Printing of this thesis was financially supported by (in alphabetical order):

Printed by University Press, Zelzate, Belgium. www.universitypress.be

ISBN: 978-90-5864-312-4

Multimodal analgesic approaches for stifle surgery in dogs. A study on the efficacy and possible side effects of the combined administration of a non-steroidal anti-inflammatory drug, opioids and/or a local anaesthetic.

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Omslagillustratie: *Perception of pain in animals does occur*, Tim Bosmans, 2012; alternatieve interpretatie van de pijngewaarwording zoals die beschreven werd door Descartes (1664).

Multimodal analgesic approaches for stifle surgery in dogs.

A study on the efficacy and possible side effects of the combined administration of a non-steroidal anti-inflammatory drug, opioids and/or a local anaesthetic.

Tim Bosmans

Proefschrift voorgedragen tot het behalen van de graad van

Doctor in de Diergeneeskundige Wetenschappen,

Faculteit Diergeneeskunde, Universiteit Gent

2012

Promotoren:

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Voor Vita

Je was ‘een proefhond’, maar in mijn gedachten was je reeds geadopteerd, want dit zou je laatste deelname aan een onderzoek zijn. Je hebt nu nooit de kans gekregen om te genieten van een vrij leven en dat is oneerlijk en moeilijk te dragen. We deden alles en meer om je te kunnen redden, maar helaas. Het spijt me.

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List of abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	invasive arterial blood pressure
APTT	activated partial thromboplastin time
ASA	American Society of Anesthesiologists
BDNF	brain derived neurothrophic factor
BMBT	buccal mucosal bleeding test
BUN	blood urea nitrogen
CaO_2	arterial blood oxygen content
CGRP	calcitonin gene-related peptide
CNS	central nervous system
CrCLR	cranial cruciate ligament rupture
CVP	central venous pressure
COX	cyclo-oxygenase
CRI	continuous rate infusion
DAP	diastolic arterial pressure
DNIC	diffuse noxious inhibitory control
DO_2	oxygen delivery
EPSP	excitatory post-synaptic potential
ET	end-tidal
$F_{E}´ISO$	end-tidal isoflurane concentration/percentage
$F_{E}´ISO_{60}$	end-tidal isoflurane concentration/percentage after 60 minutes of anaesthesia
$F_{I}´ISO$	inspired isoflurane fraction/percentage
FIO_2	inspired oxygen fraction/percentage
FR	formatio reticularis
f_R	respiratory rate
GABA	gamma-aminobutyric acid

List of abbreviations

GI	gastro-intestinal
GVGB	gescheurde voorste gekruiste band
Hb	haemoglobin
HES	hydroxyethylstarch 6%
HR	heart rate
HS	Horner's syndrome
IASP	international association for the study of pain
IL	interleukin
IPPV	intermittent positive pressure ventilation
IT	imbrication technique
IV	intravenous
LA	local anaesthetic
LOR	loss of resistance method
LOX	lipoxygenase
MAC	minimum alveolar concentration
MAC_{ISO}	minimum alveolar concentration of isoflurane
MAP	mean arterial pressure
MFPS	multifactorial pain scale
mGluR	metabotropic glutamate receptors
NGF	neurotrophic growth factor
NMDA	N-methyl-D-aspartate
NSAID	non-steroidal anti-inflammatory drug
NS	nociceptive specific
PAG	periaqueductal grey matter
$PaCO_2$	arterial tension of carbon dioxide
PaO_2	arterial tension of oxygen

List of abbreviations

PCV	packed cell volume
$P_{E'}CO_2$	end-tidal carbon dioxide partial pressure
PO	per os
PT	prothrombin time
SID	once daily
SAP	systolic arterial pressure
sAP	systemic arterial pressures
SC	subcutaneous
SpO_2	arterial oxygen saturation
SV	stroke volume
SVR	systemic vascular resistance
TTA	tibial tuberosity advancement
TNF-α	tumor necrosis factor α
UMPS	University of Melbourne Pain Scale
$\dot{Q}t$	cardiac output
VAS	visual analogue scale
WDR neuron	wide dynamic range neuron

General Introduction

Adapted from:

Bosmans T, Doom M, Gasthuys F, Simoens P, Van Ham L, Polis I (2009) Perioperative pain: physiology and pathophysiology. Vlaams Diergeneeskundig Tijdschrift 5, 302-314.

1 Introduction

Descartes postulated in the 17th century that animals were *automata* and therefore unable to experience pain (Ablondi 1998). Up until the second half of the 20th century, this theory was still accepted by science, resulting in undertreatment or even ignorance of pain in veterinary medicine (Rollin 1989). Today, most veterinarians have no problem admitting that animals do experience pain. However, several studies evaluating the attitudes of veterinarians towards postoperative pain worldwide, suggest that the use of perioperative analgesics is still inconsistent and tends to be rather low after many surgical interventions (Hewson et al. 2006). One of the possible explanations for the lack of appropriate analgesic treatment seems to be the inability of veterinarians and veterinary nurses to recognize pain in the wide variety of species referred for treatment. In a questionnaire about analgesia in dogs and cats distributed among French veterinarians, 14.3% of the respondents considered their knowledge of pain recognition to be inadequate and more than 50% reported that their methods of pain quantification and monitoring were insufficient (Hugonnard et al. 2004). Apart from their inability to recognize pain, veterinarians are often relatively reluctant to use perioperative analgesics, mainly because of a lack of familiarity with the available drugs, major concerns about the side effects and practical objections related to the recording of controlled substances such as opioids (Lascelles et al. 1999; Muir & Woolf 2001; Wright 2002).

Effective management of perioperative pain remains an ethical responsibility for veterinary practitioners. Over the last decade, major scientific improvements in the understanding of the pathophysiological processes involved in pain transmission resulted in a renewed awareness towards the need for analgesic treatment in animals (Woolf 2000). This positive evolution has led to newer theories in the domain of the optimal use of perioperative analgesics. Terms such as “pre-emptive analgesia”, where analgesics are administered before the occurrence of the noxious stimulus (Woolf 1983; Dahl & Kehlet 1993; Lascelles et al. 1997; Moiniche et al. 2002), and “multimodal analgesia”, where analgesic drugs with different modes of action are combined (Kaneko et al. 1994; Slingsby & Waterman Pearson 2001), have emerged and gained interest in daily veterinary practice. Consequently, in order to select the best “analgesic plan” for an individual patient, it is mandatory that veterinarians become familiar with the physiology and pathophysiology of pain (Woolf 2000).

2 Definition(s) of pain

The International Association for the Study of Pain (IASP) defines pain as "*an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage*" (Kelly et al. 2001). Pain requires integration of nociceptive and other sensory information at the cortical level and is therefore a conscious awareness of acute or chronic discomfort in variable degrees (Lemke 2004). Pain can be the result of injury, disease or emotional distress and is characterized by biological and/or behavioural changes. This subjective experience is accompanied by feelings of fear, anxiety, and even panic, which elicit protective motor actions, resulting in learned avoidance and possible modification of species-specific behaviour (Hellyer et al. 2007). Finally, pain is considered to consist of three key components. The *sensory-discriminatory* component provides information on the onset, location, intensity, type and duration of the stimulus. The *motivational-affective* component is closely associated with the autonomic nervous system's cardiovascular, respiratory and gastro-intestinal changes, and disturbs the feeling of well-being in the individual, thus triggering certain actions in an animal (Hellyer et al. 2007). The magnitude of quality (e.g. stabbing/pounding, mild/severe) has been quantified by the *cognitive-evaluative* component and includes also the effects of prior experience, anxiety, attention and conditioning (Hellyer et al. 2007; Price & Nolan 2007).

Different classifications of pain have been reported in the literature. The classification can be done on the basis of an anatomical (somatic *versus* visceral pain), a temporal (acute *versus* chronic pain) or an etiological approach (inflammatory *versus* neuropathic pain) (Lemke 2004).

Perioperative pain is probably best divided into two types based on the physiological/adaptive and the pathological/maladaptive concepts (Woolf & Chong 1993). Physiological pain informs the individual that something "out there" is harmful and needs to be avoided (Woolf 2000). It is defined as a type of pain that is experienced abruptly over a short period of time. Additionally, it requires a high threshold noxious input, is well localized and transient and serves a protective function (Woolf 2000; Muir & Woolf 2001; Lemke 2004). Pathological or maladaptive pain, on the other hand, is defined as the pain following severe trauma, persisting beyond the usual course of an acute disease or beyond the reasonable time required for an injury to heal. Beyond that, this kind of pain can be associated with chronic pathological processes persisting or recurring for months and even years. It

requires low threshold input, results in extended discomfort and abnormal sensitivity and therefore does not have a protective function (Muir & Woolf 2001; Lemke 2004; Hellyer et al. 2007). Under clinical conditions, pathological pain has been categorized in terms of the most likely mechanisms responsible, including inflammation and neuropathy. Nerve transsection and compression are possible causes of neuropathic pain, whereas surgical procedures, trauma, ischemia, osteoarthritis, infection, and abscess formation induce inflammatory pain. Head trauma, vertebral disc prolapse, amputation, total ear canal ablation, cancer and some specific inflammatory processes (e.g. pancreatitis) can be accompanied by elements of both inflammation and neuropathy (Muir & Woolf 2001).

Under normal conditions, the main goal of the biological imperative is to assist and repair the healing process after damage of tissues (Woolf 2000). Severe injuries and chronic pathological pain states can lower the threshold required to initiate pain: this phenomenon has been defined as *hypersensitivity*. Hypersensitivity induces the development of exaggerated responses to noxious stimuli, which is defined as *hyperalgesia*. It can also lead to *allodynia*, which is pain arising from normally non-painful perceptions (Muir & Woolf 2001). The processes of peripheral and central sensitization can explain the physiological background for the occurrence of both hyperalgesia and allodynia (*vide infra*). To understand the meaning of these pathophysiological pain terms, basic knowledge of the physiology of pain is essential.

3 Processes in the sensory pathway

Transduction, transmission, modulation and perception are the four major processes involved in the sensory pathway of pain (Fig. 1) (Kelly et al. 2001). The nociceptive pathway is a 3-way neuron chain in its simplest form, in which the first or the primary afferent neuron is responsible for transduction of noxious stimuli and transmission of signals from the periphery to neurons in the dorsal horn of the spinal cord, where modulation takes place. The second or projection neuron receives input from the first neuron and projects the signal to neurons in higher centers of the brain (medulla, pons, midbrain, thalamus and hypothalamus). In these centers, third order supraspinal neurons integrate signals from the spinal neurons and project them to subcortical and cortical areas where pain is finally perceived (Lemke 2004).

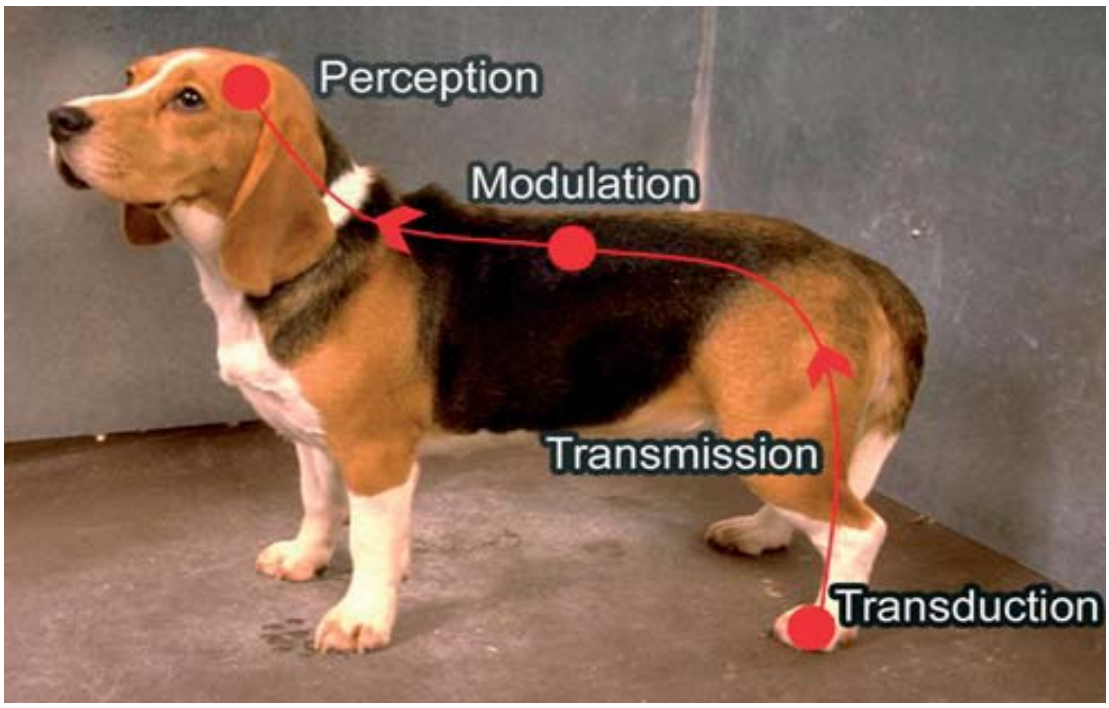

Fig. 1 Schematic representation of the processes in the sensory pathway. The arrows indicate the afferent direction of pain signaling.

3.1 Transduction

Transduction is the neurophysiological process whereby noxious thermal, chemical or mechanical stimuli (nociceptive signals) are transduced into action potentials by high-threshold pain receptors, defined as nociceptors (Kelly et al. 2001; Muir & Woolf 2001; Lemke 2004). Nociception provides information about the state of the environment near the individual and transmits it to the central nervous system (CNS). The encodement of the noxious stimulus by the nerve endings of afferent sensory pain fibers is based on the modality, intensity, duration and location of the stimulus, with intensity being the most important factor for the final determination of the severity of pain (Muir & Woolf 2001).

The primary afferent *nociceptors* are the distal terminals of the Aδ and C nerve fibers. The cell bodies of these nerve fibers are located in the dorsal root ganglia on the dorsal root of the spinal nerves originating in the body. For the trigeminal, facial, glossopharyngeal and vagal nerves originating from the head, the cell bodies are located in the trigeminal ganglia (Woolf 2000; Kelly et al. 2001; Muir & Woolf 2001). Nociceptors are distributed in skin and deep tissues in varying densities and can specifically detect a particular type of stimulus such as touch, temperature, pain, etc.

Four classes of nociceptors have been described up to now. *Mechanical* and *thermal* nociceptors respond respectively to intense pressure and extreme temperatures. These 2 types of nociceptors are classified together as Aδ mechano-thermal nociceptors (Lemke 2004). *Polymodal* nociceptors respond to noxious chemical, mechanical and thermal stimuli (Raja et al. 1988; Basbaum & Jessel 2000; Hellebrekers 2000). Finally, Schaible & Schmidt (1983) reported that many nociceptors, and most likely all of them, are inactive and rather unresponsive under normal circumstances. These *silent or sleeping* nociceptors are activated by inflammatory mediators and only respond to mechanical and thermal stimuli when activated (Schaible & Schmidt 1985; Greenspan 1997; Willis & Westlund 1997; Basbaum & Jessel 2000; Hellebrekers 2000).

3.2 Transmission

After transduction, the electrical stimulus must be transmitted to superficial and deeper layers of the dorsal horn of the spinal cord. Nociceptors that respond to thermal or mechanical stimuli transmit their information through large diameter, myelinated Aδ nerve fibers. These fibers have a high threshold and high conductive speed (5-30 m/s) and are related to "*first*" pain, which is defined as sharp, prickling and injurious pain. The signals of polymodal and silent nociceptors are transported by small diameter, unmyelinated, slowly conducting (0.5-2 m/s) C nerve fibers. These fibers are responsible for "*second*" pain, characterized by the occurrence of dull, aching and visceral pain (Basbaum & Jessel 2000; Hellebrekers 2000; Kelly et al. 2001; Lemke 2004; Price & Nolan 2007). Both types of nociceptive fibers innervate the skin (superficial pain) as well as the deep somatic or visceral structures (deep pain) (Hellyer et al. 2007). A third class of nerve fibers, the Aβ-fibers, are activated by low threshold stimuli including touch, leading to an innocuous sensation (Hellebrekers 2000). However, in cases of peripheral sensitization, they will contribute to the transmission of nociceptive signals (Price & Nolan 2007). This phenomenon will be explained under the item peripheral sensitization.

A variety of *neurotransmitters,* including excitatory amino acids (glutamate and aspartate), neuropeptides (substance P and neurokinin A) and calcitonin gene-related peptide (CGRP), are released by the first order neuron after stimulation (Kelly et al. 2001; Lemke 2004). Normal afferent input results in the release of glutamate, which binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate ligand-gated ion channels (Woolf & Salter 2000; Lemke 2004). The binding of glutamate on the AMPA

receptor generates fast post-synaptic potentials, which last for several milliseconds. The neuropeptides on the other hand bind to neurokinin receptors (Lemke 2004). Under normal conditions, N-methyl-D-aspartate (NMDA) ion channels (receptors) remain blocked by magnesium ions (Mg^{2+}) (Muir & Woolf 2001).

After entering the grey matter of the spinal cord using the so-called ”Lissauer’s tract”, a dense system of predominantly propriospinal fibers extending longitudinally from the periphery of the dorsal horn to the cord’s surface, first order nociceptive fibers synapse with second order nociceptive neurons in the dorsal horn of the spinal cord (Fine & Ashburn 1998). The grey matter of the spinal cord can be divided into several layers, which were initially described in the cat and are named “*Rexed laminae*” (Fig. 2 & 3) (Rexed 1954). The laminae consist of functionally distinct cells that form columns extending over the complete length of the spinal cord. As the borders between these laminae are not clearly separated, an overlap is present. Aδ-fibers synapse in laminae I, II and V, while C-fibers connect in lamina II (Kelly et al. 2001; Muir & Woolf 2001; Price & Nolan 2007) and send branches to laminae I en V (Fine & Ashburn 1998; Muir & Woolf 2001; Lemke 2004). The large Aβ sensory nerve fibers terminate on neurons located in laminae III, IV and V, projecting to the brain and integrating sensory input with descending information from the brain (Doubell et al. 1999).

Three types of second order neurons, the projection neurons, interneurons and propriospinal neurons, have been described in the dorsal horn. Two distinct types of projection neurons are primarily responsible for the further signaling of pain sensations to supraspinal third order neurons (Dahl & Moiniche 2004). Projection neurons in lamina I receive input directly from nociceptive Aδ- and C-fibers and are therefore defined as *Nociceptive Specific* (*NS*) neurons. Other neurons, which are mainly located in lamina V but also in laminae I and II (Jänig 1987), receive nociceptive and non-nociceptive (Aβ-fibers) information and are classified as *Wide Dynamic Range* (*WDR*) neurons (Dahl & Moiniche 2004; Lemke 2004). A *WDR* neuron has a typical large receptive field with a central area responsive to noxious and tactile stimuli (Aβ-fibers), while the periphery is responsive only to noxious stimuli (Mendell 1966; Fine & Ashburn 1998; Lemke 2004). The activity of *WDR* neurons is determined by the balanced total effect of excitatory and inhibitory inputs from their respective peripheral nerve fibers, local circuit excitatory and inhibitory neurons, and descending inputs from supraspinal sites (Dahl & Moiniche 2004).

The "*Rexed laminae*" also contain a large number of second order excitatory and inhibitory interneurons (lamina II) receiving multiple (non) nociceptive inputs from surrounding laminae and sending outputs to the brain and the ventral (motor) horn (Rexed 1952). These interneurons play a key role in gating and modulating nociceptive input (see central sensitization) (Lemke 2004).

Propriospinal neurons finally extend over several spinal segments and are responsible for segmental reflexes associated with nociception (Lemke 2004).

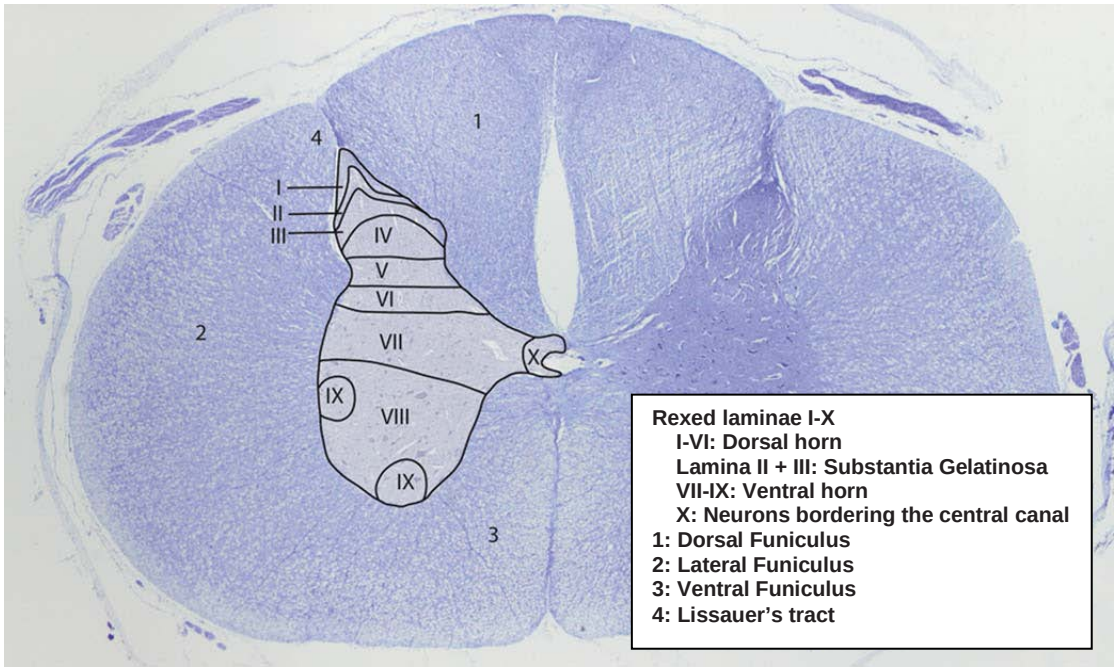

Fig. 2 Cross section of the second cervical spinal cord segment of a cat, with a schematic representation of the Rexed Laminae projected on top (Nissl stain, x4).

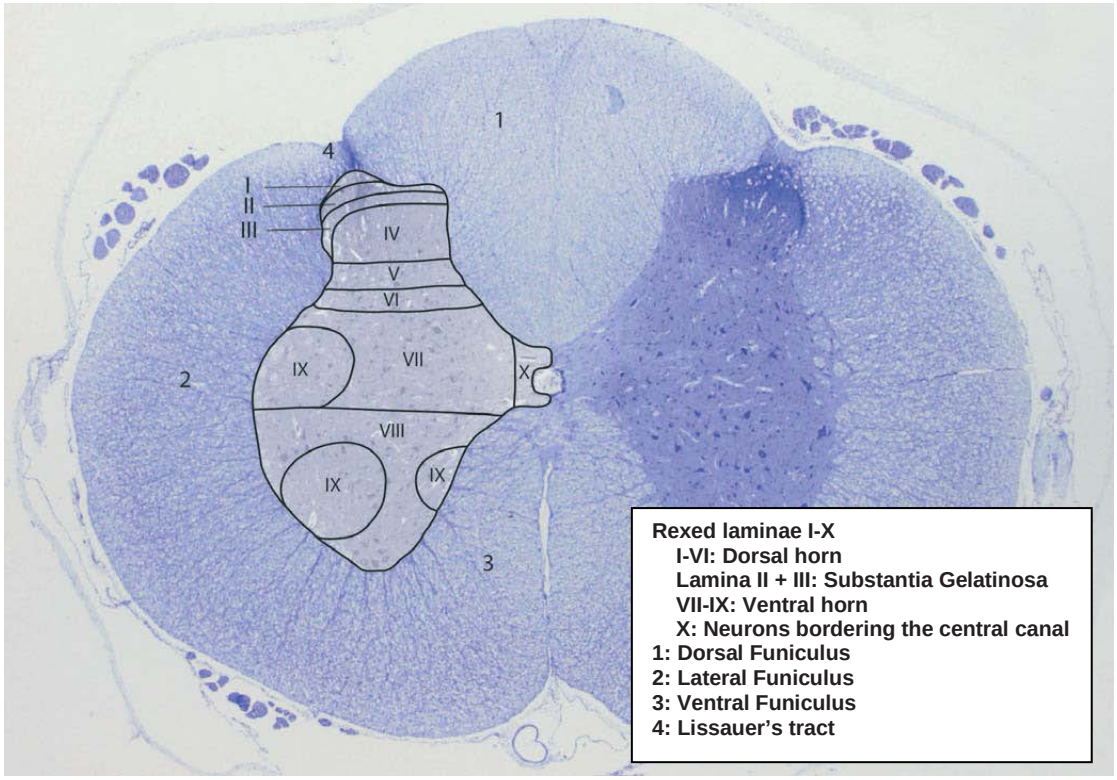

Fig. 3 Cross section of the fourth lumbar spinal cord segment of a cat, with a schematic representation of the Rexed Laminae projected on top (Nissl stain, x4).

3.3 Modulation/Modification of pain

Somatosensory pathways cannot be conceived of as “hard wired” electrical circuits that respond to stimuli in predictable ways and consistently produce a sensory perception in accordance with the stimulus in the periphery (Hellyer et al. 2007). On the contrary, pain is an active process generated partly in the periphery and partly in the central nervous system by multiple plastic changes determining the gain of the system (Woolf & Salter 2000). Nociceptive signals will launch a cascade of alterations (modulations) in the somatosensory system. This can take place in the periphery, spinal cord, brain stem and even at higher centers (Woolf & Salter 2000; Hellyer et al. 2007). Alterations in the somatosensory system have been defined by Woolf & Chong (1993) as neural plasticity or the ability of the nervous system to modify its function in response to different environmental stimuli (Lemke 2004).

The plasticity responsible for clinical pain hypersensitivity has two general forms namely modulation and modification. *Modulation* represents posttranslational and reversible changes in the excitability of neurons through the phosphorylation of receptors, ion channels, or associated regulatory proteins, resulting in altered intrinsic functional properties or cell-surface expression of channels in primary sensory and dorsal horn neurons. *Modification* stands for long lasting changes in the expression of transmitters / receptors / ion channels (Woolf & Salter 2000) and can result in altered gene expression (Muir & Woolf 2001). Both modulation and modification result in a distortion of the normal stimulus response characteristics (Woolf & Salter 2000).

Surgical procedures will induce tissue damage and injury of nerve fibers. When limited perioperative tissue trauma and inflammation occur, the pain will be discrete, proportionate and protective. It will resolve once the inflammatory response has subsided (see definition of physiological pain). In contrast, extensive or chronic trauma, inflammation and neuropathic pain (nerve damage) will induce varying degrees of peripheral and central sensitization. Consequently, the animal will experience a diffuse, disproportionate, debilitative pain, which continues beyond the resolution of the inflammatory process (see definition of pathological pain) (Lemke 2004).

3.3.1 Peripheral sensitization

Peripheral sensitization is caused by the increased sensitivity of the nociceptors resulting from extensive trauma and inflammation (Raja et al. 1988). Initially, this will result

in a decreased pain threshold, a subsequent exaggerated response to noxious stimuli, and often spontaneous pain at the site of injury (Kelly et al. 2001; Muir & Woolf 2001). This phenomenon has been defined as *primary* hyperalgesia (Raja et al. 1988; Levine et al. 1993; Muir & Woolf 2001). Peripheral sensitization will also lead to a reduction in the intensity of the stimulus necessary to initiate pain, causing pain resulting from innocuous stimuli (allodynia) (Muir & Woolf 2001; Muir 2007). S*econdary* hyperalgesia on the other hand, refers to changes in the area surrounding the tissue injury (Raja et al. 1988), which cannot be explained by peripheral sensitization, since no changes in nociceptor transduction were found outside the area of primary hyperalgesia (Muir & Woolf 2001).

At the cellular level, trauma leads to the release of sensitizing chemical mediators from different inflammatory cells. Neutrophils, macrophages and lymphocytes produce cytokines (interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF)-α) (Muir 2007), while degranulation of mast cells induces a release of histamine and serotonin (5-HT). Histamine stimulates the sensory neurons, causing pain and itching. It can even evoke the release of other neuropeptides and prostaglandins, leading to an acceleration of the inflammatory process (Kelly et al. 2001). Also serotonin, which is released by mast cells and platelets, plays a role in the early phases of some types of acute inflammatory responses (Garcia Leme et al. 1973). Although bradykinin can activate nociceptors directly, it induces sensitization primarily through prostaglandin synthesis (Levine et al. 1993). The pro-inflammatory effects of bradykinin include the release of prostaglandins, cytokines and free radicals, and the degranulation of mast cells (Kelly et al. 2001). Damaged tissue cells release also potassium ions and adenosine triphosphate and trigger the production of cyclo-oxygenase-2 (COX-2) in inflammatory cells, leading to the production of inflammatory mediators such as prostaglandins and leukotrienes, possibly in the nerve terminals themselves (Vane et al. 1998; Muir 2007). These inflammatory mediators cause further sensitization of the peripheral receptors, thereby reducing their activation threshold and increasing the responsiveness to other stimuli (Levine et al. 1993). Neurotrophic growth factors (NGF's), which are released during tissue damage or by inflammatory cells, sensitize the transducers to subsequent stimuli (Woolf & Salter 2000). Nociceptive input will activate the sympathetic nervous system as well, resulting in the release of norepinephrine, which in turn accelerates sensitization of the nociceptors (Dray 1995; Dahl & Raeder 2000; Hellebrekers 2000; Kelly et al. 2001). Stimulation of nociceptors also leads to antidromal (reverse) activation of nociceptive nerve terminals and subsequent release of the neuropeptides CGRP and substance P. These

neuropeptides, together with proteases, induce mast cell degranulation, vasodilatation and oedema, as well as further activation of nociceptors and adjacent sensory nerve fibers (neurogenic inflammation). This will lead to hypersensitivity of the non-injured surrounding tissue, which is defined as secondary hyperalgesia (Hellebrekers 2000; Kelly et al. 2001; Muir 2007).

In the end, the free nerve endings of the nociceptive afferents will be "bathed" in an environment of inflammatory mediators, the so-called "inflammatory soup", consisting of the above described vasoactive amines, ions, neuropeptides and different products of the arachidonic acid cycle (Hellebrekers 2000).

In conclusion, both neural and non-neural cellular elements are necessary for different mediators to act upon primary afferent nociceptors. Some of these mediators directly sensitize these nociceptors, while others act on different cells, which in turn release a hyperalgesic agent acting directly on the primary afferent nociceptor (Levine et al. 1993).

3.3.2 Central sensitization

The IASP defines central sensitization as "*an enhanced responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input*" (IASP Task Force 1994). These nociceptive neurons have very distinct and even antagonistic functions, not all of which are related to the perception of pain. For example, they may project towards other areas in the brain (not related to pain perception) or towards motorneurons or they can even be interneurons. Enhanced responsiveness of different nociceptive neurons may have distinct and perhaps opposing consequences in terms of pain. Consequently, central sensitization may lead to hyperalgesia and/or allodynia. On the other hand this sensitization may also lead to stronger feedback inhibition or endogenous pain control (Sandkühler 2007).

Sensory homeostasis within the spinal cord is maintained by a balance between neural inputs and descending excitatory and inhibitory influences from the brain. The "*gate control theory*", first proposed by Melzack & Wall (1965), suggested that low-threshold Aβ-fibers and high-threshold C-fibers modulate the activity of the inhibitory interneurons located in the spinal cord. Activation of the low-threshold Aβ-fibers, which normally transmit innocuous stimuli, increases inhibitory interneuron effects (Hellyer et al. 2007; Muir 2007) by inducing both tonic and phasic inhibitory effects upon nerve impulses and their projection to the brain (Gjerstad et al. 2001). The inhibitory action is mediated by gamma-aminobutyric acid

(GABA) and glycine acting on $GABA_A$ and $GABA_B$ receptors (Yaksh 1989; Woolf & Salter 2000). Activation of Aδ-fibers and C-fibers on the other hand results in inhibition of the inhibitory interneurons (Muir 2007). Many contemporary pain researchers however, object to the use of the term "*gate control theory*" (Hellyer et al. 2007).

Another modulation model is the "*Diffuse Noxious Inhibitory Control*" (DNIC) concept. This concept is based on counterirritation, where one painful stimulus reduces the pain caused by a concurrent noxious stimulus somewhere else in the body (Talbot et al. 1989). For example, pain induced by intraneural electrical stimulation at C-fiber strength can be substantially reduced by vibration of the skin within the projected pain region (Bini et al. 1984). The mechanism of DNIC involves inhibition of the activity of WDR neurons in the dorsal horn (Chapman & Nakamura 1999).

Various neuromodulators can adapt the excitatory and inhibitory synaptic transmission in the spinal cord. Opioids, GABA, serotonin and norepinephrine inhibit excitatory transmission, while ATP, substance P and prostanoids facilitate the excitatory transmission. In contrast, serotonin, norepinephrine and acetylcholine facilitate inhibitory synaptic transmission (Muir 2007).

As stated before in the discussion of transmission in the dorsal horn, normal afferent input will induce the release of glutamate, which binds to AMPA receptors. Glutamate's action on the AMPA receptor is responsible for the generation of fast excitatory post-synaptic potentials (EPSP's), which last for milliseconds. These potentials signal the onset, duration, intensity and location of the stimulus (Duggan et al. 1990). Central excitatory sensitization will occur when the repetitive thermal or mechanical stimulation of peripheral nociceptors (partially dependent on the development of peripheral sensitization) continuously stimulates neurons in the dorsal horn of the spinal cord, resulting in temporal summation and cumulative depolarization of dorsal horn neurons (Woolf & Thompson 1991). This leads to the sustained release of glutamate, substance P and the "*Brain Derived Neurothrophic Factor"* (*BDNF*) (Lemke 2004; Hellyer 2007), which will then produce slow EPSP's lasting for tens of seconds (Thompson et al. 1990). When substance P is released from high-threshold fibers, CGRP is released simultaneously, which in turn extends the spinal cord zone from which substance P is released (Skofitsch & Jacobowitz 1985). This phenomenon contributes to the increased excitability (Schaible et al. 1994) and activation of additional types of glutamate receptors, such as NMDA and metabotropic glutamate receptors (mGluR), resulting in enhanced

synaptic transmission (Muir & Woolf 2001; Lemke 2004). The magnitude of these events is proportional to the stimulus intensity and is responsible for the removal of magnesium blocking the NMDA receptor. Consequently, a prolonged enhancement of dorsal horn neurons to glutamate or NMDA will occur (Willcockson et al. 1984; Dougherty & Willis 1991; Muir & Woolf 2001), which causes calcium influx into postsynaptic neurons, followed by persistent changes in the excitability of the neuron (Dray 1995). The whole of NMDA receptor activation (i.e. making the receptors more available for activation by glutamate through the removal of the magnesium block) and the increased excitability of projection neurons is called "*wind-up*", which is supposed to be the physiological trigger for central sensitization (Woolf 1996; Muir & Woolf 2001). This is fundamentally different from peripheral sensitization because central sensitization enables low-intensity stimuli to produce pain sensations (Muir & Woolf 2001). This mechanism also allows Aβ-sensory fibers to induce pain by altering spinal cord sensory processing and by increasing spinal neuron excitability (Baba et al. 1999).

3.4 Ascending spinal pathways and pain perception

After modulation of the nociceptive signal in the dorsal horn of the spinal cord, the signal is further projected through ascending pathways to higher centers (see Fig. 4 for localization of centers), where conscious and subjective perception of the stimulus takes place as the final result of its successful transduction, transmission and modulation (Muir & Woolf 2001). The white matter of the spinal cord of domestic mammals is organized in functional tracts (Fig. 5). Among these tracts, there are multiple nociceptive pathways. None of these is exclusively involved with pain transmission and all have fibers conducting tactile information as well (Hellyer et al. 2007). Spinal pain pathways also differ between animal species, but the common ones include the spinothalamic, the spinoreticular and the spinomesencephalic pathways.

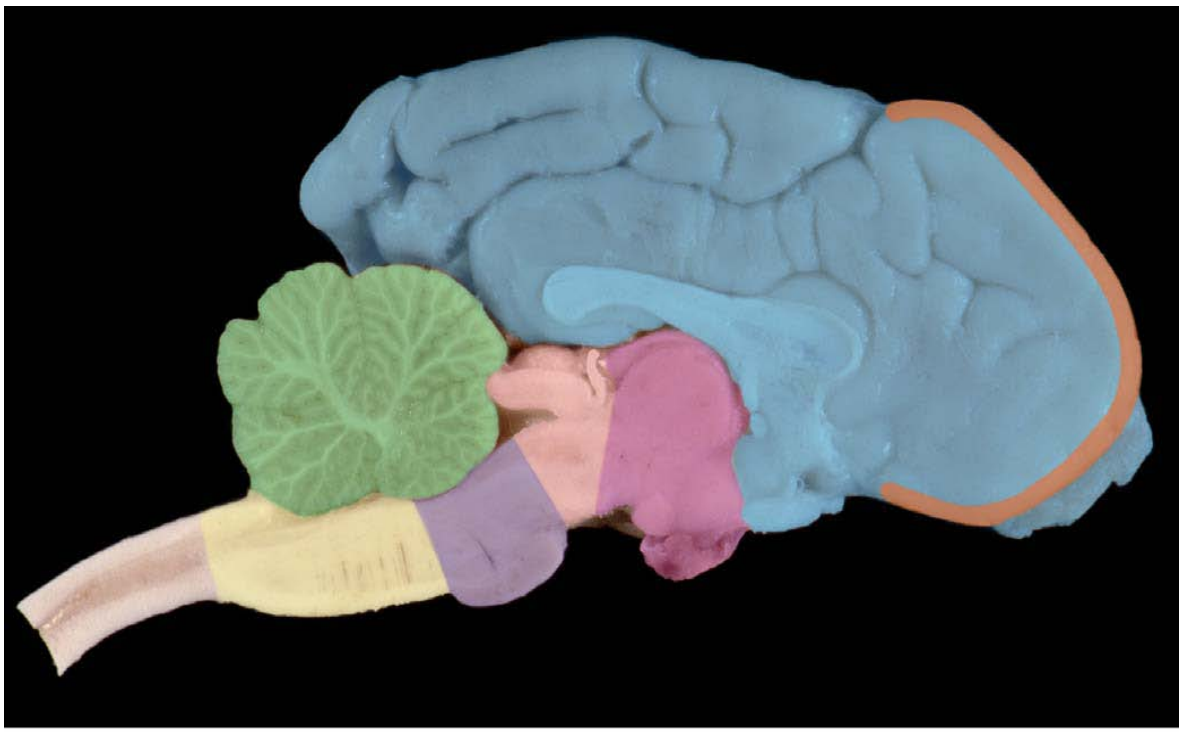

Fig. 4 Median section of the canine brain (medial view of the left hemisphere)

Telencephalon

Somatosensory cortex

Cerebellum

Diencephalon: contains thalamus, hypothalamus, hypophysis and nuclei of formatio reticularis (FR)

Mesencephalon: contains substantia grisea centralis (periaqueductal grey) and nuclei of FR

Metencephalon: contains locus coeruleus and nuclei of FR

Medulla oblongata: contains nucleus raphe magnus and nuclei of FR

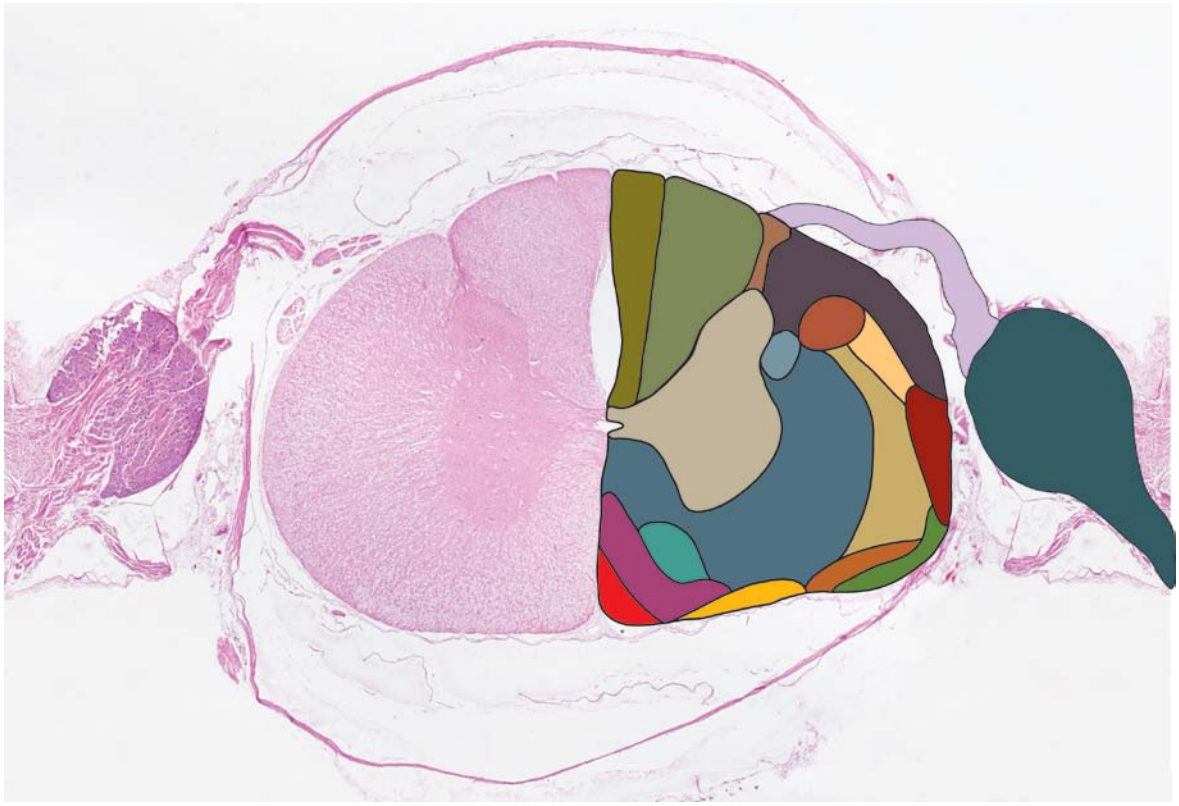

Fig. 5 Topography of the main tracts within the white matter of the feline spinal cord at the level of the second cervical segment (HE stain, x4).

Gray matter

Dorsal root ganglion

Sensory input via dorsal root

Fasciculus gracilis

Fasciculus cuneatus

Dorsolateral tract (Tract of Lissauer)

Tractus spinocerebellaris dorsalis

Tractus spinocerebellaris ventralis

Tractus spinocerebellaris rostralis

Tractus spinothalamicus (lateral part)

Tractus spinothalamicus (medial part)

Tractus spinoreticularis

Tractus pyramidalis (Tractus corticospinalis lateralis)

Tractus rubrospinalis

Tractus Spinocervicalis

Fasciculus proprius

Fasciculus longitudinalis medialis

Tractus vestibulospinalis

Tractus spinoolivaris

3.4.1 Spinothalamic pathway

The spinothalamic pathway is considered important for the transmission of deep pain and temperature (Burke & Colter 1990). It originates from laminae I and IV to VII of the spinal cord and contains axons of NS and WDR neurons (Muir 2007). These are distributed to the lateral funiculus on both sides of the spinal cord, forming a bilateral pathway (Fig. 5). They may re-enter the grey matter and make synapses with new neurons. The axons of these new neurons return to the spinothalamic tract either on the same or the other side of the spinal cord. Consequently, the spinothalamic pathway in domestic mammals is both crossed and uncrossed, and is multisynaptic with multiple interruptions (Kennard 1954; King 1987; Thomson 2006). It is also connected with the propriospinal system (King 1987).

There is a huge difference between the spinothalamic pathway anatomy of animals and of humans. In humans, all ipsilateral axons are projected across the midline and ascend in the contralateral spinothalamic tract uninterrupted to the formatio reticularis (FR) or the thalamus. Hemisection of the cord in man causes contralateral loss of pain and temperature sensation distal to the level of the lesion. Animals do not demonstrate such contralateral hemianalgesia. A loss of deep pain sensation conveys a grave prognosis in animals, since deep pain is the product of a diffuse and resilient system, which is difficult to disturb (Burke & Colter 1990).

The spinothalamic pathway is further divided into medial and lateral components (Fig. 5). The medial component projects to medial thalamic nuclei and then, via third order neurons, to the limbic system and is responsible for the transmission of ascending nociceptive input involved with the affective-motivational aspect of pain. On the other hand, the lateral component projects to lateral thalamic nuclei and subsequently to the somatosensory cortex. This component is responsible for the transmission of nociceptive input involved with the sensory-discriminative aspect of pain (Lemke 2004).

Of special importance in carnivores is *the spinocervicothalamic pathway*, which is believed to be the primary conscious pain pathway in these species (Kennard 1954; Ha & Liu 1966). This pathway is responsible for the transmission of superficial pain and tactile sensations (Ha & Liu 1966) and exhibits a high degree of somatotopy, enabling the animal to precisely determine the location of the painful stimulus (Hellyer et al. 2007). Secondary afferents (starting from the dorsal horn) run in an ipsilateral tract and project to and synapse in the lateral cervical nucleus located in spinal cord segments C_1 and C_2. The fibers originating from the cervical nucleus decussate and project to the thalamus. Some collaterals will

terminate in the FR. Finally, fibers project from the thalamus to the somatosensory cortex (Hellyer et al. 2007).

3.4.2 Spinoreticular pathway

The primary afferents of the spinoreticular pathway diverge immediately after entering the spinal cord, sending collaterals into several segments rostral and caudal to the segment of entry. This is necessary to participate in intersegmental reflexes. Second-order neurons are located in the dorsal horn and their axons are bilaterally present in the lateral and ventral funiculi (Fig. 5). These axons decussate diffusely throughout the long axis of the spinal cord (Hellyer et al. 2007). The spinoreticular pathway is mainly responsible for the transmission of deep pain and visceral sensations and projects to the FR in the medulla and pons, which is critical for the integration of nociceptive input (Milne et al. 1981; Ammons et al. 1985; Lemke 2004; Price & Nolan 2007). For this reason, somatotopy is not well defined in this pathway (Lemke 2004). Some ascending projections go directly to the thalamus and then to the cortex, but most projections of deep pain arrive in the somatosensory cortex via diffuse reticular projections to the thalamus (Hellyer et al. 2007). Ascending information increases cortical activity and activates the limbic system, which is associated with emotional responses to pain in humans and determines the aversive quality of the pain experience (Chapman 1996; Lemke 2004; Hellyer et al. 2007). Descending reticular activity blocks other sensory activity. There is also a direct link between reticular arousal centers and the dorsal horn (Fine & Ashburn 1998).

3.4.3 Spinomesencephalic pathway

After synapsing within the superficial layers of the dorsal horn (laminae I and V) (Muir 2007), axons of the spinomesencephalic pathway decussate and are situated on the contralateral side of the spinal cord (Livingston & Chambers 2000). They project to the FR and the periaqueductal grey matter (PAG) (midbrain). The PAG plays a central role in the integration and modulation of pain at the supraspinal level (Lemke 2004). These axons also project to the limbic system and the hypothalamus (Muir 2007). It has been suggested that the spinomesencephalic pathway activates a system of descending pain inhibition, beginning at the PAG (Fine & Ashburn 1998).

3.4.4 Spinohypothalamic pathway

This smaller tract projects to autonomic control centers in the hypothalamus and is responsible for the autonomic cardiovascular and neuroendocrine responses to noxious stimuli. It probably mediates some of the autonomic changes in heart rate, arterial blood pressure and respiratory rate in anaesthetized animals undergoing surgery, since the body activates the sympathetic nervous system via the hypothalamus (Chapman & Nakamura 1999; Lemke 2004).

3.5 Descending spinal pathways

Descending antinociceptive pathways begin at the supraspinal level and project to neurons in the dorsal horn of the spinal cord (Fine & Ashburn 1998; Lemke 2004). The most important descending system appears to begin in the PAG situated in the midbrain (Basbaum & Fields 1984; Lemke 2004). These neurons receive direct input from the thalamus, hypothalamus and FR and indirect input from the cerebral cortex (Basbaum & Fields 1984; Willis & Westlund 1997; Lemke 2004; Hellyer et al. 2007). Some neurons in the mesencephalic PAG project directly to the spinal cord, but most of the connections are indirect, projecting to the midline nucleus raphe-magnus (rostroventral medulla) and then to neurons in the dorsal horn (Castiglioni et al. 1978; Cameron et al. 1995; Fine & Ashburn 1998; Lemke 2004). The PAG may also send input to the locus coeruleus (pons), which projects directly to the dorsal horn neurons as well (Cameron et al. 1995; Lemke 2004). Axons that originate in the nucleus raphe-magnus release serotonin in the dorsal horn and comprise the "*serotonergic*" pathway. However, axons that originate in the locus coeruleus release norepinephrine in the dorsal horn and comprise the "*noradrenergic*" pathway (Lemke 2004). Axons of both pathways synapse in the dorsal horn with opioid-containing (endorphin, enkephalin, and dynorphin) inhibitory interneurons (Muir 2007). Activation of these antinociceptive pathways by the supraspinal release of opioid peptides is thought to be responsible for "*stress-induced analgesia*". Inhibition of these pathways is caused by the supraspinal release of GABA (Lemke 2004; Muir 2007). Paradoxically and more important in chronic pain states , release of the opioid peptides stimulates the GABA-mediated inhibition of the antinociceptive pathways, leading to local disinhibitory effects and a potential increase in pain perception (Lemke 2004; Muir 2007).

In summary, descending fibers that modulate nociception occur at various sites throughout the central nervous system. The most important inhibitory pathways appear to be

serotonergic and noradrenergic, originate in the PAG and relay through medullary reticular nuclei to the dorsal horn (Fine & Ashburn 1998).

4 Conclusions and Clinical Relevance

Non-elective surgery patients, with extensive tissue trauma and inflammation present for several days, will show a "pathological" response to pain, since at the end, peripheral and central sensitization will increase the responsiveness of dorsal horn neurons to sensory inputs (hyperalgesia and allodynia) and expand the receptive field. These phenomena are believed to be responsible for the discomfort and agony produced by severe injury (Muir & Woolf 2001). Furthermore, central sensitization can lower the threshold for perception of pain induced by future injuries, the so called pain memory. Additionally, animals are incapable to communicate directly about pain sensations and no objective method is available for the assessment of animal pain (Capner et al. 1999). This combined knowledge stresses the need for adequate analgesia in the peri-operative setting.

Osteoarthritis pain in dogs with cranial cruciate ligament rupture (CrCLR), as subject of the present thesis, is often chronic and has an important inflammatory component (Hayashi & Muir 2010). This requires a more aggressive and tailored analgesic therapy. Therefore, and especially when additional surgical pain is expected, it is of particular interest to establish the degree of present and expected inflammation as well as peripheral and central sensitization before setting up an analgesic plan to treat the individual patient. Ideally, the analgesic plan should be pre-emptive and multimodal, so that pain is tackled early and at the different levels of the nociceptive pathway. The rationale behind the pre-emptive administration of analgesics is to reduce post-operative pain intensity compared to the pain intensity observed after administration of analgesics following surgery only. Additionally, it can also lead to a shorter hospital stay (Duellman et al. 2009). A multimodal approach incorporates analgesics with different mechanisms and sites of action along the described pain pathway (*vide supra*), resulting in better analgesia and subsequently lowering the therapeutically effective dose, thereby minimizing the adverse effects of each individual drug (Lascelles et al. 2008).

The prevention and treatment of pain related to stifle surgery for the correction of a CrCLR in dogs, as part of the present thesis, should therefore be approached as such. This can be achieved, amongst other techniques, by administration of a non-steroidal anti-

inflammatory drug (NSAID) in combination with a parenteral opioid and/or by the epidural administration of the combination of an opioid and a local anaesthetic (Bergmann et al. 2007).

As a consequence of multimodal therapy, the side effects associated with the combination of these different types of analgesics is expected to differ from the sole administration of each analgesic (cfr. aims of multimodal analgesia). The investigation of the occurrence of side effects associated with (combined) drug administration is of major importance. Former surveys on the use of peri-operative analgesics have shown that practitioners expressed particular concerns about the side effects associated with the administration of NSAIDs and opioids (Capner et al. 1999; Lascelles et al. 1999; Williams et al. 2005), thereby limiting the use of both treatment modalities.

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Scientific aims

In dogs, cranial cruciate ligament rupture (CrCLR) has been shown to result in instability of the stifle joint inducing severe joint pain, lameness, inflammation and progressive degenerative changes within the affected joint. Different methods of stifle surgery including the imbrication technique (IT) and tibial tuberosity advancement (TTA) have been developed in an attempt to restore the biomechanical function and to limit the development of osteoarthritis after CrCLR in dogs. Regardless of the surgical technique used, it can be assumed that orthopaedic surgery causes severe and prolonged pain (Hellyer et al. 2007).

The first aim of the present thesis was to study the analgesic effects of different pre-emptive and multimodal analgesia protocols in dogs undergoing 2 types of stifle surgery to correct a CrCLR (IT and TTA). This orthopaedic pathology was selected for the present research, particularly because the evaluation of pain after orthopaedic surgery is more straightforward compared to the evaluation of e.g. visceral pain. The presence of well localized pain in the case of orthopaedic surgery also facilitated the comparison of analgesia between the pre- and post-operative situation.

Two clinical studies (chapter 1 & 2) were performed to assess:

- the *intra-operative* analgesic efficacy of the combination of pre-emptive oral tepoxalin administration + pre-emptive intravenous (IV) methadone administration compared to pre-emptive IV methadone administration alone in CrCLR-dogs undergoing unilateral stifle imbrication.
- the *post-operative* analgesic efficacy of the combination of pre-emptive oral tepoxalin administration + post-operative IV administration of buprenorphine compared to post-operative IV buprenorphine administration alone in CrCLR-dogs undergoing unilateral stifle imbrication.
- the *intra-* and *post-operative* analgesic efficacy of the pre-emptive administration of epidural methadone or ropivacaine 0.75%/methadone with or without pre-operative oral tepoxalin in CrCLR-dogs undergoing unilateral TTA-surgery.

The administration of analgesics can be associated with adverse effects, that can additionally vary when different classes of drugs are combined. All NSAIDs can potentially induce side effects on the gastro-intestinal, the hepatic, the renal and the coagulation system (KuKanich et al. 2012) while the epidural administration of opioids and local anaesthetics has the potential of inducing adverse effects, mainly on the cardiovascular system (Torske & Dyson 2000).

Therefore, the second aim of the present thesis was to study the side effects associated with the above described analgesic protocols. The short-term side effects related to the use of tepoxalin were evaluated in the 2 clinical studies (chapter 1 & 2). Clinical cardiovascular effects of epidural methadone and/or ropivacaine 0.75% were studied in chapter 2. However, to study the cardiovascular side effects associated with the epidural protocols more in depth, an experimental cross-over study was performed (chapter 3). Since hypotension related to the epidural administration of ropivacaine 0.75% was most likely to occur, the effects of a vascular preload of hydroxyethylstarch 6% (200 kDa/0.5) on the cardiovascular system were also investigated (chapter 4).

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Clinical and experimental studies

Chapter 1

Intra- and post-operative analgesic efficacy of the respective administration of oral tepoxalin/intravenous methadone and oral tepoxalin/intravenous buprenorphine in dogs undergoing imbrication surgery of the stifle.

Adapted from:

Bosmans T, Gasthuys F, Duchateau L, de Bruin T, Verhoeven G, Polis I (2007) A Comparison of Tepoxalin-Buprenorphine Combination and Buprenorphine for Post-Operative Analgesia in Dogs: A Clinical Study. Journal of Veterinary Medicine series A 54, 364-369.

Abstract

Objective To compare the intra-operative analgesic effect of oral tepoxalin/ intravenous methadone administration to that of methadone alone and the post-operative analgesic effect of oral tepoxalin/intravenous buprenorphine administration to that of buprenorphine alone in the 24h post-operative period in dogs undergoing imbrication surgery of the stifle.

Study Design Prospective, randomized, blinded, clinical study.

Animals Twenty client owned dogs of different breeds.

Materials and Methods The dogs (n=10 treatment^{-1}) were randomly assigned to receive orally (PO) either a lyophilisated placebo tablet (treatment P) or a lyophilisated tepoxalin tablet (10 mg kg^{-1}) (treatment T) before premedication. All dogs received methadone 0.1 mg kg^{-1} intravenously (IV) as part of the premedication. Induction and maintenance of anaesthesia were performed in a similar way for all dogs. Intra-operative rescue analgesia (fentanyl 1µg kg^{-1} IV) was administered when heart rate increased > 10% compared to baseline values due to surgical stimulation. At the time of extubation, all dogs received IV buprenorphine (10 µg kg^{-1}) and subsequently every 6 hours until 24 hours after surgery. Post-operative analgesia was evaluated using a visual analogue scale (VAS) and a multifactorial pain scale (MFPS), by an anaesthetist blinded from treatment. Potential side effects of tepoxalin were investigated by blood analyses before premedication and 24 hours after extubation, a buccal mucosal bleeding time test and recording of vomiting, diarrhoea and adverse effects at the surgical site.

Results The need for intra-operative rescue analgesia did not differ significantly between treatments. Analysis of the overall VAS-scores showed a significant decrease over time in both treatments. However, the decrease in the two treatments was not significantly different from each other (p=0.059). No significant differences were found between the MFPS-scores of both protocols. Analysis of the blood parameters showed that fibrinogen levels were overall higher 24 hours after surgery in both protocols, but were significantly more elevated in treatment T. No significant differences were found for the other blood parameters.

Conclusions and Clinical Relevance Statistically, it could not be demonstrated that tepoxalin improved intra-operative analgesia induced by methadone and post-operative analgesia provided by buprenorphine. However, the trend for a faster linear decrease of VAS-scores in the dogs receiving tepoxalin compared with placebo (p=0.059), may be suggestive of a positive trend reffering to a supplemental analgesic effect of tepoxalin. There was no

convincing evidence that the administration of tepoxalin was devoid of gastro-intestinal side effects. There were no significant adverse effects on renal function and primary haemostasis.

Introduction

Research concerning the mechanisms of nociception has shown that it is better to prevent pain than to treat it (Woolf 1983; Woolf 1989). This so-called concept of 'pre-emptive analgesia', where analgesics are administered before noxious stimulation occurs, prevents adverse central nervous system changes induced by the stimulus and renders the animal more comfortable after a surgical intervention (Lascelles et al. 1995; Lascelles et al. 1998; Dobromylskyj et al. 2000). Together with the pre-emptive approach, multimodal analgesia techniques, involving non-opioid and opioid analgesic drugs can markedly enhance pain relief in the perioperative period, both in man and in animals (Joshi & White 2001; Lemke 2004; Lascelles et al. 2005). Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, alone or in combination, are the most commonly used analgesics to treat surgical pain. The use of opioids in clinical veterinary practice for post-operative pain relief has already been well established (Taylor 1985; Hall et al. 2000). However, for a mild type of pain, guidelines of pain therapy suggest to start with the administration of a NSAID (Zuckerman & Ferrante 1998). A majority of NSAIDs inhibit cyclo-oxygenase 1 (COX-1) and COX-2 in varying degrees, ultimately reducing the production of prostaglandins (Mathews 2000). On the other hand, leukotrienes, as part of the lipoxygenase (LOX)-pathway, have highly potent hyperalgesic/pro-inflammatory effects as well, which make them possibly even more important in the pathogenesis of inflammatory diseases (Argentieri et al. 1994; Knight et al. 1996). Still, due to the potential risks of gastric ulceration, renal injury, decreased wound healing and increased haemorrhage related to the platelet dysfunction, there has been reluctance to pretreat small animal patients with NSAIDs before surgery.

Tepoxalin (Zubrin; Schering-Plough Animal Health, U.K.), a pyrazole derivative, is a dual COX and 5-LOX inhibitor registered for dogs. Several clinical and preclinical studies have pointed out that tepoxalin can be used in dogs with osteoarthritis or other musculoskeletal disorders, whereby a rapid inhibition of the COX/LOX system in inflamed tissues assures a high anti-inflammatory efficacy, as well as a fast and effective pain relief (Argentieri et al. 1994; Willburger et al. 1996, 1998; Agnello et al. 2005). Moreover, tepoxalin was associated with a high gastro-intestinal (GI) safety profile partially induced by the LOX inhibition, supporting its long-term use for chronic pain relief (Wallace et al. 1991; Kirchner et al. 1997; Bertolini et al. 2001). The aims of the present study were to investigate the possible improvement of intra-operative analgesia by 'pre-emptive' tepoxalin

administration added to an opioid-based (methadone) analgesia protocol, as well as to investigate the additive effect of tepoxalin on post-operative analgesia induced by another opioid (buprenorphine). Potential side effects on the GI tract, renal function and blood coagulation were also evaluated.

Materials and Methods

Inclusion Criteria

Twenty client owned dogs referred for cranial cruciate ligament (CrCL) repair were included in this clinical trial. Written informed owner consent was obtained for each case. Eleven breeds with sex distribution of 14 females and 6 males with an age of 7.1 ± 3.2 years (mean ± SD) and a mass of 29.9 ± 15.5 kg (mean ± SD) were included. Dogs with renal or hepatic insufficiency, GI disease, blood coagulation disorders, pregnant or lactating bitches, or dogs previously (within 1 week) treated with NSAIDs or glucocorticosteroids were excluded from the study.

Study design

The study was conducted as a randomized, blinded, prospective clinical trial.

Premedication and Anaesthesia

After diagnosis of ruptured cranial CrCL, dogs were scheduled for surgery. All dogs were fasted for at least 8 hours before surgery. The dogs were randomly assigned to 2 treatments. Treatment P (n=10), received a lyophilisated tablet per os (PO) as placebo (Placebo; Schering-Plough Animal-Health, U.K.) before premedication, whereas dogs of treatment T (n=10) received tepoxalin 10 mg kg^{-1} PO (Zubrin; Schering-Plough Animal Health, U.K.) prior to premedication. Pretreatment with tepoxalin or the placebo was blinded for the observer. Before premedication, a baseline heart rate (HR) (mean of 3 subsequent measurements) was recorded.

Premedication of all dogs included acepromazine 0.01 mg kg^{-1} IV (Placivet; Codifar, Belgium) and methadone 0.1 mg kg^{-1} IV (Mephenon; Denolin, Belgium), administered 15 minutes before induction of anaesthesia. Anaesthesia was induced with propofol 4-6 mg kg^{-1} IV to effect (Rapinovet; Schering-Plough, Belgium) and was maintained after endotracheal intubation with isoflurane (Forene; Abbott, Belgium) vaporized in 100% oxygen and delivered by a semi-closed circle system (Spiromat 656, Dräger, Germany). All dogs received

amoxicillin 10 mg kg^{-1} IV (Clamoxyl; GlaxoSmithKline n.v., Belgium) and a long acting amoxicillin 10 mg kg^{-1} (Clamoxyl LA; Pfizer Animal Health s.a., Belgium) subcutaneously (SC) after induction of anaesthesia.

Intra-operative rescue analgesia was performed if the HR increased (>10% compared to baseline HR) due to surgical stimulation, using fentanyl 1 µg kg^{-1} IV (Fentanyl-Janssen; Janssen-Cilag, Belgium).

Lactated Ringer's solution (Hartmann; Baxter, The Netherlands) was administered continuously during anaesthesia at a rate of 10 mL kg^{-1} $hour^{-1}$. The durations of anaesthesia and surgery were recorded.

At the time of extubation and subsequently every 6 hours until 24 hours after surgery, dogs of both treatments received buprenorphine 10 µg kg^{-1} IV (Temgesic; Schering-Plough, U.K.).

Surgery

Surgery was performed by one of 4 experienced surgeons. Imbrication was performed in each case according to standard surgical principles, with either meniscal release or partial meniscectomy, depending on meniscal pathology.

Post-operative Monitoring and Pain Scoring

Pain scores were always allocated by the same anaesthetist unaware of treatments at the time of extubation and 1, 2, 6, 12 and 24 hours post-surgery. An adapted multifactorial pain scale (MFPS) (range 0-13) (Table 1) based on 5 variables was used (adapted from Pibarot et al. 1997). Post-operative pain was considered mild, moderate, or severe if the total pain score was between 0 and 5, 6 and 9, 10 and 13 respectively. Afterwards, skin pressure was applied close to the surgical incision and pain was again assessed using a visual analogue scale (VAS) (range 0-100) (Murrin & Rosen 1985). Only the MFPS results were used as a guide for the administration of rescue analgesia. If they were higher than 9, an extra dose of 10 µg kg^{-1} buprenorphine IV was administered on top of the 6 hour interval IV dose of buprenorphine. Total number and time of these additional buprenorphine doses were recorded.

Adverse effects including GI and blood coagulation disorders, the occurrence of haematoma formation at the surgical site, persistent bleeding from the surgical incision,

vomiting, diarrhoea and signs of abdominal pain in the immediate post-operative period were recorded.

Table 1 Multifactorial pain scale used to evaluate pain in 20 dogs after imbrication of the stifle [adapted from Pibarot et al. (1997)]. The pain scoring system was based on changes in heart rate (compared to pre-anaesthetic heart rate), respiratory pattern, vocalization, agitation, and response to manipulation of the surgical incision (score range 0-13).

Parameters		Score
Heart rate	less than 10% increase	0
	between 11-30% increase	1
	between 31-50% increase	2
	more than 50% increase	3
Respiratory pattern	normal	0
	mild abdominal assistance	1
	marked abdominal assistance	2
Vocalization	no crying	0
	crying, responds to calm voice	1
	crying, does not respond to calm voice	2
Agitation	asleep or calm	0
	mild agitation	1
	moderate agitation	2
	severe agitation	3
Response to manipulation	no response	0
	minimal response, tries to move away	1
	turns head towards site, slight vocalization	2
	turns head with intention to bite	3
Total Pain Score		**0-13**

Blood Sampling and Buccal Mucosal Bleeding Time Measurement

Venous blood samples from a jugular vein were collected in citrated and dry tubes before premedication and 24 hours after extubation for analysis of fibrinogen, platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), blood urea nitrogen (BUN) and creatinine. A buccal mucosal bleeding time (BMBT) was performed before administration of tepoxalin or placebo and 24 hours later using a spring-loaded disposable device (Simplate R; Biomérieux, France).

Statistical Analysis

The VAS-scores were analyzed by a mixed model with dog as random effect, treatment as categorical fixed effect and time and treatment by time interaction as continuous fixed effects using the F-test for testing the fixed effects factors. The MFPS-scores were summed over the entire period and analyzed by a t-test. For the blood parameters, the difference after and before the treatment was used as response variable and compared between the two treatments by the Wilcoxon test due to the fact that the data were not normally distributed. The data for intra-operative fentanyl administration were also not normally distributed and were analyzed by the Mann-Whitney U test. A significance level of 5% was applied for all statistical methods.

Results

The duration of anaesthesia was 109 ± 35 minutes (mean ± SD), with a duration of surgery of 67 ± 29 minutes. Fifteen dogs received fentanyl as a supplemental analgesic during surgery (7 dogs of treatment T, 8 dogs of treatment P), with a total dose of 2.37 ± 1.74 µg kg^{-1}. This need for intra-operative rescue fentanyl did not differ significantly between treatments (p=0.876), and was 1 (0-4) µg kg^{-1} [median (range)] and 1 (0-5) µg kg^{-1} for treatment P and T respectively. The timing of intra-operative fentanyl administration was variable, but in general supplemental analgesia was needed at the time of incision of the joint capsule.

Analysis of the pain scores showed no significant differences for the overall mean MFPS-scores (p=0.43), with scores of 3.3 ± 1.4 and 3.7 ± 1.2 for respectively treatments P and T (Fig. 1). Mean VAS-scores in both treatments decreased significantly over time (p<0.0001). However, the decrease between the two treatments was not significantly different from each other (p=0.059), with a linear decrease of –0.687 per hour (SE=0.16) in treatment P, compared to –1.12 per hour (SE=0.16) in treatment T (Fig. 2). Overall mean VAS-scores

were 37.7 ± 6.1 and 34.2 ± 12.0 for respectively treatments P and T. After surgery one dog of treatment P with a MFPS-score above 9 needed additional buprenorphine (10 µg kg^{-1} IV) at the time of extubation.

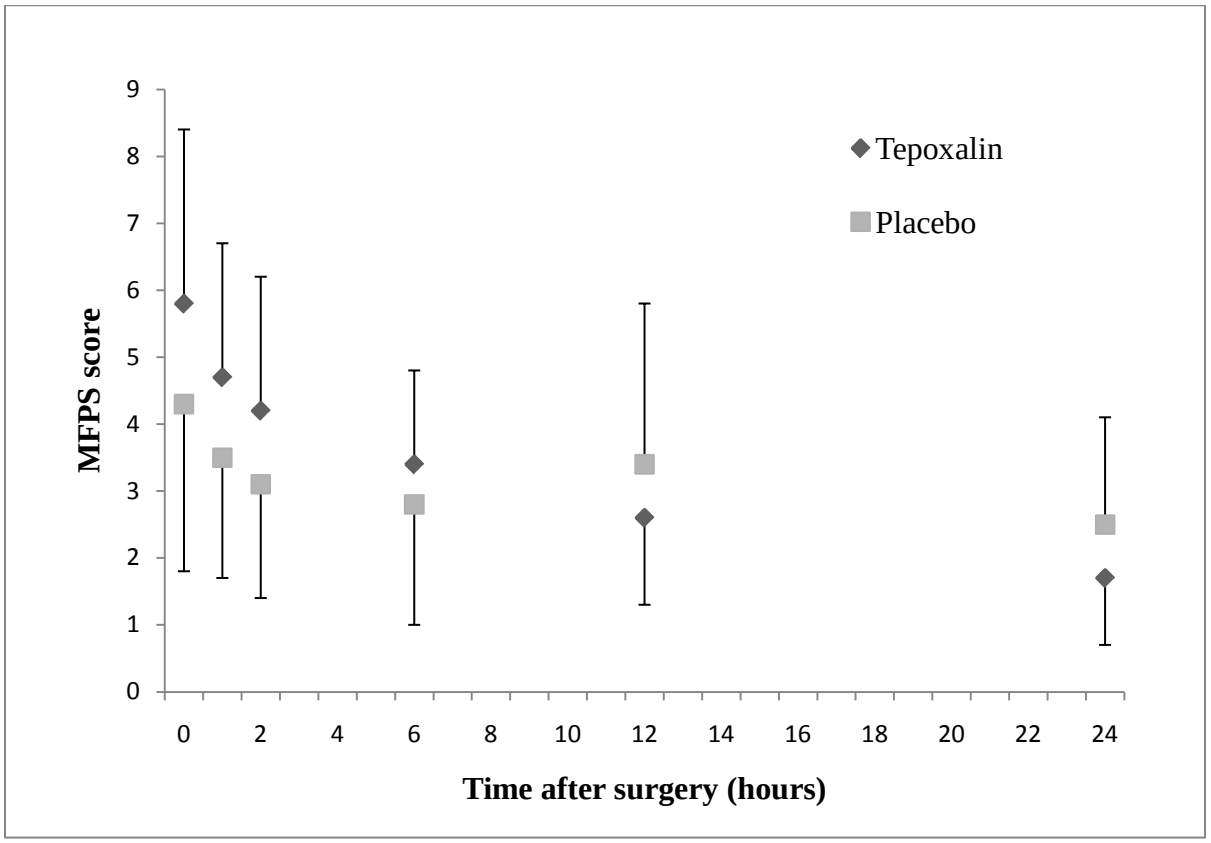

Fig. 1 Multifactorial Pain Scale-scores (MFPS) (range 0-13) displayed as (mean + or - SD), during the 24 hour post-operative period, in 20 dogs undergoing imbrication surgery of the stifle and receiving either buprenorphine (10 µg kg^{-1} IV) (Placebo) (*n*=10), or tepoxalin (10 mg kg^{-1} PO)-buprenorphine (10 µg kg^{-1} IV) (Tepoxalin) (*n*=10).

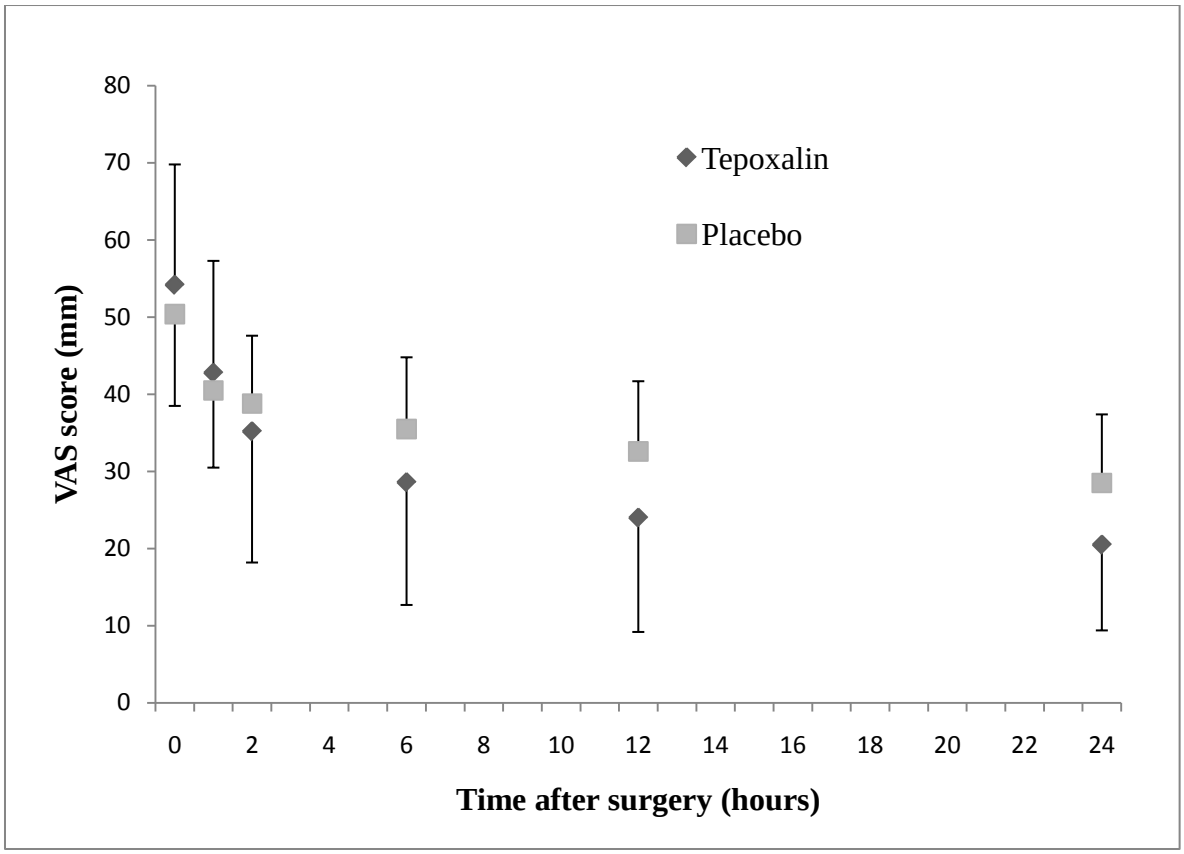

Fig. 2 Visual Analogue Scale-scores (VAS) (range 0-100) displayed as (mean + or - SD), during the 24 hour post-operative period, in 20 dogs undergoing imbrication surgery of the stifle and receiving either buprenorphine (10 µg kg^{-1} IV) (Placebo) (*n*=10), or tepoxalin (10 mg kg^{-1} PO)-buprenorphine (10 µg kg^{-1} IV) (Tepoxalin) (*n*=10).

Tepoxalin induced no renal or GI adverse effects over 24 hours, except for one dog of treatment T suffering from haemorrhagical diarrhoea 6 hours after extubation, which normalized without therapeutic intervention at T12. No dog vomited or showed signs of severe abdominal pain. One dog had a higher pre-anaesthetic BUN level than normal, but was free of clinical signs indicative of renal failure. This dog was not excluded from the trial.

In general fibrinogen levels were higher in both treatments 24 hours after surgery compared to the preoperative values. The change in fibrinogen levels differed significantly (p=0.0283) between the two treatments, with higher values for treatment T (Table 2). There were no significant differences for PT, APTT, BUN, creatinine, total protein, and BMBT.

Table 2 Blood parameters taken before premedication (pre) and 24h after surgery (post), including the difference between the two measurements (δ pre-post) for dogs undergoing imbrication surgery of the stifle and receiving either buprenorphine (10 µg kg^{-1} IV) (P) (*n*=10), or tepoxalin (10 mg kg^{-1} PO)-buprenorphine (10 µg kg^{-1} IV) (T) (*n*=10). All values are displayed as median with minimum and maximum ranges. Significance level of $p<0.05$.

Parameter	Treatment	Pre	Post	δ Pre-Post	*p*-value
Hematocrit (%)	P	47.3 (38.8-54.4)	44.5 (40.9-49.1)	-3.7 (-7.6-2.1)	0.57
	T	51.3 (42.0-56.8)	48.4 (34.2-53.1)	-3.7 (-15.0-0.1)	
Erythrocytes (million cm^{-2})	P	6.95 (5.32-7.49)	6.37 (6.04-7.28)	-0.47 (-1.06-0.75)	0.72
	T	7.44 (6.33- 8.12)	6.64 (5.10-7.91)	-0.47 (-2.30- -0.14)	
Thrombocytes (x1000 cm^{-2})	P	383 (254-1465)	350 (244-655)	-6 (-1093-44)	0.81
	T	372 (261-568)	346 (207-526)	-18 (-78.00-38)	
Protrombin time (seconds)	P	6.85 (4.70-8.90)	6.95 (5.60-9.80)	0.55 (-1.30-2.90)	0.31
	T	7.25 (5.10-8.20)	7.05 (5.40-7.50)	-0.35 (-1.30-2.40)	
APTT (seconds)	P	13.75 (11.20-21.20)	15.40 (8.20-24.60)	2.10 (-3.00-7.00)	0.87
	T	17.70 (10.20-35.30)	20.20 (10.00-32.30)	1.15 (-9.40-12.10)	
Fibrinogen (mg dL^{-1})	P	209 (117-238)	413 (102-523)	225 (-43-301)*	0.03*
	T	205 (167-391)	474 (433-684)	283 (206-386)*	
BUN (mg dL^{-1})	P	26.5 (19.0-132.0)	23.0 (15.0-137.0)	-5.0 (-18.0-6.0)	0.31
	T	32.5 (24.0-39.0)	24.5 (13.0-32.0)	-7.0 (-22.0-5.0)	
Creatinine (mg dL^{-1})	P	1.04 (0.52-2.48)	0.82 (0.58-2.62)	-0.25 (-0.36-0.14)	0.65
	T	0.88 (0.70-1.28)	0.65 (0.47-1.37)	-0.18 (-0.47-0.09)	
Total Protein (g dL^{-1})	P	7.05 (5.90-8.10)	7.05 (6.10-7.80)	0.05 (-0.30-0.40)	0.36
	T	6.85 (6.00-7.70)	6.75 (5.90-7.50)	-0.05 (-1.10-0.30)	
BMBT (seconds)	P	112 (49-162)	118 (56-207)	-5 (-37-89)	0.11
	T	94 (83-138)	114 (75-250)	27 (-32-148)	

APTT = activated partial thromboplastin time, BUN = blood urea nitrogen, BMBT = buccal mucosal bleeding time.

*$p<0.05$

Discussion

The major aims of the present study were to investigate the possible additional analgesic effects of pre-emptively administered tepoxalin both to intra-operative analgesia provided by pre-operative administration of methadone and post-operative analgesia induced by repeated post-operative administration of buprenorphine.

During surgery, 7 dogs of treatment T and 8 dogs of treatment P received 1 or more doses of fentanyl according to the rescue treatment protocol (by an anaesthetist blinded from treatment). However, no significant difference in total dose was observed between both treatments, which suggests that tepoxalin did not improve intra-operative analgesia provided by pre-emptive administration of IV methadone at the dose used in the current study. These results also suggest that, given the high incidence of rescue analgesia administered in both treatments (15/20 dogs), administration of pre-operative methadone at the dose used in the current study does not provide sufficient intra-operative analgesia for this type of surgery in dogs. The timing of administration of fentanyl was variable, but supplemental analgesia was mostly needed at the time of incision of the joint capsule which was apparently the most painful procedure during the surgical intervention. Methadone was selected as the premedication opioid, because clinical experience has shown that intra-operative administration of fentanyl is ineffective when buprenorphine is administered in the premedication. Indeed, buprenorphine has a high μ-receptor affinity and can displace full μ-agonists, such as fentanyl from the μ-receptor, which might result in unacceptable intra-operative pain (Kerr 2010). However, it seems unlikely that pre- and intra-operative administration of respectively methadone and fentanyl interfered with post-operative pain scoring, since buprenorphine (administered at the time of extubation) has a very high binding affinity for the μ-receptor and readily displaces other opioids from the receptor, while preventing others from binding (Welsh et al. 2008). Additionally, fentanyl has a short duration of action, with a peak effect lasting less than 30 minutes (Thurmon et al. 1996) and low dosages were used in this study.

Since Brodbelt et al. (1997) concluded that buprenorphine is a suitable analgesic for post-operative analgesia for elective arthrotomy in dogs, this opioid was included in both protocols as a positive control for post-operative analgesia. The inclusion of a control group without any analgesics was considered to be unethical. In dogs buprenorphine was proven to have a long elimination half-life of about 24 h, with a slow onset of action of 20-30 minutes

(Lemke, 1999) and an estimated duration of action of 4-8 h (Garrett & Chandran, 1990). A limitation of the study was the timing of administration of the first post-operative dose of buprenorphine. Considering the slow onset of action of buprenorphine it is likely that the pain scores obtained at the time of extubation were still influenced by methadone. Only one dog of the placebo treatment needed a bolus of buprenorphine (10 µg kg^{-1} IV) as rescue analgesia after extubation, due to a MFPS-score above 9. It is possible that the slow onset of action of buprenorphine and/or the absence of tepoxalin resulted in a higher pain score in this particular animal. The mean overall VAS and MFPS scores obtained in both treatments of the current study were at the lower end of the score range. This reflected in a low requirement for post-operative rescue analgesia and thus suggests both a good quality of post-operative analgesia provided by both treatments and sufficient post-operative analgesia provided by buprenorphine alone for this type of surgery in dogs.

Considering the post-operative pain scoring, two methods, the MFPS and the VAS, were used in the present study to exclude possible differences in nociception between the two treatments and to obtain a more accurate overall impression of pain. The main hypothesis of the present study was that buprenorphine and tepoxalin might cause improved post-operative analgesia after a standard surgical intervention in dogs, compared to buprenorphine as a sole analgesic. Still, no significant differences in MFPS-scores were observed between the 2 treatments. A possible explanation for the lack of significant differences between the treatments using the MFPS, might be found in the method of categorization in this scoring system, since this was reported to limit the sensitivity (Hoelzler et al. 2005). The assignment of an equal value for all variables can also account for the present findings, because physiologic variables often correlate poorly with pain (Cambridge et al. 2000; Hansen 2003). A practical problem encountered in the present study, was the occurrence of high baseline values in HR in some nervous dogs, likely induced by the new environment. As a relative increase in HR compared with these baseline values was used in the MFPS, some dogs with abnormally high baseline HR's had a lower score for increase in HR over all time periods, most likely masking a certain level of pain.

The VAS was reported to have a good correlation with mild-to-moderate pain in people and is also more sensitive and reliable than a categorical approach such as MFPS (Scott & Huskissen 1976; Grossman et al. 1991; Sammarco et al. 1996; Fowler et al. 2003). However, the decrease in VAS scores observed in the 2 treatments failed to be significantly different from each other (p=0.059). This might have resulted from inability of the evaluator

to identify animals in pain using the VAS, or possibly because the patients had indeed sufficient analgesia and comfort produced by the scheduled doses of buprenorphine. Indeed, the standard administration of buprenorphine in both treatments might have masked improved analgesia induced by tepoxalin, resulting in no statistical evidence for the analgesic superiority of the tepoxalin-buprenorphine combination over buprenorphine alone. Hence, additional analgesic effects induced by tepoxalin can not be ruled out using our statistic results. The faster linear decrease of VAS scores in the dogs receiving tepoxalin compared with placebo, although not statistically significant, may be suggestive of a positive trend referring to a supplemental analgesic effect of tepoxalin. Studies with a larger number of patients and evaluation of analgesia for a period beyond 24 hours are warranted to confirm this trend.

The second aim of the study was to look for possible adverse effects induced by tepoxalin, especially on the GI tract, the kidneys and primary haemostasis. The most prevalent side effect associated with the use of NSAIDs is gastric irritation and even ulceration, caused by inhibition of prostaglandin synthesis in the GI tract. The preclinical toxicity of tepoxalin in rats and dogs has been thoroughly assessed and a relative lack of GI side effects has been reported (Knight et al. 1996; Kirchner et al. 1997). Within the therapeutic dose range, tepoxalin distinguished itself from most commercially available anti-inflammatory drugs and was well tolerated for up to 6 months in rats and dogs at dosages in excess of the ED_{50} (Wallace et al. 1991). In the present study the oral administration of tepoxalin before surgery at a dose of 10 mg kg^{-1}, was not associated with GI side effects (vomiting, diarrhoea and signs of abdominal pain) during a period of 24 hours, with the exception of one dog of treatment T, which suffered from haemorrhagical diarrhoea 6 hours after extubation. This abnormality resolved spontaneously within 6 hours.

The influence of tepoxalin on renal function and blood coagulation over a 24 hour peri-operative time period was also investigated in the present study. Tepoxalin is excreted almost exclusively (>99%) in the GI tract (faeces), whereby less than 1% of the administered dose can be detected in the urine decreasing the risk of drug accumulation in renally impaired dogs (Tepoxalin Technical Monograph, Schering-Plough 2003). Knight et al. (1996) and Kay-Mugford et al. (2004) could not demonstrate adverse renal effects in dogs. In another study, tepoxalin did not alter renal function even when administered together with an angiotensin-converting enzyme inhibitor for 7 or 28 days (Fusellier et al. 2005). Renal function in that study was based on the measurement of the glomerular filtration ratio by means of renal

scintigraphy and plasma clearance of ^{99m}Tc-DTPA. In the present study, also no significant differences were observed for the renal parameters (blood urea nitrogen and creatinine). In a study involving healthy humans, Waldman et al. (1996) demonstrated that tepoxalin inhibits platelet function (platelet secretion and aggregation) after single oral administration. Knight et al. (1996) also reported a decrease in red blood cell count, haemoglobin, packed cell volume together with an increase in platelet counts in a preclinical evaluation of tepoxalin in rats and dogs, and an increase in PT and APTT in rats. The study of Kay-Mugford et al. (2004) showed no significant effects on haemostasis and hepatic functions, when a single preoperative dose of tepoxalin was administered to young healthy dogs undergoing anaesthesia and surgery. In the present study, a significant difference was only observed for fibrinogen. Fibrinogen levels were generally higher 24 hours after surgery in both treatments, most likely due to the fact that fibrinogen is a positive acute phase protein whereby the concentration increases in cases of trauma such as surgical interventions (Cerón et al. 2005). Fibrinogen levels in the tepoxalin dogs were significantly more elevated than in the placebo treatment, however, without an obvious explication. To the best of the authors' knowledge, there is no important clinical implication resulting from elevated fibrinogen levels in healthy dogs, in comparison to the high risk for development of atherosclerosis associated with chronic hyperfibrinogenemia in man (Mauriello et al. 2000; Turaj et al. 2006).

As a general conclusion of this study, it could not be demonstrated that tepoxalin improved intra-operative analgesia induced by methadone and post-operative analgesia induced by buprenorphine. This, however, does not mean that it does not provide analgesia at all. Further research with a larger case load and evaluation of analgesia for a period beyond 24 hours is needed to confirm the trend to a faster decrease in post-operative pain scoring in the tepoxalin-treated dogs. Due to the small sample size, the study does not provide convincing evidence that the administration of tepoxalin is devoid of GI side effects. However, administration of tepoxalin showed no significant adverse effects on renal function and primary haemostasis.

Conflict of interest statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript. Schering-Plough Animal Health kindly agreed to provide free of charge samples of oral tepoxalin and placebo tablets to be used in the study.

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Chapter 2

Analgesic efficacy of epidural methadone or ropivacaine 0.75%/methadone with or without pre-operative oral tepoxalin in dogs undergoing tibial tuberosity advancement surgery.

Adapted from:

Bosmans T, Piron K, Oosterlinck M, Gasthuys F, Duchateau L, Waelbers T, Samoy Y, Van Vynckt D, Polis I (2012) Comparison of analgesic efficacy of epidural methadone or ropivacaine/methadone with or without pre-operative oral tepoxalin in dogs undergoing tuberositas tibiae advancement surgery. Veterinary Anaesthesia and Analgesia. doi: 10.1111/j.1467-2995.2012.00744.x.

Abstract

Objective To investigate the clinical efficacy of four analgesia protocols in dogs undergoing tibial tuberosity advancement (TTA).

Study Design Prospective, randomized, blinded study.

Animals Thirty-two client owned dogs undergoing TTA-surgery.

Methods Dogs (n=8 treatment^{-1}) received an oral placebo (PM and PRM) or tepoxalin (10 mg kg^{-1}) tablet (TM and TRM) once daily during 1 week before surgery. Epidural methadone (0.1 mg kg^{-1}) (PM and TM) or the epidural combination methadone (0.1 mg kg^{-1})/ropivacaine 0.75% (1.65 mg kg^{-1}) (PRM and TRM) was administered after induction of anaesthesia. Intra-operative fentanyl requirements (2 μg kg^{-1} IV) and end-tidal isoflurane concentration after 60 minutes of anaesthesia ($FE´ISO_{60}$) were recorded. Post-operative analgesia was evaluated hourly from 1 to 8 and at 20 hours post-extubation with a visual analogue scale (VAS) and the University of Melbourne Pain Scale (UMPS). Whenever the VAS- and/or the UMPS-scores exceeded respectively 50 and 10, rescue methadone (0.1 mg kg^{-1}) was administered IV. Analgesic duration (time from epidural until post-operative rescue analgesia) and time to standing were recorded. Normally distributed variables were analyzed with an F-test (α=0.05) or t-test for pairwise inter-treatment comparisons (Bonferonni adjusted α=0.0083). Non-parametric data were analyzed with the Kruskall-Wallis test (α=0.05 or Bonferonni adjusted α=0.005 for inter-treatment comparison of post-operative pain scores).

Results More intra-operative analgesia interventions were required in PM [2 (0-11)][median (range)] and TM [2 (1-2)] compared to PRM (0) and TRM (0). The $FE´ISO_{60}$ was significantly lower in (PRM+TRM) compared to (PM+TM). Analgesic duration was shorter in PM (459 ± 276 minutes) (mean ± SD) and TM (318 ± 152 minutes) compared to TRM (853 ± 288 minutes), but not to PRM (554 ± 234 minutes). Times to standing were longer in the ropivacaine 0.75% treatments compared to TM.

Conclusions and Clinical Relevance Inclusion of epidural ropivacaine 0.75% resulted in reduction of $FE´ISO_{60}$, avoidance of intra-operative fentanyl administration, a longer duration of post-operative analgesia (in TRM) but a delay in time to standing compared to TM.

Introduction

The prevention and treatment of pain associated with orthopaedic surgery, such as tibial tuberosity advancement (TTA) is best achieved using a multimodal analgesic approach with opioids, local anaesthetics (LA) and non-steroidal anti-inflammatory drugs (NSAIDs) (Lemke 2004).

Epidural analgesia is commonly used for hind limb orthopaedic surgery. When administered epidurally, opioids exert fewer side effects and provide a longer duration of analgesia compared to systemic administration (Smith & Kwang-An Yu 2001). Methadone, a synthetic μ-opioid, is equipotent to morphine, and also exerts adrenergic (α_2) agonist and *N*-methyl-D-aspartate (NMDA) receptor antagonist actions (Codd et al. 1995; Matsui & Williams 2010). It is commercially available as an aqueous preservative-free racemic mixture and has been used epidurally in humans, horses, cows, goats, and dogs (Beeby et al. 1984; Kedlaya et al. 2002; Prieto-Alvarez et al. 2002; Bergadano et al. 2006; Iff et al. 2009; Van Loon et al. 2010; Bosmans et al. 2011). Studies on the analgesic effects of epidural methadone in dogs remain sparse (Leibetseder et al. 2006; Monteiro et al. 2008a).

Although opioids can be used as a sole analgesic epidural treatment, their combination with a long acting LA agent is preferred, because of the reported synergistic analgesic effects (Wang et al. 1993). Ropivacaine is a long-acting amino-amide LA, structurally related to bupivacaine and mepivacaine (Duke et al. 2000). In humans, it is a valuable alternative to epidural bupivacaine, mainly because of reduced neuro- and cardiotoxicity induced by accidental intravascular injection (Casati & Putzu 2005; Leone et al. 2008). Additionally, a better differentiation between sensory and motor block was observed, which can be clinically useful if early mobilization is required (Danelli et al. 2004; Leone et al. 2008; Koltka et al. 2009). Epidural ropivacaine 0.75% (1.65 mg kg^{-1}) has provided a good block of the dermatomes L5-L7 in dogs (Duke et al. 2000).

Tepoxalin (Zubrin; Schering-Plough Animal Health, UK) is a pyrazole derivative NSAID, which acts as a dual cyclo-oxygenase (COX) and lipoxygenase (LOX) inhibitor with high anti-inflammatory efficacy providing effective pain relief, together with a good gastro-intestinal (GI) safety profile, supporting its long-term use for pain relief (Argentieri et al. 1994; Agnello et al. 2005). It is used in dogs with osteoarthritis and other musculoskeletal disorders. The aims of the study were to evaluate the analgesic effects of epidural methadone

compared to methadone/ropivacaine 0.75% with or without pre-operative tepoxalin administration in dogs undergoing TTA surgery.

Materials and Methods

Animals

Dogs, of different breeds, scheduled for TTA-surgery for the management of cranial cruciate ligament rupture at the Department of Medical Imaging and Orthopaedics, Faculty of Veterinary Medicine, Ghent University between January 2008 – December 2010 were included in the study. The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2008/078). Written informed owner consent, including the option to drop out of the study at any time was obtained. Routine clinical and blood (haematology and biochemistry) examination was performed at least one week before the planned surgery. Dogs with abnormal blood results were excluded from the study. Other exclusion criteria were recent treatment with steroids or NSAIDs (< 10 days before start of the study), renal and/or GI disease, coagulopathy, pain unrelated to the stifle injury, dermatitis or history of trauma at the lumbosacral region, extreme obesity and aggression / fear.

Analgesia protocols

Thirty two dogs were randomly (blocked randomization) allocated to one of four treatments (n=8 treatment^{-1}). The observer who scored pain (TB) was unaware of the treatment given.

Dogs in group PM received an oral lyophilisated placebo tablet (Oral lyophylisate placebo tablet; Schering-Plough, Belgium) once daily (SID) for one week before surgery (the last tablet being administered immediately before premedication on the day of surgery) and epidural preservative free methadone (0.1 mg kg^{-1}) (Mephenon; Denolin, Belgium). Treatment PRM comprised pre-operative placebo treatment as in PM and epidural ropivacaine (1.65 mg kg^{-1} 0.75%) (Naropin 7.5 mg mL^{-1}; Astra Zeneca, Belgium) plus 0.1 mg kg^{-1} of preservative free methadone.

Dogs allocated to treatment TM received oral tepoxalin (Zubrin; Schering-Plough, Belgium) 10 mg kg^{-1} SID for one week before surgery, with the last dose administered before premedication and epidural preservative free methadone (0.1 mg kg^{-1}). Treatment TRM

comprised pre-operative tepoxalin as in treatment TM, with epidural ropivacaine (1.65 mg kg^{-1} 0.75%) and preservative free methadone (0.1 mg kg^{-1}).

All epidural injectates were made up to equal volumes (0.23 mL kg^{-1}) by adding NaCl 0.9% (Natrii Chloridum 0.9%; B. Braun, Germany) where necessary. The epidural injections were performed after the induction of general anaesthesia. The maximum volume for the epidural injection was set at 10 mL dog^{-1}.

Baseline measurements

One day before surgery the temperament and behaviour of each dog were assessed by the person responsible for pain scoring (TB) who specifically assessed their behavioural reactions to palpation and mobilisation of both stifles. Their pre-operative respiratory rate (f_R) (counting thorax excursions), heart rate (HR) (cardiac auscultation) and rectal temperature (digital thermometer Maximum-Thermometer; Hartmann, Belgium) were also recorded on six different time points in this specific sequence, and the mean values were recorded as baseline values for the relevant components of the University of Melbourne Pain Scale (UMPS) (appendix 1). A baseline Visual Analogue Scale (VAS) pain score [range 0 (no pain) – 100 (worst pain imaginable)] was also assessed before premedication.

Anaesthetic protocol

Premedication consisting of 0.01 mg kg^{-1} acepromazine (Placivet; Codiphar, Belgium) IV and 0.1 mg kg^{-1} of preservative free methadone IV was administered via a 20 SWG catheter placed in a cephalic vein. Fifteen minutes later, anaesthesia was induced with midazolam (0.2 mg kg^{-1} IV) (Dormicum; Roche, Belgium), immediately followed by propofol (4-6 mg kg^{-1} IV) (Propovet; Abbott Animal Health, UK) given to effect. After endotracheal intubation, anaesthesia was maintained with isoflurane (Isoflo; Abbott Animal Health, UK) (initial vaporizer setting was 2.5%) in oxygen, using a circle system and a fresh gas flow of 2 L $minute^{-1}$. Lactated Ringer's solution (Hartmann; Baxter, Belgium) was infused IV at 10 mL kg^{-1} $hour^{-1}$ throughout anaesthesia.

A 22 SWG catheter was placed in the dorsal pedal artery of the sound limb for blood pressure measurement. The lumbosacral area was clipped and aseptically prepared with the dogs in sternal recumbency and a 22 SWG spinal needle (Yale spinal needle; 3.5 inch, 0.7 x 90 mm, Becton Dickinson, Spain) was introduced into the lumbosacral epidural space, using the midline approach. After needle placement no attempts were made to aspirate cerebrospinal

fluid or blood. The needle hub was observed for 30 seconds to confirm the absence of cerebrospinal fluid and blood in the needle hub. Correct epidural placement was confirmed with the loss of resistance method (LOR) using a test injection of 0.5 mL of NaCl 0.9%. In 5 dogs, placement of the epidural needle was difficult and/or LOR was not conclusive for the puncturing of the epidural space, and the epidural needle was then placed under radiographic guidance using a test injection of 0.5 mL of Iohexol (Omnipaque 240 mg I mL^{-1}; GE Healthcare, Belgium) in right lateral recumbency. All epidural treatments were administered over 2 minutes, with continuous evaluation of the resistance to injection to detect possible displacement of the needle.

After appropriate preparation of the surgical site, dogs were transferred to the operating theatre. Anaesthesia was maintained with isoflurane in 40% oxygen. Intermittent positive pressure ventilation was initiated to maintain eucapnia [end-tidal CO_2 ($P_{E'}CO_2$) of 4.7-6 kPa]. Respiratory rate, HR, arterial oxygen saturation (SpO_2), invasive systolic (SAP), diastolic (DAP) and mean arterial (MAP) blood pressures, inspired oxygen percentage (F_IO_2), inspired isoflurane percentage ($F_{I'}ISO$), end-tidal isoflurane percentage ($F_{E'}ISO$) and $P_{E'}CO_2$ were measured continuously and recorded every 5 minutes. Body temperature was controlled with a circulating hot water mattress.

End-tidal isoflurane percentage was allowed to reach 1.4% and then the vaporizer setting was decreased by 0.2% every 5 minutes as long as the depth of anaesthesia was deemed sufficient (assessment of palpebral reflex, mandibular tone, absence of response to surgical stimulus and changes in cardiovascular parameters). If HR increased by >25% above the pre-operative value, while depth of anaesthesia was judged too light, end-tidal isoflurane concentration was increased by 0.5%. The depth of anaesthesia was re-evaluated after 5 minutes and the vaporizer setting was not reduced further. Whenever a sudden increase in HR (>25% increase above baseline) occurred together with signs of intra-operative nociception (judgement by an experienced anaesthetist), 2 µg kg^{-1} of fentanyl (Fentanyl; Janssen-Cilag, Belgium) was administered as an IV bolus, without increasing isoflurane %. When this occurred the vaporizer setting was not reduced further. The number of intra-operative fentanyl administrations over the anaesthetic period was recorded. The $F_{E'}ISO$ was recorded after 60 minutes of anaesthesia ($F_{E'}ISO_{60}$).

If HR dropped below 55 beats $minute^{-1}$, atropine (0.01 mg kg^{-1}) (Atropine Sulphate; Sterop Laboratories, Belgium) was administered IV. The number of atropine administrations over the anaesthetic period was recorded. If MAP dropped below 60 mmHg for more than 15

minutes, 7 mL kg^{-1} hydroxyethylstarch (HES) (HAES-steril 6%; Fresenius Kabi, Belgium) was infused over 30 minutes. The number of HES-administrations over the anaesthetic period was recorded.

The durations of anaesthesia and surgery, analgesia (time from epidural placement until first post-operative rescue analgesia treatment) and epidural time (time from placement epidural until extubation) were recorded.

Post-operative pain scoring

Pain scores were assessed at 1, 2, 3, 4, 5, 6, 7, 8 and 20 hours post-extubation using VAS and UMPS.

Data for the UMPS were collected in the following order: visual behavioural evaluation, and f_R, HR, rectal temperature and parameters requiring patient interaction. VAS scores were evaluated after the UMPS-scores and involved observation and interaction with the dog. If the UMPS-score exceeded 10 [cut-off value by Hancock et al. 2005] and/or the VAS-score exceeded 50 mm, methadone (0.1 mg kg^{-1}) was administered IV. Time to first administration and total number of methadone doses administered over the observation period were recorded. If rescue analgesia was required 8 hours after the extubation, it was repeated at 14 hours post-extubation.

Motor function

The animal's ability to stand was assessed after each pain assessment so that times to standing could be recorded (time from epidural injection until first post-op hour when the dog could stand without support). The time from extubation to standing was also recorded.

Side effects

The occurrence of side effects, associated with epidural drug administration (e.g. urinary retention, pruritus, vomiting) was also recorded. Additionally, the occurrence of GI side effects associated with NSAID administration (vomiting, diarrhoea) were recorded.

Statistical analysis

Analyses were performed with SAS 9.2 software (SAS 9.2 Windows Version, SAS Institute Inc., USA). The Shapiro-Wilks test was used to assess normality. Normally distributed variables are reported as mean ± standard deviation, whilst non-normally distributed variables

are reported as median (range). To evaluate the treatment effect for normally distributed variables such as most of the baseline characteristics, induction dose, durations of anaesthesia and surgery, epidural time, $F_E´ISO_{60}$, duration of analgesia and time to first rescue analgesia post-op, an analysis of variance was performed using the F-test at the 5% significance level for the global comparison and the t-test with Bonferroni-adjusted significance level (0.83 %) for pair-wise comparisons between the four treatments. The Fisher's exact test was used to compare the sex distribution between the four treatments. The Kruskall-Wallis test was used at the 5% global significance level for analysis of the variables for which the normal distribution did not hold: post-operative pain scores; time to standing; time from extubation to standing; total number of atropine and HES doses, intra-operative fentanyl and post-operative methadone administrations. Finally, the inter-treatment comparison of post-operative pain scores at the 10 time points was done at the Bonferroni-adjusted significance level of 0.5 %.

Results

There was no significant difference between groups with respect to sex, age, induction dose of propofol, body mass, baseline physiological parameters, duration of anaesthesia and surgery and epidural time (Table 1).

Table 1 Characteristics (mean ± SD) of 32 dogs (*n*=8 treatment^{-1}).

Variable	PM	PRM	TM	TRM	*p*-value*
Age (months)	60 ± 28	74 ± 26	65 ± 29	56 ± 25	0.23
Body mass (kg)	37.9 ± 6.6	40.8 ± 7.4	34.5 ± 2.9	36.8 ± 17.7	0.93
Baseline HR (beats minute^{-1})	101 ± 17	107 ± 16	102 ± 8	104 ± 25	0.75
Baseline f_R (breaths minute^{-1})	35 ± 12	34 ± 9	41 ± 18	69 ± 66	0.27
Baseline rectal temperature (°C)	38.4 ± 0.7	38.4 ± 0.3	38.4 ± 0.4	38.6 ± 0.3	0.44
Females (#/total)	6/8	4/8	5/8	4/8	0.68
Propofol induction dose (mg kg^{-1})	2.8 ± 0.5	2.8 ± 0.4	3.3 ± 0.8	3.4 ± 1.5	0.83
Duration of anaesthesia (minutes)	231 ± 49	228 ± 45	213 ± 45	213 ± 38	0.93
Epidural time (minutes)	211 ± 45	209 ± 47	191 ± 45	193 ± 42	0.88
Duration of surgery (minutes)	144 ± 49	148 ± 41	127 ± 43	129 ± 38	0.96

*Fisher's exact test for sex; F-test for other characteristics

PM = oral placebo tablet (SID) during 1 week before surgery and epidural methadone (0.1 mg kg^{-1}) after induction of anaesthesia

PRM = similar to PM, but with addition of epidural ropivacaine 0.75% (1.65 mg kg^{-1})

TM = oral tepoxalin (10 mg kg^{-1} SID), for 1 week before surgery and epidural methadone (0.1 mg kg^{-1}) after induction of anaesthesia

TRM = similar to TM, but with addition of epidural ropivacaine 0.75% (1.65 mg kg^{-1})

intra-operative assessments (Table 2)

The inclusion of ropivacaine 0.75% into the epidural protocol significantly affected $FE´ISO_{60}$ (p=0.01) with values of 0.74 ± 0.13% in the PRM + TRM treatments compared to 0.88 ± 0.17% in PM + TM treatments. There were no significant inter-treatment differences for $FE´ISO_{60}$ when single treatments were compared to each other.

The number of intra-operative fentanyl administrations was significantly greater in PM compared to either PRM or TRM (p=0.004) and in TM compared to PRM or TRM (p<0.01).

The number of intra-operative atropine administrations did not significantly differ between treatments. Two dogs in TRM received 1 dose of HES.

Table 2 Intra- and post-operative variables for 32 dogs (n=8 treatment^{-1}) undergoing unilateral tibial tuberosity advancement surgery.

	PM	**PRM**	**TM**	**TRM**
intra-operative variables				
$FE´ISO_{60}$ (%)	0.87 ± 0.17	0.69 ± 0.04	0.90 ± 0.18	0.79 ± 0.16
Fentanyl administrations (#)	2 (0-11)^{a}	0^{b}	2 (1-2)^{a}	0^{b}
Atropine administrations (#)	0 (0-2)	0 (0-2)	0 (0-1)	0.5 (0-1)
post-operative variables				
Time to standing (minutes)	340 (270-500)^{ab}	441 (335-682)^{a}	286 (175-400)^{b}	460 (315-640)^{a}
Time from extubation to standing (hours)	2.0 (1-4)^{ab}	4.5 (2-7)^{a}	1.0 (1-4)^{b}	4.0 (2-7)^{a}
Duration of analgesia (minutes)	459 ± 276^{a}	554 ± 234^{ab}	318 ± 152^{a}	853 ± 288^{b}
Time to first rescue analgesia (hours post-extubation)	4.1 ± 4.5^{a}	5.8 ± 4.2^{ab}	2.1 ± 2.4^{a}	11.0 ± 4.5^{b}
Methadone administrations (#)	3.5 (1-6)^{ab}	2.5 (1-4)^{ab}	4.0 (2-7)^{a}	1.5 (1-3)^{b}

Treatments sharing the same letter do not differ significantly from each other (p<0.0083 for $FE´ISO_{60}$, duration of analgesia and time to first rescue analgesia; p<0.05 for time to standing, time from extubation to standing and number of atropine, fentanyl and methadone administrations). Data are represented as mean ± SD or as median (range). For treatment abbreviations, please consult Table 1.

Post-operative assessments (Table 2 & 3)

Times to standing were significantly longer in the ropivacaine 0.75% treatment groups compared to TM (p=0.002). Times from extubation to standing were significantly longer in PRM and TRM compared to TM (p=0.002 and 0.003 respectively).

Duration of analgesia was significantly shorter in PM (p=0.003) and TM (p=0.0001) compared to TRM, but not to PRM. Although the duration of post-operative analgesia was shorter in PRM compared to TRM, this difference did not reach statistical significance. Post-operative rescue analgesia was administered earlier in PM (p=0.002) and TM (p=0.0001) compared to TRM. Significantly more methadone was administered in TM compared to TRM (p=0.007).

Post-operative VAS scores (Table 3) were significantly lower in TRM compared to PM and TM until 4 hours post-extubation. At 4 hours post-extubation VAS scores were significantly lower in PRM compared to PM. Post-operative UMPS scores (Table 3) were significantly lower in TRM compared to TM (at 2 hours post-extubation) and PRM (at 7 hours post-extubation).

No side effects possibly associated with the oral administration of tepoxalin or the epidural administration of methadone and/or ropivacaine 0.75% were observed in the post-operative period in any dog.

Table 3 Post-operative Visual Analogue pain Scores (VAS), represented in mm (range 0-100) and University of Melbourne Pain Scores (UMPS) (range 0-27) over time for 32 dogs (n=8 treatment^{-1}) after unilateral tibial tuberosity advancement surgery.

		Time (pre-surgery and hours post-extubation)									
Variable	Treatment	PRE	1	2	3	4	5	6	7	8	20
VAS											
	PM	24 (3-75)^{a}	31 (14-80)^{a}	43 (14-75)^{a}	38 (28-62)^{a}	42 (34-58)^{a}	40 (24-66)^{a}	43 (25-75)^{a}	44 (22-65)^{a}	40 (10-60)^{a}	45 (9-57)^{a}
	PRM	42 (20-65)^{a}	7 (4-40)^{ab}	8 (4-70)^{ab}	19 (5-60)^{ab}	27 (8-40)^{bc}	38 (27-55)^{a}	42 (26-55)^{a}	43 (26-52)^{a}	49 (40-67)^{a}	59 (34-81)^{a}
	TM	35 (25-61)^{a}	51 (21-80)^{a}	47 (24-82)^{a}	43 (30-68)^{a}	41 (27-62)^{ac}	44 (33-63)^{a}	45 (22-58)^{a}	44 (32-47)^{a}	49 (40-61)^{a}	53 (31-87)^{a}
	TRM	28 (10-60)^{a}	6 (2-16)^{b}	5 (2-28)^{b}	9 (4-32)^{b}	17 (5-40)^{b}	24 (6-40)^{a}	33 (6-50)^{a}	36 (23-50)^{a}	52 (30-60)^{a}	58 (38-74)^{a}
UMPS											
	PM	NA	4 (0-14)^{a}	6 (3-15)^{ab}	7 (2-10)^{a}	7 (3-10)^{a}	7 (0-11)^{a}	7 (2-15)^{a}	7 (1-9)^{ab}	5 (0-10)^{a}	7 (1-10)^{a}
	PRM	NA	5 (4-7)^{a}	6 (2-10)^{ab}	6 (4-11)^{a}	7 (2-10)^{a}	7 (4-12)^{a}	7 (2-11)^{a}	8 (5-11)^{a}	8 (4-13)^{a}	8 (3-11)^{a}
	TM	NA	7 (4-14)^{a}	9 (6-13)^{a}	9 (3-12)^{a}	8 (0-12)^{a}	7 (1-10)^{a}	8 (5-11)^{a}	7 (4-10)^{ab}	8 (4-12)^{a}	9 (5-13)^{a}
	TRM	NA	5 (1-6)^{a}	5 (1-9)^{b}	5 (2-9)^{a}	4 (2-8)^{a}	4 (2-10)^{a}	5 (0-9)^{a}	5 (1-7)^{b}	6 (3-9)^{a}	9 (3-11)^{a}

Treatments sharing the same letter do not differ significantly from each other (p<0.005). Data are represented as median (range). For treatment abbreviations, please consult Table 1.

Discussion

The addition of ropivacaine 0.75% to the epidural treatment protocol resulted in a significant decrease of $F_E´ISO_{60}$. Leibetseder et al. (2006) reported a reduction of $F_E´ISO$ in dogs after epidural administration of methadone compared to IV administration, whereby an $F_E´ISO$ of 1% assured stable anaesthesia during the first 20 minutes after epidural methadone injection. Epidural methadone administration in the present study also resulted in a low $F_E´ISO_{60}$ (<1 MAC). However, fentanyl administered as rescue analgesic in both PM and TM, most likely also decreased $F_E´ISO_{60,}$ therefore, it cannot be concluded that epidural methadone alone decreased $F_E´ISO$ in the present study. Tepoxalin, as a sole analgesic, did not decrease the MAC_{ISO} in dogs (Crist et al. 2007) and in the present study, no significant differences in $F_E´ISO_{60}$ were observed between PM and TM, suggesting the absence of an isoflurane sparing effect of tepoxalin. The low $F_E´ISO_{60}$ observed in treatment TM could reasonably have been the result of epidural methadone and/or fentanyl rescue analgesia.

When epidural ropivacaine 0.75% was included, intra-operative analgesic requirements were reduced to zero, supporting reports of the enhanced analgesic effect of the epidural combination opioid/LA in dogs (Troncy et al. 2002; Kona-Boun et al. 2006). By contrast, pre-operative tepoxalin did not affect intra-operative analgesic requirements. However, the number of rescue fentanyl administrations was much more variable in treatment PM compared to TM. Interestingly, Leibetseder et al. (2006) reported higher $F_E´ISO$ values needed to maintain an adequate level of anaesthesia in dogs receiving various NSAIDs pre-surgically compared to dogs which did not receive NSAIDs, with the effect being more pronounced in dogs in which methadone was administered extradurally. The higher $F_E´ISO$ values reported in that study might have indicated a lack of analgesia, since those authors did not use rescue analgesia, but rather increased the vaporizer settings based on changes in physiological parameters in response to surgery. In the present study, an additional intra-operative analgesic effect of tepoxalin could have been masked by the analgesic effects of epidural methadone and/or ropivacaine 0.75% and the use of fentanyl as rescue analgesic. So while our results suggest that pre-operative tepoxalin administration has no additional beneficial analgesic effect, an intra-operative analgesic effect of tepoxalin when given alone cannot be excluded.

Despite the isoflurane sparing effect of treatment TRM, a short period of mild intra-operative hypotension did occur in two dogs, but was quickly resolved by HES infusion.

Hypotension associated with epidural administration of ropivacaine 0.75% and ropivacaine 0.75%/methadone in anaesthetized dogs has been reported (Bosmans et al. 2011). However, those dogs were maintained at 1.4 MAC_{ISO}, which more likely contributed to hypotension. Epidural methadone alone did not induce hypotension in the present study.

The observed duration of motor block induced by ropivacaine 0.75% was markedly longer in the present study than the 133 ± 32 minutes after epidural ropivacaine 0.75% in conscious dogs reported by Feldman et al (1996). Median times to standing were 441 and 460 minutes in treatments PRM and TRM, and median times from extubation to standing of 4.5 and 4.0 hours were respectively recorded. Since the duration of motor blockade was only evaluated by observing whether or not the animal was able to stand, the presence of pain in the surgical limb could have influenced the time to standing. However, significantly shorter times to standing were observed in treatment TM compared to PRM and TRM suggesting the post-operative presence of residual motor block.

The duration of analgesia with epidural methadone (PM: 459 minutes) is similar to that reported by Leibetseder et al. (2006) of 423 minutes for a dose of 0.3 mg kg^{-1}, while Skarda & Tranquilli (2007) cited a duration of 300-900 minutes following doses of 0.05-0.15 mg kg^{-1}. The addition of epidural ropivacaine 0.75% (PRM) or oral tepoxalin (TM) to epidural methadone did not significantly alter the duration of analgesia. However, the combination of tepoxalin with epidural methadone/ropivacaine 0.75% (TRM) significantly prolonged the duration of analgesia compared to PM and TM, suggesting that there was an additive or synergistic effect between the three drugs. Additionally, significantly more post-operative rescue analgesia was necessary when ropivacaine 0.75% was not included in the tepoxalin protocols. The combined use of the three analgesic drugs resulted in better and longer post-operative analgesia, as evidenced by the fewer administrations of and the delayed need for rescue methadone administration in TRM compared to TM.

Methods of scoring pain in veterinary medicine rely on the interpretation of animal behaviour by the observer (Murrell et al. 2008) using a variety of pain scoring methods. Both the VAS and UMPS have been used with success in dogs (Firth & Haldane 1999; Hancock et al. 2005; Hoelzler et al. 2005; Bosmans et al. 2007; Abelson et al. 2011; Moll et al. 2011), and both systems were able to differentiate between treatments in the present study. Significantly lower VAS and UMPS scores were recorded for TRM, compared to the other treatments in the immediate post-operative period, while VAS scores were significantly lower in PRM

compared to PM 4 hours after extubation, with more differentiation between treatments obtained with VAS-scores compared to UMPS-scores. VAS-scores have been reported to be more sensitive and reliable than the categorical approach used by multifactorial pain scoring systems (Grossman et al. 1991; Sammarco et al. 1996; Fowler et al. 2003). A drawback of the UMPS is that it lacks determination of the relative importance of categories and descriptors on the total pain score, such that the weight of one category over another may unduly affect the total score. Additionally, changes to physiological factors such as pupil size, salivation, heart and respiratory rate are non-specific to pain. Drugs, such as methadone can reduce heart rate, alter respiratory rate and produce mydriasis in dogs (Holton et al. 1998; Holton et al. 2001; Morton et al. 2005; Monteiro et al. 2008b). Therefore, the administration of methadone as post-operative rescue analgesic in the present study could have influenced subsequent UMPS pain scores.

The addition of ropivacaine 0.75% to epidural methadone resulted in a significant reduction of $FE´ISO_{60}$, avoidance of intra-operative rescue analgesia and a delay in time to standing compared to TM. The combination of oral tepoxalin and epidural methadone/ropivacaine 0.75% delayed (compared to PM and TM) and reduced (compared to TM) the need for post-operative rescue analgesia and delayed time to standing (compared to TM). Pre-operative administration of tepoxalin enhanced post-operative analgesia only when combined with epidural ropivacaine 0.75%/methadone.

Conflict of interest statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Appendix 1 University of Melbourne Pain Scale (UMPS), used to score analgesic efficacy after tibial tuberosity advancement surgery in dogs. (Firth & Haldane, 1999)

Category	Descriptor	Score
Physiological Data		
a)	Physiological data within reference range	0
b)	Dilated pupils	2
c) *choose only one*	Percentage increase in heart rate, relative to baseline	
	> 20%	1
	> 50%	2
	> 100%	3
d) *choose only one*	Percentage increase in respiratory rate, relative to baseline	
	> 20%	1
	> 50%	2
	> 100%	3
e) rectal temperature	Exceeds reference range	1
f)	Salivation	2
Response to palpation		
a) Choose only one	No change from preprocedural behaviour	0
	Guards/ reacts[a] when touched	2
	Guards/ reacts[a] before touched	3
Activity		
a) *choose only one*	At rest-sleeping or semiconscious	0
	At rest-awake	1
	Eating	0
	Restless (pacing/getting up and down)	2
	Rolling/thrashing	3
Posture		
a)	Guarding or protecting affected area (includes fetal position)	2
b) *choose only one*	Lateral recumbency	0
	Sternal recumbency	1
	Sitting/standing, head up	1
	Standing, head hanging down	2
	Moving	0
	Abnormal posture (prayer position, hunched)	2
Vocalization[b]		
a) *choose only one*	Not vocalizing	0
	Vocalizing when touched	2
	Intermittent vocalization	2
	Continuous vocalization	3
Mental status		
a) *choose only one*	Submissive	0
	Overtly friendly	1
	Wary	2
	Aggressive	3

[a]Turning head toward affected area, biting, licking, scratching at the wound; snapping at handler; or tense muscles and a protective (guarding) posture. [b]Does not include alert barking.

Chapter 3

Cardiovascular effects of epidural administration of methadone, ropivacaine 0.75% and their combination in isoflurane anaesthetized dogs.

Adapted from:

Bosmans T, Schauvliege S, Gasthuys F, Duchateau L, Gozalo Marcilla M, Gadeyne C, Polis I (2011) Cardiovascular effects of epidural administration of methadone, ropivacaine 0.75% and their combination in isoflurane anaesthetized dogs. Veterinary Anaesthesia and Analgesia 38, 146-157.

Abstract

Objective To compare the cardiovascular effects of 4 epidural treatments in isoflurane anaesthetized dogs.

Study Design Prospective, randomized, experimental study

Animals Six female, neutered Beagle dogs (13.3 ± 1.0 kg), aged 3.6 ± 0.1 years.

Materials and Methods Anaesthesia was induced with propofol (8.3 ± 1.1 mg kg^{-1}) and maintained with isoflurane in a mixture of oxygen and air [inspiratory fraction of oxygen (FIO_2) = 40%], using intermittent positive pressure ventilation. Using a cross-over model, NaCl 0.9% (P); methadone 1% 0.1 mg kg^{-1} (M); ropivacaine 0.75% 1.65 mg kg^{-1} (R) or methadone 1% 0.1 mg kg^{-1} + ropivacaine 0.75% 1.65 mg kg^{-1} (RM) in equal volumes (0.23 mL kg^{-1}) using NaCl 0.9%, was administered epidurally at the level of the lumbosacral space. Treatment P was administered to 5 dogs only. Cardiovascular and respiratory variables, blood gases and oesophageal temperature were recorded at T-15 and during 60 minutes after epidural injection (T0).

Results Mean overall heart rate (HR in beats $minute^{-1}$) was significantly lower after treatment M (119 ± 16) (p=0.0019), R (110 ± 18) (p<0.0001) and RM (109 ± 13) (p<0.0001), compared to treatment P (135 ± 21). Additionally, a significant difference in heart HR between treatments RM and M was found (p=0.04). After both ropivacaine 0.75% treatments, systemic arterial pressures (sAP's) were significantly lower compared to other treatments. No significant overall differences between treatments were present for central venous pressure, cardiac output, stroke volume, systemic vascular resistance, oxygen delivery and arterial oxygen content (CaO_2). Heart rate and sAP's significantly increased after treatment P and M compared to baseline (T-15). With all treatments significant reductions from baseline were observed in oesophageal temperature, packed cell volume and CaO_2. A transient unilateral Horner's syndrome occurred in one dog after treatment R.

Conclusions and Clinical Relevance Clinically important low sAP's were observed after the ropivacaine 0.75% epidural treatments in isoflurane anaesthetized dogs. Systemic arterial pressures were clinically acceptable when using epidural methadone.

Introduction

Epidural analgesia is a technique frequently used to achieve an adequate pre-emptive analgesia in veterinary medicine. Many different drugs, including local anaesthetics, opioids, alpha$_2$-agonists and ketamine can be administered alone or in combination (Jones 2001). An opioid combined with a long acting local anaesthetic is the most frequently used combination in epidural analgesia in small animal medicine (Valverde 2008). Epidural analgesia allows performing surgical procedures caudally to the diaphragm in humans and animals, even without the use of general anaesthesia (Heath 1986; Gottschalk & Smith 2001). In dogs however, this is only justified in high risk patients when using adequate monitoring, oxygen supplementation and deep sedation to prevent reaction to external stimuli (Heath 1986). Consequently, epidural anaesthetics and analgesics are most frequently used as an adjunct to general anaesthetic techniques in small animals (Torske & Dyson 2000). Therefore it is of great importance to understand the cardiovascular effects of epidurally administered drugs in isoflurane anaesthetized dogs.

Most clinical studies reporting the cardiovascular effects of long-acting local anaesthetics in dogs have focused on the epidural administration of bupivacaine alone (Hendrix et al. 1996; Torske et al. 1999). However, ropivacaine, a pure S-enantiomer, structurally related to mepivacaine and bupivacaine, is a valuable alternative to bupivacaine, which has induced neurotoxicity, seizures, cardiotoxicity and even cardiac arrest following accidental intravenous (IV) injection (Albright 1979; Casati & Putzu 2005; Leone et al. 2008). Consequently, most studies investigating the cardiovascular effects of ropivacaine, have been designed with a focus on the cardiotoxicity and arrhythmogenic effects of ropivacaine in animals as a model for human medicine (Liu et al. 1982; Feldman et al. 1989; Reiz et al. 1989). The most commonly reported cardiovascular side effect of the epidural administration of local anaesthetics in animals is hypotension (Torske & Dyson 2000), which has been reported after epidural ropivacaine in conscious dogs (Hurley et al. 1991; Duke et al. 2000). To the authors' knowledge no studies have been performed investigating the cardiovascular effects of epidural ropivacaine 0.75% in isoflurane anaesthetized dogs.

Epidural administration of a small dose of an opioid produces a more profound and prolonged analgesia compared to a full parenteral dose (Skarda 1996). Additionally, the incidence of side effects of opioids in dogs is lower after epidural compared to parenteral administration (Valverde et al. 1989). Although morphine remains the classic opioid for

epidural techniques in human and veterinary medicine, several other opioids can also be used for this purpose. In humans, epidural methadone has been reported to cause less nausea, pruritus, sedation and urinary retention compared to morphine (Gedney & Liu 1998). However, information on the epidural use of methadone in the dog is sparse (Jones 2001) and only the effects of extradural and intravenous methadone on isoflurane and postoperative analgesia requirements have been reported in this species (Leibetseder et al. 2006).

The present study was performed to evaluate clinically relevant cardiovascular effects of epidurally administered methadone, ropivacaine 0.75% and the combination of both, in isoflurane anaesthetized dogs.

Materials and Methods

Animals

The study received approval from the Ethical Committee of the Faculty of Veterinary Medicine of the University of Ghent (EC 2007/042).

Six female, neutered Beagles, weighing 13.3 ± 1.0 kg (mean ± SD) and aged 3.6 ± 0.1 years, were included in the study. Dogs were classified as ASA (American Society of Anaesthesiologists) class I (normal, healthy patient), based on routine clinical, haematological and biochemical examinations. The dogs were fasted overnight with free access to water, before each experiment.

Anaesthetic and experimental protocol

The dogs were assigned randomly to receive five epidural treatments in a cross over model. A wash-out period of at least one month was allowed between treatments. The five epidural treatments were: 1) treatment P: NaCl 0.9% (Natrii Chloridum 0.9%; B. Braun, Germany); 2) treatment M: 0.1 mg kg^{-1} of preservative free methadone 1% (Mephenon; Denolin, Belgium); 3) treatment R: 1.65 mg kg^{-1} of ropivacaine 0.75% (Naropin 7.5 mg mL^{-1}; Astra Zeneca, Belgium); 4) treatment RM: 1.65 mg kg^{-1} of ropivacaine 0.75% combined with 0.1 mg kg^{-1} of preservative free methadone 1% and 5) treatment HESR: a preload of 7 mL kg^{-1} of hydroxyethylstarch 6% (HAES-steril 6%; Fresenius Kabi n.v., Belgium), administered as a continuous rate infusion over 30 minutes before epidural injection (same treatment as treatment R). The results of the comparison between treatment R and HESR are published

elsewhere (Bosmans et al. 2011). All solutions were prepared in equal volumes (0.23 mL kg^{-1}) by addition of NaCl 0.9% as required.

On the day of the experiment a 22 gauge catheter was placed aseptically in a cephalic vein to allow induction of anaesthesia and administration of fluids during anaesthesia. Blood was obtained from a jugular vein to measure plasma sodium concentration (Spotlyte Na/K/Cl analyzer; Menarini diagnostics, Italy) and also packed cell volume (PCV) (Fisher Bioblock 1-15; Sigma, Germany), which was used to calculate haemoglobin concentration [Hb (g dL^{-1}) = PCV (L L^{-1}) x 34 (Linton et al. 2000)]. The plasma sodium and blood haemoglobin concentrations then were used as required for the Lithium dilution cardiac output monitor (LiDCOplus Haemodynamic monitor; LiDCO Ltd, UK) in order to measure cardiac output ($\dot{Q}t$).

Anaesthesia was induced with propofol (Propovet; Abbott Animal Health, UK) IV to effect (8.3 ± 1.1 mg kg^{-1}). After endotracheal intubation (7 mm ID cuffed endotracheal tube), anaesthesia was maintained with isoflurane (Isoflo; Abbott Animal Health, UK) in a mixture of oxygen and air such that the fraction of inspired oxygen (FIO_2) was 40% and delivered through a rebreathing system (Cicero; Dräger, Germany). End-tidal isoflurane concentration ($FE´ISO$), was maintained at 1.8%, (equivalent to 1.4 MAC, based on a MAC_{ISO} in dogs of 1.3% (Steffey et al. 1994)). Lactated Ringer's solution (Hartmann; Baxter, Belgium) was infused IV at 10 mL kg^{-1} $hour^{-1}$ throughout anaesthesia (up to 60 minutes after epidural injection). Eucapnia [end-tidal carbon dioxide ($PE´CO_2$) between 4.7 and 6.0 kPa] and body temperature were maintained using intermittent positive pressure ventilation (IPPV) and a circulating water heating pad respectively. An intra-arterial 22 gauge catheter was placed aseptically in a dorsal pedal artery, for invasive arterial blood pressure (AP) and cardiac output measurements. Using the Seldinger technique, a 4 Fr central venous catheter (Leader-Cath; Vygon, France) was placed in the right jugular vein with the distal port located in the right atrium. Correct catheter positioning was confirmed by the characteristic pressure waveform. This catheter was used for central venous pressure (CVP) measurement and the bolus injection of lithium chloride (LiDCO Lithium Chloride® 0.15 mmol mL^{-1}; LiDCO, UK).

A multichannel physiological monitor (HP M1165A®, Hewlett-Packard, Germany) was employed to record the following parameters: electrocardiogram, oesophageal temperature, arterial oxygen saturation (SpO_2) (probe placed on the tongue), heart rate (HR),

systolic (SAP), diastolic (DAP), and mean arterial blood pressure (MAP) and CVP (during the expiration phase of ventilation). The pressure transducers for AP and CVP (ST-33; PVB Critical Care, GmbH, Germany) were calibrated before each experiment using a mercury manometer (Empire N; Rieser, Germany) and were positioned and zeroed at the level of the right atrium. Respiratory rate (f_R), $P_{E´}CO_2$, F_IO_2 and $F_{E´}ISO$ were measured using an anaesthetic multigas monitor (Capnomac Ultima; Datex Engstrom Instrumentation, Finland), which was calibrated before each anaesthesia (Quick Cal calibration gas; Datex-Ohmeda, Finland). Cardiac output was measured with the lithium dilution technique. The bolus of lithium chloride used was 0.0075 mmol kg^{-1}. Arterial blood samples were collected from the dorsal pedal artery in heparinized syringes for immediate determination of temperature corrected oxygen and carbon dioxide arterial tensions (PaO_2 and $PaCO_2$) and pH (ABL-5; Radiometer Medical, Denmark) and of PCV (Haemofuge; Heraeus instruments, Germany).

After instrumentation, the dogs were positioned in sternal recumbency with the pelvic limbs extended cranially and the head elevated. A stabilisation period of 15 minutes was respected during which the lumbosacral area was aseptically prepared. The cardiovascular measurements were commenced at time point (T) T-15. Total duration of anaesthesia before initiation of the cardiovascular measurements at T-15 (instrumentation period + stabilisation period of 15 minutes) was 49 ± 21 minutes.

At T0, a 22 gauge spinal needle (Yale spinal needle 1.5 inch, 0.7 x 40 mm; Becton Dickinson, Spain) was introduced into the lumbosacral epidural space with the needle bevel directed towards the sacrum. Correct placement of the needle was confirmed in all dogs by the presence of a distinct "popping-sensation" as a result of penetrating the *ligamentum flavum*, together with an observed "tail-flick", the lack of resistance to injection and the absence of cerebrospinal fluid and blood in the needle hub. No attempt was made to aspirate cerebrospinal fluid or blood before injection. The epidural treatment was administered over 2 minutes, while continuously evaluating the resistance to injection to rule out accidental displacement of the needle during drug administration.

Data collection and Calculations

Systemic arterial pressures (sAP) (mmHg), SpO_2 (%), oesophageal temperature (°C) and HR (beats $minute^{-1}$) were recorded at T-15 (baseline measurements), then at T0 and subsequently every 5 minutes for 60 minutes. Blood gases and $\dot{Q}t$ (L $minute^{-1}$) were obtained at T-15 (baseline measurements), T0, T15, T30, T45 and T60.

The following parameters were calculated (Gross et al. 1990; Lumb 2005):

Stroke volume (SV in mL $beat^{-1}$) = 1000 x $\dot{Q}t$ (L $minute^{-1}$) / HR (beats $minute^{-1}$)

Systemic vascular resistance (SVR in dynes second cm^{-5}) = [MAP (mmHg) - CVP (mmHg)] x 80 / $\dot{Q}t$

Arterial blood oxygen content (CaO_2 in mL L^{-1}) = [Hb concentration (g L^{-1}) x 1.39 x SaO_2] + [PaO_2 (mmHg) x 0.003]

Oxygen delivery (DO_2 in L $minute^{-1}$) = [CaO_2 (mL L^{-1}) x $\dot{Q}t$ (L $minute^{-1}$)] / 1000

Statistical analysis

A Shapiro Wilk's test showed a normal distribution of the data, justifying a parametric statistical approach of the data of this study. An ANOVA-analysis with dog as random effect and treatment as fixed effect was performed to check for differences between treatments at baseline (α=0.05). The effects of the different treatments were compared using a mixed model with dog and period nested in dog as random effect and time, treatment and their interaction as categorical fixed effects. Individual treatments were compared to each other using Tukey's multiple comparisons technique (at global α=0.05). All *p*-values were adjusted for multiple comparisons.

The effect of time within treatment was investigated comparing the parameter values at each time point with T-15 (baseline). This analysis was based on a mixed model with dog as random effect and time as categorical fixed effect. Each time point was compared with T-15 using Dunnett's multiple comparisons technique (at global α=0.05). For statistical analysis SAS 9.2 software (SAS 9.2 Windows Version; SAS Institute Inc., NC, USA) was used.

Results

At baseline, no statistically significant differences between treatments were present for any of the observed parameters.

Treatment P was administered to five dogs only, due to the death of one Beagle in treatment HESR. Data from this dog, involving treatment M, R and RM, were not excluded from statistical analysis.

Cardiovascular system

Increases in HR and sAP were observed at the time of placement of the epidural needle in all treatments (except for HR in treatment RM). In four (one, two and one animals in treatment P, M, and R respectively) out of 23 anaesthetic periods (17.4%) this nociceptive stimulation was accompanied by signs of arousal (despite IPPV, spontaneous superficial breathing and tachypnea), requiring administration of a bolus of propofol (0.9 ± 0.4 mg kg^{-1}), to allow continuing IPPV.

Results for the overall comparison between treatments

Overall HR was significantly lower after treatment M, R and RM compared to treatment P, with a mean overall difference of 16 (p=0.0019), 25 (p<0.0001) and 26 (p<0.0001) beats $minute^{-1}$ respectively (Tables 1 & 2). Additionaly, overall HR was significantly lower for treatment RM, compared to treatment M, with a mean difference of 10 beats $minute^{-1}$ (p=0.04).

In treatments R and RM, overall SAP, DAP and MAP were significantly lower compared to treatments without ropivacaine 0.75% (P and M) (Table 1). As an example, overall SAP was significantly lower after treatment R (p=0.0027) and RM (p=0.0023) compared to treatment P, with a mean difference of 40 mmHg for both comparisons. Compared to treatment M, SAP was significantly lower in treatment R (mean difference of 25 mmHg, p=0.0355) and in treatment RM (25 mmHg, p=0.0299). As these differences in systemic arterial pressures gradually increased over time, a significant interaction effect between time and treatment was detected (p<0.0001 for all sAP).

No significant differences between treatments could be observed for mean overall CVP and oesophageal temperature.

No significant differences between treatments were present for mean overall $\dot{Q}t$, SV, SVR, DO_2 and CaO_2.

Results for the comparison with T-15 within each treatment

After treatments P and M, HR gradually increased and was significantly different from baseline from T15 onwards for treatment P and from T45 onwards for treatment M (Tables 1 & 2). With treatment R, the HR increase only reached significance at T55. Heart rate did not

change significantly throughout the observational period after treatment RM, although a non-significant decrease in HR was observed from T0-T35.

With treatment P, systemic arterial pressures increased from T0 onwards. The difference with baseline was significant from T5 onwards. The maximum increase was observed at T40 for all sAP. In treatment M, SAP, DAP and MAP were significantly higher at T0, and from T15 onwards. In treatment R, following the significant increase of sAP's at epidural injection (T0), values returned to baseline measurements and further increases in sAPs were non-significant. In treatment RM, sAP's at the different time points did not significantly differ from baseline.

After treatment P, CVP was significantly lower at T5-T10 than at baseline.

A significant increase in $\dot{Q}t$ was observed after treatments M and RM at T60. A significant increase from baseline in SV was observed at T60 in treatment RM only. No significant changes from baseline in SVR were observed in any of the treatments. Arterial oxygen content was significantly lower compared to baseline from T30-T45 after treatments P and M and from T15 onwards after treatments R and RM. Oxygen delivery significantly increased at T60 with treatments M and RM.

Oesophageal temperature decreased over time ($p<0.0001$) and was significantly lower than at baseline from T20 onwards for treatment P; from T5 onwards for treatments R and RM.

Table 1 Heart rate (HR), systolic (SAP), diastolic (DAP), mean arterial pressures (MAP), central venous pressure (CVP) and oesophageal temperature, in six dogs anaesthetized with isoflurane (1.4 MAC), receiving one of four epidural injection treatments: saline (P (*n*=5)), methadone 0.1 mg kg^{-1} (M), ropivacaine 0.75% 1.65 mg kg^{-1} (R) and ropivacaine 0.75% 1.65 mg kg^{-1} + methadone 0.1 mg kg^{-1} (RM) (all equal volumes of 0.23 mL kg^{-1}).

	Time (minutes)														
	T-15	T0	T5	T10	T15	T20	T25	T30	T35	T40	T45	T50	T55	T60	Overall
HR (beats $minute^{-1}$)															
P	108 ± 24	117 ± 28	126 ± 28	131 ± 26	138 ± 33$^{£}$	138 ± 30$^{£}$	140 ± 28$^{£}$	139 ± 26$^{£}$	145 ± 25$^{£}$	140 ± 19$^{£}$	142 ± 18$^{£}$	140 ± 17$^{£}$	140 ± 17$^{£}$	139 ± 18$^{£}$	135 ± 21
M	108 ± 24	119 ± 20	113 ± 16	110 ± 19	115 ± 20	115 ± 21	118 ± 19	119 ± 17	119 ± 15	122 ± 13	125 ± 14$^{£}$	126 ± 15$^{£}$	130 ± 19$^{£}$	130 ± 21$^{£}$	119 ± 16*
R	106 ± 15	118 ± 16	106 ± 14	105 ± 21	106 ± 22	109 ± 21	110 ± 20	107 ± 22	108 ± 21	109 ± 22	110 ± 22	116 ± 18	119 ± 17$^{£}$	115 ± 20	110 ± 18*
RM	110 ± 18	107 ± 17	101 ± 12	99 ± 9	100 ± 6	104 ± 7	106 ± 10	107 ± 14	109 ± 16	114 ± 21	113 ± 19	116 ± 25	118 ± 28	118 ± 28	109 ± 13* $^{\#}$
SAP (mmHg)															
P	92 ± 11	106 ± 14	116 ± 21$^{£}$	120 ± 23$^{£}$	125 ± 27$^{£}$	128 ± 29$^{£}$	135 ± 18$^{£}$	139 ± 15$^{£}$	146 ± 22$^{£}$	148 ± 17$^{£}$	142 ± 11$^{£}$	142 ± 8$^{£}$	142 ± 5$^{£}$	142 ± 12$^{£}$	130 ± 13
M	81 ± 13	109 ± 21$^{£}$	97 ± 16	92 ± 21	103 ± 24$^{£}$	112 ± 21$^{£}$	118 ± 22$^{£}$	123 ± 21$^{£}$	124 ± 21$^{£}$	124 ± 24$^{£}$	126 ± 24$^{£}$	132 ± 26$^{£}$	135 ± 29$^{£}$	135 ± 27$^{£}$	115 ± 19
R	88 ± 13	109 ± 25$^{£}$	85 ± 15	84 ± 7	84 ± 6	82 ± 6	83 ± 5	85 ± 12	86 ± 12	90 ± 13	92 ± 15	98 ± 14	99 ± 16	102 ± 17	90 ± 10* $^{\#}$
RM	84 ± 15	94 ± 25	80 ± 16	79 ± 28	81 ± 29	80 ± 19	83 ± 22	90 ± 26	94 ± 31	96 ± 28	98 ± 29	96 ± 29	100 ± 30	100 ± 28	90 ± 22* $^{\#}$
DAP (mmHg)															
P	50 ± 6	63 ± 13	68 ± 19$^{£}$	72 ± 22$^{£}$	75 ± 21$^{£}$	76 ± 23$^{£}$	81 ± 18$^{£}$	84 ± 16$^{£}$	86 ± 15$^{£}$	87 ± 12$^{£}$	86 ± 11$^{£}$	86 ± 11$^{£}$	84 ± 13$^{£}$	84 ± 11$^{£}$	77 ± 13
M	47 ± 9	66 ± 13$^{£}$	56 ± 10	54 ± 15	62 ± 16$^{£}$	63 ± 17$^{£}$	67 ± 15$^{£}$	70 ± 14$^{£}$	71 ± 13$^{£}$	74 ± 14$^{£}$	75 ± 15$^{£}$	73 ± 13$^{£}$	73 ± 14$^{£}$	73 ± 16$^{£}$	66 ± 13
R	47 ± 6	64 ± 16	45 ± 11	46 ± 7	46 ± 5	45 ± 6	46 ± 7	46 ± 10	48 ± 11	49 ± 11	50 ± 13	53 ± 12	54 ± 13	55 ± 14	50 ± 9* $^{\#}$
RM	49 ± 9	59 ± 20	46 ± 10	45 ± 15	47 ± 12	46 ± 9	48 ± 11	52 ± 13	55 ± 17	55 ± 16	58 ± 17	52 ± 13	54 ± 11	54 ± 11	51 ± 10* $^{\#}$
MAP (mmHg)															
P	62 ± 8	75 ± 13	82 ± 3$^{£}$	86 ± 24$^{£}$	90 ± 24$^{£}$	92 ± 25$^{£}$	98 ± 19$^{£}$	101 ± 16$^{£}$	104 ± 16$^{£}$	105 ± 13$^{£}$	103 ± 11$^{£}$	102 ± 10$^{£}$	102 ± 10$^{£}$	102 ± 11$^{£}$	93 ± 14
M	56 ± 10	79 ± 17$^{£}$	66 ± 13	63 ± 17	73 ± 19$^{£}$	76 ± 19$^{£}$	82 ± 17$^{£}$	84 ± 16$^{£}$	88 ± 16$^{£}$	90 ± 16$^{£}$	91 ± 17$^{£}$	90 ± 16$^{£}$	92 ± 18$^{£}$	93 ± 19$^{£}$	80 ± 15
R	57 ± 6	77 ± 19$^{£}$	56 ± 11	56 ± 8	55 ± 6	54 ± 6	56 ± 7	56 ± 11	58 ± 12	59 ± 13	62 ± 14	65 ± 13	67 ± 14	67 ± 15	60 ± 9* $^{\#}$
RM	58 ± 10	65 ± 19	54 ± 11	54 ± 19	56 ± 16	54 ± 11	57 ± 13	62 ± 17	64 ± 20	66 ± 19	66 ± 21	65 ± 19	68 ± 19	67 ± 17	61 ± 14* $^{\#}$
CVP (mmHg)															
P	3.8 ± 3.7	2.4 ± 2.5	1.6 ± 3.0$^{£}$	1.8 ± 2.3$^{£}$	2.4 ± 3.0	2.4 ± 2.5	2.4 ± 2.5	2.4 ± 2.5	3.0 ± 1.4	2.6 ± 2.1	2.8 ± 2.2	2.4 ± 1.9	2.4 ± 1.9	2.4 ± 1.5	2.5 ± 2.2
M	3.2 ± 1.3	2.7 ± 1.8	2.7 ± 1.4	2.7 ± 1.2	3.2 ± 1.5	3.0 ± 1.4	3.0 ± 1.3	3.3 ± 2.1	3.0 ± 1.8	2.7 ± 2.5	2.5 ± 2.1	2.2 ± 1.9	2.3 ± 1.9	2.5 ± 2.2	2.8 ± 1.6
R	2.7 ± 2.2	2.7 ± 2.2	3.0 ± 2.2	3.2 ± 2.1	2.7 ± 2.2	3.0 ± 1.7	3.0 ± 2.1	3.0 ± 2.0	2.8 ± 2.3	3.2 ± 1.8	3.0 ± 2.1	3.2 ± 2.1	2.8 ± 1.9	2.7 ± 2.2	2.9 ± 2.0
RM	3.8 ± 2.9	2.7 ± 2.0	3.8 ± 1.9	3.8 ± 1.9	3.7 ± 2.1	3.5 ± 2.1	3.3 ± 2.3	3.3 ± 2.3	3.3 ± 2.0	3.0 ± 2.0	2.5 ± 2.1	2.5 ± 2.1	2.7 ± 2.1	2.5 ± 2.1	3.2 ± 1.8
oesophageal temp (°C)															
P	37.6 ± 0.4	37.4 ± 0.4	37.3 ± 0.3	37.3 ± 0.3	37.3 ± 0.3	37.2 ± 0.3$^{£}$	37.2 ± 0.3$^{£}$	37.2 ± 0.3$^{£}$	37.2 ± 0.4$^{£}$	37.2 ± 0.4$^{£}$	37.2 ± 0.3$^{£}$	37.2 ± 0.4$^{£}$	37.2 ± 0.4$^{£}$	37.1 ± 0.4$^{£}$	37.3 ± 0.3
M	36.9 ± 0.9	36.8 ± 0.8	36.7 ± 0.7	36.7 ± 0.7	36.7 ± 0.6	36.7 ± 0.6	36.6 ± 0.5	36.6 ± 0.5	36.5 ± 0.5	36.5 ± 0.5	36.5 ± 0.5	36.5 ± 0.5	36.5 ± 0.5	36.5 ± 0.6	36.6 ± 0.6
R	37.5 ± 0.6	37.3 ± 0.7	37.3 ± 0.7$^{£}$	37.2 ± 0.7$^{£}$	37.2 ± 0.7$^{£}$	37.1 ± 0.7$^{£}$	37.1 ± 0.7$^{£}$	37.1 ± 0.7$^{£}$	37.1 ± 0.8$^{£}$	37.0 ± 0.8$^{£}$	37.0 ± 0.8$^{£}$	37.0 ± 0.8$^{£}$	37.0 ± 0.8$^{£}$	37.0 ± 0.7$^{£}$	37.1 ± 0.7
RM	37.6 ± 0.9	37.5 ± 0.8	37.4 ± 0.8$^{£}$	37.4 ± 0.8	37.3 ± 0.8$^{£}$	37.3 ± 0.8$^{£}$	37.3 ± 0.8$^{£}$	37.2 ± 0.8$^{£}$	37.2 ± 0.8$^{£}$	37.2 ± 0.8$^{£}$	37.1 ± 0.8$^{£}$	37.1 ± 0.8$^{£}$	37.1 ± 0.8$^{£}$	37.0 ± 0.8$^{£}$	37.2 ± 0.8

T-15 represents baseline values. T0 represents the end of epidural injection. Data are represented as mean ± SD.

*Significant differences compared to Group P for the overall comparison (global α=0.05); $^{\#}$Significant differences compared to Group M for the overall comparison (global α=0.05); $^{£}$Significant difference from T-15 within specific treatment (α=0.05)

Table 2 Cardiac output ($\dot{Q}t$), stroke volume (SV), systemic vascular resistance (SVR), arterial oxygen content (CaO_2) and oxygen delivery (DO_2) in six dogs anaesthetized with isoflurane (1.4 MAC), receiving one of four epidural injection treatments (see Table 1)

	Time (minutes)						
	T-15	T0	T15	T30	T45	T60	Overall
$\dot{Q}t$ (L minute^{-1})							
P	2.1 ± 0.6	2.2 ± 0.8	2.7 ± 1.6	2.6 ± 0.8	2.9 ± 0.9	3.0 ± 1.1	2.6 ± 0.8
M	1.7 ± 0.6	1.8 ± 0.8	2.0 ± 0.5	2.0 ± 0.9	2.1 ± 1.0	2.3 ± 1.2$^{£}$	2.0 ± 0.8
R	1.6 ± 0.9	1.8 ± 0.6	1.6 ± 0.4	1.6 ± 0.4	2.0 ± 0.3	2.2 ± 0.3	1.7 ± 0.5
RM	1.6 ± 0.5	1.5 ± 0.5	1.5 ± 0.4	1.6 ± 0.4	1.9 ± 0.5	2.2 ± 0.4$^{£}$	1.7 ± 0.4
SV (mL beat^{-1})							
P	19 ± 3	18 ± 3	20 ± 8	19 ± 2	21 ± 5	22 ± 7	20 ± 5
M	16 ± 6	15 ± 5	17 ± 2	17 ± 7	17 ± 7	19 ± 8	17 ± 6
R	16 ± 10	16 ± 6	16 ± 6	16 ± 5	20 ± 5	20 ± 4	16 ± 6
RM	15 ± 4	14 ± 5	15 ± 4	15 ± 4	18 ± 5	20 ± 4$^{£}$	16 ± 4
SVR (dynes second cm^{-5})							
P	2355 ± 537	2900 ± 793	2851 ± 974	3076 ± 495	2882 ± 754	2844 ± 751	2709 ± 589
M	2714 ± 1122	3906 ± 1480	3028 ± 750	3863 ± 2328	4388 ± 3439	3901 ± 2717	3630 ± 1929
R	3856 ± 2915	3666 ± 1531	2936 ± 1276	2815 ± 962	2291 ± 516	2311 ± 406	3193 ± 1552
RM	2841 ± 858	3607 ± 1250	2945 ± 820	3001 ± 997	2789 ± 1234	2158 ± 340	2939 ± 728
CaO_2 (mL L^{-1})							
P	182 ± 15	175 ± 15	170 ± 15	167 ± 16$^{£}$	166 ± 14$^{£}$	169 ± 14	172 ± 13
M	187 ± 9	178 ± 17	177 ± 17	170 ± 20$^{£}$	175 ± 15$^{£}$	177 ± 13	177 ± 14
R	183 ± 21	177 ± 15	171 ± 18$^{£}$	167 ± 18$^{£}$	166 ± 18$^{£}$	165 ± 19$^{£}$	172 ± 17
RM	181 ± 14	175 ± 14	166 ± 15$^{£}$	161 ± 14$^{£}$	160 ± 16$^{£}$	158 ± 19$^{£}$	167 ± 15
DO_2 (L minute^{-1})							
P	0.37 ± 0.11	0.37 ± 0.15	0.43 ± 0.22	0.46 ± 0.15	0.46 ± 0.11	0.48 ± 0.15	0.44 ± 0.12
M	0.32 ± 0.12	0.32 ± 0.15	0.35 ± 0.12	0.36 ± 0.18	0.38 ± 0.19	0.42 ± 0.22$^{£}$	0.35 ± 0.15
R	0.30 ± 0.18	0.30 ± 0.10	0.26 ± 0.67	0.26 ± 0.07	0.33 ± 0.05	0.35 ± 0.07	0.29 ± 0.08
RM	0.29 ± 0.08	0.26 ± 0.78	0.24 ± 0.73	0.26 ± 0.06	0.31 ± 0.09	0.33 ± 0.08$^{£}$	0.28 ± 0.06

T-15 represents baseline values. T0 represents the end of epidural injection. Data are represented as mean ± SD.

$^{£}$Significant difference from T-15 within the specific treatments (global α=0.05)

Blood gas analysis and packed cell volume

There were no significant mean overall differences between treatments for any of the arterial blood gases (Table 3). However, a significant interaction effect between time and treatment was detected for arterial pH (p=0.0165). Additionally, arterial pH at T0 was significantly higher than at baseline in treatment RM.

Arterial partial pressure of CO_2 in treatment RM was significantly lower compared to baseline from T0 onwards.

Packed cell volume decreased over time in all treatments, and was significantly lower compared to baseline, from T15 onwards after treatments P, R and RM and from T30-T60 after treatment M.

Adverse effects

One dog in treatment R exhibited a transient unilateral Horner's syndrome (HS) on the right side, with ptosis of the upper eyelid and miosis of the affected pupil, after recovering from anaesthesia. The signs gradually disappeared together with disappearance of ropivacaine 0.75% induced motor block.

Table 3 Arterial blood gases and packed cell volume (PCV) in six dogs anaesthetized with isoflurane (1.4 MAC), receiving one of four epidural injection treatments (see Table 1)

	Time (minutes) T-15	T0	T15	T30	T45	T60	Overall
Arterial blood							
pH							
P	7.34 ± 0.04	7.33 ± 0.02	7.36 ± 0.02	7.33 ± 0.04	7.33 ± 0.03	7.34 ± 0.03	7.34 ± 0.03
M	7.37 ± 0.04	7.37 ± 0.06	7.35 ± 0.04	7.35 ± 0.05	7.34 ± 0.03	7.35 ± 0.03	7.36 ± 0.04
R	7.33 ± 0.05	7.32 ± 0.04	7.33 ± 0.03	7.34 ± 0.04	7.33 ± 0.05	7.35 ± 0.06	7.33 ± 0.04
RM	7.32 ± 0.02	7.35 ± 0.02[£]	7.33 ± 0.03	7.33 ± 0.02	7.32 ± 0.02	7.32 ± 0.03	7.33 ± 0.02
PCO_2 kPa (mmHg)							
P	5.77 ± 0.71 (43 ± 5)	5.64 ± 0.31 (42 ± 2)	5.16 ± 0.26 (39 ± 2)	4.95 ± 1.04 (37 ± 8)	5.40 ± 0.70 (41 ± 5)	5.24 ± 0.78 (39 ± 6)	5.37 ± 0.60 (40 ± 4)
M	5.83 ± 0.49 (44 ± 4)	5.41 ± 0.79 (41 ± 6)	5.63 ± 0.64 (42 ± 5)	5.61 ± 0.76 (42 ± 6)	5.72 ± 0.73 (43 ± 4)	5.85 ± 0.59 (44 ± 4)	5.69 ± 0.60 (43 ± 5)
R	5.67 ± 0.69 (43 ± 5)	5.63 ± 0.22 (42 ± 2)	5.36 ± 0.61 (40 ± 5)	5.43 ± 0.49 (41 ± 4)	5.30 ± 0.61 (40 ± 5)	5.14 ± 0.57 (39 ± 4)	5.44 ± 0.42 (41 ± 3)
RM	5.85 ± 0.69 (44 ± 5)	5.10 ± 0.50[£] (38 ± 4)[£]	5.12 ± 0.67[£] (38 ± 5)[£]	5.28 ± 0.33[£] (40 ± 2)[£]	5.16 ± 0.51[£] (39 ± 4)[£]	5.41 ± 0.31[£] (41 ± 2)[£]	5.33 ± 0.46 (40 ± 3)
PO_2 kPa (mmHg)							
P	31.84 ± 1.75 (239 ± 13)	32.21 ± 1.74 (241 ± 13)	32.37 ± 1.41 (243 ± 11)	33.28 ± 1.22 (250 ± 9)	33.17 ± 1.12 (249 ± 8)	32.85 ± 1.27 (246 ± 10)	32.62 ± 0.64 (245 ± 5)
M	32.12 ± 2.80 (241 ± 21)	32.61 ± 1.60 (245 ± 12)	32.96 ± 1.57 (247 ± 12)	32.72 ± 1.07 (245 ± 8)	32.25 ± 1.22 (242 ± 9)	31.83 ± 2.42 (239 ± 18)	32.42 ± 1.08 (243 ± 8)
R	31.85 ± 1.69 (239 ± 13)	33.16 ± 1.54 (249 ± 12)	32.74 ± 2.19 (246 ± 16)	32.21 ± 2.81 (242 ± 21)	33.03 ± 2.61 (248 ± 20)	34.42 ± 2.88 (258 ± 22)	32.90 ± 1.67 (247 ± 13)
RM	33.85 ± 2.57 (254 ± 19)	33.21 ± 1.79 (249 ± 13)	32.74 ± 2.30 (246 ± 17)	32.98 ± 1.73 (247 ± 13)	32.56 ± 1.80 (244 ± 14)	30.86 ± 4.32 (231 ± 32)	32.70 ± 1.83 (245 ± 14)
PCV %							
P	37 ± 3	35 ± 3	34 ± 3[£]	34 ± 3[£]	34 ± 3[£]	34 ± 3[£]	35 ± 3
M	38 ± 2	36 ± 4	36 ± 4	34 ± 4[£]	36 ± 3[£]	36 ± 3[£]	36 ± 3
R	37 ± 4	36 ± 3	35 ± 4[£]	34 ± 4[£]	34 ± 4[£]	33 ± 4[£]	35 ± 4
RM	37 ± 3	36 ± 3	34 ± 3[£]	33 ± 3[£]	32 ± 3[£]	32 ± 4[£]	34 ± 3

T-15 represents baseline values. T0 represents the end of epidural injection. Data are represented as mean ± SD.

[£]Significant difference from T-15 within specific treatment (global α=0.05)

Discussion

In the present study, epidural ropivacaine 0.75% and the combination of ropivacaine 0.75% with methadone resulted in lower systemic arterial pressures, compared to epidural injection of saline and methadone in isoflurane anaesthetized dogs. In contrast, epidural methadone administration was not associated with clinically important cardiovascular changes.

Clear increases in HR and sAP (although not always significantly different from baseline values) were observed during placement of the epidural needle in all treatments. It was hypothesized that these reactions were mainly a consequence of the lack of premedication, which is routinely used under clinical circumstances and most often includes an analgesic. As a result, the placement of the epidural needle and the injection, which represented noxious stimuli, induced an increase in sympathetic tone (Lemke 2004). In some dogs, these cardiovascular changes were accompanied by signs of inadequate depth of anaesthesia (such as "fighting the ventilator"). Consequently, in those dogs the administration of an additional bolus of propofol was necessary to allow continuing artificial ventilation. The administration of a bolus of propofol at the time of epidural needle placement is certainly one of the limitations of the present study, since propofol causes well known cardiovascular effects (Lerche et al. 2000; Auckburally et al. 2008; Enouri et al. 2008). Wouters et al. (1995) described a decrease in MAP of 18% and 45% (for up to 10 and 15 minutes respectively) following induction of anaesthesia with 7.5 and 15 mg kg^{-1} propofol in experimental dogs, together with a decrease in SVR in the high dose group (up to 15 minutes) although $\dot{Q}t$ did not change significantly. Their findings suggest a dose-dependent cardiovascular effect of propofol in non-premedicated dogs, whereby a lower dose produces less cardiovascular depression of shorter duration. The additional doses of propofol used in the present study (0.9 ± 0.4 mg kg^{-1}) were much smaller than the lowest dose reported in the study of Wouters et al. (1995). However, in that study propofol was administered to awake dogs, in contrast to the present study, where isoflurane administration might be expected to enhance the cardiovascular depressant effects of the administered doses of propofol. Despite this, it appears likely that the additional doses of propofol, administered in the present study, caused only minor and short lasting cardiovascular effects. Additionally, the distribution of the small doses of propofol over the different protocols in the present study was quite similar (1 in treatment P, 2 in treatment M, and 1 in treatment R), whereby their overall interference with mean values per group was estimated to be relatively low.

Another limitation of the present study was the method used to confirm correct extradural placement of the needle. Because dogs were instrumented, ventilated and under a stable plane of anaesthesia, it was technically not possible to confirm correct epidural needle placement radiographically after injection of a radio-opaque contrast agent. However, epidural needles were placed by an experienced person and their placement was accompanied in all dogs by a distinct "popping-sensation" as a result of penetrating the *ligamentum flavum*, together with a "tail-flick". Correct positioning of the needle was tested also by the absence of cerebrospinal fluid and blood at the needle hub and the subsequent "loss of resistance" to injection of the epidural treatment. In all dogs receiving ropivacaine 0.75%, paralysis of the hindlimbs was observed during the recovery period after the anaesthetic procedure, thus confirming correct positioning of the needle in these groups at least. Although different success rates for the "lack of resistance" method to confirm extradural needle placement have been reported, Iff and colleagues (2007) achieved 100% correct placement in 18 dogs. In another study, the success rate for experienced anaesthetists was reported to be 95% (in a total of 53 epidurals) (Iff & Moens 2010). Additionally, the loss of resistance test was found to have a specificity of 90% in 54 dogs (Garcia-Pereira et al. 2010), but this study did not specify the experience of the anaesthetist.

After treatment P, HR and sAP gradually increased over time, together with non-significant increases in $\dot{Q}t$, SV and SVR. Although no cardiovascular effects resulting from the placebo epidural treatment were expected, the observed cardiovascular stimulatory effects over time were most likely due to a gradual decrease in the cardiovascular depressant effect of the inhalant agent. Even though the authors could not find evidence for this hypothesis in dogs, a similar effect has been described in spontaneously breathing and mechanically ventilated horses (Dunlop et al. 1987; Steffey et al. 1987a; Steffey et al. 1987b; Gasthuys et al. 1990). The uncomfortable position (legs extended cranially in the sternally recumbent dogs) for a long time-period, might be an alternative explanation for the observed cardiovascular stimulatory effects over time in the placebo treatment in the present study.

Leibetseder and colleagues (2006) reported no significant decreases in median MAP values after epidural administration of 0.3 mg kg^{-1} methadone. In the present study, HR and sAP increased gradually following epidural administration of methadone. These changes were consistently smaller compared to the placebo treatment at comparable time points. Additionally, mean overall HR was lower after treatment M compared to treatment P, which suggests an effect of epidurally administered methadone on HR. The documented systemic

absorption of opioids from the epidural space may explain the cardiovascular changes observed in the present study. With increasing lipophilicity (morphine < methadone < fentanyl), epidurally administered opioids are expected to cause analgesia of faster onset but of shorter duration, because of a more rapid absorption from the epidural space. As a result, effects after epidural administration of lipophilic opioids are similar to those observed after systemic administration (Valverde 2008). The analgesic effect of methadone has been reported to be comparable to IV administration 10 minutes after epidural injection (Leibetseder et al., 2006).

The addition of methadone to the ropivacaine 0.75% protocol did not induce significant differences in mean overall cardiovascular variables when compared to epidural ropivacaine 0.75% alone.

Following epidural administration, local anaesthetics may induce hypotension by blocking pre-ganglionic sympathetic efferents, hereby reducing vasoconstrictor activity in affected areas. The degree to which this occurs depends on the balance between sympathetic and parasympathetic activity, the extent of sympathetic block, the effect of systemically absorbed local anaesthetics, the degree of cardiac filling and the adequacy of cardiac function (Veering & Cousins, 2000). Additionally, the concurrent administration of anaesthetic drugs, such as inhalant anaesthetics which exert hypotensive effects (Merin et al. 1991) can impair the compensatory haemodynamic response (vasoconstriction in unblocked areas of the body) to neuraxial administration of local anaesthetics (Stanton-Hicks et al. 1975) and result in a greater risk of hypotension in anaesthetized subjects. In the present study, systemic arterial pressures were indeed significantly lower after treatment R and RM compared to treatments P and M. Nevertheless, in the within-treatment comparison with baseline values, no significant decreases in sAP were observed after treatments R and RM. This is in contrast with the study of Hurley et al. (1991), which reported significant decreases in sAP after epidural ropivacaine and bupivacaine in awake dogs. It is probable that, in the present study, epidural ropivacaine 0.75% did not cause further reductions in arterial blood pressure because baseline values were already low due to the hypotensive effects of isoflurane (Merin et al. 1991). However, after treatments RM and R, MAP remained below a clinically acceptable level of 60 mmHg (Haskins 1996) for 30 and 45 minutes respectively, whereas cardiovascular function gradually improved over time in treatments P and M. Under clinical conditions, additional fluid therapy, inotropic therapy and dose reduction of isoflurane should therefore be considered to prevent or treat hypotension after epidural ropivacaine administration. In the study of Hurley and

colleagues (1991), Q̇t and CVP decreased, while SV and SVR were not affected. It appears likely that hypotension in those dogs resulted from a decrease in cardiac output and reduction in venous return, as evidenced by a decrease in CVP. In the present study, despite of the use of IPPV, which can cause an additive decrease in venous return (Hartsfield 2007), no significant decrease in CVP was observed. An alternative hypothesis for the lower sAP after both ropivacaine 0.75% treatments might be an extensive cranial spread of epidurally administered local anaesthetic. However, the expected associated extensive sympathetic block would cause considerable vasodilation, evidenced by a decrease in SVR and CVP, which were not observed in the present study. Despite of this, an extensive cranial spread would also explain the presence of a transient unilateral Horner's syndrome in one dog during the recovery. This case has been described in detail elsewhere (Bosmans et al. 2009). Such cranial spread can result in a block of cardiac sympathetic fiber activity to the heart, which is translated in small, but significant changes in heart rate (Otton & Wilson, 1966). In the present study, HR was indeed significantly lower after both ropivacaine 0.75% treatments than after saline injection. This probably contributed to the low values for $\dot{Q}t$ (and therefore sAP) observed after ropivacaine 0.75% administration. A more extensive cranial distribution of the local anaesthetic in the epidural space can be caused by rapid administration, volume overdosing, inadvertent subarachnoidal injection, a high water solubility of the drug and anatomical changes in the epidural space (Skaredoff & Datta 1981; Lee et al. 1989; Haskins 1992; Torske & Dyson 2000; Narouze et al. 2002). Ropivacaine is more hydrophilic than bupivacaine, so a cranial spread in the extradural space is more likely to occur (Lee et al. 1989). Hurley and colleagues (1991) observed a similar significant reduction in HR following epidural ropivacaine, but not bupivacaine administration, which is suggestive for a greater cranial spread of ropivacaine.

In the present study, no significant decreases in SVR over time were observed following epidural ropivacaine 0.75% administration. This is in agreement with the observations of Hurley and colleagues (1991). Since all dogs receiving epidural ropivacaine in the present study were paralyzed on the hind limbs following anaesthetic recovery, neuraxial administration of ropivacaine 0.75% was confirmed. Therefore it can be concluded that epidural ropivacaine 0.75% did not elicit a significant decrease in sympathetic tone at the dose and volume used in this study.

The blood loss in the present study, due to the lithium dilution cardiac output measurements was estimated to be between 30 and 40 mL per anaesthestic period. The significant decrease in CaO_2 observed in all treatments in the present study, was most probably induced by the concurrent decreases in PCV at respective time points, since PaO_2 and SaO_2 always remained within the expected range.

The drop in oesophageal temperature occurred most quickly in the ropivacaine 0.75% treatments. A possible cause for this rapid fall is the vasodilation in the area blocked by local anaesthetic.

Unfortunately, one dog was euthanized following unsuccesful rescuscitation after complications during treatment HESR. In this dog, severe hypotension was observed immediately following epidural injection. Although cardiovascular function improved upon discontinuation of anaesthesia, the dog did not regain consciousness, nor spontaneous breathing, despite resuscitation attempts lasting for 10 hours. Although uncertain, the cause of death may have been related to the treatment protocol, which included intravenous hydroxyethylstarch 6% and epidural ropivacaine 0.75% administration and the possibility exists that this injection was accidentally administered in the subarachnoidal space. However, as the results from treatment HESR are not part of the present report, details are described and possible causes discussed elsewhere (Bosmans et al. 2011). Although the animal did not receive the placebo treatment, the data from the other 3 treatments in this dog were not excluded, since the applied mixed model procedure conveniently corrects for missing values in the set up of an experiment (Duchateau & Janssen 1997).

In conclusion, epidural administration of ropivacaine 0.75% (1.65 mg kg^{-1} in a total volume of 0.23 mL kg^{-1}) and the combination of ropivacaine 0.75% and methadone (1.65 mg kg^{-1} + 0.1 mg kg^{-1} respectively, in equal volume) in ventilated, isoflurane anaesthetized dogs (F_E´ISO 1.8%), did not further aggravate pre-existing hypotension, but initially resulted in clinically important low sAP's with a delayed return to normotension, compared to epidural saline (equal volume) or methadone (0.1 mg kg^{-1}, in equal volume) administration. However, in a clinical setting, a successful epidural technique would probably lead to lowering of the inhalant vaporizer setting, resulting in decreased haemodynamic effects of the inhalant agent, which might compromise to a lesser degree the compensatory mechanisms responsible for minimising the effect of local vasodilation on systemic blood pressure. Epidural methadone

(0.1 mg kg^{-1}, in equal volume) was not associated with clinically important cardiovascular changes.

Conflict of interest statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Chapter 4

Influence of a preload of hydroxyethylstarch 6% on the cardiovascular effects of epidural administration of ropivacaine 0.75% in anaesthetized dogs.

Adapted from:

Bosmans T, Schauvliege S, Gasthuys F, Duchateau L, Steblaj B, Gadeyne C, Polis I (2011) Influence of a preload of hydroxyethylstarch 6% on the cardiovascular effects of epidural administration of ropivacaine 0.75% in anaesthetized dogs. Veterinary Anaesthesia and Analgesia, 38, 494-504.

Abstract

Objective To evaluate the cardiovascular effects of a preload of hydroxyethylstarch 6% (HES), preceding an epidural administration of ropivacaine 0.75% in isoflurane anaesthetized dogs.

Study Design Randomized experimental cross-over study (washout of 1 month)

Animals Six female, neutered Beagle dogs (mean 13.3 ± SD 1.0 kg; 3.6 ± 0.1 years).

Materials and Methods Anaesthesia was induced with propofol and maintained with isoflurane in oxygen/air. All dogs were anaesthetized twice to receive either treatment HESR [continuous rate infusion (CRI) of 7 mL kg^{-1} HES started 30 minutes (T-30) prior to epidural administration of ropivacaine 0.75% 1.65 mg kg^{-1} at T0] or treatment R (no HES preload and similar dose and timing of epidural ropivacaine administration). Baseline measurements were obtained at T-5. Heart rate (HR), mean (MAP), diastolic (DAP) and systolic (SAP) invasive arterial pressures, cardiac output (lithium dilution and pulse contour analysis) and derived parameters were recorded every 5 minutes for 60 minutes. Statistical analysis was performed on 5 dogs, due to the death of one dog.

Results Clinically relevant decreases in mean arterial pressure (< 60 mmHg) were observed for 20 and 40 minutes following epidural administration in treatments HESR and R respectively. Significant decreases in mean and diastolic arterial pressures were present after treatment HESR for up to 20 minutes following epidural administration. No significant within-treatment and overall differences were observed for other cardiovascular parameters. A transient unilateral Horner's syndrome occurred in two dogs (1 in each treatment). One dog died after severe hypotension, associated with epidural anaesthesia.

Conclusions and Clinical Relevance A CRI of 7 mL kg^{-1} of HES administered over 30 minutes before epidural treatment did not prevent hypotension induced by epidural ropivacaine 0.75%. Intended epidural administration of ropivacaine 0.75% in isoflurane anaesthetized dogs was associated with a high incidence of adverse effects in this study.

Introduction

Ropivacaine is a newer, long acting amino-amide local anaesthetic, structurally related to mepivacaine and bupivacaine. In contrast to the older racemic solutions, ropivacaine is a pure S(-) isomer. In human medicine, ropivacaine has been recommended over bupivacaine for epidural anaesthesia/analgesia and peripheral nerve blocks, mainly due to reduced neuro- and cardiotoxicity associated with accidental intravascular injection (Casati & Putzu 2005; Leone et al. 2008). Additionally, ropivacaine allows a better differentiation between sensory and motor block, which can be useful clinically if early mobilization is desired (Ogun et al. 2003; Danelli et al. 2004; Leone et al. 2008; Koltka et al. 2009).

In spite of the favourable characteristics of ropivacaine, hypotension induced by the intrathecal/epidural administration of local anaesthetics in humans and animals remains the most common side effect, regardless of the type of local anaesthetic (Casati et al. 1997; Torske & Dyson, 2000; Riesmeier et al. 2009; Teoh & Sia 2009). In human medicine and especially for Caesarean sections, the administration of an intravenous (IV) preload of crystalloids or colloids before intrathecal anaesthesia is commonplace to prevent hypotension (Nishikawa et al. 2007; Siddik-Sayyid et al. 2009; Tamilselvan et al. 2009). However, there is little to no consensus on the type and dose of fluids, or the efficacy of preloading. Indeed, a preload of crystalloids was reported to be unable to prevent hypotension (Rout et al. 1992; Jackson et al. 1995; Rout & Rocke 1999), while colloids were suggested to be more effective than crystalloids for maintaining blood pressure (Baraka et al. 1994; Riley et al. 1995; Ueyama et al. 1999). Nevertheless, colloid preloads before intrathecal anaesthesia, were also reported to have little protective effects on blood pressure (Muray et al. 1989; Karinen et al. 1995; Riesmeier et al. 2009; Siddik-Sayyid et al. 2009; Tamilselvan et al. 2009).

To the authors' knowledge, the subject of preloading before epidural anaesthesia has not been studied in dogs. The aim of the current study was to evaluate whether or not a preload of hydroxyethylstarch 6% (200 kDa/0.5) 7 mL kg^{-1}, administered as a constant rate infusion (CRI) over 30 minutes immediately before the epidural administration of ropivacaine 0.75%, was able to prevent ropivacaine induced hypotension in anaesthetized dogs.

Materials and Methods

Animals

Six female, neutered Beagles with a mean body weight of 13.3 ± 1.0 kg (mean ± SD) and aged 3.6 ± 0.1 years were included in the study, which was approved by the Ethical Committee of the Faculty of Veterinary Medicine of the University of Ghent (EC 2007/042). Dogs were classified as ASA (American Society of Anesthesiologists) 1 (healthy, no systemic disease) based on routine clinical, haematological and biochemical examinations. Food, but not water, was withheld for 12 hours prior to anaesthesia.

Anaesthetic and experimental protocol

The current experiment was part of a larger study, in which the dogs were randomly scheduled in a non-blinded cross-over design, including five epidural treatments, with a wash-out period of at least 1 month between treatments (Bosmans et al. 2011). In the present paper a comparison is made between treatment HESR: a preload of 7 mL kg^{-1} of hydroxyethylstarch 6%, 200 kDa/0.5 (HES) (HAES-steril 6%; Fresenius Kabi n.v., Belgium), administered as a continuous rate infusion (CRI) over 30 minutes immediately before epidural injection of 1.65 mg kg^{-1} of ropivacaine 0.75% (Naropin 7.5 mg mL^{-1}; Astra Zeneca, Belgium) and treatment R: same epidural treatment as treatment HESR, but without vascular preload. The total volume of the epidural injectate was 0.23 mL kg^{-1} in both treatment groups.

At the start of each experiment, a blood sample was taken from a jugular vein to determine the packed cell volume (Fisher Bioblock 1-15; Sigma, Germany), and plasma sodium concentration (Spotlyte Na/K/Cl analyzer; Menarini diagnostics, Italy). Both parameters were required to obtain cardiac output ($\dot{Q}t$) data using the Lithium dilution cardiac output monitor (LiDCOplus Haemodynamic monitor; LiDCO Ltd, UK).

A 22-gauge catheter was placed aseptically in a cephalic vein of the unpremedicated dogs. Anaesthesia was induced with propofol (Propovet; Abbott Animal Health, UK) intravenously (IV) to effect (mean 8.3 ± SD 0.9 mg kg^{-1}) and endotracheal intubation (7 mm ID cuffed endotracheal tube) was performed. Anaesthesia was maintained with isoflurane (Isoflo; Abbott Animal Health, UK) in a mixture of oxygen and air (F_IO_2 40%) using a rebreathing system (Cicero; Dräger, Germany). End-tidal isoflurane concentration (F_E´ISO) was maintained at 1.4 MAC (F_E´ISO of 1.8%), based on a MAC_{ISO} in dogs of 1.3% (Steffey

et al. 1994). Eucapnia [end-tidal carbon dioxide partial pressure ($P_{E'}CO_2$) between 4.7 and 6.0 kPa] and body temperature were maintained respectively using intermittent positive pressure ventilation and a water-heated pad. Lactated Ringer's solution (Hartmann; Baxter, Belgium) was infused IV at 10 mL kg^{-1} $hour^{-1}$ throughout anaesthesia in all dogs (up to 60 minutes after epidural injection). Additionally, a preload of HES 7 mL kg^{-1} was administered in treatment HESR, as a CRI over 30 minutes immediately prior to epidural administration of ropivacaine 0.75%, using a syringe driver (Ohmeda 9000 Syringe Pump; Ohmeda, UK).

For invasive arterial blood pressure (AP) and $\dot{Q}t$ measurements, an intra-arterial 22-gauge catheter was placed aseptically in a dorsal pedal artery. Using the Seldinger technique, a 4 Fr central venous catheter (Leader-Cath; Vygon, France) was placed in the right jugular vein with the distal port located in the right atrium. Correct catheter positioning was confirmed by the characteristic waveforms. The central venous catheter was used for central venous pressure (CVP) measurement (during the expiration phase of respiration) and bolus injection of lithium chloride 0.0075 mmol kg^{-1} (LiDCO Lithium Chloride 0.15 mmol mL^{-1}; LiDCO, UK).

A multichannel monitor (HP M1165A; Hewlett-Packard, Germany) was used to record the following parameters: electrocardiogram, oesophageal temperature, arterial oxygen saturation (SpO_2) (probe placed on the tongue), heart rate (HR), systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP) and CVP. The pressure transducers for AP and CVP (ST-33; PVB Critical Care, GmbH, Germany) were calibrated before each experiment using a mercury manometer (Empire N; Germany) and were positioned and zeroed at the level of the right atrium. Inspiratory fraction of oxygen (F_IO_2), $P_{E'}CO_2$, $F_{E'}ISO$ and respiratory rate (f_R) were measured using an anaesthetic multigas monitor (Capnomac Ultima; Datex Engstrom Instrumentation, Finland), which was calibrated before each anaesthesia (Quick Cal calibration gas; Datex-Ohmeda, Finland).

After instrumentation, the dogs were positioned in sternal recumbency with the pelvic limbs extended cranially and the head elevated. A variable equilibration period was respected until a stable plane of anaesthesia was reached ($F_{E'}ISO$ of 1.8% and loss of eyelid reflexes). The duration of instrumentation and equilibration (until T-30) was 51 (27-83) minutes (mean and range).

At T-30 (which commenced after the equilibration period) the experiment was started in both treatments. The HES CRI was started in treatment HESR. The first LiDCO-

measurement was performed at T-15 in both treatments and was subsequently repeated every 15 minutes until the end of the anaesthetic period (T60) (total of 6 recordings). These LiDCO-measurements accounted for calibration of the subsequent pulse contour measurements which were recorded every 5 minutes. The pulse contour measurements were used for statistical analysis.

Systemic arterial pressures (sAP in mmHg), HR (beats $minute^{-1}$), $\dot{Q}t$ (L $minute^{-1}$), systemic vascular resistance (SVR in dynes second cm^{-5}), stroke volume (SV in mL $beat^{-1}$), CVP (mmHg), SpO_2 (%), and oesophageal temperature (°C) were recorded starting from T-15 and subsequently every 5 minutes for 60 minutes.

Baseline cardiovascular measurements were obtained at T-5, which was five minutes before the epidural injection.

A 22-gauge spinal needle (Yale spinal needle, 1.5 inch, 0.7 x 40 mm, Becton Dickinson, Spain) was introduced into the lumbosacral epidural space at T-2 with the needle bevel directed towards the sacrum. Correct placement of the needle was confirmed by the presence of a distinct “popping-sensation” as a result of penetrating the ligamentum flavum, the lack of resistance to injection and the absence of cerebrospinal fluid and blood in the needle hub. No attempt was made to aspirate cerebrospinal fluid or blood before injection. When cerebrospinal fluid or blood egressed from the needle hub, the spinal needle was removed and replaced. The epidural treatment was administered over 2 minutes, while continuously evaluating the resistance to injection to rule out accidental displacement of the needle during drug administration. The first cardiovascular measurements immediately following epidural treatment were recorded at T0.

Statistical analysis

A paired t-test was performed to compare the two treatments at baseline (T-5) (α=0.05) and to compare the total duration of the instrumentation and equilibration periods (duration of anaesthesia until T-30) between the two treatments (α=0.05). A Shapiro Wilk’s test showed a normal distribution of the data, justifying a parametric statistical approach of the data of this study. The effects of treatment R and HESR were compared using a mixed model with dog and period nested in dog as random effect and time, treatment and their interaction as categorical fixed effects, comparing both treatments globally (at α=0.05) and at 6 selected time points T-5, T0, T15, T30, T45 and T60 (at Bonferroni adjusted α=0.008). For

each treatment separately, an analysis was made for the difference between T-5 (baseline) and each subsequent time point. This analysis was based on a mixed model with dog as random effect and time as categorical fixed effect. Each time point was compared with T-5 using Dunnett's multiple comparisons technique (at global α=0.05).

Results

Baseline comparison (T-5) between treatments

At baseline, no statistically significant differences between treatments were present for any of the observed parameters.

Comparison of duration of anaesthesia until T-30 between treatments

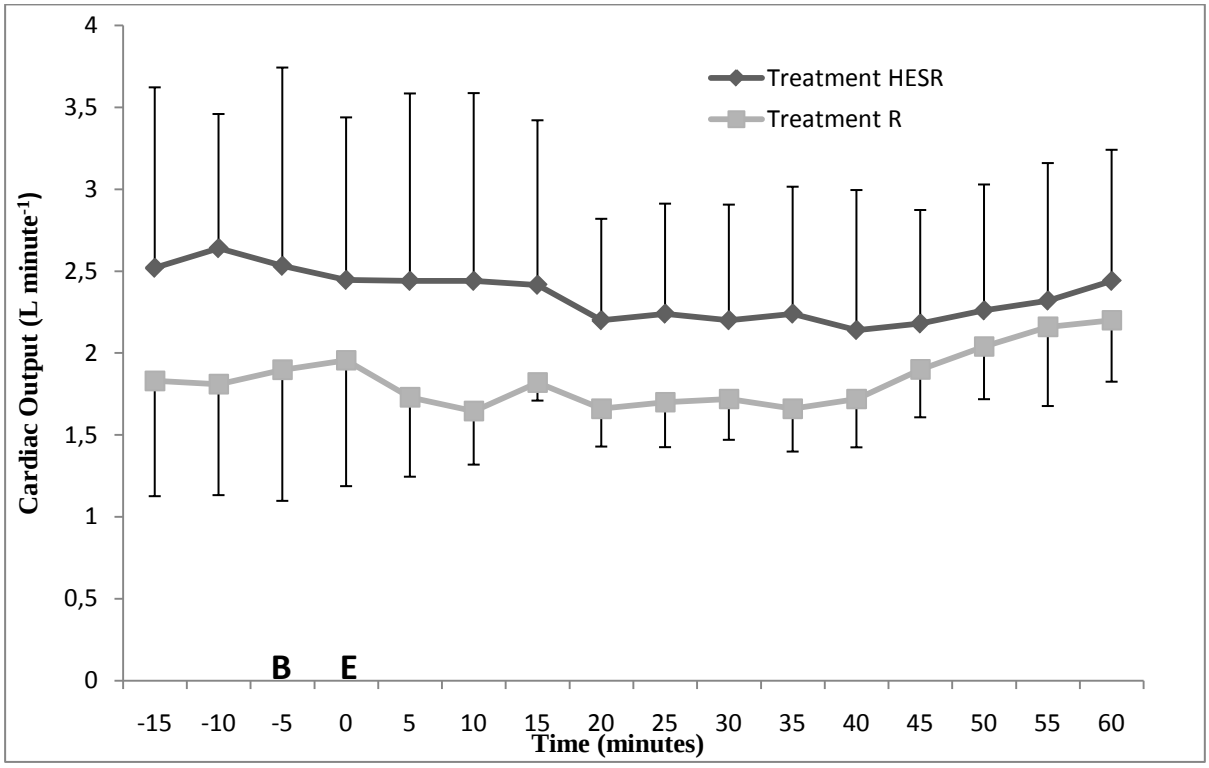
There was no statistically significant difference (p=0.23) in the total length of instrumentation and equilibration period between treatment R (43 ± 19 minutes) and treatment HESR (58 ± 18 minutes).

Comparison between treatments

No significant overall differences between treatments were observed for any of the recorded variables (examples see Figs. 1, 2 & 3).

Similarly, no significant differences between treatments were observed for any of the variables recorded at the selected time points (T-5, T0, T15, T30, T45 and T60).

Fig. 1 Cardiac output over time in five isoflurane anaesthetized Beagles (1.4 MAC), receiving an epidural injection of ropivacaine 0.75% 1.65 mg kg^{-1} (0.23 mL kg^{-1} total epidural volume). Data are represented as mean + or - SD.



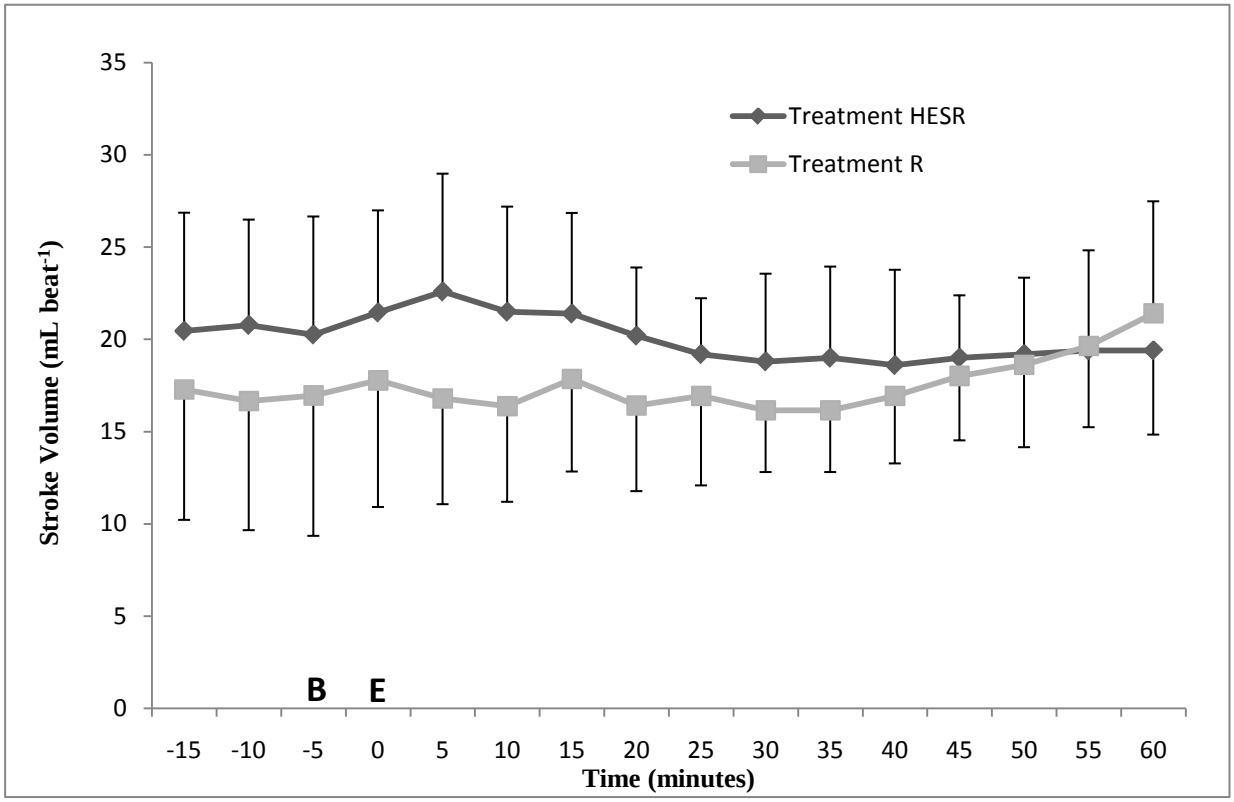
Treatment HESR: epidural treatment preceded by a preload of hydroxyethylstarch 6% 7 mL kg^{-1}, administered as a CRI, started at T-30 and ended at T0

Treatment R: epidural treatment without preload

B: time of baseline recordings before epidural administration

E: time of epidural treatment

Fig. 2 Stroke volume over time in five isoflurane anaesthetized Beagles (1.4 MAC), receiving an epidural injection of ropivacaine 0.75% 1.65 mg kg^{-1} (0.23 mL kg^{-1} total epidural volume). Data are represented as mean + or - SD.



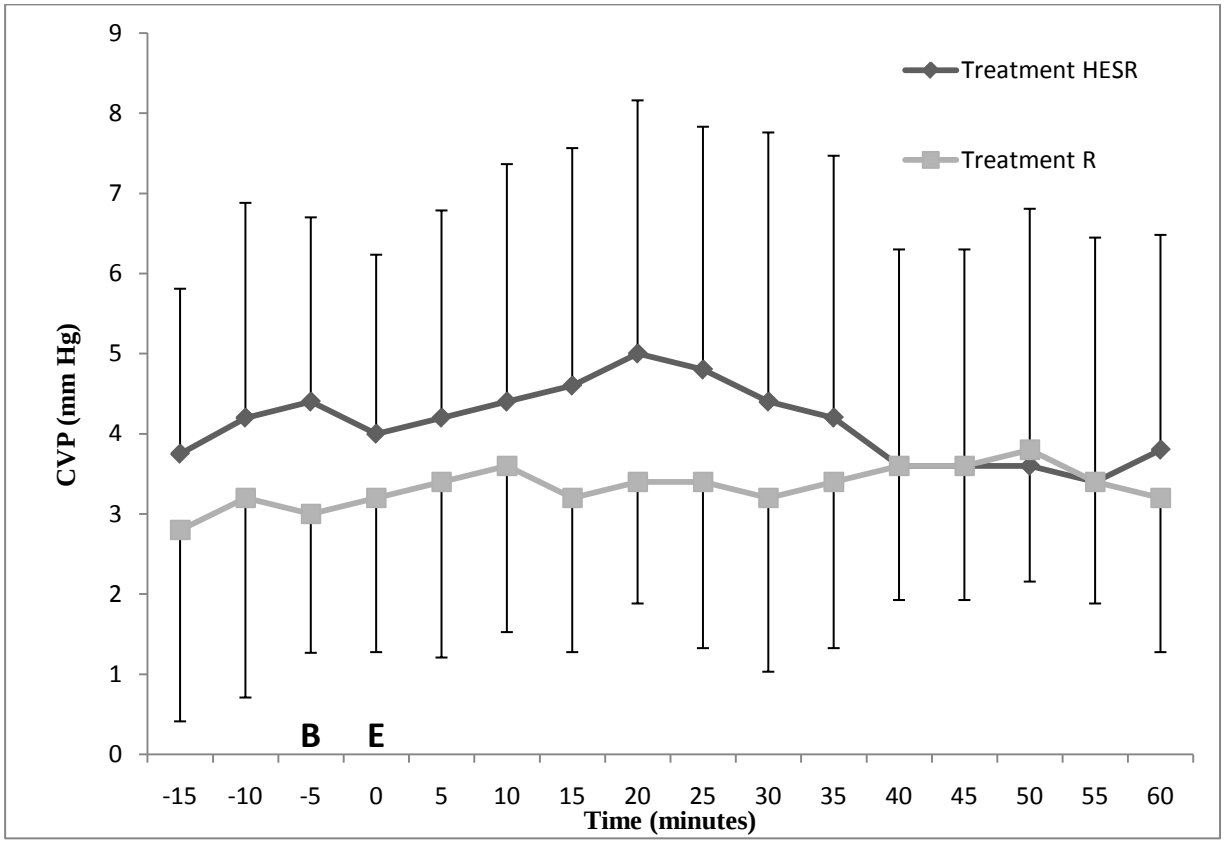
Treatment HESR: epidural treatment preceded by a preload of hydroxyethylstarch 6% 7 mL kg^{-1}, administered as a CRI, started at T-30 and ended at T0

Treatment R: epidural treatment without preload

B: time of baseline recordings before epidural administration

E: time of epidural treatment

Fig. 3 Central venous pressure over time in five isoflurane anaesthetized Beagles (1.4 MAC), receiving an epidural injection of ropivacaine 0.75% 1.65 mg kg^{-1} (0.23 mL kg^{-1} total epidural volume). Data are represented as mean + or - SD.



Treatment HESR: epidural treatment preceded by a preload of hydroxyethylstarch 6% 7 mL kg^{-1}, administered as a CRI, started at T-30 and ended at T0

Treatment R: epidural treatment without preload

B: time of baseline recordings before epidural administration

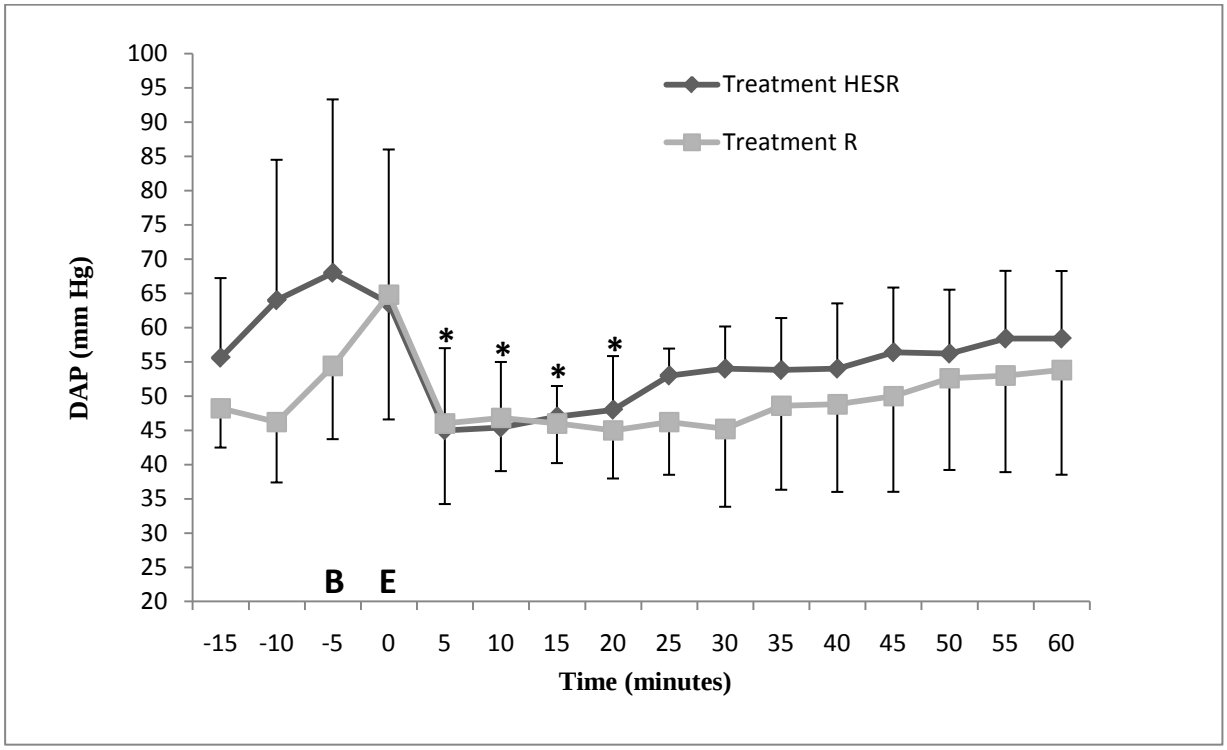
E: time of epidural treatment

Comparison with baseline (T-5) within each treatment

Following epidural administration of ropivacaine 0.75% in treatment HESR, DAP was significantly lower compared to baseline at T5 (p=0.003), T10 (p=0.003), T15 (p=0.007) and T20 (p=0.01) respectively (Fig. 4). A similar significant decrease in MAP from baseline (Fig. 5) was recorded at T5 (p=0.005), T10 (p=0.003), T15 (p=0.007) and T20 (p=0.009). Mean

arterial pressure was lower than 60 mm Hg from T5-T20 in treatment HESR and from T5-T40 in treatment R (Fig. 5). A significant decrease in SVR from baseline of 1130 dynes second cm^{-5} (p=0.04) was observed at T5 in treatment HESR (Fig 6).

Fig. 4 Diastolic arterial pressure over time in five isoflurane anaesthetized Beagles (1.4 MAC), receiving an epidural injection of ropivacaine 0.75% 1.65 mg kg^{-1} (0.23 mL kg^{-1} total epidural volume). Data are represented as mean + or - SD.



Treatment HESR: epidural treatment preceded by a preload of hydroxyethylstarch 6% 7 mL kg^{-1}, administered as a CRI, started at T-30 and ended at T0

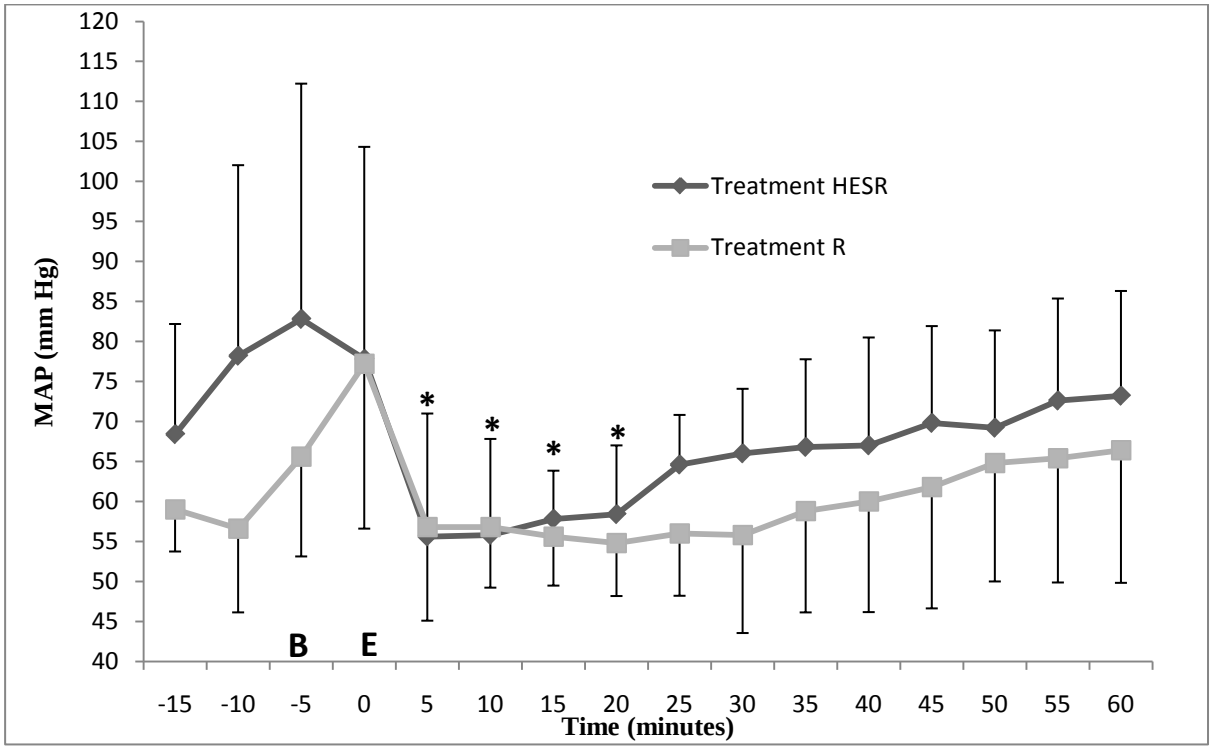
Treatment R: epidural treatment without preload

B: time of baseline recordings before epidural administration

E: time of epidural treatment

*Significant difference (p<0.05) from T-5 within specific treatment

Fig. 5 Mean arterial pressure over time in five isoflurane anaesthetized Beagles (1.4 MAC), receiving an epidural injection of ropivacaine 0.75% 1.65 mg kg^{-1} (0.23 mL kg^{-1} total epidural volume). Data are represented as mean + or - SD.



Treatment HESR: epidural treatment preceded by a preload of hydroxyethylstarch 6% 7 mL kg^{-1}, administered as a CRI, started at T-30 and ended at T0

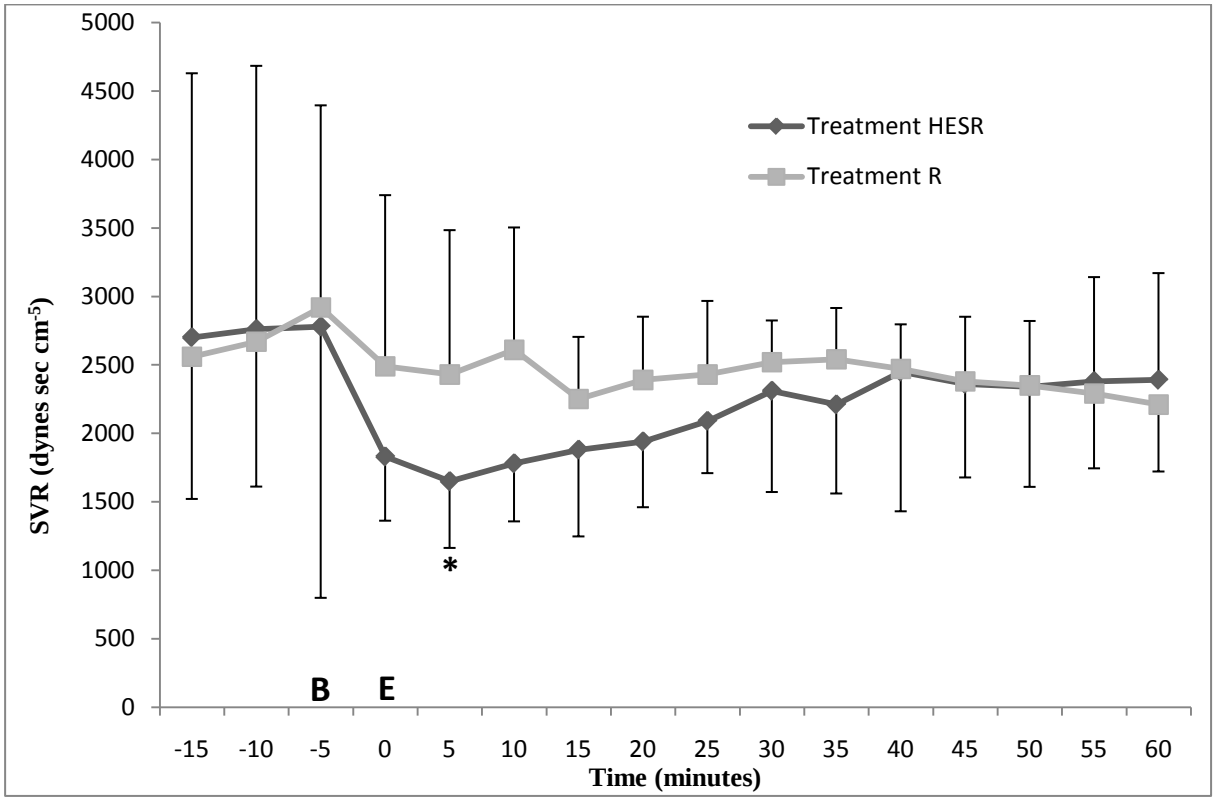
Treatment R: epidural treatment without preload

B: time of baseline recordings before epidural administration

E: time of epidural treatment

*Significant difference (p<0.05) from T-5 within specific treatment

Fig. 6 Systemic vascular resistance over time in five isoflurane anaesthetized Beagles (1.4 MAC), receiving an epidural injection of ropivacaine 0.75% 1.65 mg kg^{-1} (0.23 mL kg^{-1} total epidural volume). Data are represented as mean + or - SD.



Treatment HESR: epidural treatment preceded by a preload of hydroxyethylstarch 6% 7 mL kg^{-1}, administered as a CRI, started at T-30 and ended at T0

Treatment R: epidural treatment without preload

B: time of baseline recordings before epidural administration

E: time of epidural treatment

*Significant difference (p<0.05) from T-5 within specific treatment

Significant decreases from baseline in oesophageal temperature were observed from T20 until T60 in treatment R. However, oesophageal temperature was not significantly different between treatments at selected time points, nor at the overall comparison between treatments (p=0.1), with a mean overall temperature of 37.4 ± 0.4 °C and 36.6 ± 0.8 °C for treatment R and HESR respectively.

In all dogs a "tail-flick" was observed at placement of the epidural needle. All dogs exhibited hind limb paralysis after the anaesthetic recovery, confirming correct neuraxial administration of ropivacaine 0.75% in all dogs.

A transient unilateral Horner's syndrome was present in two dogs after recovery (one right sided in treatment R and one left sided in treatment HESR). Details of the dog with the left sided Horner's syndrome are discussed in detail elsewhere (Bosmans et al., 2009).

During the first attempt at placement of the spinal needle, blood (1 dog in treatment HESR) or cerebrospinal fluid (1 dog in each treatment) egressed from the needle hub. Subsequent needle replacement was successful in all cases. In the 2 dogs with Horner's syndrome, no blood or cerebrospinal fluid was observed during epidural needle placement.

One dog showed a severe hypotension immediately following epidural administration of ropivacaine 0.75% (MAP<40 mmHg for 20 minutes) during treatment HESR. Consequently, depth of anaesthesia was decreased. Upon discontinuation of the anaesthetic procedure, normotension was restored easily using inotropic therapy, but different attempts at weaning the dog from the ventilator were unsuccessful and the dog failed to regain consciousness. Initially anisocoria was observed, which evolved over time to needlepoint miosis in both eyes. After 10 hours of supportive therapy and different resuscitation attempts, the dog was euthanized. The data obtained for this dog were excluded from statistical analysis. A complete necropsy was performed in this dog. This revealed a focal bleeding (3cm) in the vertebral canal at the level of the lumbosacral space. Histological examination revealed a small amount of haematoidin and haemosiderin macrophages at the level of the lumbosacral meninges and a focal intraspinal bleeding dorsal to the ependymal canal. Synaptophysin-immunohistochemical examination (for neuronal degeneration) was negative for the braincortex, hippocampus, cells of Purkinje and several segments of the spinal cord.

Discussion

In the present experimental study, a preloading of HES administered as a CRI of 7 mL kg^{-1} over 30 minutes immediately prior to epidural ropivacaine 0.75% failed to mitigate the decrease in blood pressure observed after epidural administration of ropivacaine 0.75 %. However, the results suggest but do not prove a faster recovery from hypotension in treatment HESR. The intended epidural administration of ropivacaine 0.75% in isoflurane anaesthetized dogs was associated with a high incidence of adverse effects in the present study.

Preloading of patients to mitigate the hypotension induced by an epidural or intrathecal anaesthetic procedure remains controversial in human medicine. Different doses, rates, and types of starches with different molecular masses and ratios of C2/C6 substitution of the glucose molecules have been studied in human anaesthesiology for this purpose. Some authors suggested the use of a fixed volume prior to epidural treatment, regardless of the patient's weight. The quantity (from 0.5 to 1 L) and quality (70 kDa/0.5-1.0 to 130 kDa/0.4) of HES and also the period of time in which HES is infused (from 10 to 30 minutes) vary greatly between studies (Karinen et al. 1995; Vercauteren et al. 1996; Ueyama et al. 1999; Riesmeier et al. 2009; Siddik-Sayyid et al. 2009; Tamilselvan et al. 2009). Some other authors have proposed HES doses based on bodyweight, most commonly 15 mL kg^{-1}, but again with a wide variation in molecular weight and degree of substitution (70 kDa/0.7 to 130 kDa/0.4) and a wide variation in infusion rates (over 10 minutes to rapid administration at the time of identification of cerebrospinal fluid) (Nishikawa et al. 2007; Teoh & Sia 2009). Apparently there is no real consensus in human medicine regarding the kind of preloading colloid, nor on the dose and dosing rate. A prudent dose recommendation was made in an editorial of the British Journal of Anaesthesia, stating: "*We still believe that in regard to elective cases where time is available, a modest amount of preload of up to 10 mL* kg^{-1} *would seem not to be harmful*" (Rout & Rocke 1995).

Preloading veterinary patients before an epidural anaesthesia has gained little to no attention. The first problem in the present study was the determination of an acceptable dose of the preloading infusion. Since there is no reference to preload dosing in dogs and a wide variation exists in the doses applied in human medicine, the dosing of HES was arbitrarily set at 7 mL kg^{-1} and infused as a CRI over 30 minutes immediately prior to epidural treatment. This dose is approximately one third of the daily infusion rate of 20 mL kg^{-1} day^{-1} recommended for dogs in intensive care units (Hughes 2001). The application of a higher dose and administration rate was in our opinion hard to justify, because the experimental dogs used in the present study were normovolaemic and did not show complementary risk factors for severe hypotension (e.g. gravidity with reduced venous return as observed in obstetric human studies).

The overall goal of administering colloids before an epidural/intrathecal anaesthesia is to increase venous return and maintain central blood volume and cardiac output, in an attempt to mitigate the occurring hypotension, which is mainly induced by a reduction in systemic vascular resistance and an increase in venous capacitance (Baraka et al. 1994; Riley et al.

1995; Ueyama et al. 1999; Nishikawa et al. 2007). In some human studies, Q̇t and SV were reported to increase following the preload procedure, but these increases only occasionally prevented the hypotension (Ueyama et al. 1999), while no effect was observed in other reports (Riesmeier et al. 2009; Tamilselvan et al. 2009). Additionally, Karinen and colleagues (1995) reported an increase in CVP after preloading, that returned to baseline values following intrathecal anaesthesia and which was unable to prevent intrathecal anaesthesia induced hypotension.

In contrast to the above described increases in Q̇t, CVP and SV following HES administration as a preload before intrathecal treatment in other studies, the HES infusion administered in the present experimental study did not result in a significant increase in Q̇t, CVP and SV (figure 1, 2 & 3) at baseline (T-5) compared to treatment R. This might be the consequence of the rather low dose and slower rate of administration of HES used in the present study compared to human studies. Following epidural administration of ropivacaine 0.75%, a period of clinically important low MAP values (< 60 mmHg) was present after epidural administration in both treatments of the present study, again suggesting that the colloid preloading procedure was unable to prevent or counteract the occurring hypotension. Controversially, the decrease from baseline in MAP and DAP only reached statistical significance in treatment HESR. This might be explained by the fact that slightly (but not significantly) higher baseline values were recorded near the end of preload in this treatment compared to treatment R, resulting in a more pronounced difference in the comparison between baseline values and subsequent time points. Additionally, the period of clinically low MAP after the epidural treatment was shorter in treatment HESR (20 minutes) compared to treatment R (40 minutes). This observation suggests but does not prove a faster recovery from epidural induced hypotension, most likely due to the application of the HES preload.

Oesophageal temperature decreased significantly in treatment R. Because hypothermia has been shown to decrease MAC of inhalant agents due to a diminished metabolic rate (Eger et al. 1965; Steffey & Mama 2007), it may be hypothesized that anaesthetic depth was not the same in both treatment groups. However, oesophageal temperature was not significantly different between treatments, neither in the overall comparison, nor in the comparisons at specific time points. It would therefore seem unlikely that oesophageal temperature altered the MAC_{ISO}-equivalence between treatments.

In the present study, one dog in treatment HESR showed severe hypotension immediately following epidural administration of ropivacaine 0.75% (MAP<40 mmHg for 20 minutes). Upon discontinuation of anaesthesia, normotension was quickly restored using a CRI of dobutamine (DobutrexMylan, Hoeilaart, Belgium, 5 µg kg^{-1} $minute^{-1}$). However, the dog failed to regain consciousness and to breathe spontaneously. Intermittent positive pressure ventilation was continued for 10 hours, but unfortunately no improvement was observed and the dog was euthanized. Although the causative factor(s) remain(s) unknown, it may be hypothesized that this uncommon complication resulted from an unexpected brain stem anaesthesia by ropivacaine 0.75% or an adverse anaphylactoid reaction to HES. Indeed, accidental intrathecal injection of ropivacaine 0.75% could have resulted in an extensive cranial spread of the local anaesthetic, affecting the brain stem and thereby explaining the observed unconsciousness and apnea. The time of onset of the severe hypotension, namely immediately after the supposed epidural ropivacaine 0.75% administration, favours this hypothesis. In humans, intrathecal injections of large doses of local anaesthetic into the lumbar spine can result in hypotension, bradycardia, apnoea and loss of consciousness (Griffiths & Gillies 1948; Hamilton 1985). Brain stem anaesthesia was also reported as a complication of local anaesthetic retrobulbar block (Hamilton 1985; Wang et al. 1989). However, in contrast with the dog in the present study, resuscitation was successful in all reported human patients. In the dog in the present study, blood was emerging from the needle hub of the spinal needle following the first attempt to place the epidural needle. This bleeding explained the necropsy finding of a focal bleeding in the vertebral canal at the level of the lumbosacral space. The needle was therefore replaced at the level of L6-L7 and the epidural treatment was subsequently injected. Although no cerebrospinal fluid was observed before injection, suggesting a correct epidural placement, a possible intrathecal injection might have occurred. The resulting volume overdosing might have facilitated cranial spread and subsequent brainstem anaesthesia. The necropsy finding of a focal intraspinal bleeding dorsal from the ependymal canal might support the theory of accidental intrathecal injection in this dog. In light of this intraspinal bleeding, it should be stated that the occurrence of a “tail-flick” of the dog when placing a spinal needle, although not under voluntary control of the person placing the spinal needle, might be dangerous, since this results from needle contact with either nerves or the spinal cord. To avoid accidental intrathecal administration, aspiration before injection of the epidural treatment to detect cerebrospinal fluid, should be performed.

The finding of anisocoria in the dog that died and as a symptom of a unilateral Horner's syndrome in two other dogs in the present study (one in each treatment) also suggests a more cranial spread of ropivacaine 0.75%. The discussion of the relation between epidural spread of ropivacaine 0.75% and the occurrence of a unilateral Horner's syndrome is described in depth elsewhere (Bosmans et al. 2009).

A substantial histamine release during preloading with HES can induce severe anaphylactoid reactions (Wiedermann 2004) and subsequent hypotension. It is unlikely that this occurred in the present fatality when considering the time of onset of hypotension. In humans, anaphylactoid reactions such as bronchospasm, brady- or tachycardia, tachypnea, erythema, oedema and urticaria were reported to occur within a few minutes after the initiation of a HES infusion (Cullen & Singer 1990). The incidence of anaphylactoid reactions to colloid solutions was also estimated to be relatively low (Ring & Messmer 1977; Ring 1985) and a smaller allergic risk has been assigned to HES compared to gelatins (Laxenaire et al. 1994).

One of the limitations of the present study was the small number of dogs included. This was exacerbated by the loss of data due to the death of one dog. A second limitation, in light of clinical implication of the results, was the choice for 1.4 x MAC_{ISO}. To avoid any influence of premedication on the cardiovascular effects of epidural ropivacaine 0.75%, it was preferred not to premedicate the dogs in the experimental protocol. As a result of this, a high $F_{E}'ISO$ was needed to avoid reactions to epidural needle placement. In contrast, a reduction in the required dose of isoflurane would be expected following epidural administration of a local anaesthetic before surgery in a clinical setting. This dose reduction might be expected to attenuate some of the cardiovascular effects encountered in the current experimental protocol. No specific measurements were taken in the current study to avoid/treat hypotension before the epidural treatment. As a result, some dogs suffered hypotension even before the epidural ropivacaine 0.75% was administered.

In conclusion, the results of this study did not demonstrate that preloading with a commonly used colloid, hydroxyethylstarch 6% administered as a CRI of 7 mL kg^{-1} over 30 minutes prior to epidural administration of 1.65 mg kg^{-1} of ropivacaine 0.75%, was able to mitigate the decrease in blood pressure induced by ropivacaine 0.75% in isoflurane anaesthetized dogs. However, the recovery from hypotension in treatment HESR might have occurred more rapidly. In a clinical setting, a successful epidural technique should lead to a

MAC_{ISO} reduction, preventing extensive hypotension. The intended epidural administration of ropivacaine 0.75% in isoflurane anaesthetized dogs was associated with a high incidence of adverse effects in the present experimental protocol.

Future studies involving a larger study population and applying other doses and rates of administration of HES are justified to further elucidate the role of HES preloading before epidural anaesthesia in dogs.

Conflict of interest statement

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Chapter 5

Transient unilateral Horner's syndrome after epidural ropivacaine 0.75% in a dog.

Adapted from:

Bosmans T, Schauvliege S, Gasthuys F, Gozalo Marcilla M, Polis I (2009) Transient unilateral Horner's syndrome after epidural ropivacaine in a dog. Veterinary Anaesthesia and Analgesia 36, 401-406.

Abstract

Observations

A left sided Horner's syndrome (ptosis, prolapse of the nictitating membrane and miosis) was observed in a four year old female, neutered Beagle dog after epidural injection of 0.22 mL kg^{-1} ropivacaine 0.75% in 0.01 mL kg^{-1} of saline during isoflurane anaesthesia. Clinical signs disappeared gradually, and resolved completely 4 hours and 10 minutes after injection.

Conclusions

An intended epidural injection of ropivacaine 0.75% in 0.01 mL kg^{-1} of saline during isoflurane anaesthesia caused unilateral (left) Horner's syndrome in a four year old female, neutered Beagle dog.

History

The cardiovascular effects of epidural ropivacaine 0.75% were studied in a four year old, neutered female Beagle, weighing 14.0 kg in a project approved by the Ethical Committee of the faculty of Veterinary Medicine, University of Ghent, Belgium (N° 2007/042). The dog was healthy, based on routine clinical, haematological and biochemical examinations.

Observations and interventions

The animal was fasted overnight and had free access to water. On the day of the experiment, a 22-gauge over-the-needle catheter was placed aseptically in a cephalic vein. No pre-anaesthetic medication was administered and anaesthesia was induced with propofol (Propovet; Abbott Animal Health, UK) intravenously (IV) to effect (7.9 mg kg^{-1}). This was followed by endotracheal intubation (7 mm ID cuffed ETT). Anaesthesia was maintained with isoflurane (Isoflo; Abbott Animal Health, UK) delivered to ensure end-tidal concentrations (F_E´ISO) of 1.8% [= 1.4 x MAC; MAC_{ISO} in dogs = 1.3% (Steffey et al. 1994)] vaporized in a mixture of oxygen and air (F_IO_2 40%) using a rebreathing system. Lactated Ringer's solution (Hartmann; Baxter, Belgium) was infused IV at 10 mL kg^{-1} $hour^{-1}$ throughout anaesthesia (up to 60 minutes after epidural injection). Intermittent positive pressure ventilation was initiated to achieve normocapnia [end-tidal carbon dioxide (P_E´CO_2) between 4.7 and 6.0 kPa; 35-45 mmHg]. An intra-arterial 22-gauge catheter was placed in the dorsal pedal artery, for invasive arterial blood pressure (AP) and cardiac output measurements. Using the Seldinger technique, a central venous catheter was placed without difficulties during the first attempt in the right jugular vein for central venous pressure (CVP) measurement and the injection of lithium chloride (0.15 mmol mL^{-1} LiDCO Lithium Chloride; LiDCO, UK) at a dose of 0.0075 mmol kg^{-1} body mass. After instrumentation the dog was placed in sternal recumbency with the pelvic limbs extended cranially and the head elevated above the rest of the body. The lumbosacral area was aseptically prepared. Anaesthetic monitoring included an electrocardiogram, oesophageal temperature, arterial oxygen saturation (SpO_2), heart rate (HR), systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP) as well as CVP measurements, using a multichannel physiological monitor (HP M1165A; Hewlett-Packard, Germany). The pressure transducers for AP and CVP were positioned at the level of the right atrium. P_E´CO_2, inspiratory fraction of oxygen (F_IO_2), F_E´ISO and respiratory rate (f_R) were measured using an anaesthetic multigas-monitoring device (Capnomac Ultima; Datex Engstrom Instrumentation, Finland). Cardiac output ($\dot{Q}t$),

systemic vascular resistance (SVR) and stroke volume (SV) were measured by means of the lithium dilution technique (LiDCO plus; LiDCO, UK). Arterial oxygen and carbon dioxide pressures (PaO_2 and $PaCO_2$), pH and standard bicarbonate concentration were taken every 15 minutes (ABL-5; Radiometer Medical, Denmark). Prior to the experiment the pressure measurement modules of the multichannel physiological monitor and the anaesthetic multigas monitoring device were calibrated with a mercury-manometer (Empire N; Germany) and a standard gas mixture (Quick Cal calibration gas; Datex-Ohmeda, Finland). For the LiDCOplus monitor, lithium dilution cardiac output measurements (every 15 minutes during the experiment), accounted for calibration of the pulseCO-measurements.

Thirty minutes before the epidural injection, a commercial plasma-expander, hydroxyethylstarch 6% (HES) (HAES-steril 6%; Fresenius Kabi n.v., Belgium) 7 mL kg^{-1} was infused IV over 30 minutes using a syringe-driver. Baseline cardiovascular measurements were performed at the start of the HES-infusion (T-30).

At T0, a 22-gauge spinal needle (Yale spinal needle; 1.5 inch, 0.7 x 40 mm, Becton Dickinson, Spain) was introduced into the lumbosacral epidural space in the sternally recumbent dog and an interval of 1 minute allowed to ensure that no blood or cerebrospinal fluid egressed from the needle. A dose of 0.22 mL kg^{-1} ropivacaine 0.75% (Naropin 7.5 mg mL^{-1}; AstraZeneca, Belgium) combined with 0.01 mL kg^{-1} saline (NaCl 0.9%; B. Braun Medical n.v./s.a., Belgium) was injected over 2 minutes using the absence of resistance to injection to indicate continued extradural positioning of the needle. The needle bevel was directed towards the sacrum. No attempt was made to aspirate cerebrospinal fluid or blood before injection.

Cardiovascular variables during anaesthesia are shown in Table 1. Decreases in SAP, DAP, MAP, $\dot{Q}t$, and HR were observed after epidural injection, which persisted throughout anaesthesia. The observed drop in SV on the other hand, occurred only 15 minutes after epidural injection and was less pronounced, whilst the SVR initially decreased (first 10 minutes) and thereafter increased. Total anaesthesia time was 143 minutes and recovery from anaesthesia was uneventful.

When the dog regained consciousness it was able to hold its head normally, although no signs of voluntary pelvic limb movement were noted. However, clinical signs of ptosis, miosis and prolapse of the nictitating membrane of the left eye pointed to the possibility that a unilateral Horner's syndrome (HS) was present. No specific treatment was instigated and the dog was

examined at regular time intervals thereafter. These signs waned gradually, with ptosis and prolapse of the nictitating membrane being the first to disappear completely. The last anomaly observed was a mild anisocoria, with the affected, miotic pupil exhibiting a positive direct pupillary light reflex. All signs had disappeared 250 minutes after epidural injection. At this time, there was full restoration of motor function in the pelvic limbs as well.

Table 1 Cardiovascular variables over time in a female anaesthetized Beagle dog [mixture of oxygen (F_IO_2: 40%) and air, $F_E'ISO$: 1.8 %, $P_E'CO_2$: 4.7-6.0 kPa], receiving an epidural injection of 0.22 mL kg^{-1} ropivacaine 0.75% + 0.01 mL kg^{-1} saline.

	Time (minutes)																
	T-30	**T-15**	**T-10**	**T-5**	**T0**	**T5**	**T10**	**T15**	**T20**	**T25**	**T30**	**T35**	**T40**	**T45**	**T50**	**T55**	**T60**
T (°C)	37.4	37.2	37.1	37.0	36.9	36.9	36.8	36.8	36.7	36.6	36.6	36.5	36.5	36.5	36.5	36.4	36.4
SpO_2 (%)	94	95	93	98	97	97	97	97	97	96	96	97	97	97	97	97	97
HR (beats $minute^{-1}$)	123	114	120	140	135	111	107	103	100	98	98	98	97	97	97	98	98
SAP (mmHg)	102	113	149	163	155	90	87	92	92	86	88	86	84	98	91	101	97
DAP (mmHg)	53	66	88	99	91	51	50	53	51	49	51	50	47	52	50	53	52
MAP (mmHg)	63	78	105	116	108	61	59	63	60	58	60	58	56	63	60	65	63
$\dot{Q}t$ pulseCO (L $minute^{-1}$)	NA	2.6	3.1	3.1	2.4	2.4	2.1	1.8	1.8	1.7	1.7	1.8	1.7	1.8	1.9	2.0	2.1
SV pulseCO (mL $beat^{-1}$)	NA	21	24	22	21	24	20	18	18	17	18	18	18	19	19	20	16
SVR pulseCO (dynes sec cm^{-5})	NA	2850	2700	2800	2000	1750	1950	2450	2450	2500	2450	2400	2400	2300	2300	2300	3100
$\dot{Q}t$ LiDCO (L $minute^{-1}$)	NA	2.60	NA	NA	2.55	NA	NA	1.86	NA	NA	1.70	NA	NA	1.75	NA	NA	1.65
CVP (cmH_20)	3	4	4	4	4	4	4	5	5	5	5	6	5	5	6	5	6

T, body temperature in °C; SpO_2, arterial oxygen saturation in %; HR, heart rate in beats $minute^{-1}$; SAP, invasive systolic arterial pressure in mmHg; DAP, invasive diastolic arterial pressure in mmHg; MAP, invasive mean arterial pressure in mmHg; $\dot{Q}t$ pulseCO, cardiac output measured by means of pulsecontour analysis in L $minute^{-1}$; SV pulseCO, stroke volume measured by means of pulsecontour analysis in mL $beat^{-1}$; SVR pulseCO, systemic vascular resistance measured by means of pulsecontour analysis in dynes sec cm^{-5}; $\dot{Q}t$ LiDCO, cardiac output measured by means of the lithium dilution method in L $minute^{-1}$ (baseline for calibration of pulseCO); CVP, central venous pressure in cmH_20; NA, not applicable. T-30 represents the start of a HAES-Steril 6%-infusion of 7 mL kg^{-1} over 30 minutes. T0 represents the start of epidural anaesthesia.

Discussion

Horner's syndrome is a disorder of the sympathetic nerves supplying the eye (de la Calle et al. 2004). Clinical signs in humans include ptosis, miosis, anhydrosis, enophthalmus and conjunctival and facial congestion (Hogagard & Djurhuus 2000; Theodosiadis et al. 2006). In animals, including the dog, prolapse of the nictitating membrane can also be observed (Holland 2007; Skarda & Tranquilli 2007).

Horner's syndrome has been reported in humans after spinal/epidural blockade, with an increased incidence in obstetric procedures (Narouze et al. 2002; de la Calle et al. 2004; Theodosiadis et al. 2006) where it may be as high as 1.33% after epidural analgesia during labor and 4% after epidural anaesthesia for cesarean operation (Clayton 1983). In contrast, there are only a few case reports describing the syndrome as a complication of lumbar epidural injection for non-obstetric operations (Dahlgren & Tornebrandt 1995).

Kern et al. (1989) describe brachial plexus avulsion and thoracic neoplasia as possible causes of HS due to second-order neuron disorder in dogs and cats, while Jones (2001) and Skarda & Tranquilli (2007) mentioned the syndrome as a possible complication of epidural anaesthesia although the basis of this citation is not referenced. Furthermore, we could find no reports on HS in dogs caused by epidural local anaesthetic injection.

In the present case, disappearance of HS signs occurred together with restoration of motor function of the pelvic limbs, making the epidural injection the most likely cause of the complications observed. Horner's Syndrome occurring after epidural local anaesthetic injection in humans is thought to be caused by interruption of ocular preganglionic sympathetic neurons as they leave the spinal cord from C8 to T1 ventral roots (Hogagard & Djurhuus 2000; Narouze et al. 2002). In dogs, the spinal sympathetic innervation of the eyes synapses with neurons of the intermediolateral grey column nuclei (preganglionic nuclei) at lower cord levels (level T1-T3). The axons of these nuclei join the vagosympathetic trunk and travel up the neck forming synapses in the cranial cervical ganglion near the base of the skull. From here, postganglionic neurons project through the tympano-occipital fissure, and the middle ear cavity, and exit the skull as components of the ophthalmic nerve, a branch of the trigeminal nerve (Dewey 2003).

Reported causes of epidural-related HS in humans have included the cranial spread of different local anaesthetic solutions (lidocaine, bupivacaine, ropivacaine) in both the

extradural and subdural spaces (Hogagard & Djurhuus 2000; Narouze et al. 2002; Theodosiadis et al. 2006) and the enhanced sensitivity of sympathetic preganglionic B fibres to local anaesthetics (Heavner & de Jongh 1974). In the present case, extensive cranial spread of epidural ropivacaine 0.75%, resulting in high sympathetic blockade (level T1-T3) would appear to be the most likely cause. Volume overdosing, a fast injection rate, inadvertent intrathecal injection, a higher water solubility of the local anaesthetic and anatomical changes in the epidural space can all account for more extensive cranial distribution (Skaredoff & Datta 1981; Lee et al. 1989; Haskins 1992; Torske & Dyson 2000; Narouze et al. 2002). In the present case, the injected volume of ropivacaine 0.75% (0.22 mL kg^{-1}) was based on a study by Duke et al. (2000) in which no signs of abnormal spread were mentioned. However, an additional volume of 0.01 mL kg^{-1} of saline was added in the present case, making the total injection volume 0.23 mL kg^{-1} or 3.2 mL. This represents a total volume of 1 mL per 4.4 kg in the involved animal. In clinical cases, the authors normally use an epidural injection volume of 1 mL per 4.5 kg, so far without complications. A maximum volume of 6 mL for extradural injection in any dog was reported to be safe by Torske & Dyson (2000), although this citation was not referenced. Therefore it seems unlikely that the increased volume (0.01 mL kg^{-1} saline) accounted for the problem. Cranial spread is also said to result from a high injection rate (Haskins 1992). The normal recommendation for speed of epidural injection has been reported to be 30 to 60 seconds (Jones, 2001), although this citation was not referenced. In the referred study of Duke et al. (2000), a constant rate injection of 3 mL $minute^{-1}$ was used. If applied to the present case, this would stand for a recommended total injection time of 64 seconds for the total of 3.2 mL. However, the epidural injection in the current study was performed over 120 seconds, which exceeds injection time of both references. Accidental intrathecal injection would also account for more cranial spread, but in the current case, no cerebrospinal fluid was noticed in the spinal needle before injection. However, since no aspiration was done before injection, accidental intrathecal or intravascular injection cannot be completely excluded. Ropivacaine is 2 to 3 times more water soluble than bupivacaine, which can possibly account for more cranial epidural distribution, even when using the correct dose (Lee et al. 1989). Finally, the dog had already received six epidural punctures over the last 4 years. Possible anatomical changes, such as fibrosis/ adhesions or haemorrhage in the epidural space induced by this may explain the problem, especially because the signs were only observed unilaterally. Acute epidural/subdural and subarachnoid haemorrhage have been reported in humans, although usually in association with abnormal coagulation (Adler et al. 2001). Also, anatomical changes in the extradural space of pregnant women have been

held responsible for HS, although these are usually related to epidural venous congestion and increased epidural pressure secondary to uterine contractions (Skaredoff & Datta 1981; Sprung et al. 1991). Conclusively, anatomical changes in the epidural space, possibly elevating the epidural pressure are likely in the present case. In the authors' opinion, the other described possible causes cannot fully explain a unilateral block, since the dog was placed in sternal recumbency throughout the procedure and this normally accounts for bilateral effects of the epidural solution (Jones 2001).

The possibility that extensive cranial epidural spread of ropivacaine 0.75% caused the clinical signs in the present case is supported by the cardiovascular changes observed. Epidurally administered local anaesthetics reduce vaso- and venoconstrictor activity in affected areas by blocking preganglionic sympathetic fibres. More cranial spread will cause an extended vasodilatory effect and block cardiac sympathetic fibre activity to the heart itself (Otton & Wilson 1966; Veering & Cousins 2000; Narouze et al. 2002), causing small but significant reductions in HR (Bromage 1967; Dohi et al. 1983; Tanaka et al. 1991) and left ventricular contractility (Reiz et al. 1980; Hotvedt et al. 1984; Goertz et al. 1993). In the present case, reductions in AP, $\dot{Q}t$, and HR were observed immediately after epidural injection, followed by a less pronounced reduction in SV at T15. These changes persisted throughout anaesthesia and can be explained by vasodilatation with decreased chronotropy and inotropy. The reduction in SVR observed during the first 10 minutes after epidural injection may have corresponded to extended vasodilatation. Although it began to increase shortly thereafter, it did not reach baseline values until T=60.

Although HES is reported to have a clinical effect lasting 4 to 8 hours in humans (Anonymous 2004) its infusion in the present case failed to correct hypotension. This may have been due to vasodilatation caused by sympathetic block. The infusion of larger volumes, in combination with inotropic agents or vasopressors might have remedied this. This treatment was not instigated in the present case, because of the experimental protocol.

The precautions (elevation of the head, slow injection rate, caudally directed needle tip) taken to avoid cranial spread, did not prevent signs of HS in the present case. However, all signs disappeared gradually over 250 minutes after injection and corresponded with the restoration of motor function in the pelvic limbs. This supports the belief that the syndrome was caused by epidural ropivacaine 0.75%.

In conclusion, intended epidural injection of ropivacaine 0.75% (0.22 mL kg^{-1}) in saline (0.01 mL kg^{-1}) during isoflurane anaesthesia, after repeated epidural injections, could possibly cause a transient unilateral HS, as well as cardiovascular depression in dogs, because of a high sympathetic block (T1-T3).

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General Discussion

Part 1: Analgesia

In the introduction of the present PhD thesis the physiology and pathophysiology of pain were reviewed in depth. This review shows clearly that un- or undertreated pain associated with surgery is likely to deteriorate towards pathological pain and highlighted the importance of a pre-emptive and multimodal analgesic approach when surgery is to be performed. Therefore, the first and main focus of this thesis was to evaluate the effects of yet unstudied pre-emptive and multimodal analgesia protocols in dogs undergoing stifle surgery for correction of cranial cruciate ligament rupture (CrCLR) in a clinical setting.

In general, elective surgery allows a good setting for pre-emptive analgesia because the timing of noxious stimulation is known (Woolf & Chong 1993). However, the pathophysiological processes involved in the development of CrCLR are responsible for the presence of pain before surgery. Either CrCLR induces a severe inflammation process of the affected stifle joint, or the presence of chronic synovitis results in degenerative changes in the CrCL and other joint structures, responsible for the CrCLR (Hayashi & Muir 2010). In both processes, the inflammation is most likely the trigger for the pain. Therefore, per definition, the applied analgesia protocols in the present thesis were not fully pre-emptive, since pain was already present in each patient before surgery. However, long lasting beneficial analgesic effects have been clearly demonstrated in humans when epidural analgesia or intravenous/oral opioids were administered several days before surgical amputation of an already painful lower extremity, confirming the benefit of pre-emptive analgesia in human patients suffering pain before surgery (Bach et al. 1988).

Analgesia for surgical interventions can be obtained by different methods. In human and veterinary medicine, opioids remain the primary analgesic agents of choice to treat acute peri-operative pain (Mathews 2000; Buvanendran & Kroin 2009). Additionally, regardless of the procedure, opioids should be included in the analgesic protocol because of their well known anaesthetic sparing effects (Hellyer et al. 2007). However, dose related opioid-associated adverse effects (e.g. ileus, bradycardia) are an important issue (Buvanendran & Kroin 2009; Dugdale 2010). Therefore it is now common practice to incorporate opioids into a multimodal or balanced analgesic approach to treat pain associated with major surgery. As already mentioned, a multimodal analgesic plan incorporates drugs with different mechanisms of action, resulting in an additive or synergistic analgesic effect. It also aims at reducing the side-effects associated with each individual drug (Buvanendran & Kroin 2009). Different

multimodal analgesic combinations of tepoxalin [non-steroidal anti-inflammatory drug (NSAID)], methadone and buprenorphine (opioids) and ropivacaine 0.75% [local anaesthetic (LA)] were investigated in the clinical part of the thesis (chapter 1 & 2). The combination of these 3 classes of drugs is frequently preferred for the treatment of pain associated with orthopaedic surgery of the hind limbs, though the analgesic effects of the combination of these drugs have not yet been studied in depth (Fowler et al. 2003; Lemke 2004).

Analgesia provided by tepoxalin

Non-steroidal anti-inflammatory drugs may have an important drug sparing effect in major surgical interventions, because they reduce the inflammatory response in the periphery by inhibiting prostaglandin and/or leukotriene synthesis, thereby limiting the transduction and transmission of the nociceptive stimulus (first and second step in the pain pathway, *vide supra*), which decreases sensitization of peripheral nociceptors (McCormack & Brune 1991). Additionally, recent research indicates that central inhibition of cyclooxygenase 2 (COX-2) may also play an important role in modulating nociception (third step in the pain pathway, *vide supra*) (Buvanendran et al. 2006). This property confirms earlier findings from *in vivo* animal studies (Jurna & Brune 1990; Malmberg & Yaksh 1992, 1993; Björkman 1995). Despite the multiple mechanisms of action, the sole administration of NSAIDs is believed to lack the potency for adequate analgesia in the post-operative period after major orthopaedic surgery in dogs (Sackman 1991; Gentry & Mann 1993; Fowler et al. 2003). This hypothesis, is in the opinion of the author quite empirical, since several studies on pre-emptive administration of carprofen, an NSAID registered for pre-operative administration in dogs, did prove that the drug was effective for post-operative analgesia after orthopaedic surgery (Lascelles et al. 1994; Welsh et al. 1997; Grisneaux et al. 1999; Laredo et al. 2004; Bergmann et al. 2007). However, most of these studies did not compare its efficacy against a placebo control.

In spite of the existing controversy, some studies have already suggested the beneficial effect of a multimodal analgesic plan which included NSAIDs. Meloxicam was demonstrated to exert an additional post-operative analgesic effect when combined with the epidural combination of morphine and mepivacaine in dogs undergoing CrCLR repair (Fowler et al. 2003). Bergmann et al. (2007) reported superior post-operative analgesia after orthopaedic surgery in dogs when the combination of carprofen and pre-emptive epidural mepivacaine

was compared to sole administration of carprofen, as evidenced by significantly lower pain scores and subsequent need for rescue analgesia at various time points in the post-operative period for the combination treatment. Unfortunately, a group that only received pre-emptive epidural mepivacaine was not included. Consequently, the superior analgesia from the combination treatment may have been due to sufficient alone standing analgesia provided by epidural mepivacaine.

The results of the obtained *intra-operative* analgesia induced by tepoxalin in surgically treated canine patients of the present thesis confirmed the hypothesis that NSAIDs alone fail to provide sufficient intra-operative analgesia for major orthopaedic surgeries.

In the first clinical study (chapter 1), the *intra-operative* need for rescue analgesia was not significantly different in the tepoxalin treatment compared to the placebo treatment, suggesting a lack of additive intra-operative analgesic effect of tepoxalin when methadone is administered as a premedication before imbrication surgery. Possible explanations can be found in the timing of administration of the drug and the feeding state of the patients. Tepoxalin was administered only once before premedication to fasted patients, which might have resulted in an incomplete effect of the drug at the time of maximum surgical stimulation (observed at the time of incision of the joint capsule). Indeed, tepoxalin plasma concentrations in dogs have been reported to peak after 2.3 ± 1.9 hours (mean ± SD) (Tepoxalin Technical Monograph, Schering-Plough, 2003). Additionally, the maximum plasma concentration of tepoxalin after oral administration was demonstrated to decrease by 50% in fasted animals compared to animals that were fed (Homer et al. 2005). In a second series of patients, tepoxalin was administered for one week before surgery with the last tablet administered before premedication, assuring therapeutical plasma concentrations of the drug at the time of surgical stimulation (chapter 2). Nevertheless, the lack of additional *intra-operative* analgesia by tepoxalin found in chapter 1, was more or less confirmed in the second clinical study of chapter 2, where oral tepoxalin combined with epidural methadone did not lower intra-operative rescue analgesia requirements compared to epidural methadone alone. However, the total number of intra-operative rescue analgesia administrations was less variable in the combination treatment compared to the epidural methadone treatment alone. Therefore it could not be concluded that tepoxalin was devoid of any intra-operative analgesic effect.

The *post-operative* analgesia induced by tepoxalin was different in the two clinical studies of the present thesis. On one hand, we were not able to demonstrate a significant

additive effect of pre-emptive tepoxalin administration on top of the post-operative analgesia provided by buprenorphine administered every 6 hours (chapter 1). Similar results were reported when carprofen was combined with buprenorphine (Shih et al. 2008). Beside the possibility of tepoxalin not having an additional analgesic effect, the results from our study might have been induced by the inability to differentiate pain with the pain scoring systems used, or by the sufficient analgesia provided by buprenorphine since only one dog in the buprenorphine group needed post-operative rescue analgesia. A trend towards a faster linear decrease of VAS-scores in the tepoxalin group compared to the placebo group was found (p = 0.059), suggesting a possible additive analgesic effect of tepoxalin. However, further research in a larger group of dogs is required to confirm this trend. In the second clinical study, tepoxalin was demonstrated to prolong the duration of post-operative analgesia only when combined with epidural ropivacaine 0.75% and methadone, suggesting an additive or synergistic analgesic effect of the combination of the 3 drugs. This finding is in agreement with the studies reporting the beneficial effects of NSAIDs in a multimodal analgesic plan for post-operative analgesia (Fowler et al. 2003; Bergmann et al. 2007) (*vide supra*).

Analgesia provided by epidural methadone and/or ropivacaine 0.75%

The pre-emptive epidural administration of a LA alone or in combination with opioids is one of the most applied techniques to assure analgesia during orthopaedic surgery of the hind limbs of dogs and cats (Jones 2001; Valverde 2008). This technique is supposed to provide one of the best means of blocking noxious stimuli (step 2 in the pain pathway, *vide supra*) and preventing central sensitization (step 3 in the pain pathway, *vide supra*) (Gottschalk & Smith 2001). This last hypothesis was confirmed by one of the earliest studies on the efficacy of pre-emptive epidural analgesia for limb amputation in humans, whereby pre-emptive administration of epidural analgesia dramatically lowered the incidence of phantom limb pain and stump pain evaluated one year after surgery (Bach et al. 1988).

The main sites of action of epidural LA's include the intradural spinal nerve roots and the periphery of the spinal cord (Jones 2001). The effect of the LA depends on its lipid solubility, pKa, pH of the solution and the tissues and the protein binding capacity of the drug (Jones 2001). The site of action of epidural opioids is the dorsal horn of the spinal cord, where they prevent the release of substance P (pre-synaptic action) and hyperpolarize cells (post-synaptic action) (Yaksh 1981; Jones 2001). They also increase the activity in the descending

inhibitory pain pathways (Yaksh 1981). Both epidural LA's and opioids are capable of modulating the nociceptive signal (step 3 in the pain pathway). The epidural administration of the combination of morphine and bupivacaine in dogs has been widely studied with promising results (Hendrix et al. 1996; Hoelzler et al. 2005; Novello & Corletto 2006; Kona-Boun et al. 2006; Gomez de Segura et al. 2009; Campoy et al. 2012). This combination is still most commonly used in clinical situations as well. In the present thesis it was chosen to investigate the analgesic effects of epidural methadone alone, or combined with ropivacaine 0.75% as a single epidural technique, or as a part of a multimodal analgesic approach together with pre-emptive oral administration of tepoxalin in dogs undergoing tibial tuberosity advancement (TTA) surgery (chapter 2). Methadone was preferred because of its multiple receptor activity, while ropivacaine 0.75% was included in the protocol because of its reduced potential for adverse effects compared to bupivacaine and the reported better differentiation between sensory and motor block. The obtained results suggest that this combination can indeed be used in a clinical situation. When the epidural combination of ropivacaine 0.75%/methadone was used in the current study, *intra-operative* analgesia was superior compared to epidural methadone alone, evidenced by no need for rescue analgesia together with the presence of an isoflurane sparing effect. This is in contrast with the literature, since Adami et al. (2012) reported a need for rescue analgesia in dogs receiving epidural ropivacaine 0.5 and 1% or the combination of ropivacaine 0.5%/sufentanil for tibial plateau leveling osteotomy surgery.

In the current study, the inclusion of ropivacaine 0.75% did not significantly increase the duration of *post-operative analgesia* provided by epidural methadone alone. Superior *post-operative* analgesia was only observed when pre-emptive tepoxalin was administered concurrently with the epidural combination of methadone and ropivacaine 0.75%, suggesting a synergistic effect of the 3 drugs (*vide supra*) and thereby confirming the efficacy of a multimodal analgesic approach in this kind of surgery. Superior post-operative analgesia resulting from an epidural combination of drugs (ropivacaine 0.5% + epinephrine + sufentanil) compared to epidural administration of ropivacaine 0.5% alone was also demonstrated in the study of Adami et al. (2012).

Part 2: Adverse effects

As a second part of the conclusion from the general introduction of the present thesis, the usefulness of investigating the side effects associated with the combination of different types of analgesics was included, since several studies pointed out that the use of NSAIDs and opioids in particular is limited amongst veterinarians because of concerns for the side effects (Capner et al. 1999; Lascelles et al. 1999; Williams et al. 2005). Additionally, the epidural use of opioids and local anaesthetics has been associated with side effects, some of them even fatal, both in veterinary and human medicine (*vide infra*).

Adverse effects associated with the administration of tepoxalin

All NSAIDs can induce side-effects mainly on the gastro-intestinal (GI), hepatic, renal and coagulation system (Lascelles et al. 2007; Papich 2008; Sparkes et al. 2010; KuKanich et al. 2012). A direct irritant effect on the GI tract (weak acids), inhibition of prostaglandin E_2 and I_2 synthesis resulting in decreased protective mucus secretion in the stomach, decreased mucosal blood flow, decreased bicarbonate production, increased acid secretion and decreased turnover of GI epithelial cells can induce haemorrhage and GI ulcerations (Papich 2008; KuKanich et al. 2012). As a consequence of the interaction with the hepatic system, intrinsic or idiosyncratic reactions can occur with any veterinary NSAID at an equal risk rate (KuKanich et al. 2012). Inhibition of renal prostaglandin synthesis can result in decreased renal perfusion, decreased tubular function and sodium and fluid retention. Dehydration, anaesthesia, shock, or pre-existing renal disease are important risk factors that contribute to NSAID-induced renal toxicity (Papich 2008; Sparkes et al. 2010). Finally, inhibition of TXA_2 leads to decreased platelet aggregation and inhibition of PGI_2 may lead to increased thrombosis (Lascelles et al. 2007).

In the search for NSAIDs with the least side effects, the quest has gone from non-selective COX inhibitors over dual COX/LOX inhibitors, to preferential and highly selective-COX-2 inhibitors. The former belief that COX-2 is only an inducible enzyme that does not contribute to homeostasis in contrast to COX-1, the so-called “constitutive COX enzyme”, was proven to be incorrect, since the COX-2 enzyme also yields homeostatic functions in certain situations (*vide infra*). Moreover, the tendency to use highly selective COX-2 inhibitors in order to prevent adverse effects by preserving COX-1 function, only applies to

the potential decrease of GI adverse effects in healthy GI tissues but not to GI adverse effects on diseased GI mucosae, and renal or hepatic adverse effects. In fact, the renal, coagulation and GI adverse effects of diseased GI tissue can be worsened when highly selective COX-2 inhibitors are used (Mattia & Coluzzi 2005). For example, COX-2 selective inhibitors may adversely affect the kidney during conditions of decreased perfusion (e.g. anaesthesia induced hypotension) (Papich 2008). Highly selective COX-2 inhibition may lead to increased thrombosis due to decreased synthesis of PGI_2 (Egan et al. 2004). Additionally, COX-2 is also upregulated in damaged and healing tissues of the GI tract, inducing angiogenesis at the edge of gastric ulcers (Wooten et al. 2010). Its inhibition in this situation can worsen the GI disease which might lead to ulcer perforation (Lascelles et al. 2007; Goodman et al. 2009).

The benefit of additional inhibition of 5-LOX by dual COX/LOX inhibitors, such as tepoxalin, is situated in the reduced formation of leukotrienes responsible for chemotaxis, adhesion and degranulation of neutrophils, which is important in the prevention of GI inflammation. Leukotrienes are likely to be synthetized when only COX-inhibiting drugs are used, because the 5-LOX pathway has more substrate available to form leukotrienes. There will be a shift in the production of inflammatory mediators from the COX to the 5-LOX pathway (Kirchner et al. 1997; Sparkes et al. 2010). By preventing this shift, 5-LOX inhibition might be beneficial in protecting the GI mucosa from damage attributable to COX inhibition, due to a better balance between COX and 5-LOX products (Bertolini et al. 2001; Goodman et al. 2008).

Because of its promising GI profile and the balanced inhibition of COX and LOX, we chose to investigate the analgesic and the short term adverse effects of tepoxalin, the only dual COX and LOX inhibiting NSAID licensed in Belgium for the treatment of osteoarthritis in dogs. Unfortunately, it is no longer available anno 2012 due to commercial reasons.

In the first clinical study with a 24 hour observation period (chapter 1), haemorrhagical diarrhoea was observed in only one tepoxalin treated dog at 6 hours following extubation. It normalized without therapeutical intervention within 6 hours. Although it was not certain that tepoxalin administration was the causative factor, this possibility could not be excluded either. No other adverse GI effects were noted in the other 9 dogs nor in the 16 tepoxalin treated dogs in the second clinical study (8 day observation period: 6 days at home; 2 days hospitalized). These results are in agreement with the literature, were a good GI safety

profile of tepoxalin in dogs was suggested (Knight et al. 1996; Kirchner et al. 1997; Goodman et al. 2009).

In chapter 1, the effects of tepoxalin administration before anaesthesia on blood biochemistry parameters and buccal mucosal bleeding time (BMBT) were additionally studied. There were no significant differences for the coagulation times, BMBT, blood urea nitrogen, creatinine, and total protein. These findings were also in agreement with the literature (see chapter 1). The tepoxalin treated group had higher levels for post-operative fibrinogen compared to the placebo group, however, without a clear explanation.

All findings of the present thesis confirmed the safety of administering tepoxalin before anaesthesia. However, since anaesthesia related hypotension increases the risk for NSAID induced renal adverse effects (*vide supra*), it must be stressed that blood pressure needs to be maintained within normal limits during anaesthesia whenever NSAIDs are applied before or during anaesthesia.

Adverse cardiovascular effects associated with the epidural administration of methadone and/or ropivacaine 0.75%

The cardiovascular effects of the epidural administration of both methadone and ropivacaine 0.75% in dogs in a clinical situation have not been studied in depth. The most commonly reported cardiovascular side effect of epidural administration of local anaesthetics is without any doubt hypotension (Torske & Dyson 2000), but also bradyarrhythmias (Iff & Moens 2008) have been reported. Additionally, in human medicine cardiotoxicity and even cardiac arrest due to accidental intravenous injection (Albright 1979) have been described. Therefore, the cardiovascular effects associated with the epidural administration of methadone and/or ropivacaine 0.75% were studied in isoflurane anaesthetized healthy Beagles in several parts of the present thesis.

In chapter 3, the overall comparison between the epidural treatments demonstrated lower heart rates (HR) in all treatments compared to the placebo treatment. Additionally an overall lower HR in the combined methadone/ropivacaine 0.75% treatment was observed compared to the methadone treatment. When ropivacaine 0.75% was included into the epidural treatment, the overall systemic blood pressure was significantly lower compared to other treatments, without significant differences for the overall comparison of other

cardiovascular parameters. The most important within treatment differences from baseline were an increase in HR and systemic arterial pressures (sAP's) induced by epidural methadone and clinically important low blood pressures in the ropivacaine 0.75% containing treatments, with mean arterial pressure (MAP) values lower than 60 mm Hg, the lower limit for MAP in clinical conditions. This last result suggests that ropivacaine 0.75% induces hypotension when applied epidurally. Hurley et al. (1991) also reported significant decreases in sAP's with epidural administration of ropivacaine in awake dogs. However, the dogs included into the study of chapter 3 were kept at a relatively deep anaesthetic level (1.4 x MAC_{ISO}), which might also have contributed to the low baseline sAP's and this due to the hypotensive effects of isoflurane. The negative influence of isoflurane on baseline and subsequent blood pressure values could most likely have been prevented by premedicating the dogs, since premedication usually results in an isoflurane sparing effect. This hypothesis was confirmed in the clinical canine patients of chapter 2, where as a result of the use of premedication, it was possible to use lower end-tidal isoflurane concentrations, whereby only a short period of hypotension was observed in a minority (2/16) of the dogs receiving epidural ropivacaine 0.75%. This last finding suggests that epidural ropivacaine 0.75%-induced hypotension can be prevented in a clinical situation by using premedication and lower ET isoflurane concentrations.

Epidural methadone was never associated with clinically important cardiovascular changes in dogs in both studies of the present thesis (chapter 2 & 3) which was in agreement with the data available in the literature (Leibetseder et al. 2006).

Since the occurrence of hypotension following epidural administration of ropivacaine 0.75% in isoflurane anaesthetized dogs was expected, the effects of preloading the vascular system with a colloid [hydroxyethylstarch 6% (200 kDa/0.5) (HES)], a technique commonly used with variable success in human medicine (Tamilselvan et al. 2009), was further investigated as a possible therapeutic precaution. A dose of 7 mL kg^{-1} HES, administered over 30 minutes immediately prior to epidural administration of ropivacaine 0.75% was unable to prevent the hypotension effectively, but was indicative for a faster recovery from hypotension (chapter 4). In contrast, the same HES dose and rate of administration did restore hypotension after the epidural administration of the combination of methadone/ropivacaine 0.75% in clinical canine patients in the second chapter. A major difference between the two studies was the influence of isoflurane concentrations on the magnitude of hypotension (*vide supra*) and also the timing of the administration of HES. Future studies should focus on other doses, rates and timing of

the administration of colloids to prevent/treat hypotension associated with epidural administration of ropivacaine 0.75% in dogs.

Unfortunately, one dog suffered from a cardiorespiratory and neurological complication (hypotension, persistent apnea, coma) following intended epidural injection of ropivacaine 0.75%, which was unresponsive to the installed treatment (see chapter 4). Since we did not use a 100% objective method for identification of the epidural space, it was hypothesized that accidental intrathecal injection of the epidural dose of ropivacaine 0.75% caused a fatal brainstem anaesthesia. Brainstem anaesthesia following intrathecal injection of LA's has also been described in human medicine but was always reversible. Up to now, this complication had not been reported in dogs, until very recently (2012) a similar clinical situation occurred where a dog suffered severe hypotension and apnea following intended epidural injection of bupivacaine 0.5% under isoflurane anaesthesia in our clinic. Although hypotension was treated successfully and spontaneous respiration was finally restored, severe central neurological abnormalities did persist for several days and the dog was finally euthanized. Epidural drug administration was confirmed in the experimental studies of this thesis by means of subjective "clinical techniques", such as the combined appearance of a popping sensation as a result of penetration of the *ligamentum flavum* during needle placement, the subsequent absence of cerebrospinal fluid and blood at the needle hub before injection and the "loss of resistance" to injection of the epidural treatment or saline during injection. We did not aspirate from the epidural needle before injection. The occurrence of "a tail-flick", as described in the dogs during the experimental studies from this thesis, does not always occur and is not under voluntary control of the person placing the needle. Therefore it should not be interpreted as "a test" to identify the epidural space. In fact, the occurrence of "a tail-flick" associated with neuraxial needle placement can be dangerous (see chapter 4), since it results from needle contact with either nerves or the spinal cord. However, from personal clinical experience, the unintended appearance of a sudden tail movement of very short duration at placement of the needle suggests that the needle is most likely in the right place to perform a neuraxial anaesthesia technique, but should always be followed by proper subjective or objective methods for the identification of either the epidural or intrathecal space. In the absence of objective methods the importance of aspirating from the epidural needle before injection, to make sure that the injection is performed epidurally cannot be stressed enough in the author's opinion. This technique is now common practice at our facility. Objective methods for identification of the epidural space are contrast radiography,

fluoroscopy, ultrasonography and detection of pressure changes on entering the epidural space which can be combined with an acoustic device (Grau et al. 2003; Naganobu & Hagio 2007; Iff & Moens 2010; Iff et al. 2011). These techniques are not frequently performed because they need additional equipment. Since the dogs in the experimental studies of this thesis were instrumented and needed extensive monitoring at the time of epidural injection, it was impossible to move them to our medical imaging department for confirmation of epidural needle placement by means of contrast radiography, a technique that is performed in a clinical setting when epidural positioning of the needle is inconclusive.

Other adverse effects associated with the epidural administration of methadone and/or ropivacaine 0.75%

Reported side effects related to epidural administration of LA's other than cardiovascular complications are Horner's syndrome (HS), hypothermia, hypoventilation and apnea, increased bleeding at the surgical site, introduction of infection at the site of injection, coma and convulsions, and post-puncture headache which has been reported in man only (Jones 2001; Dugdale 2010; Barbara et al. 2011; Goel & Burkat 2011). Additionally, pruritus, urinary retention, nausea, vomiting, respiratory depression and delayed hair regrowth or white hair regrowth at the injection site are complications associated with epidural administration of opioids in dogs (Jones 2001; Troncy et al. 2002; Dugdale 2010).

The occurrence of HS associated with epidural administration of LA's had only been mentioned anecdotally in veterinary medicine (Jones 2001), whereas in human medicine it is a frequently reported complication (Barbara et al. 2011; Goel & Burkat 2011). During the research for the present thesis, 2 dogs receiving epidural ropivacaine 0.75% showed a transient HS (right and left sided respectively). Additionally, anisocoria was initially present in the dog that suffered the fatal complication observed after epidural/intrathecal ropivacaine 0.75%. In contrast, none of the dogs included into the clinical study presented post-operative signs of HS. Overall, the total incidence of HS related to epidural ropivacaine 0.75% administration in the present thesis was relatively high namely 7.14 % (2/28). The exact incidence of epidural induced HS in human medicine is unknown but ranges only between 0.13-4% (Clayton 1983; Biousse et al. 1998; Rabinovich et al. 2010). The possible explanations for the transient left sided HS observed in 1 of the dogs in the immediate post-experimental period after epidural ropivacaine 0.75% administration were further elaborated

on in chapter 5. It was concluded that the HS most likely resulted from an extensive unilateral cranial spread of ropivacaine 0.75%, probably caused by anatomical changes in the epidural space, since the dog already had 6 epidurals at previous occasions. This hypothesis might explain the discrepancy between the incidence of HS observed in the experimental compared to the clinical studies of the present thesis.

Urinary retention, pruritus and vomiting in the 20 hour post-operative period were not observed in the clinical study of chapter 2.

General Conclusions and Clinical Relevance:

Based on the results of the present thesis, it can be concluded that *intra-operative analgesia* for major orthopaedic surgery of the hind limbs (TTA) in dogs which were premedicated with methadone, was superior using the combination of pre-emptive epidural ropivacaine 0.75% and methadone compared to pre-emptive epidural administration of methadone with or without pre-emptive administration of tepoxalin (7 day administration). Oral tepoxalin (1 day administration) could not be demonstrated to improve the *intra-operative* analgesia provided by intravenous methadone as premedication in dogs undergoing imbrication surgery.

Post-operative analgesia for TTA surgery in dogs was found to be superior when the 3 drugs (oral tepoxalin + epidural methadone and ropivacaine 0.75%) were included in the multimodal analgesia protocol compared to epidural methadone with or without oral tepoxalin administration. The addition of epidural ropivacaine 0.75% to epidural methadone did not improve post-operative analgesia. A trend for better post-operative analgesia was found for the combination of oral tepoxalin and intravenous buprenorphine in dogs undergoing imbrication surgery.

Oral tepoxalin administration (1 or 7 day treatment) before anaesthesia in dogs undergoing surgery for CrCLR repair was devoid of serious side effects. This suggests that dogs that are already on tepoxalin therapy do not necessarily have to be switched to another NSAID therapy before surgery.

Epidural methadone was not associated with side effects in the present study. In contrast, epidural ropivacaine 0.75%, with or without epidural methadone was associated with

several side effects including per-anaesthetic hypotension, HS and even the death of 1 dog. When interpreting these results, one should differentiate between the clinical study, where only mild hypotension was reported as a side effect, and the experimental study. It was suggested that possible anatomical changes induced by the multiple injections into the epidural space might have contributed to the HS and accidental intrathecal injection of ropivacaine 0.75%. Additionally, the influence of isoflurane anaesthesia on hypotension was more pronounced in the experimental study.

As an overall clinical conclusion it can be stated that the multimodal analgesic combination of oral tepoxalin, epidural methadone and ropivacaine 0.75% provided superior total analgesia compared to the other studied analgesic combinations for stifle surgery in dogs. However, the use of epidural ropivacaine 0.75% during isoflurane anaesthesia can result in hypotension and possibly HS. The need for aspiration from the epidural needle after placement and before injection of the epidural must be stressed, since accidental intrathecal injection of ropivacaine 0.75% can be fatal. Epidural administration of methadone is safe and provided similar post-operative analgesia as epidural methadone with ropivacaine 0.75% when tepoxalin was not administered pre-operatively.

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Appendix

Reflections on statistical power analysis in relation to the results of this study.

Nickerson (2000) suggested that the term "statistical power" is often not understood, and as a result neglected in many research papers, resulting in underpowered scientific studies. With no information on the statistical power of the study for a certain studied parameter, one is not able to conclude that there is no effect of the treatment when the null hypothesis (mostly: no significant difference between the means of a parameter in the treatment groups of a study) cannot be rejected. In those cases incorrect affirmation of the null hypothesis (type II error) might lead to a false negative result. Only when a statistical test has a known sufficient power, failure to reject the null hypothesis can be considered as an affirmation of the null hypothesis (Cashen & Geiger 2004).

Statistical power is defined as 1-β, where β is the probability of failing to reject the null hypothesis when it is actually false (type II-error). Statistical **power** is determined by three parameters: **the significance level α, the sample size and the effect size** (Cashen & Geiger 2004). When three of the four elements are known, the missing one can be calculated. Ideally, the significance level, a level of power and the effect size of each parameter to be studied should be specified before commencing a study. From this information the ideal sample size can be calculated so that one is able to draw valid conclusions when the null hypothesis cannot be rejected. It is easy to understand that when the number of observations in a sample increases, the reliability of the sample value approximating the population value also increases (Cohen, 1977).

The level of significance α (type I-error) denotes the probability to reject the null hypothesis when it is actually true (Cashen & Geiger 2004). Mostly a significance level of 0.05 is set. This means that there is a 5% chance that the observed difference between the means of a certain parameter in different treatments is due to coincidence (false positive result) (Cashen & Geiger 2004).

The level of β is ideally set at the same level of the type I-error. This means that if α is set at 0.05, β is set at 0.05, resulting in a level of power of 0.95 (Sedlmeier & Gigerenzer 1989).

The last parameter necessary to calculate sample size is the effect size. Therefore it is important to determine when an effect is large enough to be considered of importance (for

example, what is a clinically important measured difference in blood pressure between two treatment groups after administration of a drug) (Cashen & Geiger 2004). If many parameters are to be tested, an effect size for each parameter should ideally be determined. The smaller the effect size, the larger the required sample size (Cashen & Geiger 2004).

In this thesis, all the statistical methods were discussed with a statistician before the start of the studies. However, no a priori power studies (i.e. volume of sample size studies) were performed for the studied parameters. Mainly, this was due to a lack of info about the expected effect sizes.

Considering the results of chapter 1, we were not able to demonstrate that tepoxalin improved intra-operative analgesia provided by methadone and post-operative analgesia provided by buprenorphine. Despite the lack of a priori power calculations, we correctly mentioned that these results do not implicate that the analgesic effect from tepoxalin administration is missing, because affirmation of the null hypothesis (meaning there is no effect from the administration of tepoxalin) in this case might be incorrect and might lead to a false negative result (see above). A trend to a faster decrease in post-operative pain scores was observed and it was stated in the discussion that further research with a larger case load is needed to confirm this trend. Essentially, this means that the study may have been underpowered.

In chapter 2, a lot of significant results were obtained, confirming the power of the used statistical tests for those variables and enabling us to correctly affirm the alternative hypotheses (meaning that there was a significant difference between the means of the studied variables of the different treatments). Most likely, an even higher number of significant results would have been found as a result of inclusion of more patients per treatment group. For example, there was a huge observed difference between the duration of analgesia in treatments PRM (554 ± 234 minutes) and TRM (853 ± 288 minutes) with clinical relevance, however this difference did not reach statistical significance. Blind acceptation of the null hypothesis in this example would most likely lead to a false negative result.

In chapter 3, significant results were found for most of the studied variables, both for in-between and inter-treatment comparisons, confirming the statistical power for most of the studied variables. We therefore think that the most important conclusion (the clinically important low systemic arterial pressures associated with epidural ropivacaine 0.75% and the combination of epidural ropivacaine 0.75%/methadone compared to epidural saline or

methadone) is a correct conclusion, even though we did not perform power calculations. In the general discussion we mentioned that the incidence of Horner's syndrome reported in our studies was much higher than the incidence in human medicine (7.14% versus 0.13-4%). Of course this result must be evaluated taking into consideration the small sample size representing the total population in our studies.

Since no significant differences between treatments were found in chapter 4, the study might have been underpowered. Therefore, the lack of results between treatments should be interpreted carefully. Although we were not able to demonstrate that the hydroxyethylstarch 6% preload resulted in improved cardiovascular function, this does not necessarily mean that it is devoid of these effects (in essence, the lack of affirmation of the alternative hypothesis cannot result in blind acceptance of the null hypothesis).

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Summary

Summary

In the general introduction the physiology and pathophysiology of pain were reviewed. The different physiological steps in the pain pathway (transduction, transmission, modulation and perception) were explained. This is of particular importance for the understanding of the theory of multimodal analgesia, where analgesics with different modes of action, affecting the different steps in the pain pathway are combined to prevent or treat pain more adequately compared to sole administration of an analgesic drug. The pathophysiology of pain was also explained. This is of particular importance for the understanding of the rationale behind pre-emptive analgesia, where analgesics are administered before a noxious stimulus occurs in order to prevent peripheral and central sensitization, which can lead to hyperalgesia, pain arising from normally non-painful perceptions (allodynia) and even chronic pain.

In this thesis, two clinical studies were conducted to evaluate the analgesic effects of different multimodal analgesic approaches in dogs undergoing two types of stifle surgery for the correction of cranial cruciate ligament rupture, respectively imbrication and tibial tuberosity advancement surgery. Additionally, possible side effects linked to the administration of the used analgesic combinations were studied.

In **chapter 1**, the intra-operative analgesic efficacy of the combination of pre-emptive tepoxalin (10 mg kg^{-1}, single oral administration) with pre-operative methadone administration (0.1 mg kg^{-1} IV) was studied in dogs undergoing imbrication surgery. Additionally, the post-operative analgesic efficacy of tepoxalin and post-operatively administered buprenorphine (10 µg kg^{-1} IV q 6 hours until 24 hours after surgery) was evaluated in the same dogs. Ten dogs received oral tepoxalin immediately before premedication; the remaining 10 dogs received a placebo tablet. Intra-operative rescue analgesia (fentanyl 1 µg kg^{-1} IV) was administered whenever heart rate increased more than 10% compared to baseline. The need for intra-operative rescue fentanyl did not differ significantly between groups. It was concluded that tepoxalin did not improve intra-operative analgesia provided by methadone. Post-operative analgesia was scored at extubation and at 1, 2, 6, 12 and 24 hours post-extubation, using a visual analogue scale and a multifactorial pain scale. There were no significant differences between groups for the overall multifactorial pain scale scores. However, there was a faster decrease of the visual analogue scores in the tepoxalin treated animals compared to the placebo treatment, which did nearly reach statistical significance. The possible additive post-operative analgesic effect of tepoxalin on post-operative buprenorphine induced analgesia needs to be examined more extensively, before a conclusion on this matter can be made.

The side effects associated with a single administration of tepoxalin in dogs undergoing imbrication surgery were also studied by means of blood analyses (biochemistry and coagulation parameters) and a buccal mucosal bleeding time before premedication and 24 hours after surgery. There were no significant differences between treatments for the coagulation times, blood urea nitrogen, creatinine, total proteins and buccal mucosal bleeding time. Additionally, the occurrence of side effects associated with gastro-intestinal damage (vomiting, diarrhoea) was recorded. Haemorrhagic diarrhoea was observed in 1 tepoxalin treated dog at 6 hours following extubation which normalized without therapeutical intervention within 6 hours. No other adverse gastro-intestinal effects were noted.

In **chapter 2**, the analgesic efficacy of 4 analgesic protocols was studied in 32 dogs (*n*=8 per treatment) undergoing tibial tuberosity advancement surgery. All dogs received methadone (0.1 mg kg^{-1} IV) as a part of the premedication. Sixteen dogs received oral tepoxalin (10 mg kg^{-1} once daily for 7 days before surgery), whereas the others received a placebo tablet. After induction of anaesthesia, the dogs received either methadone 1% (0.1 mg kg^{-1}) or the combination of methadone 1% (0.1 mg kg^{-1}) with a local anaesthetic; ropivacaine 0.75% (1.65 mg kg^{-1}) epidurally. The need for intra-operative rescue analgesia (fentanyl 2 µg kg^{-1} IV) was evaluated based on increases in heart rate and other signs of intra-operative nociception. Additionally, the isoflurane sparing effect of the analgesic combinations was recorded. The epidural combination of ropivacaine 0.75% and methadone resulted in superior intra-operative analgesia and an isoflurane sparing effect compared to the epidural administration of methadone with or without addition of tepoxalin. Post-operative analgesia was scored using a visual analogue scale and the University of Melbourne Pain Scale at 1 to 8 hours (hourly) and at 20 hours post-extubation. Post-operative analgesia was superior when the 3 drugs (oral tepoxalin and epidural methadone/ropivacaine 0.75%) were included in the multimodal analgesia protocol compared to epidural methadone irrespective of oral tepoxalin administration. The addition of epidural ropivacaine 0.75% to epidural methadone did not improve post-operative analgesia.

The occurrence of side effects, associated with epidural drug administration (e.g. intra-operative hypotension, urinary retention, pruritus, vomiting) was also recorded. Two dogs receiving epidural ropivacaine 0.75%/methadone suffered mild intra-operative hypotension that responded well to a continuous rate infusion of hydroxyethylstarch 6% (200 kDa/ 0.5) of

7 mL kg^{-1} infused over 30 minutes. Additionally, no gastro-intestinal side effects were recorded after 7 days of tepoxalin administration. Regarding the occurrence of side effects related to oral administration of tepoxalin in **chapter 1 & 2**, it could be concluded that oral tepoxalin administration (1 or 7 day treatment) before anaesthesia in dogs undergoing surgery for cranial cruciate ligament repair was devoid of serious side effects. This suggests that dogs that are already on tepoxalin therapy do not necessarily have to be switched to another NSAID therapy before surgery. Additionaly, the results of the tibial tuberosity advancement-study showed that pre-operative administration of tepoxalin improves post-operative analgesia when combined with epidural methadone/ropivacaine 0.75% and the results of the imbrication study were suggestive for improved post-operative analgesia in dogs when pre-operatively administered tepoxalin was combined with post-operative administration of buprenorphine.

In **chapter 3**, the cardiovascular effects of the epidural administration of saline (placebo), methadone 1% (0.1 mg kg^{-1}), ropivacaine 0.75% (1.65 mg kg^{-1}) and their combination (similar doses) were studied in 6 isoflurane anaesthetized experimental dogs in a cross-over study. This was done by means of the lithium dilution and pulse contour analysis method. After induction of anaesthesia in unpremedicated dogs, catheterization and placement of monitoring and a stabilization period of 30 minutes, the epidural treatment was administered (T0). Systemic arterial pressures, arterial oxygen saturation, oesophageal temperature, heart rate and central venous pressure were recorded at T-15 (15 minutes before epidural injection), and subsequently at T0 and every 5 minutes for 60 minutes. Blood gases and cardiac output were obtained at T-15, T0, T15, T30, T45 and T60. Stroke volume, systemic vascular resistance, arterial oxygen content and oxygen delivery were calculated at the same time points.

A significant reduction in overall mean heart rate following epidural administration of methadone, ropivacaine 0.75% and their combination compared to the placebo treatment was demonstrated. Additionally, overall mean heart rate was significantly lower after epidural ropivacaine 0.75%/methadone administration compared to epidural methadone administration. After both ropivacaine 0.75% containing treatments, the systemic arterial pressures were significantly lower compared to other treatments. Moreover, the mean arterial pressure was lower than 60 mm Hg (which is defined as the lower limit for mean arterial pressure in clinical conditions) in the ropivacaine 0.75% containing treatments. No significant overall

differences between treatments were present for central venous pressure, cardiac output, stroke volume, systemic vascular resistance, oxygen delivery and arterial oxygen content. Heart rate and systemic arterial pressures significantly increased after epidural placebo and epidural methadone administration compared to baseline. With all treatments significant reductions from baseline were observed in oesophageal temperature, packed cell volume and arterial oxygen content. The most important conclusion from this study was that epidural ropivacaine 0.75% administration was associated with hypotension. The epidural administration of methadone without ropivacaine 0.75% was not associated with clinically important cardiovascular changes in dogs in both studies (**chapter 2 & 3**) of the present thesis.

In **chapter 4**, the influence of a preload of hydroxyethylstarch 6% (200 kDa/0.5) (administered as a continuous rate infusion of 7 mL kg^{-1} over 30 minutes prior to epidural administration) on the cardiovascular effects of epidural administration of ropivacaine 0.75% (1.65 mg kg^{-1}) was studied by means of a cross-over study in anaesthetized experimental dogs, whereby on one occasion epidural ropivacaine 0.75% administration was preceded by the hydroxyethylstarch preload (started at T-30) (treatment HESR). On the other occasion the preload was not administered (treatment R). The cardiovascular baseline measurements were obtained at T-5 and the epidural treatment was administered at T0. Heart rate, mean, diastolic and systolic arterial pressures, cardiac output (pulse contour analysis) and derived parameters were recorded every 5 minutes for 60 minutes. Statistical analysis was performed on 5 dogs, due to the unexpected death of one dog during treatment HESR.

Clinically relevant decreases in mean arterial pressure (< 60 mmHg) were recorded for 20 and 40 minutes following epidural administration in treatments HESR and R respectively. Significant decreases in diastolic arterial pressure were present after treatment HESR for up to 20 minutes following epidural administration. Although the hydroxyethylstarch preload was not able to mitigate the hypotension induced by epidural ropivacaine 0.75% in anaesthetized dogs, the results suggest a faster recovery from epidural ropivacaine 0.75% induced hypotension when a hydroxyethylstarch preload is administered.

Considering the combined data of **chapters 2, 3 and 4**, it can be concluded that hypotension induced by epidural administration of ropivacaine 0.75% is less severe when an isoflurane sparing effect can be achieved due to effective premedication in a clinical situation.

In **chapters 3 & 4**, a transient unilateral Horner's syndrome was observed in 2 experimental dogs (right sided in treatment R; left sided in treatment HESR). The relation to epidural ropivacaine 0.75% administration was clear since signs disappeared together with waning of ropivacaine 0.75% induced motor block. The possible explanations for the occurrence of Horner's syndrome related to epidural ropivacaine 0.75% administration were discussed in a case report (**chapter 5**). Most likely, anatomical changes (fibrosis/adhesions or haemorrhage) in the epidural space, induced by multiple epidural injections, were responsible for an extensive cranial spread of epidural ropivacaine 0.75% in these dogs, which might explain the occurrence of Horner's syndrome. This hypothesis is strengthened by the fact that no dogs from the clinical study (**chapter 2)** suffered Horner's syndrome.

One dog in the experimental study from **chapter 4**, suffered a severe hypotension immediately following epidural administration of ropivacaine 0.75% (mean arterial pressure<40 mmHg for 20 minutes) during treatment HESR. Despite a reduction of the anaesthetic depth and the restoration of normotension by means of adequate inotropic therapy, the animal was unable to breathe spontaneously. After a 10 hour resuscitation and stabilisation attempt, the dog was euthanized. Following necropsy and histological examination of the spinal cord, an undeliberate intrathecal anaesthesia with an extensive cranial spread of ropivacaine 0.75% resulting in brainstem anaesthesia was thought to be the most likely explanation for this fatal complication.

As an overall clinical conclusion it could be stated that the multimodal analgesic combination of oral tepoxalin, epidural methadone and ropivacaine 0.75% provided superior total analgesia compared to the other studied analgesic combinations for stifle surgery in dogs. However, the use of epidural ropivacaine 0.75% during isoflurane anaesthesia can result in hypotension and possibly Horner's syndrome. The need for aspiration of the epidural needle after placement and before injection of the epidural must be stressed, since accidental intrathecal injection of ropivacaine 0.75% can be fatal. When tepoxalin was not administered pre-operatively, the epidural administration of methadone provided similar post-operative analgesia as epidural methadone with ropivacaine 0.75%. Additionally, the epidural administration of methadone was safe.

Samenvatting

De fysiologie en pathofysiologie van pijn werden in deze thesis vooraf belicht aan de hand van een overzichtsartikel. Hierin werden de verschillende fysiologische aanknopingspunten in het ontstaansproces van pijn besproken (transductie, transmissie, modulatie en perceptie). Het begrijpen van deze aanknopingspunten is onontbeerlijk om het begrip 'multimodale analgesie' te kunnen bevatten. Bij multimodale analgesie worden analgetica met een verschillend werkingsmechanisme gecombineerd, die elk ingrijpen op verschillende aanknopingspunten in het ontstaansproces van pijn, met het ultieme doel de pijn beter te bestrijden dan na toediening van slechts één analgeticum. De kennis van de pathofysiologie van pijn is belangrijk om het nut van 'pre-emptive analgesia' te kunnen situeren. Bij het gebruik van de 'pre-emptive analgesia' strategie worden analgetica toegediend vooraleer een pijnlijke stimulus plaatsvindt. Deze strategie is uitermate belangrijk in de preventie van perifere en centrale sensitisatie en kan finaal het ontstaan van hyperalgesie, allodynia en chronische pijn verhinderen.

In deze thesis werd het analgetisch aspect van verschillende analgetische combinaties onderzocht aan de hand van twee klinische studies bij honden, waarbij imbricatie en 'tibial tuberosity advancement', 2 types van chirurgische correctie van een gescheurde voorste gekruiste band, uitgevoerd werden. Tevens werden mogelijke nevenwerkingen van de gebruikte analgetische combinaties geëvalueerd.

In **hoofdstuk 1** werd het intra-operatieve analgetische effect van 'pre-emptive' toegediende tepoxaline (eenmalige orale toediening van 10 mg kg^{-1}) na een premedicatie met 0.1 mg kg^{-1} methadon bestudeerd bij honden die een imbricatie ondergingen. Tevens werd in deze studie het post-operatieve analgetische effect van tepoxaline en post-operatief toegediende buprenorphine (10 µg kg^{-1} IV q 6 uur tot 24 uur na de chirurgie) bestudeerd. Aan 10 honden werd tepoxaline oraal toegediend onmiddellijk voor de premedicatie; de andere 10 patiënten kregen een placebo toegediend. Intra-operatief werd als bijkomende analgesie fentanyl (1 µg kg^{-1} IV) toegediend wanneer de hartfrequentie meer dan 10% steeg ten opzichte van de uitgangswaarde genomen voor de chirurgie. Er kon geen significant verschil in behoefte aan bijkomende analgesie bekomen door fentanyl aangetoond worden tussen de 2 groepen. Als conclusie werd gesteld dat toediening van tepoxaline niet resulteerde in een verbetering van de intra-operatieve analgesie bekomen door de methadon toediening. De post-

operatieve analgesie werd tevens beoordeeld na extubatie en 1, 2, 6, 12 en 24 uur volgend op de extubatie met behulp van een 'visual analogue score' en een multifactorieel pijnscoresysteem . Er werden geen significante verschillen gevonden tussen de groepen voor de multifactoriële pijnscores bekomen over de gehele tijdsperiode. Voor de 'visual analogue scores' werd een snellere daling over de tijd aangetoond bij de honden behandeld met tepoxaline vergeleken met de placebo behandeling. Deze daling was echter net niet statistisch significant. Het eventuele additieve post-operatieve analgetische effect van tepoxaline bovenop de analgesie verkregen door post-operatieve toediening van buprenorphine, dient verder onderzocht te worden om hier verder uitsluitsel over te kunnen geven.

De neveneffecten die mogelijks geassocieerd kunnen worden met een eenmalige toediening van tepoxaline bij honden die imbricatie chirurgie ondergaan, werden eveneens bestudeerd en dit aan de hand van een bloedonderzoek (biochemie en stollingsparameters) en een 'buccal mucosal bleeding time' test, uitgevoerd voor de premedicatie en 24 uur na de chirurgie. Er kon alleen een significant hogere fibrinogeen concentratie aangetoond worden in de honden die tepoxaline toegediend kregen. De oorzaak voor deze hogere waarde kon echter niet verklaard worden. Bijkomend werd gekeken naar het voorkomen van neveneffecten die geassocieerd worden met gastro-intestinale schade (braken, diarree). Eén hond, behandeld met tepoxaline, had 6 uur na de extubatie een bloederige diarree, die spontaan verdween binnen 6 uur.

In **hoofdstuk 2** werd de efficiëntie van 4 analgetische protocols bij 'tibial tuberosity advancement' chirurgie bestudeerd bij 32 honden (*n*=8 per protocol). Alle patiënten kregen methadon (0.1 mg kg^{-1} IV) als deel van de premedicatie. Zestien honden kregen oraal tepoxaline (10 mg kg^{-1} eenmaal daags gedurende 7 dagen voorafgaand aan de chirurgie) toegediend; bij de andere dieren werd een placebo toegediend. Na inductie van de anesthesie werd het respectievelijk epidurale analgetische protocol toegediend: methadon 1% (0.1 mg kg^{-1}) al dan niet in combinatie met een lokaal anestheticum; ropivacaïne 0.75% (1.65 mg kg^{-1}). De intra-operatieve bijkomende toediening van fentanyl (2 µg kg^{-1} IV) als additionele analgesie was gebaseerd op een stijging van >25% van de hartfrequentie ten opzichte van de uitgangswaarde samen met klassieke tekenen van het optreden van intra-operatieve nociceptie. Tevens werd het sparende effect van de analgetische combinatie op het verbruik van het gebruikte volatiele anestheticum, zijnde isofluraan, nader bestudeerd. De epidurale

combinatie van ropivacaïne 0.75% en methadon resulteerde in superieure intra-operatieve analgesie en in een isofluraansparend effect ten opzichte van epidurale toediening van methadon met of zonder tepoxaline. De post-operatieve analgesie werd geëvalueerd aan de hand van een 'visual analogue scale' en de 'University of Melbourne Pain Scale', uitgevoerd van 1 tot 8 uur (elk uur) en 20 uur na de extubatie. De post-operatieve analgesie was beter wanneer orale tepoxaline samen met de epidurale combinatie van methadon en ropivacaïne 0.75% werden aangewend dan wanneer epidurale methadon met of zonder orale tepoxaline werd toegediend. De toevoeging van ropivacaïne 0.75% aan epidurale methadon zonder orale tepoxaline verbeterde de post-operatieve analgesie echter niet.

Het voorkomen van neveneffecten (intra-operatieve hypotensie, urineretentie, pruritus en braken), mogelijks geassocieerd met de toegediende epidurale medicatie werd eveneens opgevolgd. Twee honden die ropivacaïne 0.75%/methadon epiduraal toegediend kregen vertoonden een milde intra-operatieve bloeddrukdaling, welke echter snel gecorrigeerd werd door middel van een toediening van hydroxyethylstarch 6% (200 kDa/ 0.5) aan een dosering van 7 mL kg^{-1} over 30 minuten. Bijkomend werden geen GI neveneffecten waargenomen na toediening van tepoxaline gedurende 7 dagen. Globaal over de 2 studies heen kan gesteld worden dat orale tepoxaline toediening gedurende 1 tot 7 dagen voor chirurgische correctie van een gescheurde voorste gekruiste band bij de hond geen ernstige neveneffecten veroorzaakt. Deze bevinding suggereert dat het niet nodig is om de behandeling van honden die al tepoxaline toegediend krijgen vóór chirurgie te staken. Daarenboven toonden de resultaten van de 'tibial tuberosity advancement'-studie aan dat de pre-operatieve toediening van tepoxaline de post-operatieve analgesie verbetert wanneer het gecombineerd wordt met epidurale methadon/ropivacaïne 0.75% en was er een trend tot betere post-operatieve pijnbestrijding wanneer pre-operatief toegediende tepoxaline werd gecombineerd met post-operatieve toediening van buprenorphine bij honden met GVGB die imbricatie chirurgie ondergingen.

De cardiovasculaire effecten van de epidurale toediening van een fysiologische zoutoplossing (placebo), methadon 1% (0.1 mg kg^{-1}), ropivacaïne 0.75% (1.65 mg kg^{-1}) en de combinatie van de laatste twee (zelfde doseringen) werden verder bestudeerd aan de hand van een cross-over studie bij 6 experimentele honden in **hoofdstuk 3**. Hiervoor werd de lithium dilutie en 'pulse contour' analyse methode gebruikt om een correcte inschatting te krijgen van de cardiovasculaire veranderingen. Na inductie van de anesthesie bij niet-gepremediceerde honden, plaatsing van alle noodzakelijke monitoring en catheters en een stabilisatieperiode

van 30 minuten, werd de epidurale behandeling toegediend (T0). De systemische arteriële bloeddrukken , arteriële zuurstofsaturatie, oesophagale temperatuur, hartfrequentie en centraal veneuze druk werden eerst genoteerd op T-15 (15 minuten voor toediening van de epidurale) en vervolgens op tijdstip T0 en iedere 5 minuten gedurende 60 minuten. De uitgangswaarden voor de bloedgasparameters en het hartdebiet werden bekomen op tijdstip T-15 en deze parameters werden vervolgens genoteerd op tijdstippen T0, T15, T30, T45 and T60. Het slagvolume, de systemisch vasculaire weerstand, arteriële zuurstof inhoud en het zuurstoftransport werden berekend op dezelfde tijdstippen.

Na de epidurale toediening van methadon, ropivacaïne 0.75% of hun combinatie trad er ten opzichte van de placebo behandeling een significante daling op van de gemiddelde hartfrequentie genomen over alle tijdspunten heen. Deze hartfrequentie was tevens significant lager na de epidurale combinatie toediening vergeleken met de epidurale methadon toediening. De systemische arteriële bloeddrukken waren significant lager wanneer ropivacaïne 0.75% werd geïncorporeerd in de epidurale behandeling (zowel afzonderlijk als in de combinatie met methadon) vergeleken met de andere behandelingen. Bovendien was de gemiddelde bloeddruk lager dan 60 mm Hg (de lage limiet voor gemiddelde bloeddruk in klinische condities) wanneer ropivacaïne 0.75% werd gebruikt. Er waren geen significante verschillen over alle tijdspunten heen voor centraal veneuze druk, hartdebiet, slagvolume, systemisch vasculaire weerstand, arteriële zuurstof inhoud en zuurstoftransport. De hartfrequentie en de systemische arteriële bloeddrukken stegen significant volgend op epidurale placebo en methadon toediening vergeleken met de uitgangswaarden. In alle behandelingen werd een daling waargenomen van de oesophagale temperatuur, hematocriet en de arteriële zuurstofinhoud ten opzichte van de uitgangswaarden. Als belangrijkste conclusie gerelateerd aan de epidurale toediening van ropivacaïne 0.75% werd de klinisch belangrijke lage bloeddruk weerhouden. De epidurale toediening van methadon zonder ropivacaïne 0.75% was niet geassocieerd met klinisch belangrijke cardiovasculaire veranderingen bij de hond (**hoofdstuk 2 en 3**).

In **hoofdstuk 4** werd het effect van de toediening van hydroxyethylstarch 6% (200 kDa/ 0.5) op de cardiovasculaire veranderingen geïnduceerd door de epidurale toediening van ropivacaïne 0.75% (1.65 mg kg^{-1}) bij geanestheseerde experimentele honden verder onderzocht door middel van een cross-over studie, waarin de epidurale injectie wel

(behandeling HESR) of niet (behandeling R) werd voorafgegaan door de hydroxyethylstarch preload (7 mL kg^{-1} toegediend over 30 minuten voorafgaand aan de epidurale injectie). De cardiovasculaire uitgangswaarden werden bekomen op tijdstip T-5. De hartfrequentie, systemische arteriële bloeddrukken, hartdebiet ('pulse contour' analyse) en afgeleide parameters werden vervolgens iedere 5 minuten genoteerd gedurende 60 minuten. De statistische analyse van deze studie werd slechts uitgevoerd op 5 honden, omwille van de onverwachte dood (gerelateerd aan het studieprotocol) van één hond in het HESR protocol.

Een klinisch relevante daling van de gemiddelde bloeddruk (< 60 mmHg) gedurende 20 en 40 minuten na de epidurale toediening van ropivacaïne 0.75% werd opgemerkt tijdens respectievelijke behandelingen HESR en R. Verder kon er een significante daling van de diastolische bloeddruk worden aangetoond gedurende de eerste 20 minuten volgend op epidurale ropivacaïne 0.75% toediening in behandeling HESR. Alhoewel de preload de bloeddrukdaling niet kon verhinderen, suggereren deze resultaten dat de bloeddrukdaling zich sneller normaliseert na een bolus HES.

Wanneer gekeken werd naar alle data bekomen in **hoofdstukken 2, 3 en 4** kon geconcludeerd worden dat de bloeddrukdaling veroorzaakt door epiduraal toegediende ropivacaïne 0.75% minder uitgesproken is wanneer een isofluraansparend effect mogelijk is door een efficiënte premedicatie onder klinische omstandigheden.

Een unilateraal syndroom van Horner van voorbijgaande aard werd beschreven bij twee proefhonden (rechts na behandeling R; links na behandeling HESR) in **hoofdstukken 3 en 4**. Er was een causaal verband tussen de epidurale toediening van ropivacaïne 0.75% en het HS, omdat de symptomen simultaan verdwenen met de uitwerking van de motorische blokkade veroorzaakt door ropivacaïne 0.75%. De mogelijke verklaringen voor het ontstaan van dit HS werden toegelicht in **hoofdstuk 5** en dit onder de vorm van een casusbespreking. Hoogstwaarschijnlijk lagen anatomische veranderingen (fibrose/ adhesies of bloedingen) in de epidurale ruimte, ten gevolge van multipele epidurale injecties, aan de basis van een unilaterale verdere craniale spreiding van ropivacaïne 0.75% bij de honden van onze studies, wat het Horner syndroom kon veroorzaken. Deze hypothese werd versterkt doordat in de klinische studie (**hoofdstuk 2)** geen enkele van de 16 honden die ropivacaïne 0.75% epiduraal kregen toegediend symptomen van Horner syndroom vertoonde.

Tijdens behandeling HESR **(hoofdstuk 4)** trad bij één hond een zeer ernstige bloeddrukdaling op (gemiddelde bloeddruk < 40 mmHg gedurende 20 minuten) onmiddellijk na de epidurale toediening van ropivacaïne 0.75%. Ondanks de snelle vermindering van de anesthesiediepte en het herstel van de gemiddelde bloeddruk met behulp van een aangepaste inotrope therapie, kon dit dier niet meer zelfstandig ademen. Na reanimatie en stabilisatiepogingen gedurende 10 uur werd de hond geëuthanaseerd en gelijkschouwd. Een accidentele intrathecale injectie van ropivacaïne 0.75%, waardoor een anesthesie van de hersenstam geïnduceerd werd, weerhouden als de meest waarschijnlijke oorzaak van deze fatale complicatie.

Als algemene klinische conclusie kon gesteld worden dat voor kniechirurgie bij de hond de totale analgesie verkregen door de multimodale analgetische combinatie van orale tepoxaline met epidurale methadon/ropivacaïne 0.75% superieur was aan de andere onderzochte analgetische combinaties voor kniechirurgie bij de hond. Desalniettemin kan de epidurale toediening van ropivacaïne 0.75% gedurende isofluraan anesthesie leiden tot een belangrijke bloeddrukdaling en Horner syndroom. De noodzaak om te aspireren vanaf de naald alvorens de epidurale injectie toe te dienen moet benadrukt worden, omdat een onvrijwillige intrathecale overdosis van ropivacaïne 0.75% potentieel fataal is. Wanneer geen pre-operatieve tepoxaline werd toegediend, resulteerde de epidurale toediening van methadon in een goede post-operatieve analgesie vergelijkbaar met deze na epidurale toediening van methadon/ropivacaïne 0.75%. Bovendien was de epidurale toediening van methadon veilig.

Curriculum Vitae

Curriculum Vitae

Tim Bosmans werd geboren op 18 juni 1977 te Brasschaat. Na het behalen van het diploma secundair onderwijs, richting Wiskunde-Wetenschappen aan het Gemeentelijk Instituut Brasschaat, startte hij in 1995 met de studies Diergeneeskunde aan het RUCA. In 2002 behaalde hij met onderscheiding het diploma van dierenarts aan de UGent.

In het daaropvolgende jaar werkte hij als zelfstandig dierenarts ad interim in verschillende praktijken, maar bleef verbonden met de Vakgroep Geneeskunde en Klinische Biologie van de Kleine Huisdieren door op vrijwillige basis één dag per week mee te lopen op de dienst anesthesie. In augustus 2003 werd hij 50% praktijkassistent aan dezelfde dienst. Vanaf oktober 2004 werd hij voor de overige 50% wetenschappelijk medewerker. In februari 2007 werd hij vervolgens in dienst genomen als voltijds assistent. Sinds oktober 2010 volgt hij ook het residency programma van de European College of Veterinary Anaesthesia and Analgesia, met als doel het behalen van de titel van Europees specialist in dit vakgebied.

Tim Bosmans is auteur of mede-auteur van 30 publicaties in nationale en internationale tijdschriften en nam deel aan verschillende nationale en internationale congressen. Hij heeft verschillende post-universitaire bijscholingen voor practici en vakdierenartsen gegeven en werd reeds verscheidene malen als gastspreker op nationale en internationale symposia uitgenodigd. Hij wordt op regelmatige basis gevraagd als reviewer voor internationale diergeneeskundige tijdschriften.

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Dankwoord

Dankwoord

"Verspil vooral geen tijd, want voor je het weet is het tijd om je doctoraat te verdedigen." Dat is zowat één van de eerste dingen die Professor Gasthuys tegen me zei toen we indertijd samenkwamen om het onderwerp van mijn doctoraat te bespreken. En gelijk had hij, de tijd is werkelijk voorbij gevlogen en mijn doctoraat loopt op zijn einde. Graag had ik dan ook een aantal mensen willen bedanken voor hun bijdrage aan dit werk.

Als laatstejaarsstudent, nu reeds tien jaar geleden, heb ik voor de eerste keer een doctoraatsverdediging bijgewoond. Het was de verdediging van Ingeborgh, de toenmalige promotor van mijn scriptie. Achteraf vroeg ik een kopie van haar doctoraatsboekje, omdat ik toen reeds geïnteresseerd was in alles wat met anesthesie te maken had. Op het eerste blad schreef ze "Ik hoop dat je het nog ver brengt." Het is pas onlangs dat ik deze zin teruggelezen heb en ik moet zeggen dat er een spontane glimlach verscheen, want mijn droom om in de anesthesie verder uit te groeien is toch al wel op goede weg. Ingeborgh, dankjewel voor het vertrouwen en alle kansen die je me tot hiertoe in de anesthesie hebt gegeven, voor de hulp met het doctoraat en voor het luisterende oor wanneer ik een keertje moest ventileren.

Ook mijn co-promotor, Professor Frank Gasthuys wil ik graag bedanken voor de tijd en moeite die hij in mijn doctoraatsonderzoek heeft gestoken. Vooral uw supersnelle verbeteringen van de artikels (dikwijls enkele uren na het doormailen) zullen mij altijd bijblijven. Ik apprecieer het ook heel erg dat u de moeite deed om 's avonds nog langs te komen toen ik bij Vita (de hond met de fatale complicatie) moederziel alleen bij de ventilator zat te hopen op een spectaculair herstel, helaas. Ook erg bedankt voor het vertrouwen dat u in me stelt als resident.

Stijn, "de slimste mens ter wereld" (misschien moeten we je eens inschrijven voor de gelijknamige quiz), bedankt voor alle hulp bij het op touw zetten en bij de uitvoering van de experimentele proeven, voor jouw kritische commentaar op publicaties en mijn thesis in zijn geheel en vooral in het vertrouwen in mij als jouw resident.

Professor Claerebout, bedankt voor het in goede banen leiden van de laatste stappen van mijn doctoraat.

Hilde, bedankt om in mijn leescommissie te willen zetelen, voor de nuttige commentaren en ook voor het vertrouwen dat je altijd in mij hebt gehad, reeds vanaf mijn eerste stappen in de praktijk. Het bewijst dat chirurgen en anesthesisten toch vriendschappelijk kunnen overeenkomen.

Professor Moens, het is altijd een plezier om u te ontmoeten op AVA-meetings en ik ben vereerd dat u in mijn leescommissie wou zetelen. Hartelijk dank om vanuit Wenen naar hier te willen afzakken om mijn openbare verdediging bij te wonen.

Bernadette, ik mag van jou niet langer Professor Van Ryssen zeggen, eerst en vooral bedankt voor het aanleveren van de patiënten voor mijn klinische onderzoeken. Ook bedankt voor de bemoedigende woorden over mijn onderzoekswerk en de aangename samenwerking.

Geert, ik ben altijd content als ik nog eens een keertje Antwerps kan klappen met u. Bedankt voor je deskundige TTA en imbricatie chirurgieën, input in mijn doctoraat en voor de collegialiteit en vriendschap.

Antwerps klappen, dat kan ik natuurlijk ook met mijn eigenste collega Tim, al moet ik toegeven dat we het beide een beetje beginnen af te leren nu we al enkele jaren Oost-Vlaming geworden zijn (not!). Tim, bedankt voor de hulp met mijn doctoraat, zowel praktisch bij de TTA-studie als inhoudelijk. Ook hartelijk dank voor je input als lid van de examencommissie en voor het beantwoorden van al mijn praktische vragen gerelateerd aan het regelen van de rompslomp rond de verdediging. Ik heb altijd zeer graag met jou samengewerkt en ik wens je zeer veel succes in je toekomstige job. We gaan je hier zonder twijfel allemaal erg missen.

Dr. Foubert, bedankt voor uw enthousiaste positieve reactie op de vraag of u deel wou uitmaken van de examencommissie van dit doctoraat. Ik apprecieer ten zeerste de tijd en het werk dat u stak in het nalezen van mijn doctoraat en ben vereerd dat u aanwezig wou zijn op de verdediging. Na mijn studies heb ik altijd een klein beetje spijt gehad dat ik geen humane geneeskunde gestudeerd heb, omdat jullie anesthesie (en zeker uw vakgebied, cardio-anesthesie) nog veel meer mogelijkheden biedt dan de diergeneeskundige anesthesie, alhoewel ik de waaier aan diersoorten waarschijnlijk wel zou missen.

Naast de leden van de examencommissie, mag ik natuurlijk alle andere mensen die hebben bijgedragen tot het welslagen van mijn doctoraat niet vergeten te bedanken.

Zonder statistiek zijn er geen resultaten om te bespreken. Professor Duchateau, hartelijk dank voor uw deskundige analyse van de gegevens en voor de hulp bij de interpretatie ervan.

Mijn collega anesthesisten wil ik allemaal bedanken voor de vriendschap en de jarenlange aangename samenwerking. Koen, bedankt voor de uitgebreide hulp bij de TTA-

studie. Iemand moest er immers voor zorgen dat ik volledig geblindeerd was voor de behandelingen. Het overtuigen van de eigenaars om te participeren in de TTA-studie heeft je ongetwijfeld veel werk bezorgd. Miguel, thanks for helping me out with the experimental studies on the Beagles, it is much appreciated my friend. De andere anesthesisten: Caroline, Sanne, Barbara, Sofie, Inge, Annika en Mariella hebben allemaal op hun manier bijgedragen tot het welslagen van mijn doctoraat. Dit ging van overnemen van wachten, over bemoedigende woorden tot het verrichten van extra kliniekwerk wanneer ik met mijn onderzoeken of doctoraatsvoorbereiding bezig was. Hartelijk dank hiervoor.

De ZAP-leden van onze vakgroep wil ik speciaal bedanken omdat ze me de kans hebben gegeven om een residency in de anesthesie te kunnen starten, gelijktijdig met mijn doctoraatsvoorbereidingen. De andere leden van onze vakgroep en ook van de andere vakgroepen waarmee ik nauw samenwerk wil ik vooral bedanken voor de jarenlange aangename samenwerking en vriendschap en voor hun begrijpende en troostende woorden na het verlies van Vita. Ook bedankt voor de schouderklopjes tijdens de laatste loodjes van dit doctoraat. De interns, Peggy en de studenten wil ik speciaal bedanken voor het noteren van de baseline waarden gedurende de avonden dat de TTA honden gehospitaliseerd waren in afwachting van de operatie de volgende ochtend. Alle mensen die er dagelijks voor zorgen dat de Beagles goed verzorgd worden verdienen uiteraard ook een pluim. Maarten, bedankt voor je expertise met de drukplaat (spijtig genoeg hebben we de data niet kunnen gebruiken in mijn doctoraat, maar je hebt er dan toch een eigen publicatie uitgehaald). Piet, Delphine, Yves, Eva, Lynn, Evelien, Hanna, Seppe en David wil ik graag bedanken voor hun medewerking met de twee klinische studies. Stijn, bedankt om er altijd voor te zorgen dat ik ’s morgens onmiddellijk kon beginnen met de TTA-anesthesieën, zodat ik niet tot een stuk in de nacht op de faculteit moest blijven. Marjan, bedankt voor het maken van de histologische coupes, die resulteerden in de mooie figuren. De medische beeldvormers wil ik bedanken voor hun hulp met de sporadische contrastradiografieën wanneer de epidurales moeilijk te plaatsen waren en voor de kosteloze MRI van Vita’s hersenen.

Bart en Ilse, bedankt voor jullie onvoorwaardelijke steun, zowel vriendschappelijk als daadwerkelijk (onder andere het meermaals opvangen van de kindjes). Een mens kan maar geluk hebben om zo’n toffe buren/vrienden te hebben.

Kurt, maat, bedankt voor jouw onvoorwaardelijke vriendschap. Veel woorden moet ik hier niet aan vuil maken, want je weet wel wat ik bedoel.

Dankwoord

Nathalie, Frederic, Liesbeth, Ann, Kim, Karen, Sharon, Vanessa, Marijke, Kris, Mathieu en Nathalie, David, Caroline, Yves en Ils, Ilse, Erika en Bert bedankt voor de leuke tijd die we hadden tijdens onze studies of tijdens het werk. Ik ben blij dat we elkaar na al die jaren toch nog regelmatig ontmoeten.

Ongetwijfeld zal ik wel iemand vergeten zijn in dit dankwoord. Mocht dit het geval zijn dan is dit zeker niet met opzet gebeurd.

Graag wil ik naast al deze mensen ook nog mijn familie bedanken. Eerst en vooral ben ik mijn ouders uiterst dankbaar dat ze mij destijds de kans hebben gegeven om te studeren voor dierenarts. Ik kan echt zeggen dat ik een zorgeloze jeugd gehad heb en ik hou dan ook heel mooie en leuke herinneringen over aan mijn periode in Brasschaat. Mama en papa, bedankt voor alles!!! Mijn kleine, grote broer moet ik natuurlijk ook bedanken. Bro, we hebben tot op de dag van vandaag een zeer goede band en ik ben zeker dat dit in de toekomst zo blijft. Bedankt om in mij te geloven. Katrien, bedankt voor je interesse in mijn werk. Veel succes met je nieuwe job in het Antwerpse. Verder wens ik jou en mijn broer nog heel veel succes met de verbouwingen in jullie huis, het zal er zeker fantastisch uitzien wanneer alles af is. Bovendien is het veel te groot om alleen in te wonen en Noa en Finn zouden wel graag een neefje en/of nichtje hebben (hint, hint). Sarah, bedankt voor de vele keren dat je op de kindjes hebt gelet, zodat ik in de laatste weken voor mijn doctoraatsverdediging nog wat kon nalezen. Ik plaag je veel, maar ik zie je wel heel graag hoor. Fernand en Vera, bedankt voor de interesse die jullie altijd voor mijn werk getoond hebben en voor de bezoekjes en lekkere etentjes, die zorgden voor voldoende afleiding.

Tenslotte blijven er nog drie mensen over die alles voor mij betekenen en die ervoor zorgen dat al de rest eigenlijk relatief onbelangrijk is. Cristine, Noa en Finn, ik had me geen beter gezinnetje kunnen voorstellen. Bedankt dat jullie er voor me zijn en dat jullie me de kans gaven om dit te bereiken. Noa, je wordt al een flinke kerel en eerlijkheid, zachtheid en leergierigheid zijn je beste troeven. Ik ben dan ook heel trots op je en ben zeker dat dit zo zal blijven. Finneke, mijn klein beerke, even lief als zijn grote broer, maar een klein beetje koppiger. Een groot bazeke in een klein lichaampje dus. Ik ben blij dat je zo graag naar school gaat en vind het fantastisch om je zo snel te zien evolueren de laatste tijd (hij kan zelfs al ‘doctoraat’ zeggen). Je super schattig glimlachje heeft al vele harten doen smelten. Ik kan alleen maar trots op je zijn. Cristine, een doctoraat maken kost tijd en die tijd moest ergens vandaan komen. Ik heb je dan ook dikwijls alleen gelaten met de gasten omdat ik nog bezig

was met mijn onderzoek. Bedankt voor je geduld en begrip hiervoor. Je weet dat ik er nog niet ben en dat er ons nog enkele moeilijke jaren, qua studeren dan, te wachten staan, maar ik beloof je dat ik dat later goed zal maken. Ik zie je graag!