DISSERTATION

EVALUATION OF POTENTIAL ANALGESIC DRUGS USING NEW MODELS TO STUDY PAIN IN DOGS AND CATS

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ABSTRACT

EVALUATION OF POTENTIAL ANALGESIC DRUGS USING NEW MODELS TO STUDY PAIN IN DOGS AND CATS

Pain remains an important health issue in both humans and animals. To improve the management of pain and understand the underlying mechanisms, animal models of pain have been generated over the past few decades. This dissertation presents two new models of acute pain developed to evaluate drug effects on nociceptive responses in cats and dogs. The first model determines the MAC sparing effect of an agent during visceral noxious stimulus of the ovary and ovarian ligament in the anesthetized cat. This technique was developed for dogs and modified subsequently to investigate the anesthetic sparing effect of different drugs in cats. The second method evaluates the efficacy of analgesic medications in conscious dogs using nociceptive threshold testing devices. One thermal and two mechanical nociceptive threshold testing devices were utilized to evaluate the antinociceptive effect of different drugs such as buprenorphine in dogs.

Both models are promising to test the analgesic effect of different drugs. Maropitant, NK-1 antagonist, reduced significantly the anesthetic requirements during the ovary and ovarian ligament stimulation in cats. This indicates that maropitant may have the antinociceptive properties encouraging and supporting further investigation of this agent in clinical trials. Orotransmucosal buprenorphine increased thermal and mechanical nociceptive thresholds in dogs using the three testing devices. These findings show potential of the OTM route as an alternative administration of buprenorphine for pain treatment in dogs.

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TABLE OF CONTENTS

CHAPTER 1: PAIN AND PAIN NEUROPHYSIOLOGY

Introduction

Pain is defined by International association for the study of pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain mechanisms serve as a natural protective function of organisms against noxious stimuli by changing the physiology and behavior to reduce or avoid further damage, and promote recovery. People with a loss of pain function appear to have recurrent injuries such as burns, repeat fractures, and self-injuries (Ma & Turner 2012). Many of them do not survive childhood because without feeling pain they cannot learn self-awareness necessity to avoid danger. Additional pain terminology is provided in **Table 1.1**.

In humans the pain experience consists of three dimensions: sensory- discriminative, motivational-affective and cognitive-evaluative (Melzack & Casey 1968). The sensory discriminative aspect provides information about the noxious stimulus. The motivational-affective dimension conveys the unpleasant nature of the experience and triggers responses to escape the unpleasantness. The cognitive-evaluative component summarizes the effects of social values, prior experience, and conditioning. This last dimension relies on self-reporting; hence in non-verbal subjects, such as animals, it is debated as to whether the pain experience has a cognitive-evaluative component.

Table 1.1: Pain terminology defined by International association for the study of pain (http://www.iasp-pain.org)

Terminology	Definition
Pain	An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.
Allodynia	Pain due to a stimulus that does not normally provoke pain.
Hyperalgesia	Increased pain from a stimulus that normally provokes pain.
Neuropathic	Pain caused by a lesion or disease of the somatosensory nervous system.
Nociceptive	Pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors.
Pain threshold	The minimum intensity of a stimulus that is perceived as painful.
Sensitization	Increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally subthreshold inputs.
Central sensitization	Increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input.
Peripheral sensitization	Increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields.

Neurophysiology and pathways of pain

Nociception or the normal processing of nociceptive stimuli involves detection and transmission of noxious information from the peripheral to the central nervous system. It is composed of four components: transduction, transmission, modulation, and perception. During transduction, a noxious stimulus (mechanical, thermal or chemical) is converted into an electrical impulse which is propagated through nerve fibers (mostly Aδ or C fibers) of the first-order neurons leading from the peripheral nociceptors to the spinal cord. Modulation takes place mainly in the dorsal horn of the spinal cord where the first-order neurons synapse with second-order neurons. Nociceptive input can be amplified or attenuated at this site by a number of neuropeptides released from neighboring neurons and descending pathways. Second-order neurons project from here to third-order neurons in supraspinal structures in the ascending pathway of the spinal cord. The third-order neurons link to the cerebral cortex where further processing results in perception.

Peripheral pathways

Nociceptors are naked nerve endings of first-order afferent neurons that have cell-bodies in dorsal root ganglions. They are distributed broadly in skin and deep tissues. Some nociceptors are activated by a specific type of noxious stimulus such as mechanical, thermal, or chemical while most of them are polymodal or activated by multiple types of noxious stimuli. Nociceptors have a threshold of activation, and respond progressively according to the intensity of the stimulus to generate action potentials which are conducted along nerve fibers to the dorsal horn of the spinal cord.

According to pain concepts in most textbooks, thinly myelinated Aδ fibers and unmyelinated C fibers are two types of sensory fibers conducting most of the nociceptive signals to the dorsal horn while the large myelinated Aβ fibers transmit other sensory information to the central nervous system. Aδ fibers are associated with sharp and pricking pain, rapidly conduct impulse (5-20 m/s). C fibers are associated with dull, burning pain and slowly conduct impulse (0.5-1 m/s). Both fiber types innervate skin (associated with superficial pain) and deep somatic/visceral structures (associated with deep pain) but in different ratios. The ratio of Aδ to C fibers is 1:1 to 1:2 in cutaneous nerves, and 1:8-10 in visceral nerves (Dugdale 2010). However, evidences reviewed by Djouhri and Lawson in 2004 indicate the existence of Aβ nociceptors. A population of somatic afferent A-fiber nociceptors in guinea pig, mouse, rat, cat and monkey conducts impulse in the Aβ conduction velocity range (Djouhri & Lawson 2004). Hence it is possible that some of Aβ afferents may play a role in transmitting the somatic nociceptive signals.

Nevertheless, a substantial population of Aβ fibers that conduct signals of non-nociceptive sensory to the central nervous system may also have a significant role in pain circuitry as proposed in the Gate control theory by Melzack and Wall in 1962. The idea of the gate control theory is that noxious input is modulated by both noxious and non-noxious stimuli at the level of the spinal cord. The theory suggests that firing of the Aβ non-nociceptive fibers by non-noxious stimuli activates the inhibitory interneurons which may inhibit the activity of projection neurons (postsynaptic inhibition) or reduce the release of neurotransmitter from the nociceptive fiber (presynaptic inhibition) (Hellyer et al. 2007). As a result, nociceptive transmission is interrupted and this information cannot be sent to the central nervous system (Melzack & Wall 1965).

Peripheral sensitization

Under normal conditions, pain caused by an acute stimulus dissipates rapidly. Under conditions where the stimulus may be ongoing, inflammatory mediators released from damaged cells and injured tissue can sensitize nociceptors. These sensitized nociceptors evoke a stronger response to any given stimulus than in normal state and their thresholds may be reduced such that even innocuous stimuli can activate them. Additionally silent nociceptors, which are not activated in normal state now respond to noxious stimuli. Hence these processes collectively term result in the two clinically relevant conditions of hyperalgesia and allodynia.

Molecular mechanisms of nociceptor activation

Nociceptors use signal-transduction mechanisms to control excitability and sensitization of primary sensory neurons. Noxious stimuli physically, chemically, or thermally stimulate the sensory nerve ending which causes the opening of ion channels to allow the influx of cations which produce depolarization. If this depolarization is strong enough the voltage-gated Na^+

channels will open to trigger an action potential and recruit neighboring Na^+ channels to conduct the pain signals along the axons of the neurons. While Na^+ channels are necessary for the action potential generation and conduction, K^+ and Ca^{2+} assist in controlling the excitability of neurons. Other important receptors, such as transient receptor potential (TRP) receptors and acid-sensing ion channels, are involved in processing information in the periphery. Inflammatory mediators such as bradykinin and prostaglandin also play an important role in signal transduction.

Voltage-gated sodium channels

Na^+ channels including $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are expressed exclusively in nociceptive neurons. $Na_v1.7$ is blocked by tetrodotoxin (TTX-sensitive (S)), while $Na_v1.8$ and $Na_v1.9$ are tetrodotoxin resistant (TTX-R). Both TTX-S and TTX-R sodium channels are believed to be essential for the generation and conduction of action potentials and nociceptive processing based on preclinical studies (Baker & Wood 2001). A loss of function or mutation of $Na_v1.7$ results in insensitivity to pain (Cox et al. 2006). Additional compelling evidence supports the relationship between pain and TTX-S and TTX-R Na^+ channels, i.e, knock-out of the TTX-S or TTX-R channels in rodents attenuates hypersensitivity and hyperalgesia following nerve injuries and inflammation (Amaya et al. 2006; Gold 2008).

TRP receptors

Transient receptor potential cation channel vanilloid subfamily V member 1 (TRPV1) is a permeable, nonselective cation channel that is found in both neuronal, such as brain tissue and dorsal root ganglia, and nonneuronal tissues, such as skin and urinary bladder (Hayes et al. 2000; Immke & Gavva 2006). This receptor is believed to serve as an integrator of multiple noxious stimuli, including capsaicin, heat, acid, products of lipoxygenase, anandamide, and polyamines (Cortright & Szallasi 2004; Van Der Stelt & Di Marzo 2004; Ahern et al. 2006; Wong & Gavva 2009). The receptor opens in response to noxious thermal and chemical stimuli (Caterina et al. 1997; Tominaga et al. 1998). In addition, many inflammatory mediators, such as bradykinin, extracellular ATP, prostaglandins, nerve growth factor, glutamate, and activated phospholipase C have been reported to be able to modulate the activity of TRPV1 (Premkumar & Ahern 2000; Lee et al. 2005; Immke & Gavva 2006). Various TRPV1 antagonists have been shown to reverse nociceptive responses in rodents with inflammatory conditions such as those associated with complete Freund adjuvant-induced thermal or mechanical hyperalgesia at the plantar surface of the hind paw (Honore et al. 2005; Immke & Gavva 2006).

Other TRP members that may be involved in nociceptive transduction include TRPV2 (TRP vanilloid 2), TRPV4 (TRP vanilloid 4), TRPA1 (ankyrin 1) and TRPM8 (melastatin 8) (Schaible et al. 2011). TRPA1 and TRPM8 are candidates involved in mechanisms of cold nociception (Peier et al. 2002; Reid 2005). TRPV2 is likely to be involved in thermal nociception because it is activated at temperatures higher than 52 °C (Tominaga & Caterina 2004). TRPV 4 function may be related to the transduction of mechanical stimuli (Zhang et al. 2008; Alessandri-Haber et al. 2009).

Acid-sensing ion channels

Acid-sensing ion channels (ASICs) belong to the epithelial sodium channel (ENaC)/degenerin (DEG) superfamily of ion channels. They are depolarizing cationic channels with high Na^+ permeability following the stimulation of low extracellular pH. ASICs are found in sensory neurons of dorsal root ganglia, nociceptive fibers, as well as in vagal and trigeminal ganglia of central nervous system supporting a role in detection of pain of the channels. Several subunits of ASICs have been reported, including ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4 (Deval et al. 2010). ASIC1a and ASIC3 are targets of interest for pain treatment. ASIC3 appears to be involved in development of somatic inflammatory pain and visceral pain in both heart and gastrointestinal tract (Immke & McCleskey 2001). ASIC1a may play a role in central sensitization of second-order sensory neurons and be involved inhibition on the endogenous enkephalin pathway (Deval et al. 2010). Thus in addition to peripheral sensitization, ASICs may be potential targets for management of central sensitization.

Purinergic ion channels and ATP

Purinergic (P) receptors containing the P2X3 subunit (P2X3 homotrimeric and P2X2/3 heterotrimeric) are members of the P2X family of ATP-gated ion channels. These receptors are non-selective cation channels localized on Aδ and C fibers, and in both peripheral and central terminals of the sensory neurons in the dorsal root and cranial sensory ganglia. ATP forms a ligand for these receptors and is released from various cells as a consequence of tissue injury (Ford 2012). A high concentration of ATP is also released from the terminals of primary afferent neurons following nociceptive stimulus (Wirkner et al. 2007). P2X3 and P2X2/3 are, therefore, believed to participate in nociceptive signaling. This assumption is also supported by genetic and pharmacological studies. P2X3 gene disruption results in a hypoalgesic phenotype in rodents; in agreement, P2X3 receptor antagonists in animal models of pathological pain have provided reduction of pain behavior (Jarvis 2003; Ford 2012).

Neuropeptides

Neurogenic inflammation encompasses a series of inflammatory responses activated by neuropeptides, such as tachykinin peptides and the calcitonin gene related peptide (CGRP), which are mainly released from endings of primary sensory neurons in response to noxious stimulation.

Tachykinin peptides: The tachykinin family of peptides are expressed in the nervous system and in many organs. The most notable tachykinin is substance P, which is postulated to be involved in sensitivity of pain. Substance P is synthesized in small sensory afferents and released in response to noxious thermal, mechanical and chemical stimuli (Duggan et al. 1988). This tachykinin acts by binding to neurokinin-1 (NK-1) receptors which are expressed in both peripheral and central terminals of primary afferent neurons, dorsal root ganglion neurons, trigeminal ganglion neurons, and throughout the brain (Maeno et al. 1993). To date, NK-1 receptors are believed to play an important role in central sensitization of the spinal cord, but may not be necessary for acute nociceptive transmission (De Felipe et al. 1998).

Calcitonin gene related peptide (CGRP) is a neuropeptide released from peripheral and central neurons in response to inflammation. It is a peptide vasodilator that may play a role in pain transmission (Benemei et al. 2009). At periphery CGRP causes vasodilation and smooth muscle relaxation, as well as it is involved in migraine pathogenesis in the central nervous system (Benemei et al. 2009). In the dorsal horn of the spinal cord CGRP facilitates evoked activity (Biella et al. 1991). In CGRP knockout mice secondary hyperalgesia did not develop secondary to joint inflammation (Zhang et al. 2001).

Inhibitory peptides

Endogenous inhibitory peptides demonstrated in dorsal root ganglia and in peripheral sensory neurons include peripheral opioids (Stein et al. 2009), somatostatin, and cannabinoids. These peptides act on their specific receptors in sensory neurons to produce antinociception. Somatostatin neurotransmitter is distributed throughout the body and also localized in the dorsal root ganglion cells. Somatostatin receptors are believed to maintain a tonic inhibitory control over nociceptors and activation inhibits pain responses in both humans and animals. (Carlton et al. 2001a; Carlton et al. 2001b). The endocannabinoid system has been found to function as an antinociceptive system. Endogenous cannabinoids and cannabinoid agonists diminish responses to noxious stimuli via CB1 and CB2 G_i-protein coupled receptors (Malan et al. 2001; Pertwee 2001).

Inflammatory mediators

Inflammation of peripheral tissue and nerves can induce peripheral sensitization as mentioned previously. Many inflammatory mediators (i.e., bradykinin, prostaglandins), cytokines (interleukins), and neurotrophins (nerve growth factor) are involved in mechanisms behind the sensitization. These mediators are released during the inflammation and act on their receptors in nociceptive neurons to enhance the neuronal activity.

Prostaglandins and bradykinin are marked sensitizers of nociceptors. Prostaglandin E_2 binds to G_s-protein-coupled receptor resulting in an increase of the second messenger cyclic adenosine monophosphate (cAMP) which activates protein kinase A in cells. This pathway enhances excitability of neurons by sensitizing ion channels in membrane such as TRPV1 receptors and Na^+ channels (Schaible et al. 2011). Bradykinin can activate neurons and sensitize them to mechanical and thermal stimuli to evoke an action potential even with a subthreshold stimulus (Liang et al. 2001). Bradykinin (B2) receptors are coupled to G_q-proteins which activate phospholipase C (PLC) and in turn protein kinase C (PKC) which results in sensitization of sensory ion channels (Linley et al. 2010).

Cytokines are important mediators of peripheral sensitization. In mice, intradermal interleukin (IL)-1β, keratinocyte-derived chemokine (KC), tumor necrosis factor-alpha (TNFα), IL-8, IL-12, IL-15 and IL-18 have provided intense and sustained mechanical sensitization (Stein et al. 2009). Thus these cytokines are likely to play a role in inflammatory pain.

Nerve growth factor (NGF), a neurotrophin, is produced in large amounts during inflammation. Tyrosine receptor kinase A (TrkA) is a main receptor of NGF expressed in primary afferent neurons. This NGF: 1) it increases currents through TRPV1 receptors to reduce the thermal nociceptive threshold, 2) increases expression of TRPV1, bradykinin receptors, P2X receptors, Na^+ channels, and synthesis of substance P and CGRP with long term exposure and 3) induces inflammatory mediator release from inflammatory cells (Schaible 2007; Stein et al. 2009; Schaible et al. 2011).

Central pathways

Spinal cord

Central axons of first-order neurons synapse on second-order neurons in the dorsal horn of the spinal cord. They terminate predominantly in laminae I, II, and V of the dorsal horn on projection neurons and local interneurons. Laminae I and II receive direct primary afferent input from Aδ and C fibers. Wide Dynamic Range (WDR) neurons in laminae V respond to both noxious and non-noxious stimuli which are transmitted by the Aδ, C and Aβ fibers. Consequently, WDR neurons can play a role in the segmental suppression of pain in the Gate Control Theory (Almeida et al. 2004).

Aδ and C fibers release neurotransmitters including the excitatory amino acids aspartate and glutamate as well as substance P to activate dorsal horn neurons which contain pharmacological ionotropic glutamate receptors, such as N-methyl-D aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (Dingledine et al. 1999; Traynelis et al. 2010). Glutamate binding activates these receptors to allow the flow of Na^+, K^+ and Ca^{2+} resulting in an excitatory postsynaptic current. This depolarizing may trigger an action potential; this action potential propagates the excitatory signals along the axon ascending to supraspinal structures.

Inhibitory neurons in the dorsal horn are also activated by firing of the Aδ, C and Aβ fibers. Following stimulation the inhibitory neurons release gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system to regulate nociceptive activity by interacting with GABA receptors in the projection neurons and the primary afferents. There are three classes of GABA receptors involved in the modulation: $GABA_A$, $GABA_B$ and $GABA_C$. Activation of $GABA_C$ receptors induces antinociception (Reis & Duarte 2007); however the receptors are expressed predominantly in the retina and play an important role in visual signaling (Qian & Ripps 2009). After binding to $GABA_A$ and $GABA_C$, chloride-permeable ion channels are activated to hyperpolarize neurons and to impair the propagation of excitatory signals; $GABA_B$ receptors are G protein-coupled receptors and their activation leads to an increase of K^+ conductance resulting in cell hyperpolarization (Bormann 2000; Chen et al. 2005). Activation of $GABA_A$ and probably $GABA_C$ receptors generates inhibitory postsynaptic potentials while $GABA_B$ receptors have a role in both postsynaptic and presynaptic inhibition (Zhu & Lo 1999; Yang et al. 2001; Lemke 2007; Labrakakis et al. 2009).

Central sensitization

Peripheral sensitization induced as discussed earlier, results in increased nociception input to the central nervous system. This in turn can lead to central sensitization. WDR neurons are important cells in the expression of this spinal facilitation of pain, or so-called wind up. As a consequence of the prolonged firing of primary nociceptive afferents, the increased release of glutamate neurotransmitter as well as the release of substance P and brain-derived neurotrophic factor onto the second-order neurons produces a sustained and augmented post-synaptic depolarization which activates NMDA receptors by relieving the magnesium ion (Mg^{2+}) block of NMDA in WDR neurons. These activated NMDA receptors then allow influx of Ca^{2+} and Na^{+} ions into the cells, and bring the postsynaptic membrane potential closer to threshold. Thus subsequent neurotransmitter release is more likely to produce action potentials in the postsynaptic neurons. Furthermore, calcium ions can produce long-lasting changes in the postsynaptic cells that have a lower threshold for excitation over longer periods of time. Consequently, innocuous stimuli transmitted along Aβ fiber may be interpreted as noxious and result in allodynia; excitatory signals from Aδ and C fibers may also be amplified resulting in hyperalgesia.

Recently it has been recognized that the spinal cord glial cells are activated by release of proinflammatory products, such as cytokines and chemokines, caused by peripheral inflammation and injury. These products increase neuronal excitability by activating receptors directly, upregulating the actions of excitatory amino acid receptors, or downregulating the actions of inhibitory receptors, such as GABA receptors (Watkins et al. 2007). This sensitization may outlast the stimuli that triggered it, and has been suggested as a possible causal mechanism for chronic hyperalgesia and allodynia (Kidd & Urban 2001).

Afferent nociceptive pathways of the spinal cord

Axons of second-order neurons (projection neurons) form afferent bundles to transmit the nociceptive impulses to supraspinal structures including two that are important in pain transmission and recognition namely the thalamus and reticular formation of the medulla. There are many ascending nociceptive pathways that have been described in the spinal cord. However, the spinocervicothalamic tract conveying somatic and superficial pain information, and the spinoreticular tract transmitting deep pain signals and visceral organ information are considered the most important.

The spinocervicothalamic tract is the primary pain pathway for transmission of superficial pain and tactile sensations. After receiving impulses from primary afferents, the secondary afferents in spinal cord both mediate local reflexes and project cranially via an ipsilateral tract in the lateral funiculus of the spinal cord close to the white matter. The axons synapse in the lateral cervical nucleus of spinal C1 and C2 segments. From this nucleus, nerve fibers decussate through the C1 spinal segment and caudal medulla and travel up to the thalamus from where fibers project to the somatosensory cortex. Some collaterals of the ascending fibers also terminate in the reticular formation (Hellyer et al. 2007).

The spinoreticular tract (spinoreticulothalamic) transmits deep pain and visceral sensations. The pathway begins with axons of first-order neurons entering the cord and immediately diverging to send collaterals to segments cranial and caudal to the segment entry.

This spreading of information results in coordination of multiple intra- and inter-segmental reflexes in response to the nociceptive input. Axons of projection neurons then travel diffusely in the lateral and ventral funiculi, close to the grey matter of the cord, and information ascends bilaterally throughout the spinal cord. In the brainstem most ascending projections terminate in the reticular formation, from where fibers project to multiple destinations including thalamus and limbic system. The thalamus passes the information indirectly to cerebral cortex which results in pain perception, and the limbic system resulting in evoked emotional responses to noxious stimulation. As a result of the multisynaptic and diffuse manner of the spinoreticular pathway, deep and visceral pains are always poorly localized involved (Hellyer et al. 2007).

Descending pathway

The periaqueductal grey matter (PAG) and rostral ventromedial medulla (RVM) play a role in modulation of pain (Gebhart 2004). The PAG is the grey matter located in midbrain. Following receipt nociceptive input from the dorsal horn of the spinal cord, it provides input to the hypothalamus, parabrachial nucleus (PBN), nucleus tractus solitaries (NTS) and RVM or the supraspinal structures which give rise to the descending pathways. The PAG also has connections to corticolimbic structures including the frontal cortex and amygdala (Millan 2002). RVM is a group of neurons located on the floor of medulla; in rats RVM includes the nucleus raphe magnus, nucleus reticularis gigantocellularis pars alpha, and nucleus reticularis paragigantocellularis lateralis (Fields et al. 1991). Projection of neuronal impulses from PAG to RVM is the major pathway for mediating descending inhibition. After receiving the ascending nociceptive input the PAG releases endorphins onto the nucleus raphe magnus of RVM, other medullary reticular nuclei and the dorsal horn of the spinal cord. Input from the PAG further activates monoaminergic pathways in the nucleus raphe magnus that release serotonin (5-HT) onto inhibitory interneurons in the spinal cord to inhibit nociceptive transmission (Hellyer et al. 2007). The PAG also communicates with the noradrenergic locus coeruleus which contacts the RVM and transmits descending noradrenergic inhibitory projections to the spinal cord (Ossipov et al. 2010). The RVM contains two types of cells believed to cause descending inhibition and facilitation of spinal nociceptive transmission. First, Off-cells are excited by opioids and inhibited by nociceptive input (Millan 2002). They are thought to trigger descending inhibition because decrease in firing of these cells are correlated with increasing nociceptive transmission, whereas increase in their activity results in reducing of nociception (Schaible 2007). In contrast, the second group of cells known as On-cells are inhibited by opioids and excited by nociceptive input. Hence On-cells seem to facilitate nociceptive transmission in spinal cord (Millan 2002; Schaible 2007).

Pharmacology

Peripheral targets for analgesic medications

Targets for analgesic medications include receptors, channels and mediators involved in peripheral nociceptive transduction and transmission. Long standing peripheral analgesics include non-steroidal anti-inflammatory drugs (NSAIDs), local anesthetics, and opioids. NSAIDs are used commonly to treat inflammatory pain, such as arthritis and musculoskeletal pain, but they may be used to treat neuropathic pain in some cases. The NSAIDs inhibit both peripheral and central cyclooxygenases (COX), in particular COX-2 which is responsible for the production of prostaglandins at the site of inflammation (Warner & Mitchell 2004). Although these drugs are effective at relieving pain of inflammatory origin, chronic treatment with NSAIDs could increase the risk of side effects such as gastrointestinal hemorrhage and ulcers and renal damage. Local anesthetics, such as lidocaine and bupivacaine, block Na^+ channels which are essential for the generation and conduction of action potential in processing of nociceptive transduction and transmission. These compounds are used to prevent or reduce the firing of nociceptive fibers resulting in pain relief. Opioids are another group of analgesics that may produce peripheral analgesia in addition to their central antinociceptive effects. However, the mechanisms underlying the peripheral action of opioids are still unclear (Cunha et al. 2010).

In attempts to develop novel analgesics, other promising molecules with both genetic and pharmacological properties have been investigated such as TRPV1 antagonists, NGF antagonists and selective Na channel blockers.

TRPV1 is considered as a promising target for pain modulation due to activation by a variety of noxious physical and chemical stimuli (Willis 2009) associated with inflammation processes and has resulted in the clinical development of the vanilloid class of drugs. Following peripheral inflammation, TRPV1 upregulation appears to occur at central as well as peripheral terminals of DRG neurons, leading to pre-synaptic augmentation of glutamatergic signaling in the spinal cord (Premkumar & Sikand 2008). However both TRPV1 agonists and antagonists may be able to reduce pain. Since TRPV1 is a highly Ca^{2+} permeable channel, activation by a TRPV1 agonist can induce sustained influx of Ca^{2+} resulting in desensitization and inhibition of the generation of action potential, as well as nerve terminal degeneration leading to long-lasting pain relief (Kissin 2008). On the other hand TRPV1 antagonists inhibit the receptor and prevent the generation of action potential at both the spinal and peripheral terminals. Side effects such as hyperthermia caused by TRPV1 antagonists need to be considered and avoided.

NGF, (as mentioned previously), is believed to play a role in inflammatory and neuropathic pain mechanism. Increased NGF levels have been found in animal models of inflammatory and neuropathic pain, while the NGF sequestration reduced hyperalgesia (Watson et al. 2008). Recently, a novel NGF receptor antagonist (ALE-0540) has been investigated and has shown anti-allodynic properties in rat models of neuropathic pain and thermally-induced inflammatory pain (Owolabi et al. 1999). In human clinical trials Tanezumab (RN-624), a first-in-class recombinant humanized monoclonal antibody targeting NGF, has demonstrated favorable results. Phase I and II clinical trials of Tanezumab in people with osteoarthritic pain and chronic lower back pain showed efficacy in reducing pain as well as a good safety and tolerability profile (Cattaneo 2010). Hence NGF and its receptor (TrkA) show promise as therapeutic targets in the management of chronic inflammatory pain.

$Na_v1.8$ channels (TTX-R Na^+ channel) provide an interesting target for treatment of both inflammatory and neuropathic pain. A-803467, a selective $Na_v1.8$ sodium channel blocker, reduced mechanical allodynia in a variety of rat pain models including spinal nerve ligation, sciatic nerve injury, capsaicin-induced secondary mechanical allodynia, and thermal hyperalgesia after intraplantar complete Freund's adjuvant injection (Jarvis et al. 2007). Similarly, another $Na_v1.8$ sodium channel blocker (A-887826) demonstrated positive results in a rat model of neuropathic pain. Following oral administration of A-887826, rats demonstrated a reduced behavior of tactile allodynia in the spinal nerve ligation model (Zhang et al. 2010). This early evidence supports the role of $Na_v1.8$ channels in pathological pain states of both neuropathic and inflammatory pain.

Bradykinin receptors are one of targets of interest for novel analgesics. B1 and B2 are the two types of bradykinin receptors. Both of them are coupled to G-proteins and activated by bradykinin in different states. In normal tissue B1 is dormant, while B2 can respond to bradykinin immediately and contributes to acute pain state (Rodger 2009). In inflammatory conditions, B1 is activated and stimulated by bradykinin further contributing to pain and enhancing inflammation (Millan 1999). To date there are only a small number of studies looking at pharmacologic antagonists for B1 and B2 to support a role of bradykinin antagonists in the treatment of pain. Further pharmacological investigation is needed.

Cannabinoids are inhibitory peptides that have analgesic properties via CB1 receptor in sensory neurons. Endogenous ligands for the CB receptors include arachidonylethanolamide, 2-arachidonylglycerol and palmitoylethanolamide (Rodger 2009). Recently the analgesic effect of a CB agonist, tetrahydrocannabinol (THC), has been investigated in a clinical trial. Following oral administration of THC, patients with peripheral neuropathic pain demonstrated a reduction in pain intensity scores; however, sedative and gastrointestinal side effects were observed (Nurmikko et al. 2007).

Central targets for analgesic medications

Opioids are commonly used for treatment of pain in both human beings and animals. Opioid receptors (μ, δ, κ) are G protein-coupled receptors distributed throughout the central nervous system. Activation of opioid receptors by endogenous or synthetic opioids results in closing of voltage sensitive calcium channels, K^+ efflux leading to hyperpolarization; and inhibition of adenylyl cyclase to produce cAMP. This results in reduced neuronal excitability and a reduction in transmission of nerve impulses and release of excitatory neurotransmitters (McDonald & Lambert 2008).

α_2-adrenoceptor agonists mediate their analgesic properties by mimicking endogenous norepinephrine which participates in descending inhibitory pathways. The α_2-adrenergic receptor is a G_i protein-coupled receptor located throughout both central and peripheral nervous systems. Activation of α_2-adrenergic receptors appears to interrupt the nociceptive transmission principally at the spinal cord and locus coeruleus. It inhibits the release of excitatory neurotransmitters from primary afferent terminals, as well as hyperpolarizes neurons to reduce the excitability of cells (Millan 2002).

Although opioids and α_2-adrenoceptor agonists are widely used as central analgesics, side effects (e.g., cardiopulmonary depression, sedation, gastrointestinal stasis, tolerance) of these drugs are often reported in patients. Additional novel targets for analgesics such as N-type $Ca_v2.2$ calcium channel, NMDA, NK-1, GABA, Glycine, and P2X4 receptors are being evaluated as well.

N-type $Ca_v2.2$ calcium channels: Ca^{2+} entry via voltage-gated Ca^{2+} channels into primary sensory neurons is necessary for regulating neurotransmitter release which favors the transmission of the sensory information to the central nervous system (Rodger 2009). Both preclinical and clinical data have identified that N-type $Ca_v2.2$ calcium channels as play a role in the increase of neuron excitability and release of neurotransmitters whereas reduction of $Ca_v2.2$-mediated Ca^{2+}entry produces pain relief (Winquist et al. 2005). Intrathecal administrations of selective $Ca_v2.2$ antagonists, such as omega–conotoxins, ziconotide and ct-GVIA, have shown antinociceptive properties in several preclinical models of neuropathic, postoperative and arthritis pain (Winquist et al. 2005). Nevertheless, many aspects of molecular mechanisms remain to be determined to develop $Ca_v2.2$ blocker as a new analgesic with acceptable side effects.

Presently, gabapentin and pregabalin, GABA analogues, have been shown to be effective for neuropathic pain disorders. However rather than directly working at GABA receptors it is likely that they inhibit calcium currents via voltage-gated calcium channels containing the $\alpha_2\delta$-1 subunit resulting in a reduction of neurotransmitter release and an attenuation of postsynaptic excitability (Sills 2006). Evidence supports that they have analgesic properties against diabetic neuropathy and post-herpetic neuralgia (Backonja 2002; van Seventer et al. 2006).

As stated previously NMDA receptors play a role in central sensitization (Petrenko et al. 2003). Consistent with this, NMDA antagonists such as ketamine and amantadine, exhibit analgesic properties against pathological pain in both humans and animals (Robertson 2005; Lamont 2008; Lascelles et al. 2008; Muir 2010; Prommer 2012). However, clinical use of these antagonists is limited by their side effects resulting from suppression of physiological functions of NMDA in the central nervous system (Vinuela-Fernandez et al. 2007; Holtman et al. 2008). High dose of ketamine can develop psychomimetic side effects such as hallucination, sedation, nausea, dissociative reactions, muteness, dizziness, and visual distortions in humans (Sang 2000). Following administration of high-dose continuous infusion ketamine in horses, signs of excitation including exaggerated responses to movement, light and noise were reported (Fielding et al. 2006). In an effort to minimize side effects low subanesthetic doses of ketamine were used and investigated. The results suggested that the subanesthetic doses of ketamine may produce effective analgesia in acute musculoskeletal trauma in humans (Gurnani et al. 1996) and enhance the analgesic efficacy of opioids and α-2 agonists with a reduced incidence of the side effects when used as an adjunct for postoperative analgesia in humans and dogs (Schmid et al. 1999; Wagner et al. 2002; Himmelseher & Durieux 2005; Chizh 2007). Recently studies have focused on inhibiting the binding of protein tyrosine kinase Src to the NMDA receptor. Tyrosine kinase

Src binds to the NMDA receptor at NADH dehydrogenase subunit 2 (ND2) to increase NMDA activity. Disruption of the Src and NMDA interaction prevented pain responses induced by intraplantar formalin and reversed pain hypersensitivity associated with inflammation and nerve injury without the detrimental effects (Liu et al. 2008).

γ-Aminobutyric acid (GABA) and glycine are inhibitory neurotransmitters released primarily from inhibitory interneurons in the mammalian spinal cord. Activation of GABA receptors induce hyperpolarization of neurons impairing the dendritic propagation of excitatory signals. A loss of synaptic inhibition in the spinal cord from GABA inhibitory system may develop and maintain the chronic pain condition (Zeilhofer 2008). Recent studies have found that peripheral nerve damage and inflammation induce the GABA dysfunction and cause pathological pain. Nerve damage may induce apoptosis of inhibitory interneurons that release GABA. Additionally prostaglandin E2 released during inflammation blocks the action of glycine, disrupting the inhibitory pathway and allowing excitatory postsynaptic events (Ahmadi et al. 2002). Therefore, selective GABAergic agonists seem to be promising agents for the treatment of pathological pain. They do however cause sedation, amnesia and addiction when administered for treatment of chronic pain (Knabl et al. 2008). The central nervous system depressant properties of propofol (an anesthetic) and diazepam (a benzodiazepine tranquilizer) are related to the actions on GABA receptors, but the analgesic efficacy of the drugs remains controversial (Casarrubea et al. 2012; Hasani et al. 2012).

Tricyclic antidepressants (TCAs) are recommended as one of the first-line medications of neuropathic pain in humans (Attal 2012). Mechanisms of TCAs include inhibition of presynaptic reuptake of the monoamines serotonin and norepinephrine which mediate descending modulatory pathways, and blockade of NMDA receptors and sodium channels (Sindrup et al.

2005). In randomized, controlled trials in humans TCAs (i.e., amitriptyline, imipramine and clomipramine) relieved neuropathic pain such as postherpetic neuralgia and diabetic painful polyneuropathy (Sindrup et al. 2005). The common side effects of TCAs are orthostatic hypotension, dry mouth, sweating, constipation, blurred vision, urinary retention, dizziness and sedation (Attal 2012). In veterinary patients there are no published reports using the antidepressants for pain management, however, recommended dosages of amitriptyline and imipramine are provided in dogs and cats to for example urinary bladder pain such as interstitial cystitis (Mathews 2008; Grubb 2010a).

Serotonin-norepinephrine reuptake inhibitors such as duloxetine and venlafaxine are effective to alleviate diabetic painful polyneuropathy, but cause some side effects e.g., gastrointestinal disturbances and sedation in humans (Attal 2012). These drugs may enhance the activity of serotonin and norepinephrine in the descending inhibitory pathways resulting in analgesia. Tramadol, a synthetic opioid, may be classified as a serotonin-norepinephrine reuptake inhibitor based on its ability to inhibit the reuptake of these neurotransmitters. Tramadol is commonly used in combination with traditional analgesics in veterinary medicine. Pharmacokinetics of the drug are erratic and variable across individuals and species in animals, therefore, the pain management should not be relied on tramadol alone (Grubb 2010b; Rychel 2010).

The actions of substance P are mediated by NK-1 receptor. Substance P is an important neurotransmitter in both peripheral and central pain mechanisms. The NK-1 receptor is involved in central sensitization in the spinal cord making it an interesting target for treatment of pathological pain. Intrathecal administrations of NK-1 antagonists reduced the response of dorsal horn neurons evoked by noxious stimuli and diminished the hyperalgesic state induced by persistent stimulation (Holzer-Petsche & Rordorf-Nikolic 1995). Recently a systemic NK-1 antagonist was shown to decreased the minimum alveolar concentration of sevoflurane in dogs (Boscan et al. 2011) and cats (Niyom et al. 2013). A few additional studies have focused on the antinociceptive effect of NK-1 blockers, but the primary focus for this class of drug continues to be on antiemetic effects (Diemunsch et al. 2009). This may be due to the fact that NK-1 antagonists have failed to show analgesic activity in clinical trials evaluating both acute and chronic pain conditions. Discussion has centered around the appropriateness of using NK-1 antagonists for the conditions tested in those clinical trials. Nevertheless, a big gap remains between the lack of efficacy in clinical trials and experimental evidence supporting a role for substance P in pain modulation (Cervero 2009).

Besides the promising targets for analgesics mentioned previously, purine P2X3 receptor and P2X4 receptor have also received limited attention for their role in pain processing (Rodger 2009). P2X3 receptor is found on nociceptors and is activated by ATP which is released from injured tissue. As ATP can sensitize nociceptors via P2X3 receptors, therefore blocking the receptors may reduce pain. In a state of peripheral sensitization, local release of ATP in the spinal cord stimulates microglia via P2X4 receptor resulting in the formation and release of brain-derived neurotrophic factor (BDNF) (Coull et al. 2005). BDNF acts on tyrosine kinase B (TrkB) which is located close to GABA and glycine receptors. This interaction causes outward movement of Cl^- through the GABA and glycine receptors creating the membrane depolarization and sensitization (Coull et al. 2005; Rodger 2009). Thus blocking the action of BDNF at TrkB receptor site may prevent or reduce neuropathic and inflammatory pain.

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CHAPTER 2: ANIMAL MODELS OF PAIN

Introduction

Understanding of the fundamental physiology of pain has increased vastly over the past few decades. Animal models of pain have been a vital component of this progress, and remain important for understanding of the mechanisms of pain, identifying novel pharmacological targets in pain therapy, improving pain treatments, as well as finding clinical dosing of analgesic drugs (Mogil et al. 2010). Early investigative efforts focused on animal models of acute and nociceptive pain evaluating the effects of physiological pain on healthy tissue by applying quantifiable noxious stimulus to the animal until a response is evoked. Subsequently pain models have sought to explore mechanisms of pathological pain arising from inflammation as well as neuropathic pain and that caused by disease (Mogil 2009).

A critical problem of pain assays is that pain itself is very subjective and highly individualized making it difficult to assess, especially when the subjects are non-verbal. Therefore, to quantify pain in animals, nociceptive behavioral and physiological responses to noxious stimulation are utilized as indirect indicators of pain.

Nociceptive behavioral responses observed in animals may be categorized as reflexive (withdrawal), voluntary, and chronic pain behaviors.

Reflexive behaviors: Reflex actions are evoked by noxious stimuli and act as a protective mechanism to prevent tissue injury. They may be involuntary movements to noxious sensory input mediated through the spinal cord via motor nerves or a conscious response to avoid further damage. Flexor reflexes (e.g. limb flexor reflex) are commonly used in pain experiments where animals are stimulated by a noxious stimulus (e.g. heat) and assessed for a specific reflexive response. However, these responses are not specific to nociceptive stimuli and interfered by other factors; for example, an increase in surface temperature may facilitate the nociceptive R_{III} reflex from a knee-flexor muscle in humans (Plaghki et al. 1998). These reflexive withdrawals are a function of the spinal reflex arc and remain intact even in spinal animals (animals with transected spinal cords). Thus results obtained from reflexive behavior assessments are limited and may not represent the pain experience which intensely involves processing of supraspinal structures.

Voluntary behaviors: Simple purposeful innate behaviors such as vocalizing, licking, biting, skin twitching and checking a limb in response to noxious stimulation may be considered as indicators of pain. These behaviors are more complex than the spinal reflexes; however, they can be found in both decerebrate and intact animals (Woolf 1984; Matthies & Franklin 1992). Hence caution is needed in interpreting these innate behaviors.

Chronic pain behaviors: Responses to ongoing nociceptive input are expected to be prolonged and have an impact on health quality. Hyperalgesia and allodynia are characteristics of chronic pain along with systemic behaviors such as anxiety, decreased social interaction (Benbouzid et al. 2008), reduced sympathetic responsivity (Vierck et al. 2008), weight loss (Abbadie et al. 1994), and poor sleep quality (Andersen & Tufik 2003).

Physiological responses to a noxious stimulation have also been assessed and measured as pain indicators in non-verbal patients such as human infants (Raeside 2011) and animals (Bufalari et al. 2007). Physiological signs that may indicate pain include tachypnea, tachycardia, hypertension, dilated pupils and increases in plasma cortisol and epinephrine levels (Mathews 2000). Pain stimulates the hypothalamo-pituitary-adrenal axis and the sympathetic nervous system resulting in release of cortisol (Mormede et al. 2007) and catecholamines (Huskisson

1974) respectively. However other factors such as stress conditions and trauma also activate these systems, therefore, elevations of the hormone levels may be not related directly to pain (Mormede et al. 2007; Ledowski et al. 2012). An increase in glucose and lactate production in response to cortisol and catecholamine induced-glycogenolysis might be utilized to assess pain condition (Prunier et al. 2005). Plasma β-endorphin is another potential indicator which may be correlated with pain (McCarthy et al. 1993; Raekallio et al. 1997).

Pain assessment tools in animal models assessing behavior

To identify pain in animals, the two main tools used to assess pain in animals are pain scaling systems and analgesiometers. They both were developed with the objective of having accurate and validated techniques to facilitate effective pain management strategies.

Pain scaling systems

Pain scaling systems are utilized in both preclinical and clinical pain studies. In animal models the first few pain scales were modified from the human pain scales. For example, in 1978 LaMotte and Campbell compared the nociceptive responses to intensity of thermal stimulation in monkeys with a scale used on human being to assess response to thermally induced pain (LaMotte & Campbell 1978); Taylor and Houlton (1984) investigated the postoperative analgesic effect of morphine, buprenorphine and pentazocine in dogs following orthopedic surgery using a numerical rating scale (NRS) and the simple descriptive scale (SDS) (Taylor & Houlton 1984). Likewise the visual analogue scale (VAS) that has been used widely in humans was also applied in to compare the postoperative analgesic effect between pain medications in dogs (Reid & Nolan 1991; Nolan & Reid 1993).

These simple pain scales, VAS, NRS and SDS, rate the pain behavior based on the pain intensity. VAS (**Figure 2.1**) is a pain measurement instrument using a 100 mm line with the ends anchored such that 0 mm is indicating no pain and 100 mm is indicating worst pain imaginable. Investigators place a mark on the line corresponding to their perception of pain based on the animal's behavior. The SDS usually consists of 3 to 5 numerical reference points associated with different characteristic physiology/behaviors which become the pain score for the patient. Each number is assigned an expression of the animal that describes a value of pain intensity (e.g. no pain, mild, moderate or severe pain) (Hansen 2003). The NRS in animal studies usually is a numbered scale of 4- to 10- points; the end points representing the extreme of pain intensity. Observers assign a number that relates to the animal's current level of pain. Despite significant attempts at refinement, these simple subjective scales have been shown to have high intra- and inter-observer variability in the assessment of acute pain in dogs (Holton et al. 1998). They also only rely on the intensity of pain to make a determination and so scores may not represent the experience of pain which is a complex phenomenon. Attempts to develop more objective and multi-dimension pain scoring systems in animals have therefore also been initiated in 1985. Morton and Griffiths (1985) proposed a composite scale by defining species specific signs of behavior and changes of physiological parameters that indicate pain, then refining them into multiple categories and assigning a score within each. The scale consists of 4 categories including bodyweight, appearance, clinical signs, unprovoked behavior, and responses to external stimuli. The sum of these scores was interpreted as the pain status of the animals (Morton & Griffiths 1985). From there on, more pain scales that are specific to species and types of pain have been developed such as the Melbourne Pain Scale for evaluation of postoperative pain in dogs (Firth & Haldane 1999), the Glasgow Composite Pain Scale which is a behavior-based composite scale to assess acute pain in dogs (Holton et al. 2001), and a composite pain scale for assessing acute postoperative pain in cats undergoing ovariohysterectomy (Brondani et al. 2011).

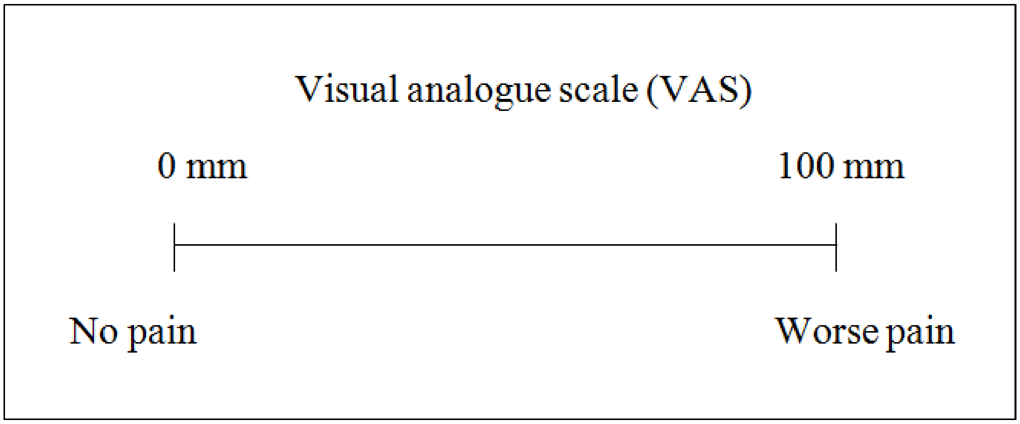

Figure 2.1 Visual analogue scale (VAS)

Analgesiometers

In trying to design objective instruments for pain studies, analgesiometry devices have been created in an effort to produce a quantifiable noxious stimulus in order to measure nociceptive threshold. The noxious stimuli applied in animal models include thermal, electrical, chemical and mechanical stimuli. Even though this has been used several times in the past, electric shock is controversial to use as a noxious stimulus because it is not a natural type of noxious stimulus and excites non-nociceptive Aβ fibers as well as nociceptive A and C fibers (Le Bars et al. 2001).

Thermal noxious stimuli may be applied using a tail immersion, a hot plate, and other heating devices which are designed to apply the noxious stimulus to the animals until a nociceptive response is elicited. Mechanical stimulating devices, such as algometry, Von Frey filaments and other force applying devices, input quantifiable pressure to stimulate the animal responses. The tests using these analgesiometers are explained below under Acute pain model and Inflammatory pain model.

Experimental models of pain

Animal pain models that have been developed in pain research can be classified into at least four categories: acute, inflammatory, neuropathic, and clinically oriented pain models (Mogil 2009).

Acute pain model

This model allows the investigators to understand the mechanisms of pain and evaluate the analgesic efficacy of pain medications in normal animals. Reflexive and voluntary behaviors responding to noxious stimuli (thermal, mechanical and chemical stimuli) are commonly used as indicators of pain in this model. Based on the application site of the noxious stimulus the model may be categorized into somatic and visceral pain testing.

Somatic pain testing

Tail-flick and hot plate tests were the first and second most common tests of nociception in rodents between 1970 and 1999 (Le Bars et al. 2001). The tail-flick latency test was first devised by D'Amour and Smith in 1941 (D'Amour & Smith 1941) for measuring pain sensation in rats. The animal tail is stimulated by radiant noxious heat until it provokes a withdrawal reflex noted by defensive movement of the tail of the animal. The reaction time of this movement known as tail-flick latency is recorded. An alternate heat source used to assess the tail-flick is hot water. Immersion of tail in hot water stimulates a strong tail movement and a flinch of the whole body (Sewell & Spencer 1976; Statile et al. 1988), similarly latency time of the reaction is recorded. The immersion test has been used mostly in rodents but reported also in monkeys (Dykstra & Woods 1986). Hot plate was first described in 1944 in the evaluation of analgesics in rodents. The subject is put into an open-ended cylindrical space with a metallic floor plate that is heated at a consistent rate of rise, and the temperature of 55°C, 60°C, 65°C and 70°C were used to test (Woolfe & MacDonald 1944). Avoidance responses such as jumping and licking are observed, and the reaction time is measured for each temperature. Hargreaves test (Hargreaves et al. 1988) or plantar test is another method measuring the thermal nociceptive threshold. The test applies a high-intensity beam of light directed at hindpaws of a freely moving animal in a clear plastic chamber. The time that the animal takes to withdraw its hindpaw is recorded as withdrawal latency. This test too has been used mainly in rodents, and can be applied to measure thermal threshold in amphibians such as the frog (Coble et al. 2011).

The first mechanical nociceptive threshold test in rodents was devised in 1929. (Bianchi & Franceschini 1954). From there on, a number of mechanical threshold testing devices in animals have been developed in attempting to improve the specificity, reliability and sensitivity of the assessments. In general the devices apply an increasing measurable pressure at the tail or paw until the withdrawal reflex is observed; the pressure that evokes the response is recorded as the threshold. The prolongation of the withdrawal reflexes and escape behaviors, as well as a higher intensity tolerated by the animal are interpreted as increases of the nociceptive thresholds.

For the tests using chemical stimulation, acetic acid has been used as an algogenic substance to investigate the analgesic effect of drugs. The lowest concentration of acetic acid is applied on the skin at a specific area and continued with increasing concentrations until the animal vigorously wipes the affected area. The first concentration of acetic acid that provokes a wiping response is considered the nociceptive threshold (Pezalla 1983; Stevens et al. 2001; Coble et al. 2011).

Analgesiometers have also been developed for acute pain testing in other animal species that differ from rodents in anatomy and behavior. In dogs and cats, nociceptive threshold testing devices have been developed using different kinds of noxious stimulus, i.e., thermal (Andrews & Workman 1941; Winter & Flataker 1953; Vaupel 1989; Ylisela & Vainio 1989; Barnhart et al. 2000; Steagall et al. 2007; Wegner et al. 2008), mechanical (Martin et al. 1964; Martin et al. 1974; Martin et al. 1976; Hamlin et al. 1988; Rudo et al. 1989; Vaupel 1989; Lascelles et al. 1997; Barnhart et al. 2000; Dixon et al. 2007; Steagall et al. 2007; Slingsby et al. 2011) and electrical stimuli (Kaymakcalan et al. 1974; Skingle & Tyers 1979; Skingle & Tyers 1980; Skingle et al. 1982; Hayes et al. 1986; Hamlin et al. 1988; Vainio et al. 1989; Brown et al. 2002; Bergadano et al. 2009; van Oostrom et al. 2011). In horses, pressure algometry, a mechanical instrument to quantify mechanical nociceptive thresholds within musculoskeletal structures in human, provides a quantitative and repeatable method for assessing musculoskeletal pain both in axial skeleton (Haussler & Erb 2006a; Haussler & Erb 2006b) and thoracic limb (Haussler et al. 2007). In farm animals (e.g., sheep, cattle, pig) the nociceptive measurements that have been used include radiant thermal stimulating devices (Nolan et al. 1987; Whay et al. 1997; Machado Filho et al. 1998; Herskin et al. 2003) and force applying devices (Nolan et al. 1987; Ley et al. 1996; Whay et al. 1997; Sandercock et al. 2009).

Visceral pain testing

To assess nociceptive responses in viscera, many visceral pain models have been devised. The writhing test is the early attempt to induce visceral pain in animals by intraperitoneal injection of irritants, such as acetic acid, and phenylquinone (Siegmund et al. 1957; Blumberg et al. 1965; Singh et al. 1983). After the administration the abdominal constrictions (writhing episodes) which are considered as nociceptive behavior in response to the irritant, are counted during a period of time. However this method lacks selectivity on the viscera and causes animal under suffering; consequently, newer methods were invented to apply a finite noxious stimulation specifically to each organ such as colon, urinary bladder, stomach, uterus and ovary. For example a balloon is inserted into the hollow organ and distended to stimulate the wall of the organ as a noxious stimulus (Ness & Gebhart 1990; Ness et al. 2001; Christianson & Gebhart 2007). Responses including skeletal muscle contraction, heart rate and blood pressure elevations are monitored and recorded. Electromyographic recordings may also be used to count and record muscle contraction. For the ovary, a new technique has been recently presented to determine the minimum alveolar concentration (MAC) in anesthetized dogs by applying a force on ovary and ovarian ligament which can evoke purposeful movements. This model has been validated for induction of visceral pain (Boscan et al. 2011).

Inflammatory pain model

The objective of this model is to induce a painful condition that mimics clinical pain of inflammatory. Inflammatory pain is a big health issue causing suffering to millions in both humans and animals especially chronic inflammation such as arthritis and inflammatory bowel disease. Unlike pain originated from acute inflammation that act as a physiological function to prevent further damage and cease after the noxious stimulus is removed, chronic inflammation pain occurs when healing persists beyond the expected time, due to ongoing of inflammatory process. The model has helped scientists understand the underlying mechanism of inflammatory pain and develop potential treatments. To induce inflammation, irritating substances or the inflammatory mediator is injected into the body part of an animal such as the hindpaws. Formalin, carrageenan, capsaicin, and complete Freund's adjuvant (CFA) are common inflammatory substances that can irritate tissue and provoke inflammatory responses. After the induction of inflammation the measurement of pain responses, such as withdrawal latency and tail-flick latency, using analgesiometry is performed over time (hours to days depending on the lasting effect of a specific substance) compared to baseline values obtained prior to the substance administration. In general tissue inflammation lowers the nociceptive threshold, and/or reduces the latency period. Allodynia may be induced in the model and evaluated using Von Frey filaments.

Von Frey filaments are a typical mechanical nociceptive threshold testing device for inflammatory pain model, both in animals and human beings. A set of Von Frey filaments consist of various calibrated nylon monofilaments of varying diameter. The filaments are pressed against the skin with force so that the filaments bend and form U shapes providing for a constant applied force in each filament size. This tool can detect mechanical allodynia in models of inflammatory and neuropathic pain.

Neuropathic pain model

Each year an estimated 4 million people in the United States suffer from neuropathic pain (Chen et al. 2004). Neuropathic pain is initiated or caused by a lesion or disease of the somatosensory nervous system as defined by International association for the study of pain. It is a complex disorder and remains a challenge to treat. Based on the location of injury neuropathic pain can be divided into peripheral neuropathic pain and central neuropathic pain. Peripheral neuropathic pain occurs following a lesion of the peripheral somatosensory nervous system while central neuropathic pain is resulted from injury at the central somatosensory neurons (Xu et al. 2012). To understand the mechanisms behind it, neuropathic pain models in animals have been developed.

Experimental anesthesia dolorosa or axotomy model is the oldest model of neuropathic pain (Wall et al. 1979). Hind limbs of the animals (rats and mice) are deafferented by complete transection of the sciatic and saphenous nerves. Following the transection some animals develop self-mutilating behaviors, such as biting and attacking the denervated hind limbs, which may reflect phantom and spontaneous pain in humans.

Chronic constriction injury, partial sciatic nerve injury, spinal nerve ligation, spared nerve ligation and common peroneal nerve ligation are techniques used more commonly for studying the peripheral neuropathic pain. Sciatic and infraorbital nerves are common targets of the chronic constriction model in rats. Placing constrictive ligatures around the nerves can produce allodynia, hyperalgesia and possibly spontaneous pain similar to what is observed in human patients.

Partial sciatic nerve injury was first developed in rats as a behavioral model of causalgiform pain disorders. At the level of the upper thigh sciatic nerve is ligated tightly with an 8-0 silicon-treated silk suture, and about 1/3 – 1/2 of the nerve thickness was trapped in the ligature (Seltzer et al. 1990). The animals in this model develop touch-evoked allodynia, hyperalgesia and sympathic dependent pain which parallel to causalgia pain in humans.

Spinal nerve ligation is another animal model for the peripheral neuropathic pain. L5 and L6 spinal nerves are ligated tightly distal to the dorsal root ganglia with silk suture. A long-lasting hyperalgesia to noxious heat and mechanical allodynia on the injured hind limbs are developed (Kim & Chung 1992).

Spared nerve ligation is a method that allows researchers to investigate relative changes in damaged nerves and neighboring intact sensory neurons by an axotomy and ligation two (tibial and common peroneal nerves) of the three terminal branches of the sciatic nerve and leaving one (sural nerve) intact (Decosterd & Woolf 2000). The spared nerve ligation has been shown to induce prolonged changes in mechanical and thermal pain sensitivity and mimic closely to the changes observed in clinical neuropathic conditions in humans (Decosterd & Woolf 2000; Erichsen & Blackburn-Munro 2002).

Ligation of common peroneal nerve assesses nociceptive responses in a neuropathic pain model without affecting motor function. This technique is less invasive but evokes long-lasting behavioral allodynia and thermal hyperalgesia in mice (Vadakkan et al. 2005).

In addition to the experiment models of neuropathic pain caused by peripheral nerve ligation and transection, models of sciatic cryoneurolysis using a cryoprobe to develop peripheral neuropathy by freezing the proximal sciatic nerve (DeLeo et al. 1994; Willenbring et al. 1995), models of sciatic inflammatory neuritis induced by injection of antigen such as zymosan (yeast cell walls) around the sciatic nerve (Chacur et al. 2001), models of neuropathy induced by chemotherapy such as vincristine (Authier et al. 2003) and paclitaxel (Polomano et al. 2001), photochemical-induced (Kupers et al. 1998) and laser-induced (Chiang et al. 2005) sciatic nerve injury have been used to study the painful neuropathy due to variable causes (Jaggi et al. 2011).

For central neuropathic pain study a number of animal models of spinal cord injury have been developed. Various techniques to induce injury at the spinal cord have been utilized, i.e., spinal cord contusion or hemicontusion, spinal cord transection or hemisection, photochemical-induced ischemia, and excitatory neurotoxins intrathecal injection models. Most animals developed thermal hypersensitivity and mechanical allodynia over different durations (week to month based on technique inducing injury) (Nakae et al. 2011). However, the models also induce motor dysfunctions, hence the result interpretation has proven to be difficult and potentially misleading.

Clinically oriented pain models

A number of diseases can induce pain, i.e., osteoarthritis, cancer, diabetes, and pancreatitis. These types of pain are typically chronic, may be severe and therefore difficult to treat. Understanding the mechanisms of pain with these diseases is necessary to develop appropriate pain management. In models of osteoarthritis many substances have been used for intra-articular injection to induce inflammation in joints of subjects; monoiodoacetate, kaolin-carrageenan, Freund's adjuvant and sodium urate. Assessment of pain can be performed by scaling systems, gait analysis, range of motion analysis, weight distribution, Hargreaves and hotplate to determine thermal hyperalgesia, force applying devices to detect mechanical hyperalgesia and Von Frey filaments to state mechanical allodynia (Neugebauer et al. 2007).

Streptozocin is administered intraperitoneally in rats to induce diabetic neuropathy (Courteix et al. 1993). This drug is a selective toxin of β cells in the pancreatic islet cells (Calcutt et al. 1996). Following the injection the animals demonstrate hyperglycemia, allodynia and hyperalgesia that may reflect signs observed in human patients with diabetic neuropathy (Courteix et al. 1994).

Rodent models of cancer pain have been developed in the last decade. Implanting cancer cells in a specific organ may be used to induce cancer in animals. Investigation of pain in these models is very useful to understand the pain mechanisms of each type of cancer and test novel drugs for cancer pain treatment. In bone cancer model, the rodents receive intra-bone (mostly in tibia medullary cavity) injections of cancer cells, such as mammary gland carcinoma , sarcoma, and fibrosarcoma cells (Pacharinsak & Beitz 2008). The cancer-induced animals develop pain behaviors, mechanical allodynia, and mechanical hyperalgesia (Medhurst et al. 2002; Mao-Ying et al. 2006). Until now the pain models of bone cancer, facial cancer (Ono et al. 2012), melanoma skin cancer (Fujita et al. 2010), and oral cancer (Nagamine et al. 2006) have been established and investigated in rodents.

Animal pain models have been proved to play a vital role in pain research. Studies can provide fundamental understanding of pain mechanisms and improve the pain treatment but translation directly from basic animal experimental findings to clinical manifestations is challenging. In particular the knowledge obtained from acute assays needs to be interpreted carefully as discussed and reviewed by Le Bars et al. (2001). Briefly, clinical pain tends to more severe than responses to the testing around the nociceptive threshold, and the responses evoked from healthy tissue in acute models and pathologic tissue in clinical patients differs. No test of nociception presently possess all performance characteristics, i.e., sensitivity, specificity, validity, reproducibility and repeatability or reliability (Le Bars et al. 2001). Although a number of contemporary animal pain models are better designed to reflect clinical pain, the experimental condition may interfere with the results of the test. For example, the investigator-animal interaction, the animal handling and the research environment that might induce stress and anxiety are likely to affect the pain tolerance and responses in the animals (Kornetsky 1954; Calcagnetti & Holtzman 1992; Rosellini et al. 1994; Chesler et al. 2002). Therefore the information obtained from the laboratory models need to be translated with caution.

Pain clinical trials are studies that evaluate clinically the analgesic efficacy of potential analgesic drugs which previously demonstrated promising results in laboratory animal models. The testing drug is administered to clinical patients. The trials reveal how the clinical pain condition responds to the treatment and determine side effects of the drug. For example, the testing of the analgesic efficacy of intrathecal resiniferatoxin, a potent capsaicin analog, in clinical canine patients with bone cancer pain (Brown et al. 2005), and the evaluation of the postoperative analgesic efficacy of low dose ketamine as an adjunct analgesic in dogs undergoing a forelimb amputation (Wagner et al. 2002). Clinical trials can translate the information from the laboratory experiments into clinical use directly. The findings obtained from the clinical trials are useful; however, ethical issues need to be considered if other analgesic modulates are denied.

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CHAPTER 3: EVALUATION OF THE ANALGESIC EFFECT OF MAROPITANT IN CATS[1]

Introduction

Maropitant (Cerenia; Pfizer Animal Health, NY, USA) is a NK-1 receptor antagonist approved for the prevention and treatment of acute vomiting in dogs with demonstrated safety and efficacy preventing and treating emesis caused by motion sickness (Benchaoui et al. 2007; Conder et al. 2008), administration of cisplatin (Vail et al. 2007), hydromorphone (Hay Kraus 2012), doxorubicin (Rau et al. 2010), and emetogens such as apomorphine and syrup of ipecac (Sedlacek et al. 2008), among others (de la Puente-Redondo et al. 2007; Ramsey et al. 2008). In cats, maropitant is well tolerated with antiemetic properties against xylazine and motion sickness induced emesis (Hickman et al. 2008).

Studies in multiple species have shown that NK-1 receptor antagonists suppress the response to noxious stimuli. For example a NK-1 receptor antagonist (CP-96,345) elevated pain thresholds significantly after intraperitoneal injection of acetic acid in mice (Nagahisa et al. 1992). Nociceptive behaviors induced by intraplantar formalin injection in rats (Yamamoto & Yaksh 1991; Smith et al. 1994; Rupniak et al. 1995), gerbils (Smith et al. 1994; Rupniak et al. 1996) and mice (Sakurada et al. 1993) were attenuated after administration of NK-1 antagonists. In genetic modes, NK-1 receptor knockout mice were less responsive to the intraplantar formalin injection (King et al. 2000), and demonstrated reductions in nociceptive responses to intracolonic administration of capsaicin (Laird et al. 2000). These results suggest a role for NK-1 receptors in regulating pain transmission.

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Recently authors reported a decrease in the sevoflurane minimum alveolar concentration (MAC) requirement during ovary and ovarian ligament stimulation after intravenous administration of maropitant in dogs (Boscan et al. 2011b), which may indicate the antinociceptive properties of this NK-1 antagonist.

A study in cats demonstrated the release of substance P, a ligand of NK-1 receptors, in spinal cord after noxious stimulation using thermal stimulation and capsaicin (Go & Yaksh 1987). In anesthetized cats, intrathecal administration of a NK-1 receptor antagonist reduced the cardiovascular responses evoked by noxious chemical (bradykinin) stimulation of the gallbladder (Pan et al. 1995). Due to these prior favorable results and in an effort to further evaluate the antinociceptive effects of maropitant we decided to use a model of ovarian stimulation previously described in dogs (Boscan et al. 2011a) to determine the MAC sparing effect of the drug in cats during visceral noxious stimulus.

Materials and methods

Animals

Twenty one client-owned, domestic, healthy female cats, greater than 12 weeks of age, weighing 2.7 ± 0.8 kg (mean ± SD) were enrolled in the study. Food was withheld overnight, but water was available at all times. The study was approved by the Animal Care and Use Committee from Colorado State University.

Experimental protocol

The study was divided into three phases. In phase 1, the ovarian stimulation model previously used in dogs (Boscan et al. 2011a) was modified for cats to determine the optimal force for ovarian stimulation that generates a response without damaging tissue. This was done by constructing a stimulus – response curve while determining the sevoflurane MAC. The second phase was designed to identify the MAC sparing effects during ovarian stimulation of two doses of maropitant. During phase 1 and 2 of the study, we identified 5 pregnant cats by observing a gravid uterus during laparoscopy. Due to the potential effects of pregnancy on MAC requirements and because the effects of pregnancy in cats on MAC requirements has not yet been published, the 5 pregnant cats were removed from phase 1 and 2 and a third phase was added to the study to evaluate the differences in MAC between pregnant and non-pregnant cats.

Phase 1

Anesthesia was induced in 5 cats with sevoflurane in oxygen using an induction chamber and a face mask until cats could be intubated. Once orotracheally intubated (3.5 – 4.5 mm internal diameter endotracheal tube), anesthesia was maintained with sevoflurane in oxygen using a circle breathing system. The cats were mechanically ventilated to maintain an end-tidal carbon dioxide ($ETCO_2$) between 25 and 35 mmHg. Lactated Ringers solution (Baxter, IL, USA) was administered at 5 ml/kg/h during anesthesia. An ECG was used to assess heart rate and rhythm. A Doppler was placed over a digital artery and used with an appropriately sized cuff on the proximal limb to assess blood pressure. An esophageal thermometer (Power Lab amplifiers from ADInstruments, CO, USA) was placed to assess core temperature and a calibrated sidestream end-tidal gas analyzer (Biochem 9100; BCI International, WI, USA) was used to measure inspired and expired O_2, CO_2 & sevoflurane and record respiratory frequency. A catheter was placed through the endotracheal tube to the level of the carina to facilitate end-tidal sampling. Core temperature was maintained between 37°C and 39°C using externally supplied heat during the study.

Laparoscopic surgery was performed to access the right ovary and ovarian ligament. Two 5 mm cannulas were placed along the midline and the abdomen was insufflated with CO_2 to pressures between 4 - 8 cmH_2O for visualization. For ovarian stimulation a 3-0 biosyn suture was placed around and through the ovary and ovarian ligament (**Figure 3.1**). The suture loose ends were exteriorized through the abdominal wall and connected to a pre-calibrated force transducer. The force transducer has a force displacement range of 0.05 – 2 Kg/mm (0.5 – 20 Newton's) and maximum load of 10 Kg (FT03; ADInstruments, CO, USA). The technique used followed a similar protocol previously described in dogs (Boscan et al. 2011a).

It has been recommended for MAC determination that a supra-maximal noxious stimulus (increase in stimulus intensity does not alter MAC) should be used to obtain reliable and repeatable results (Quasha et al. 1980; Valverde et al. 2003) as the variation of MAC is reduced when the intensity of stimulation increases (Eger et al. 1965). The highest traction force that does not harm or damage tissues is considered optimal to determine MAC in our study.

To identify the optimal traction force for further MAC comparisons, a stimulus-response curve was created using 4 stimulation forces (1.96, 3.92, 5.88 and 7.85 Newton's) tested randomly in each cat to determine the sevoflurane MAC in duplicate within each traction force. MAC was determined by applying the desired traction force for 1 minute at a given sevoflurane concentration (if no response was observed) or until purposeful movement was observed. If purposeful movement was observed, the end-tidal sevoflurane was increased by 10% for the following test. On the contrary, if no movement occurred, the end-tidal sevoflurane was decreased by 10% for the following test. At least 15 minutes were allowed for equilibration at the new sevoflurane concentration between tests. MAC was defined as the average of the concentrations generating a positive and negative response. MAC was depicted as mean ± SD, corrected for calibration values. Since the current study was performed at around 1,500 meters above the sea level, the MAC which is measured as a percentage of a volatile anesthetic at 1 atmosphere was adjusted further and is reported in the standard atmosphere at sea level (760 mmHg).

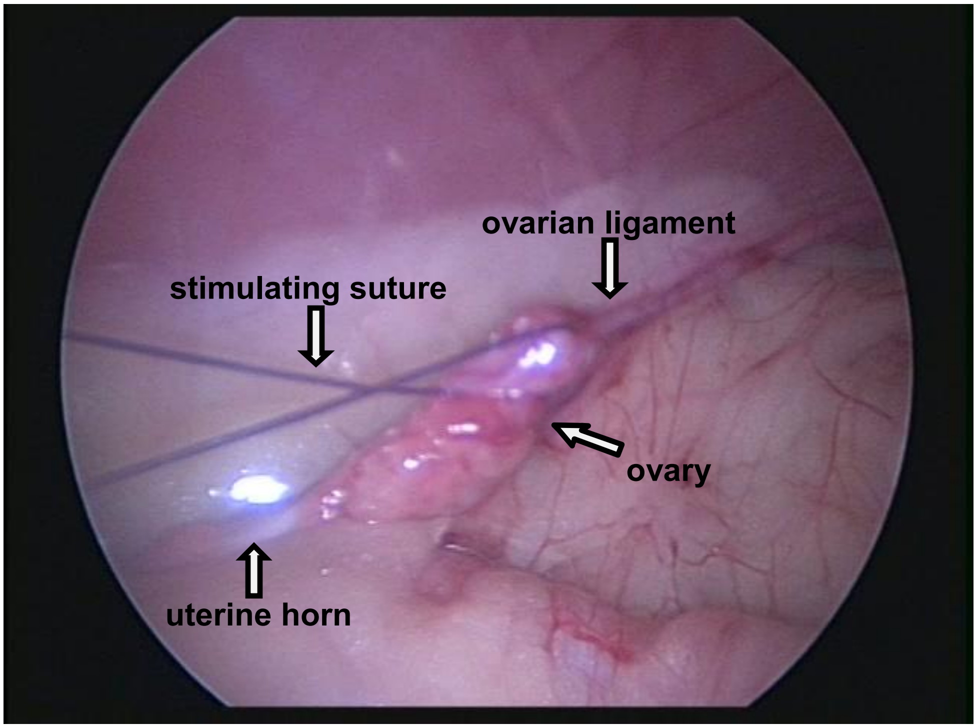

Figure 3.1 A 3-0 biosyn suture was placed through and around the right ovary and ovarian ligament for the ovarian noxious stimulation.

Phase 2

Ten cats were anesthetized and monitored as described above. Laparoscopic surgery to access the right ovary and ovarian ligament was performed as described for phase 1. MAC determinations were performed in triplicate for each cat to evaluate the anesthetic sparing effect of two maropitant doses. First, a baseline MAC determination was performed between 1 - 2 hours after anesthesia induction. A force of 4.9 Newtons was selected as the optimal force from phase 1 and was used to stimulate the ovary and ovarian ligament during phase 2. Following baseline MAC determination, a maropitant dose of 1 mg/kg was administered intravenously over 5 minutes, and then MAC was redetermined at 10 minutes after the administration. This was repeated following a maropitant dose of 5 mg/kg which again was administered intravenously over 5 minutes. Cardiorespiratory variables were recorded prior to, during and after drug administration.

Phase 3

Five pregnant cats were identified by observing a gravid uterus during laparoscopic surgery in phase 1 and 2. Owners of the cats were informed and decided to continue the surgery. The protocol of phase 1 was performed in these pregnant cats to construct the stimulus-response curve using 4 stimulation forces (1.96, 3.92, 5.88 and 7.85 Newton's). Data from six non-pregnant cats (including 5 cats in phase 1) was used for comparison.

At the end of the study (phase 1, 2 and 3), all cats were spayed laparoscopically. To prevent infection cefazolin 20 mg/kg was administered intravenously prior to recovery. Ketoprofen 1 mg/kg and buprenorphine 0.02 mg/kg were administered subcutaneously 15-30 minutes before recovery for postoperative pain management. All cats recovered and were returned to their owners without complications.

Statistical methods

Phase 1

The stimulus – response curve was constructed to determine the optimal stimulation force to study MAC by use of SAS statistical software, version 9.2. (SAS institute, Inc., NC, USA). Various non-linear growth curve models were considered to describe the dose response relationship between traction force and sevoflurane requirements. Akaike's information criterion (AIC) was used to compare the model fit between the growth curve models, and to identify a model with the best fit. Maximum likelihood estimates of the model parameters were obtained using the Quasi-Newton Raphson algorithm. The parametric bootstrap technique was used to calculate the standard error of the estimated traction force required for the curve to reach plateau, and to construct the corresponding 95% confidence intervals (Davison & Hinkley 1997). Specifically, the observed means and standard deviations of the anesthetic requirements (%) for the traction forces 1.96, 3.92, 5.88 and 7.85 Newton's were used to generate Monte Carlo samples of size m=1,000 which were drawn from a multivariate normal distribution. The standard deviations of the estimated plateau levels from the fitted growth curve models across the 1,000 simulated data were then used to estimate the standard errors of the estimated traction force required for the curve to reach plateau.

Phase 2

Data were summarized as mean ± SD by use of GraphPad Prism statistical software, version 4.03 (GraphPad Software Inc, CA, USA). A repeated measures ANOVA followed by post hoc bonferroni test were used for data comparison. The repeated-measures factor was time and the between-subject factor was treatment. Pairwise comparisons between treatments at each time point were examined using t-tests. Residuals from ANOVA were approximately normal and independent. Values of $P < 0.05$ were considered statistical significant.

Phase 3

The stimulus – response curve of 5 pregnant and 6 non-pregnant cats were constructed and the statistical models explained in the phase 1 were used for the analysis. A linear mixed effects model with repeated measurements was used for comparison between two groups. P value < 0.05 was considered significant. A power calculation was used to estimate the number of cats required in the study to show a statistically significant difference.

Results

Phase 1

The stimulation – response curve is depicted in **Figure 3.2**. The MAC obtained from using four different traction forces (1.96, 3.92, 5.88 and 7.85 Newton's) ranged between 2.69 and 4.17 % with a hyperbolic presentation.

The 3-parameter logistic growth curve model was a model with the best fit; therefore, it was used to describe the dose response relationship between sevoflurane requirements and traction forces.

The estimated traction force to reach the plateau level of the curve with a 95% confidence interval was 4.3 ± 3 Newton's (mean ± SE) which was obtained from the 3-parameter logistic growth curve formula as depicted below.

$$y_{ij} = \frac{c}{1 + \exp\left(-(a + b \cdot x_{ij})\right)} + s_i + \varepsilon_{ij}$$

y_{ij} is the sevoflurane requirements (%) for subject i at measurement point j,

s_i is the random subject effect,

ε_{ij} is the overall error,

x_{ij} is the traction force (N) for subject i at measurement point j,

a is the intercept parameter,

b is the slope parameter, and

c is the plateau of the growth curve.

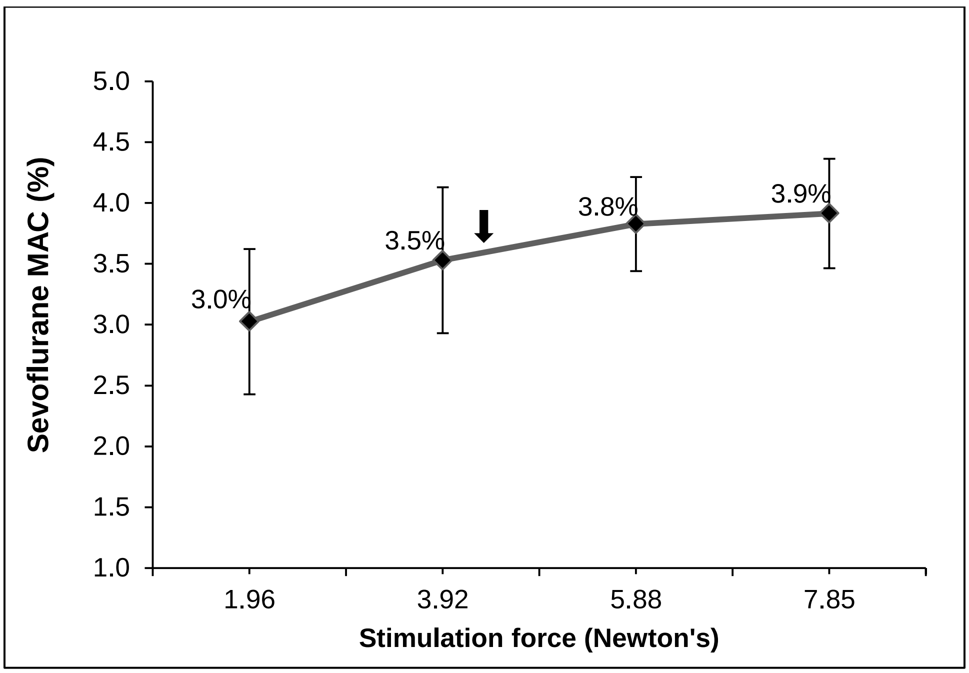

Figure 3.2: The stimulus - response curve for the sevoflurane MAC when traction forces ranging from 1.96, 3.92, 5.88 and 7.85 Newton's were applied to the right ovary and ovarian ligament in five cats. The mean ± SD of sevoflurane MAC was measured using the end-tidal sevoflurane concentration. The stimulation force was recorded using a force transducer. The arrow indicated a stimulation force of 4.3 Newton's, the estimated traction force to reach the plateau level of the curve.

Phase 2

During phase 2 the anesthesia time was 264 ± 14 min (mean ± SD). The average time spent to determine MAC for baseline, 1 mg/kg and 5 mg/kg maropitant was 51 ± 4, 53 ± 6 and 45 ± 6 min (mean ± SD), respectively.

A stimulation force of 4.9 Newtons was chosen to determine MAC during phase 2. This was the force considered to be a supra-maximal force for the model. The force was slightly greater than the estimated traction force to reach the plateau level of the curve with a 95% confidence interval in phase 1 (4.3 Newtons). Stronger traction forces should not influence the MAC value results and the stimulation force is not likely to cause tissue damage, desensitization or hyperalgesia.

As depicted in **Figure 3.3** the sevoflurane MAC in the baseline group was 2.96 ± 0.3% (mean ± SD). Maropitant administration at 1 mg/kg decreased MAC to 2.51 ± 0.3% (15%, $P < 0.01$). At higher dose (5 mg/ kg) maropitant did not reduce MAC further when compared to the low dose (2.46 ± 0.4%; $P = 0.33$).

There were no differences in Doppler blood pressure, body temperature, heart rate, respiratory rate and $ETCO_2$ between groups (**Table 3.1**). However, intravenous administration of maropitant decreased the blood pressure transiently. When 1 mg/kg and 5 mg/kg were administered, the Doppler blood pressure decreased from 88 to 61 mmHg ($P < 0.001$), and 86 to 50 mmHg ($P < 0.001$) respectively for 6 minutes or less and then returned to pre-administration values. Therefore caution is advised with intravenous maropitant administration.

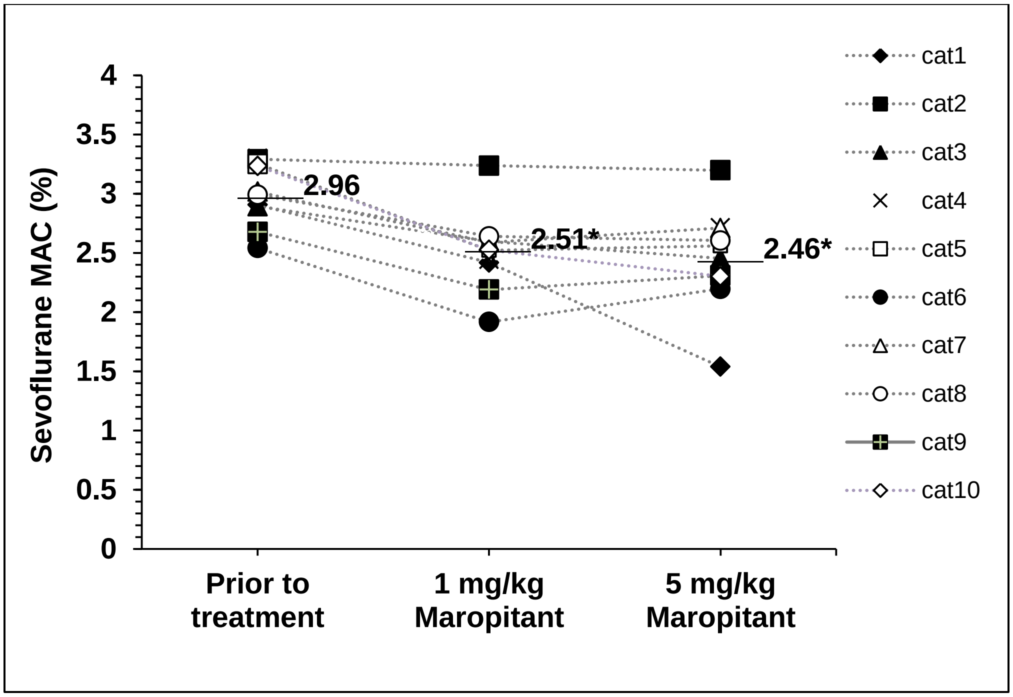

Figure 3.3: Each point represents sevoflurane MAC (%) for each cat prior to and after 1 mg/kg and 5 mg/kg of maropitant administration. Numbers mean the average MAC of each group. *Indicates significant differences compared to the MAC obtaining prior to the treatment (P < 0.05).

Table 3.1: Mean ± SD values for Doppler obtained systolic arterial blood pressure (SAP), body temperature, heart rate, respiratory rate and $ETCO_2$ in the cats prior to (baseline) and after 1 mg/kg and 5 mg/kg of maropitant administrations. Values were averaged from the MAC determination period (51 ± 4 min for MAC of baseline, 53 ± 6 min for MAC of 1 mg/kg maropitant and 45 ± 6 min for MAC of 5 mg/kg maropitant). (Niyom et al. 2013)

	Baseline	**Maropitant 1 mg/kg**	**Maropitant 5 mg/kg**
SAP (mmHg)	75 ± 4	80 ± 4	70 ± 3
Temperature (°C)	37.5 ± 0.5	38 ± 0.3	38.2 ± 0.2
Heart rate (beats/min)	157 ± 8	157 ± 11	136 ± 9
Respiratory rate (breaths/min)	14 ± 1.6	19 ± 2.8	14 ± 1.4
$ETCO_2$ (mmHg)	28.6 ± 0.76	28.9 ± 0.97	31 ± 0.9

Phase 3

The pregnant group consisted of 5 cats weighing 3.7 ± 0.6 kg and ranged from 6 months to 1.5 years of age. The non-pregnant group consisted of 6 cats weighing 2.3 ± 0.7 kg and ranged from 3 months to 2 years.

The sevoflurane MAC in the pregnant group for 1.96, 3.92, 5.88 and 7.85 N were 2.29 ± 0.09%, 2.54 ± 0.2%, 2.75 ± 0.3% and 2.91 ± 0.25% respectively while MAC in the non-pregnant group were 2.32 ± 0.4%, 2.77 ± 0.4%, 2.95 ± 0.3% and 3 ± 0.3% respectively. The stimulus – response MAC curve was not different between two groups (*P* = 0.335) but the MAC requirements in the pregnant cats were consistently lower by 5% (**Figure 3.4**).

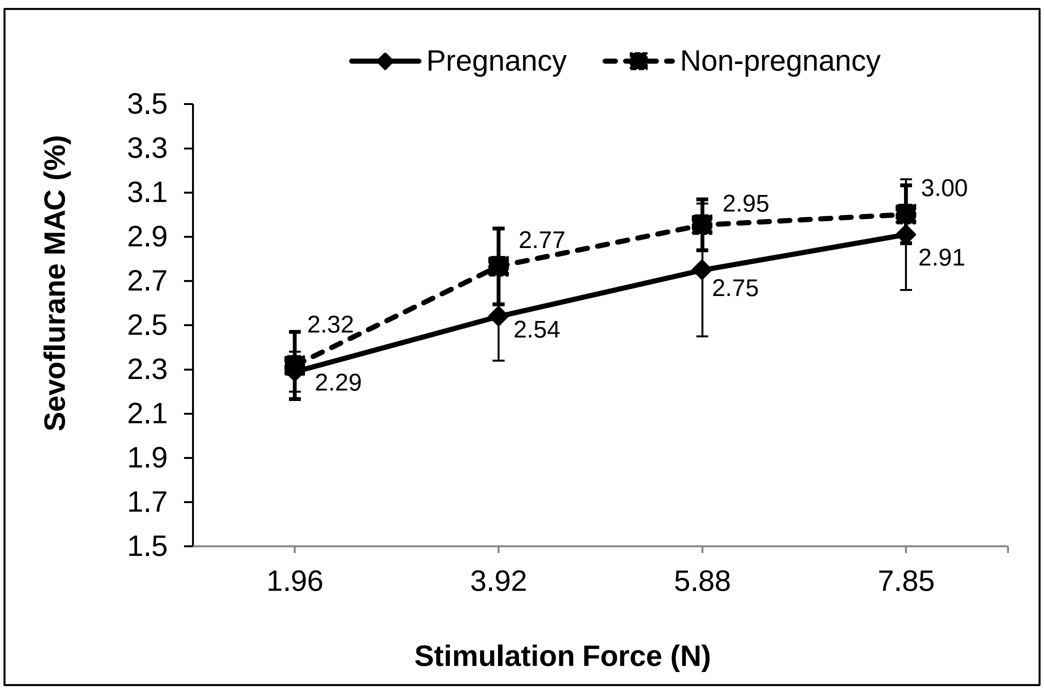

Figure 3.4: Stimulus-response curve for sevoflurane MAC using four traction forces (1.96, 3.92, 5.88 and 7.85 N) to the right ovarian ligament in five pregnant and six non-pregnant cats.

The three-parameter logistic growth curve model provided the best fit and was chosen to model the dose response relationship between traction force and anesthetic requirements. The estimated traction forces to reach the curve plateau level with 95% confidence interval were 9.2 ± 8 and 3.6 ± 1.5 N for pregnant and non-pregnant groups respectively. The sevoflurane concentration when each group reached a plateau in the stimulus – response curve was 2.99 and 2.73 for pregnant and non-pregnant groups respectively.

A power calculation using the collected data from the 11 cats determined that 18 cats per group would be needed in order to reach statistical difference at $P < 0.05$.

Discussion

In the current study maropitant, a specific NK-1 receptor antagonist, reduced the sevoflurane requirements in cats during ovary and ovarian ligament stimulation after intravenous administration, but the effect was not dose dependent over the doses of 1 mg/kg and 5 mg/kg. These findings are similar to those in a previous study evaluating the anesthetic sparing effect of maropitant in dogs (Boscan et al. 2011b). In that study maropitant at 1 mg/kg and 5 mg/kg decreased the sevoflurane MAC in dogs by 24% and 30% respectively. The anesthetic sparing effect of maropitant tends to be greater in dogs when compared to the reduction of sevoflurane MAC observed in cats (15% and 17% respectively).

The higher maropitant dose (5 mg/kg) did not significantly decrease the sevoflurane MAC further in either cats or dogs. A reason of this dose independent sparing effect is unknown. Possible explanations include 1) the maximal maropitant effect occurs at less than or equal to 1 mg/kg. If the maximal maropitant effect was between 1 - 5 mg/kg, the sparing effect would be greater at a dose of 5 mg/kg and a difference would be observed between the two doses, 2) drug – receptor affinity relationships; maropitant may have very high affinity in dogs and cats, resulting in receptor saturation at lower doses (< 1 mg/kg) as previous studies have shown that different NK-1 antagonists may have receptor affinity differences among different species (Beresford et al. 1991; Gitter et al. 1991; Barr & Watson 1993). Another possibility is that general anesthetics such as sevoflurane could be occupying and inhibiting NK-1 receptors on visceral pain transmission in the spinal cord (Wang et al. 2008b) and reducing population of receptors available for inhibition. Finally, it is possible that NK-1 receptors have limited ability to modulate immobility produced by the inhaled agent or limited ability as an analgesic and anesthetic sparing agent. Further studies are necessary to identify the dose – effect relationship in dogs and cats.

Mechanisms responsible for the MAC sparing effect of maropitant during visceral noxious stimulation remain unknown. Both, NK-1 receptors and substance P are expressed in the nociceptive pathway at many levels including nerve terminals, dorsal root ganglia, spinal cord, ascending projections and higher brain structures (Duggan et al. 1988; Mantyh et al. 1995; Quartara & Maggi 1998). In the current study, at the time of MAC determination maropitant was presumed to have enough time to reach all body compartments where NK-1 receptors may be located. Hence it is not possible to hypothesize the action site for maropitant.

The findings from the present study indicate a role of NK-1 receptors in visceral nociceptive processing which is also supported by preclinical information including 1) expression of substance P in 21% of somatic (cutaneous) versus greater than 80% of the visceral afferents (Perry & Lawson 1998), and 2) the presence of high concentrations of NK-1 receptors in spinal cord regions where visceral afferents terminate in laminae I and X (Brown et al. 1995; Laird et al. 2000).

These results however are confounded by previous studies, for example, intrathecal administration of NK-1 antagonists in rats showed analgesic effect (Chapman & Dickenson 1993; Ishizaki et al. 1997), while a study in dogs showed no benefit of epidural maropitant injection (Alvillar et al. 2012).

During the MAC measurements $ETCO_2$ in cats was maintained within 25 - 35 mmHg and the body temperature was maintained between 37°C and 39°C because of their potential effects on the MAC values. A decrease in body temperature reduces the anesthetic requirement (Quasha et al. 1980) while narcotic properties has been observed in dogs with arterial carbon dioxide partial pressure ($PaCO_2$) levels above 95 mmHg associated with arterial pH below 7.1 (Eisele et al. 1967). However a decrease of $PaCO_2$ from 42 to 14 mmHg in dogs and $PaCO_2$ of 20.8 mmHg in humans do not change MAC of halothane significantly (Eger et al. 1965; Bridges & Eger 1966) .

In addition to the anesthetic sparing effect of maropitant reported, this study represents a new approach to measure MAC in cats using visceral stimulation of the ovary and ovarian ligament. The model was adapted from a previous dog study (Boscan et al. 2011b). The application of the model to cats appeared to work well inducing predictable visceral noxious stimuli. There was no evidence of visual macroscopic tissue damage to the ovary or ovarian ligament and the response was consistent and repeatable within and between animals. This model may be of veterinary clinical interest because of its similarities to the pain response observed during ovariectomy or ovariohysterectomy surgeries.

A potential caveat in the study is that the end tidal gas samples were measured via an automated sidestream collection system. The sidestream collection systems may have produced a larger sevoflurane variability due to the higher respiratory rate and lower tidal volumes observed in cats.

An interesting observation was that intravenous administration of maropitant decreased blood pressure significantly for a short period of time. The Doppler obtained blood pressure decreased by 30% and 41% when low and high maropitant doses were administered respectively. The transient decrease of blood pressure was also noticed in dogs after the intravenous administration of the drug (Boscan et al. 2011b). Other NK-1 antagonists tested have shown diverging results. Five different pure NK-1 antagonists tested did not show any cardiovascular effects in rodents (Iyengar et al. 1997; Cellier et al. 1999; Wang et al. 2008a), ferrets (Watson et al. 1995) or dogs (Watson et al. 1995). On the contrary, the NK-1 antagonist (CP-96,345) decreased blood pressure in mice (Sakamoto et al. 1993). We do not know if there could be a direct cardiovascular effect from maropitant or the vehicles (metacresol and sulphobutylether-beta-cyclodextrin) in Cerenia®. It is possible that cresol derivatives may induce transient cardiovascular disturbance as shown in a previous study in pigs. That study reported signs of tachycardia, arrhythmias, and severe hypotension during intravenous administration of a cresol derivative in anesthetized pigs (Iaizzo et al. 1999). However, the clinical implication from this finding is unknown but we advise caution if the intravenous route is used.

In the present study we evaluated the relative analgesic efficacy of maropitant using MAC determination. Although it is not considered the best method for pain assessment and tranquilizer such as acepromazine can decrease MAC in dogs, goats and ponies (Heard et al. 1986; Doherty et al. 1997; Doherty et al. 2002) , many analgesic drugs used in veterinary medicine reduce MAC in animals such as cats and dogs (Yackey et al. 2004; Machado et al. 2006; Solano et al. 2006; Wilson et al. 2006; Ferreira et al. 2009; Ko et al. 2009; Seddighi et al.

2009; Credie et al. 2010; Monteiro et al. 2010) and some studies used MAC as a reference to test the analgesic potency of drugs (Gomez de Segura et al. 1998). This technique provides a reliable quantification of the observed effect and reduces the impact of animal handling stress and emotional responses on the results. The model may also be considered more ethical than other models in conscious animals and allow comparisons between different analgesic substances (Docquier et al. 2003).

Maropitant decreased the sevoflurane MAC requirements during visceral noxious stimulus in cats. Along with previously similar findings in dogs (Boscan et al. 2011b), this may indicate the visceral analgesic properties of maropitant in small animals. These are consistent with previous experiments that demonstrated the antinociceptive effect of NK-1 antagonists in rodents (Yamamoto & Yaksh 1991; Nagahisa et al. 1992; Sakurada et al. 1993; Smith et al. 1994; Rupniak et al. 1995; Rupniak et al. 1996). However NK-1 antagonists that have previously showed positive results in animal models had been failed to exhibit analgesic efficacy in human clinical studies of pain (Goldstein et al. 2001; Sindrup et al. 2006). Therefore, clinical trials are warranted to further evaluate the visceral analgesic effect of maropitant in cats.

The difference between sevoflurane MAC requirement of pregnant cats vs. non-pregnant cats during ovarian stimulation was small and probably of no clinical relevance (5%). However the MAC values during pregnancy were lower consistently at all forces in cats which may agree with a reduction of inhalant anesthetic requirements during pregnancy in humans (Gin & Chan 1994; Chan et al. 1996), rats (Strout & Nahrwold 1981) and sheep (Palahniuk et al. 1974; Okutomi et al. 2009). The statistical insignificance in the present study may be due to the small sample size and the small difference observed. According to the power calculation, the effect of pregnancy on MAC requirement is likely to be observed if each treatment group contained at least eighteen cats.

Hormonal changes during pregnancy may be responsible for the reduction in anesthetic requirements. In cats progesterone is increased 12 - 35 fold during pregnancy (Verhage et al. 1976); similar to women and rabbits where the ratio of progesterone plasma levels between pregnant and non-pregnant subjects are 60:1 (Datta et al. 1986) and 5:1 (Flanagan et al. 1987) respectively. Furthermore, administration of progesterone reduces halothane MAC in male dogs (Tanifuji et al. 1986) and ovariectomized rabbits (Datta et al. 1989) and decreases sevoflurane MAC in male mice (Shimizu et al. 2010). Progesterone is believed to have sedative (Soderpalm et al. 2004) and antinociceptive properties (Kuba et al. 2006), which may be the underlying mechanisms for a lower MAC requirement in pregnant subjects.

The limitations of the pregnant cat portion of the study include firstly the sample size which was low and therefore insufficient to demonstrate a significant difference. Second, blood concentrations of progesterone were not measured; therefore, the correlation of progesterone and MAC requirements in cats remains to be investigated. Third, the range of age was quite different between the two groups. Two of the 3 months-old cats were recruited in the non-pregnant cat group while the others ranged from 6 months to 2 years of age. This may be important because the anesthetic requirement changes with age (Quasha et al. 1980). Finally, using an automated side-stream system for sampling the end-tidal sevoflurane may produce large variability of anesthetic concentrations as stated earlier.

In conclusion, maropitant both at 1 and 5 mg/kg decreased the sevoflurane MAC requirements during visceral noxious stimulus in cats by 15 and 17% respectively. This may indicate the potential visceral analgesic efficacy of maropitant which warrants further investigation. The ovarian stimulation model of MAC measurements appeared to work well and produced repeatable responses to visceral noxious stimulus in cats. Pregnant cats may have lower sevoflurane requirements when compared to non-pregnant cats but the difference is small enough (5%) that it is considered clinically insignificant. Hence at this time we do not advocate the use of lower inhalant anesthetic percentages or concentrations for pregnant cats.

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CHAPTER 4: EVALUATION OF THE ANALGESIC EFFECT OF ORAL TRANSMUCOSAL BUPRENORPHINE IN DOGS USING THERMAL AND MECHANICAL NOCICEPTIVE THRESHOLD TESTING DEVICES[2]

Introduction

Buprenorphine, a partial agonist at the μ opioid receptors, is a commonly used analgesic drug in small animal practice. The orotransmucosal (OTM) administration of the drug can be used for pain management in dogs (Abbo et al. 2008; Ko et al. 2011) and cats (Robertson et al. 2003; Catbagan et al. 2011). The OTM route is easy and convenient and a pharmacokinetic and pharmacodynamic study in cats showed that a single dose of 0.02 mg/kg of buprenorphine administered via the OTM route is as effective as the same dose administered intravenously (Robertson et al. 2005a). The analgesic efficacy of buprenorphine in dogs was measurable following OTM administration (Mama et al. 2008) using a mechanical nociceptive threshold testing device (Self-built C-clamp; Colorado State University, CO, USA).

In the current study we used the mechanical nociceptive threshold testing device that we had used previously and two additional recently developed nociceptive threshold testing systems to assess the antinociceptive effect of the orotransmucosal buprenorphine in dogs. These new devices (Topcat metrology, Ely, Cambridgeshire, England) were originally constructed for use in cats (Dixon et al. 2002; Slingsby et al. 2009; Dixon et al. 2010) and were subsequently modified for use in horses (Robertson et al. 2005b; Love et al. 2012) and dogs. One system provides a measurement of mechanical nociceptive threshold while another determines thermal nociceptive threshold. The repeatability in measurements of these two devices was also investigated in the

[2] A portion of this study was published in The American Journal of Veterinary Research (Niyom et al. 2012)

present study. A strong advantage of these testing devices is that both require no or minimal animal restraint during the measurements. This minimizes stress and anxiety induced by the restraint which may further interfere with pain tolerance (Kornetsky 1954; Calcagnetti & Holtzman 1992; Rosellini et al. 1994).

Materials and Methods

Animals

Three male and three female 7- to 8- month-old healthy Walker hounds weighing between 16 and 23 kg were used. Dogs were housed individually; fresh water and commercial dry dog food were provided ad libitum. The dogs had daily interaction with study personnel for socialization. Dogs were also familiarized with the nociceptive devices and the study environment for 1 week prior to the start of the study. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Colorado State University.

Experimental protocol

The study was divided in two phases. Phase 1 was designed to evaluate the repeatability in measurements of the thermal and mechanical nociceptive threshold testing devices (Topcat metrology Ltd.) in unmedicated dogs. Phase 2 was used to evaluate the analgesic efficacy of the orotransmucosal buprenorphine in dogs using a mechanical nociceptive threshold testing device (Self-built C-clamp) and the two devices tested in phase 1.

Phase 1

To investigate the repeatability of thermal threshold measurements, the laterodorsal aspect of each dog’s thorax was clipped of hair and the thermal threshold device applied (**Figure 4.1**). This device was used to measure skin temperature before stimulation (baseline) and then remotely generate heat at a fixed rising temperature. The temperature at which the dog first responded (e.g., turned their head toward the stimulus, attempted to bite the device, tried to avoid the device) was considered the threshold. The difference between baseline skin temperature and the threshold was recorded for each measurement.

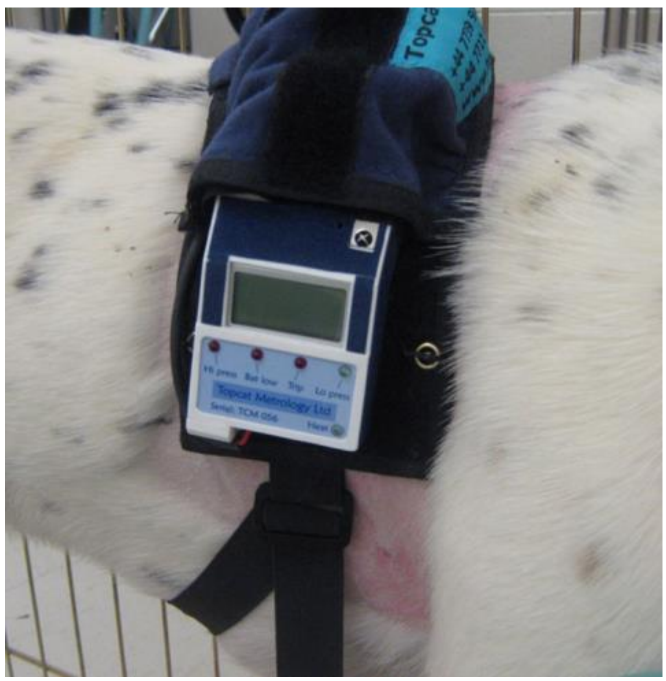
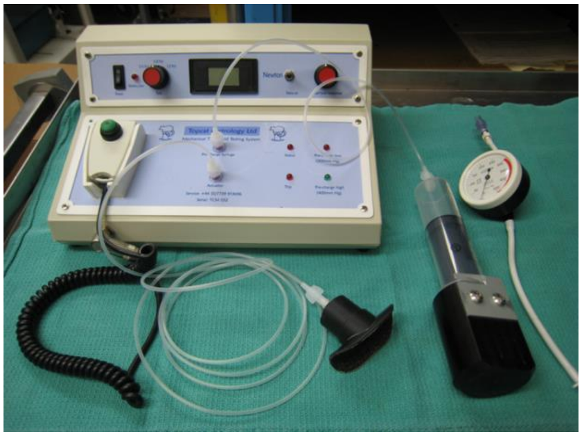


Figure 4.1: Thermal nociceptive threshold testing device (Topcat Metrology Ltd.) was applied on the latero-dorsal aspect of a dog’s thorax. The thorax was clipped of hair allowing better contact between the thermal probe and skin of the dog.

The mechanical nociceptive threshold device (**Figure 4.2**) was applied to the proximal aspect of the forelimb, specifically the dorsolateral aspect of the radius of each dog. The device consisted of a blunt ended probe attached to a force sensor that could be remotely activated with increasing force at a fixed rate. The threshold was recorded as the force at which the dog first responded (proximal mechanical threshold).

A.



B.

C.

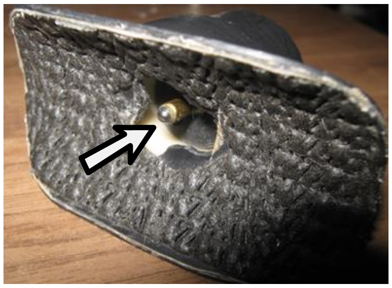


Figure 4.2: Mechanical nociceptive threshold testing device (Topcat Metrology Ltd.). The device (A) was applied to the proximal aspect of the shaved forelimb, specifically in the dorsolateral aspect of the radius of the dog. A sham device placed on the opposite limb (B); The arrow indicates the blunt ended probe of the device (C).

The dogs were brought into the study room two at a time, for mechanical and thermal threshold measurements. They were allowed to roam freely around the room during the thermal threshold assessment. However, during the measurement of mechanical threshold, some dogs needed to be slightly restrained in order to stay next to the pressure transducer.

In order to minimize tissue damage in the absence of a response to noxious stimulation, cutoffs of 60°C, and 20 Newton's were set for the thermal and proximal mechanical nociceptive devices, respectively. The thermal and proximal mechanical thresholds were assessed three times, at 7 am, 1 pm, and at 9 pm for three consecutive days. At each time point the threshold measurements were repeated at the manufacturer's recommended intervals to obtain the average of 2 - 3 readings within 10% of each other and the average of these values was used in subsequent analyses. All measurements were performed by the same investigator.

Phase 2

Following baseline measurements each dog was administered buprenorphine 0.03 mg/kg (Hospira, IL, USA) orotransmucosally. Rectal temperature, pulse rate, and respiratory rate were recorded prior to drug administration and at 1, 6, and 24 hours post drug administration. Pulse rate was measured by femoral pulse palpation and respiratory rate by observation of thoracic excursions, both over a 30-second interval.

Assessment of analgesic efficacy

Thermal and proximal mechanical nociceptive threshold testing devices were calibrated on the morning of each trial and the calibrations checked twice daily. For the device used on the distal aspect of the forelimb (Self-built C-clamp), known weights were used for calibration once before the trial due to prior experience and familiarity.

The self-built C-clamp (**Figure 4.3**) was used to apply force manually in a dorsopalmar manner just distal to the large foot pad over the metacarpal bones. The clamp was a manually applied C-clamp equipped with a calibrated 1-cm^2 force transducer connected to an electronic recorder capable of recording the peak force or pressure at which the dog first responded (distal nociceptive threshold). Values were subsequently converted from lb/cm^2 to Newton's. For the thermal and proximal mechanical threshold measurements, the protocols from phase 1 were repeated.

Figure 4.3: Mechanical nociceptive threshold device (Self-built C-clamp) was used to apply force manually in a dorsopalmar manner just distal to the large foot pad over the metacarpal bones to determine the distal mechanical nociceptive threshold.

At each measurement point, nociceptive thresholds were determined 2 to 3 times for each testing modality. Attempts were made to have 2 to 3 measurement values within 10% of each other and the mean of these values was used in subsequent analyses.

To minimize tissue damage in the absence of a response to noxious stimulation, cutoffs of 60°C, 20 Newton's, and 20 lb/cm^2 were set for devices used to determine the thermal, proximal mechanical, and distal mechanical nociceptive thresholds, respectively. All threshold measurements were obtained prior to (baseline), and at 15 minutes and 1, 2, 4, 6, 12, 18, and 24 hours after drug administration. Behavioral and physiologic data were obtained before threshold measurements were performed.

Statistical analysis

Data were summarized as mean ± SD and analyzed by use of statistical software (SAS/STAT software, version 9.2; SAS Institute Inc., NC, USA). A mixed model ANOVA was used for analysis data obtained from phase 1. Pairwise comparisons between the different time points were examined by use of *t* tests. In phase 2, a repeated measures ANOVA was used to compare between baseline data and those at the other time points for a given parameter. *t* tests were used for pairwise comparisons. Residuals from ANOVA were evaluated and confirmed to be approximately normally distributed and independent. Values of $P < 0.05$ were considered significant for all analyses.

Results

Phase 1

As shown in **Table 4.1**, the thermal nociceptive threshold was not significantly different between the three different time points during each day or between days.

The proximal mechanical threshold did not differ between the three different time points during the day. However the mechanical threshold averaged over all three time points on day 3 was significantly higher than that on day 1 (mean ± SD of 8.1 ± 2.3 vs. 7 ± 1.3 N, respectively; P = 0.014).

Table 4.1: Mean (SD) values for differences between initial skin and threshold temperature (°C) and proximal mechanical nociceptive thresholds (Newton's) in 6 Walker hounds at the three different time points (7 am, 1 pm and 9 pm) during each day for three consecutive days. Different letters denote significant ($P < 0.05$) differences between time points each day.

Nociceptive thresholds	Day 1			Day 2			Day 3		
	7:00 A	**1:00 P**	**9:00 P**	**7:00 A**	**1:00 P**	**9:00 P**	**7:00 A**	**1:00 P**	**9:00 P**
Difference between skin and threshold temperature (°C)	11.2 (1.5)[A]	10.7 (1.2)[A]	11.3 (2.6)[A]	11.1 (1.3)[A]	10.8 (2.8)[A]	9.9 (1.3)[A]	10.7 (1.8)[A]	10.3 (1.7)[A]	10.5 (1.8)[A]
Proximal mechanical nociceptive threshold (Newton's)	6.6 (1.1)[a]	7.1 (1.9)[a,c]	7.3 (0.9)[a,c]	7.5 (2.2)[a,b,c]	7.5 (1.8)[a,b,c]	7.4 (2.1)[a,b,c]	7.2 (2.1)[a,c]	8.3 (2.6)[b,c]	8.8 (2.2)[b]

Phase 2

Physiologic responses

Respiratory rate at 1, 6 and 24 hours (all were 19 ± 4 breaths/min) after buprenorphine administration were lower than at baseline (27 ± 4 breaths/min; all $P \leq 0.002$). Pulse rate was lower than the baseline value at 6 hours after drug administration (95 ± 13 vs. 117 ± 9 beats/min; P = 0.001). Rectal temperatures did not change significantly over time. (**Table 4.2**)

Table 4.2: Mean (SD) values of physiologic variables in 6 Walker hounds after administration of buprenorphine (0.03 mg/kg, OTM). *Value differs significantly (P < 0.05) from respective baseline value.

	Pulse rate (beats/min)	**Respiratory rate (breaths/min)**	**Rectal temperature (°C)**
Baseline	117 (9)	27 (4)	39.0 (0.2)
1 h	105 (13)	19 (4)*	38.8 (0.6)
6 h	95 (13)*	19 (4)*	38.5 (0.6)
24 h	109 (13)	19 (4)*	39.0 (0.2)

Behavioral responses

Responses to the nociceptive devices varied with the individual dog. Some turned their heads toward the stimulus and, in some situations, attempted to bite the device, whereas others attempted to move away from the stimulus or had a definitive skin twitch (thermal) or forelimb lift (mechanical).

Nociceptive thresholds

Results from the nociceptive threshold measurements are shown in **Figure 4.4**. The thermal threshold was significantly higher than baseline at 2 hours post drug administration (11.3 ± 3.2 vs. 16.2 ± 4.6 °C; P = 0.025).

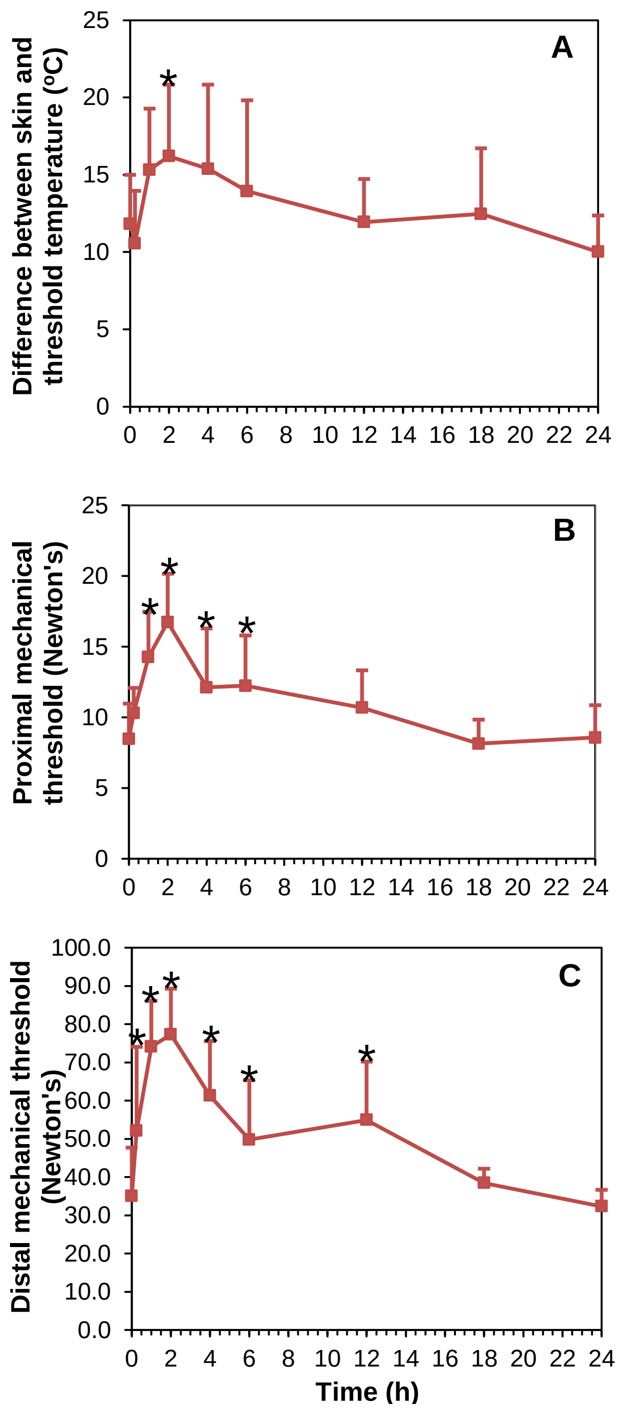


Figure 4.4: Mean ± SD values for thermal (A) proximal mechanical (B), and distal mechanical (C) nociceptive thresholds in 3 male and 3 female 7- to 8-month-old healthy Walker hounds after administration of liquid buprenorphine (0.03 mg/kg, orotransmucosally). Time 0 on the x-axis represents values measured at baseline prior to drug administration. *Value differs significantly ($P < 0.05$) from the baseline value.

The proximal nociceptive thresholds at 1 (14.3 ± 3.2 Newton's), 2 (16.7 ± 3.4 Newton's), 4 (12.1 ± 4.2 Newton's) and 6 (12.2 ± 3.6 Newton's) hours after buprenorphine treatment were significantly higher than baseline (8.5 ± 2.5 Newton's; $P < 0.001$, $P < 0.001$, $P = 0.023$ and $P = 0.019$, respectively).

The distal nociceptive thresholds were significantly higher than baseline at every time point through the 12-hour measurement.

Discussion

The test of repeatability presented no significant differences in the thermal threshold during different time points under the controlled conditions. No sign of tissue damage at any site of testing was observed. The application of the thermal and proximal mechanical threshold testing devices to dogs appeared to work well, inducing predictable responses. The response characteristics of each individual dog were consistent and repeatable. These results are in agreement with the manufacturer studies in cats using similar devices which showed that both repeatability of the nociceptive threshold pressure (Dixon et al. 2007) and temperature (Dixon et al. 2002) were considered acceptable.

However, the proximal mechanical threshold recorded during day 3 appeared to be higher than the data observed during day 1. This may reflect some degree of stress-induced analgesia in dogs as some of them needed to be slightly restrained during the assessment. It is also possible that dogs were less concerned with the stimulus from the device when the measurement was repeated multiple times.

During the study some dogs tried to remove the thermal and proximal mechanical devices by chewing and pulling them out. This was not observed in a previous study using a similar thermal device for testing the analgesic efficacy of pain medications in six adult beagles (6 - 8 years old). The beagles in that study wore the thermal device for up to 10 hours without attempting to remove or playing with the device during the evaluation (Hoffmann et al. 2012). It is likely this resulted from our use of young dogs (7-8 months old) with high levels of alertness, playfulness and responsiveness to environment compared to the adult dogs. Hence the thermal device may be more suitable in mature dogs. Another explanation is that in the beagle study, dogs had been wearing the thermal device and performed the placebo treatment over a six month period prior to the drug tests (Hoffmann et al. 2012). Therefore, the problem of less tolerance in animals may be minimized by extending the period for habituation to the devices prior to starting an experiment.

Changes in nociceptive thresholds following orotransmucosal buprenorphine in dogs were observed with all testing modalities used. However the thermal nociceptive threshold increased for only a short period of time (2 hour) following drug administration.

The thermal threshold testing device was used previously to evaluate the analgesic efficacy of buprenorphine in cats after OTM administration. The results demonstrated an increase of the thermal threshold between 30 min and 6 h after buprenorphine treatment (0.02 mg/kg) (Robertson et al. 2005a). This may indicate a longer duration of action of the drug or a better detection of the analgesic efficacy of orotransmucosal buprenorphine using the thermal device in cats compared to dogs.

Given the unequal duration of the threshold increase between the 2 mechanical threshold tests (between 1 and 6 hours for the proximal test and between 15 minutes and 12 hours for the distal test), it is likely the sensitivity of the 2 tests is different.

The device used for the proximal limb measurements has been used to evaluate the analgesic effects of buprenorphine administration in cats (Steagall et al. 2007) and the effect of butorphanol in cats and dogs (Dixon et al. 2010). An increase of the proximal mechanical threshold was found in cats at 2 hour after administration of buprenorphine (0.01 mg/kg, subcutaneously) (Steagall et al. 2007), at 30 minute after butorphanol treatment (0.2 mg/kg, intramuscularly), and in dogs between 15 and 45 minutes after administration of butorphanol (0.25 mg/kg, intramuscularly) or fentanyl (0.005 – 0.01 mg/kg, intravenously) (Dixon et al. 2010). Nonetheless, to our knowledge, the present study is the first in which the proximal mechanical testing device was used in dogs that received buprenorphine, so no data are available for comparison.

Results obtained by use of the distal mechanical nociceptive stimulus are similar to those reported for the same dose and route of buprenorphine administration in dogs (Mama et al. 2008). In that study evidence of analgesia was observed between 15 minutes and 8 hours after drug administration.

Pharmacokinetic studies of OTM buprenorphine have been performed in both dogs and cats after a dose of 0.02 mg/kg was administered. The median bioavailability in six adult cats was 116.3% (67.6 – 133.6%), the half-life was 243 min (125 – 1154 min), the maximum plasma concentration was 12.5 ng/mL (2.6 – 19.4 ng/mL), and the time of maximum concentration was 30 min (10 – 45 min) (Robertson et al. 2005a). In dogs the bioavailability was 38 ± 12% (mean ± SD), the half-life was 426 ± 72 min, the maximum plasma concentration was 2.2 ± 0.3 ng/mL, and the time of maximum concentration was 42 ± 12min (Abbo et al. 2008). These pharmacokinetic parameters seem to indicate a higher absorption of the drug in cats and consequently the better efficacy.

Even though the cutoff of 60 ºC was preset to prevent tissue damage from the thermal nociceptive threshold device, inflamed spots were noticed on the thoracic skin of some dogs in the area exposed to the thermal probe. Despite this we did not note a difference in thermal thresholds over time except at 2 hour post drug. To avoid tissue inflammation in future studies using the thermal nociceptive threshold device in dogs, the cutoff temperature level for the thermal device may need to be lower than 60 ºC.

In summary, all testing devices applied in the present study demonstrated increase of nociceptive thresholds in dogs following the OTM administration of buprenorphine. This suggests the potential usefulness of orotransmucosal buprenorphine for treatment of pain in dogs.

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CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

Pain is a subjective, individualized and complex phenomenon. To understand mechanisms of pain and improve pain treatment, animal models using analgesiometers and/or scaling systems to assess pain under individual circumstances have been developed. This dissertation presents two studies evaluating the analgesic properties of two drugs in small animal models using different approaches. Additionally the development and/or validation of these models are highlighted. The first study was designed to evaluate the antinociceptive effect of maropitant, a selective NK-1 receptor antagonist, using visceral noxious stimulus for MAC determination in cats. In the second study multiple nociceptive modalities were used to investigate the analgesic efficacy of orotransmucosal buprenorphine in dogs.

A model for ovary and ovarian ligament stimulation previously applied in dogs (Boscan et al. 2011a) was modified and used to determine the effect of maropitant on the anesthetic requirement of sevoflurane in cats. This visceral model was selected because the preclinical information showed high expression of the NK-1 receptor in neural pathway carrying visceral nociceptive signals (Brown et al. 1995; Perry & Lawson 1998; Laird et al. 2000). This model induced predictable visceral noxious stimuli that do not damage ovary and ovarian ligament tissues (Boscan et al. 2011a), and was utilized to evaluate the MAC sparing effect of maropitant in dogs (Boscan et al. 2011b).

In our study we observed that the ovarian stimulation model did work well as a visceral noxious stimulus for determination of MAC and demonstrated the anesthetic sparing effect of maropitant in cats. Intravenous maropitant both at 1 mg/kg and 5 mg/kg reduced the MAC requirements for sevoflurane significantly compared to the value obtained prior to the treatment.

The underlying mechanisms of the MAC sparing effect of maropitant remain unknown. However, as reviewed in Chapter 3, preclinical studies in multiple species suggest that NK-1 receptors may play a role in regulating visceral pain transmission. For example, nociceptive responses to intracolonic administration of capsaicin were reduced in NK-1 knockout mice (Laird et al. 2000). The responses to visceral noxious stimuli such as intraperitoneal injection of acetic acid in mice (Nagahisa et al. 1992) and gerbil (Gallantine & Meert 2004), and bradykinin stimulation of gallbladder in cats (Pan et al. 1995) were attenuated after the administration of NK-1 receptor antagonists. Hence it is possible that maropitant reduced the nociceptive responses evoked by the stimulation of the ovary and ovarian ligament in cats, and so decreased the anesthetic requirement for preventing purposeful movements during the MAC determinations.

Nevertheless MAC values are more likely to be the result from the interaction between the tested drug and inhalant anesthetic (Docquier et al. 2003), and further investigation is required to evaluate the antinociceptive effect of maropitant without combining it with other drugs.

To further evaluate the analgesic effect of maropitant, an animal model of visceral clinical pain may be of interest due to the MAC sparing effect of the drug during the visceral noxious stimulation. Recent studies reported some visceral pain conditions are associated with NK-1 receptor expression. An upregulation of the lumbosacral spinal cord NK-1 receptor was found in rats with irritated bladder-induced abdominal pain (Ishigooka et al. 2001), and a significant positive correlation between NK-1 expression in colonic lamina propria of diverticulosis patients and VAS pain scores during rectal distension in humans (Humes et al. 2012). Also a significant relationship between NK-1 receptor mRNA concentrations in pancreatic tissue and the intensity, frequency and duration of pain in humans with chronic pancreatitis has been shown (Shrikhande et al. 2001). The evidence encourages further clinical trials to evaluate the analgesic efficacy of maropitant in animals with cystitis, enteritis and pancreatitis.

The second study was designed to evaluate the analgesic efficacy of orotransmucosal buprenorphine in dogs. Following validation two mechanical and one thermal nociceptive threshold testing devices were utilized. All of the devices demonstrated increases of nociceptive thresholds at at least one time point after buprenorphine treatment, supporting that the OTM administration of buprenorphine has potential usefulness as an analgesic medication in dogs.

Two testing devices from Topcat Metrology Company constructed originally for use in cats (Dixon et al. 2002; Slingsby et al. 2009; Dixon et al. 2010) appear to be repeatable in measuring nociceptive thresholds in dogs. However, using the proximal mechanical threshold testing device over multiple time periods may promote some degree of learning and stress-induced analgesia in the animals.

Differences in analgesic duration of buprenorphine among the three testing devices (at 2 hour post treatment for thermal test, between 1 and 6 hours post drug for proximal mechanical test, and up to 12 hours post dosing for distal mechanical test) may assert the importance of using multiple modalities to evaluate the analgesic efficacy of a pain medication. The mechanism of pain is complex; therefore, the outcomes can be varied between individual modalities.

Our findings obtained from maropitant and orotransmucosal buprenorphine studies indicate the promising analgesic effect of both tested drugs. However the investigations were limited to normal animals in which the nociceptive responses were evoked from healthy tissue.

Further investigation using animal models of clinical pain are required for assessing the drug effects on naturally occurring pain.

Recently a study evaluated the postoperative analgesic efficacy of orotransmucosal buprenorphine in dogs undergoing ovariohysterectomy. In the study dogs received buprenorphine orotransmucosal 0.12 mg/kg or 0.02 mg/kg via the cheek pouch prior to anesthetic induction. The postoperative pain assessment using a pain scale demonstrated an analgesic duration of 20.3 hours and 7.3 hours, respectively (Ko et al. 2011). This is in agreement with our results where nociceptive thresholds were elevated for up to 12 hours after OTM administration of 0.03 mg/kg of buprenorphine.

Results of our studies and recent reports from broader clinical use are encouraging and support the ongoing investigation of both compounds for treatment of animal pain.

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