpredictable drug uptake thought the skin, transdermal fentanyl is not recommended as the only postoperative analgesic drug in growing pigs.

Drugs used for anaesthesia and analgesia given to growing pigs (23 ± 4 kg).

	Drugs	**Dose**
Induction	medetomidine	0.05 mg kg^{-1}, IM
	tiletamine/zolazepam	2.5 mg kg^{-1} each, IM
Maintenance	isoflurane	To effect
Analgesia	morphine	0.1 or 0.12 mg kg^{-1}, EP
	buprenorphine	0.1 mg kg^{-1}, IM
	fentanyl	50 µg h^{-1} (patch)

IM: intramuscular; EP: epidural

Future researches

The epidural morphine analgesia might be improved by using a permanent catheter. This will be enable continuous epidural morphine administration during anaesthesia and in the awake pig. This continuous epidural morphine administration might improve the cardiovascular parameters. After the general anaesthesia, this technique might result in even earlier return to the pig's regular behaviour compared to the standard epidural injection method.

Recently, there have been developed more advanced systems to evaluate behavioural effects in growing pigs. For instance, computer programmes for recording spontaneous locomotor and feed intake pattern. To refine the evaluating of analgesic effect in pigs, the use of these computer programmes could be employed in complement to the videotape recording method used in this thesis.

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