DISSERTATION

EVALUATION OF THE PHARMACODYNAMICS AND ANALGESIC EFFICACY OF TWO BUPRENORPHINE FORMULATIONS IN DOGS

Submitted by

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ABSTRACT

EVALUATION OF THE PHARMACODYNAMICS AND ANALGESIC EFFICACY OF TWO BUPRENORPHINE FORMULATIONS IN DOGS

Pain is an unpleasant and distressing experience. While it serves a protective function for a short period, untreated, ongoing pain has significant detrimental effects. Opioids are one of the most important analgesic agents used for pain management in human and veterinary medicine, with the μ opioid agonists being the most effective for moderate to severe pain. Traditional presentations of μ opioid agonists or partial agonists usually require frequent dosing, which can lead to significant stress due to frequent animal restraint, variability in the level of analgesia and can be labor intensive and time consuming for veterinary personal.

Buprenorphine has been shown to be effective in the treatment of mild to moderate pain with minimal adverse effects in dogs. Despite having a longer dosing interval than most other mu agonists, buprenorphine still requires hospitalization of clinical patients or intermittent handling of research animals. In recent years, new formulations of buprenorphine have been introduced and have the potential to provide longer duration of action after a single subcutaneous injection. This dissertation presents the pharmacodynamics and analgesic efficacy of two long-lasting buprenorphine formulations in dogs. The first formulation evaluated is a sustained-release buprenorphine (buprenorphine HCL in a proprietary sustained release biodegradable liquid

polymer matrix). The pharmacokinetics and selected behavioral, physiologic and antinociceptive effects of two doses of this sustained-release formulation was evaluated in dogs. The antinociceptive effects of this formulation were assessed using a mechanical nociceptive testing threshold device. The second formulation is a high-concentration buprenorphine, which has been approved for use in cats. Selected behavioral, physiologic and antinociceptive effects of two doses of this high-concentration formulation were evaluated in dogs undergoing neutering. The analgesic efficacy of this formulation was evaluated using 3 different pain-scoring systems.

Both formulations showed promising results. Sustained-release buprenorphine provided significant increase in mechanical nociceptive thresholds for up to 84 hours after drug administration, while the high-concentration buprenorphine provided effective analgesia with minimal side effects in dogs undergoing neutering for at least 24 hours after drug administration. These findings suggest that both formulations have the potential to be an effective and practical alternative in providing long-lasting analgesia in dogs.

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CHAPTER 1: PAIN NEUROPHYSIOLOGY AND PHARMACOLOGIC INTERVENTIONS

1.1 Introduction

In 1994, the International Association for the Study of Pain (IASP) Task Force on Taxonomy defined pain as "an unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage" and also noted that "the inability to communicate verbally does not negate the possibility that an individual is experiencing pain and is in need of appropriate pain relieving treatment". This pain definition has been extensively accepted and used in both human and veterinary medicine, but recognition that it does not address the current scientific advancements in the understanding, assessment and treatment of pain has led to the proposal of an updated definition by Williams and Craig (2016): "Pain is a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive and social components". The updated definition builds upon the original one by including cognition and social behaviors, providing a more inclusive perspective (Williams & Craig 2016). Pain experience in people comprises three dimensions namely sensory-discriminative, affective-motivational and cognitive-evaluative (Bee & Dickenson 2009). The sensory-discriminative dimension provides information on the onset, location, intensity, type and duration of pain inducing stimulus. The motivational-affective dimension consists of the unpleasant experience of pain and suffering as well as triggers the organism into action. The cognitive-evaluative dimension encompasses the effects of prior experience, social and cultural values, anxiety, attention and conditioning. In animals, this dimension may be the only one that differs significantly from humans (Hellyer et al. 2007).

Pain serves a protective function for living organisms (Muir III & Woolf 2001), allowing individuals to detect and respond to a potentially damaging stimulus (Moore 2016), leading to disuse, rest and recuperation, guarding and avoidance, thereby minimizing further injury and promoting the repair process (Muir III 2009). Although pain is beneficial for survival for the short period, ongoing pain can cause many deleterious effects to animals. For example, reduction in food and water consumption (Flecknell 2000; Valtolina & Goggs 2012), impaired respiration, reduction in mobility and self-maintenance behaviors (Flecknell 2000), precipitation of abnormal behaviors (e.g., self-trauma, hiding, aggression), immune system suppression and increase in blood viscosity (which can contribute to pathologic blood clot formation), increase in the metastatic rate of some cancers (Downing 2015), increase in rate of postoperative infection and sepsis (Grant 2006) and decreased or prolonged healing (Pigott 2017).

As described, pain affects animals in several aspects, and hence it is our responsibility to alleviate pain and ensure that animals in our responsibility have good quality of life. To do this effectively, it is important to understand the pain pathways and how medications act along these pathways.

1.2 Pain pathway

The pain pathway consists of four main mechanisms namely, transduction, transmission, modulation and perception (Grubb 2018) as illustrated in **figure 1.1**

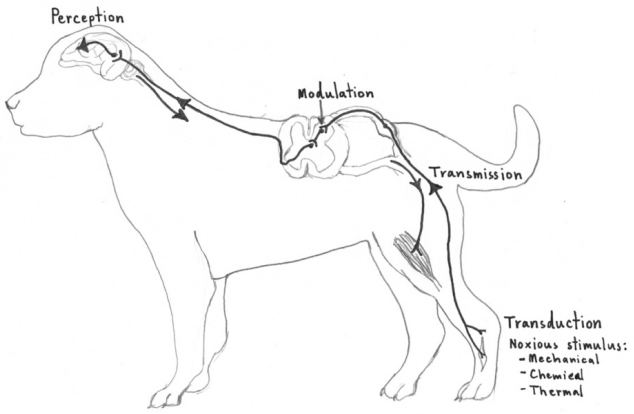

Figure 1.1: Pain pathways

1.2.1 Transduction

The conversion of a physical stimulus into electrical activity at the peripheral nociceptor is known as transduction (Palmer 2007). Nociceptors have a high threshold and normally respond only to stimuli of sufficient energy to potentially or actually damage tissue (Chan et al. 2018). When the first-order neurons, which have the naked nerve endings in the periphery and cell bodies in dorsal horn ganglia, are stimulated by noxious stimuli, they encode noxious stimuli (e.g. mechanical, chemical or thermal stimulus) into electrical activity through activation of transduction channels expressed on the axonal membrane (Ringkamp et al. 2018). Important transduction channels include transient receptor potential (TRP) channels, purinergic 2X (P2X) receptors and acid sensitive ion channels (ASICs) (Tominaga 2007).

1.2.1.1 Transient receptor potential (TRP) channels

Mammalian TRP channels are ligand-gated and voltage-sensitive, and most family members are inward rectifying channels that non-selectively conduct cations (Ca^{2+}, Na^{+}), leading to rapid changes in intracellular cation concentration (Veldhuis et al. 2015). They have a wide variety of modes of activation (temperature, chemical compounds, mechanical stimulation,

oxidative stress, pheromones, acid, osmolarity, lipids, light) and regulation (transcription, alternative splicing, glycosylation, phosphorylation), ion selectivity, broad tissue distribution and physiological functions (Levine & Alessandri-Haber 2007). TRP channels' physiological functions range from sensory functions (such as vision, nociception, taste transduction, temperature sensation and pheromone signaling) to homeostatic functions (such as Ca^{2+} and Mg^{2+} flux and osmoregulation) (Bishnoi & Premkumar 2013). Mammalian TRP channels comprise 28 members, which can be grouped based on sequence homology into 6 subfamilies, namely vanilloid (TRPV1-TRPV6), melastatin (TRPM1-TRPM8), ankyrin (TRPA1), canonical (TRPC1-TRPC7), mucolipin (TRPML1-TRPML3) and polycystin (TRPP1-TRPP3) (Wu et al. 2010; Veldhuis et al. 2015; Thiel et al. 2017; Moran & Szallasi 2018). Only some members of the subfamilies TRPV (TRPV1, TRPV3 and TRPV4), TRPM (TRPM2, TRPM3 and TRPM8) and TRPA (TRPA1) have shown ability to detect and transduce specific nociceptive modalities (Mickle et al. 2015; Mickle et al. 2016).

TRPV1 channels have gained significant attention in pain studies as inflammatory mediators (bradykinin, prostaglandins and glutamate among others) have been shown to modulate receptor activity (Premkumar & Ahern 2000; Lee et al. 2005; Immke & Gavva 2006). TRPV1 channels are found in the brain and dorsal root ganglia, as well as in skin and other non-neuronal tissues (Hayes et al. 2000; Immke & Gavva 2006). They can be activated by a wide variety of noxious stimuli (Mickle et al. 2015) with capsaicin, heat and pH being the main ones (Moran et al. 2011). TRPV3 channels may be involved in thermosensory transduction (both innocuous and noxious heat) (Moqrich et al. 2005), while TRPV4 is believed to be involved in mechanical nociception in the colon (Brierley et al. 2008; Holzer 2011a; Mueller-Tribbensee et al. 2015). TRPM2 channels are involved in visceral nociception and hypersensitivity

(Matsumoto et al. 2016), TRPM3 channels are associated with noxious heat detection (Vriens et al. 2011; Straub et al. 2013; Held et al. 2015; Krügel et al. 2017) and TRPM8 channels are involved in the detection of cold temperature (both innocuous and noxious) (Bautista et al. 2007; Colburn et al. 2007; Dhaka et al. 2007; Knowlton et al. 2010; Knowlton et al. 2013). TRPA1 channels are believed to play an important role as a transducer of irritant (both endogenous and exogenous) or proalgesic agents that elicit inflammatory pain (Bautista et al. 2006). They may also be involved in both cold (Kwan et al. 2006; Karashima et al. 2009) and mechanical nociception (Kerstein et al. 2009).

1.2.1.2 Purinergic 2X (P2X) receptors

P2X receptors are ATP gated non-selective cation channels (Rassendren & Ulmann 2014) located on Aδ- and C fibers and in sensory neurons in the dorsal root and cranial sensory ganglia. These receptors open in response to the binding of extracellular ATP (Gu & Heft 2004), which is released in response to tissue injury (Ford 2012). At least 7 subunits (P2X1-P2X7) of P2X receptors have been identified (Tsuda et al. 2010). P2X3 receptors may be involved in the transduction of various painful conditions, such as acute pain associated with tissue injury (Chizh & Illes 2001; Wirkner et al. 2007; Toulme et al. 2010), visceral pain (Burnstock 2009; Burnstock 2012; Burnstock 2013), tooth-pulp pain (Adachi et al. 2010) and inflammatory pain (Oliveira et al. 2009; de Oliveira Fusaro et al. 2010; Teixeira et al. 2010; Prado et al. 2013).

1.2.1.3 Acid-sensing ion channels (ASICs)

ASICs are sodium-selective ion channels activated by low extracellular pH, and belong to the degenerin/epithelial sodium channel (DEG/ENaC) family (Waldmann et al. 1997;

Schaible et al. 2011). They are important sensors of tissue acidosis (Schaible et al. 2011). ASICs have been described in sensory neurons of dorsal root ganglia, in vagal and trigeminal ganglia of central nervous system and in nociceptive fibers (Deval et al. 2010). At least 6 subunits of ASICs have been identified in mammals, namely ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4 (Holzer 2011b; Lee & Chen 2018). ASIC3 is the most abundant subtype expressed in the peripheral nervous system as well as being the most acid-sensitive ASIC subtype (can be activated at pH around 7.0) (Deval & Lingueglia 2015; Lee & Chen 2018). ASIC3 plays a role in pain associated with various conditions including muscle fatigue (Sluka & Gregory 2015), peritoneal pain related to acidosis (Holzer 2011b), chest pain due to cardiac ischemia and gastrointestinal pain associated with gastric acidity (Gu & Lee 2010).

Examples of drugs that are effective at pain transduction include non-steroidal anti-inflammatory drugs (NSAIDs), local anesthetics (Epstein 2018; Grubb 2018), and corticosteroids (Epstein 2018).

1.2.2 Transmission

Transmission is the propagation of nerve impulses from the periphery (site of transduction), via afferent sensory fibers, to the spinal cord (dorsal horn) and from the spinal cord to the brain, via two main ascending pathways (Tranquilli et al. 2004). There are 3 major components of the transmission system: the first-order neuron (peripheral sensory neurons), the second-order neuron (spinal neurons) and the third-order neuron (neurons of brainstem and diencephalon) (Ringkamp et al. 2018). The encoded electrical activity propagates along the afferent sensory fibers of the first-order neurons to the second-order neurons. The speed of transmission is directly correlated to the diameter and myelination of the sensory fibers (Dubin

& Patapoutian 2010). There are 3 types of afferent sensory fibers: large-diameter myelinated Aβ fibers, thinly myelinated Aδ fibers and unmyelinated C fibers (Ringkamp et al. 2018). The Aβ fibers have a fast conduction velocity of more than 20 m/s and are responsible for transmitting non-nociceptive signal (e.g., light touch, pressure, hair movement) (Ringkamp et al. 2018). The thinly myelinated Aδ fibers with conduction velocities between 2 and 20 m/s and unmyelinated C fibers with conduction velocities of less than 2 m/s are responsible for the transmission of nociceptive signals (Ringkamp et al. 2018). Aδ fibers can be further divided into type I and type II. Type I Aδ fibers respond to both mechanical and chemical stimuli, but have a high heat threshold (>50^{o}C), whereas type II Aδ fibers have a very high mechanical threshold with a much lower heat threshold than type I Aδ fibers (Hladnik et al. 2015). C fibers are further categorized into peptidergic and non-peptidergic C fibers (Shilo & Pascoe 2014; Riegelhaupt & Angst 2019). The peptidergic C fibers express substance P (SP) and calcitonin gene-related peptide (CGRP) and are sensitive to neural cell derived nerve growth factor, while the non-peptidergic C fibers express the receptor tyrosine kinase and are sensitive to glial cell line-derived neurotrophic factor (Shilo & Pascoe 2014; Riegelhaupt & Angst 2019). Aδ fibers are responsible for the first pain (a sharp, intense, tingling sensation), while C fibers are responsible for the second pain (a prolonged burning sensation) (Ringkamp et al. 2018). The Aβ fibers are involved in modulating nociceptive transmission as described by the gate control theory (Melzack & Wall 1965), which will be discussed later in this chapter. Most of Aδ fibers terminate in lamina I (with a small subset terminating in lamina V) and C fibers terminate in lamina I and II of the dorsal horn of the spinal cord (Braz et al. 2014; Riegelhaupt & Angst 2019). When the action potentials reach the central terminal of sensory neurons in the spinal cord, calcium influx through presynaptic voltage-gated calcium channels triggers the release of

pronociceptive neurotransmitters, which in turn, bind to their specific receptors to excite the second-order neurons (McGivern 2006). The second-order neurons are located in the dorsal horn of the spinal cord (Posner 2008). These second-order neurons include projection neurons (nociceptive specific and wide dynamic range), interneurons (excitatory and inhibitory) and propriospinal neurons (Shilo & Pascoe 2014). The nociceptive specific (NS) projection neurons are densely located in lamina I and scattered throughout lamina III-VI (Bell 2018). They receive input from Aδ- and C fibers (D'Mello & Dickenson 2008), thus transmit only noxious signals (Riegelhaupt & Angst 2019). The wide dynamic range (WDR) projection neurons are located in lamina I and V (Benarroch 2001; Suzuki & Dickenson 2005). They receive input from Aß-, Aδ- and C fibers (Benarroch 2001; D'Mello & Dickenson 2008; Grubb 2018) and respond to input in a graded fashion depending on stimulus intensity (D'Mello & Dickenson 2008; Grubb 2018), thus transmitting both non-noxious and noxious signals (Hellyer et al. 2007; Riegelhaupt & Angst 2019). The WDR projection neurons receive both somatic and visceral inputs, permitting the convergence of viscera-somatic signals leading to the perception of referred pain (e.g., myocardial ischemia causing shoulder pain) in humans (Riegelhaupt & Angst 2019). The projection neurons transmit information about noxious stimuli to the higher centers in the central nervous system (CNS) (Takazawa & MacDermott 2010) via ascending spinal tracts within the spinal cord. The spinocervicothalamic and the spinoreticular tracts are the most important from a clinical perspective. The spinocervicothalamic tract is the dominant ascending nociceptive pathway in carnivores (Ha & Liu 1966) and conveys the sensation of touch and superficial pain and is considered discriminative as it allows for the exact location of the pain stimuli by the animal (Hellyer et al. 2007). The spinoreticular tract transmits information regarding deep and visceral pain and because it's a more diffuse pathway, it is non-discriminative. In humans (and

likely in animals), this tract is associated with the emotional component of the pain perception (Hellyer et al. 2007).

Several channels and receptors are involved in the transmission of nociceptive signal, for example voltage-gated sodium channels (VGSCs), voltage-gated calcium channels (VGCCs), potassium channels (K^+ channels) (Gohar 2005; Xiao et al. 2018), glutamate receptors (Gohar 2005) and neurokinin-1 receptors (NK-1receptors). (Shilo & Pascoe 2014).

1.2.2.1 Voltage-gated sodium channels (VGSCs)

VGSCs open upon depolarization by generator potentials, leading to sodium ion influx (Lampert et al. 2014) and the initiation and propagation of action potentials in excitable cells (Catterall et al. 2005; Peters & Ruben 2014), including nerve, muscle and neuroendocrine cell types (Catterall et al. 2005). The family comprises of 9 isoforms ($Na_v1.1$-$Na_v1.9$) (Yu & Catterall 2003; Bagal et al. 2014), which can be further divided based on their susceptibility to being blocked by tetrodotoxin (TTX) (Wood et al. 2004). Three isoforms ($Na_v1.7$-$Na_v1.9$) are preferentially expressed in peripheral sensory neurons (Yu & Catterall 2003; Dib-Hajj et al. 2015) and involved in pain transmission (Dib-Hajj et al. 2010; Levinson et al. 2012). $Na_v1.7$, which is TTX-sensitive, plays an important role in setting the threshold for generation of action potentials in small diameter nociceptive neurons (Cummins et al. 2007). It is normally expressed on both peripheral and central terminals of nociceptive neuron (Xiao et al. 2018) and its absence (as seen in the global $Na_v1.7$ knockout mice) results in complete insensitive to pain (Gingras et al. 2014). In addition, people with a loss-of-function mutation of $Na_v1.7$ suffer from congenital indifference to pain (CIP) (Cox et al. 2006; Goldberg et al. 2007). $Na_v1.8$ and $Na_v1.9$ are TTX-resistant channels. $Na_v1.8$ plays a major role in underlying the upstroke of action potentials and

continuous firing activity during sustained depolarizations in small-diameter peripheral sensory neurons (Renganathan et al. 2001). It's involved in noxious heat nociception, mechanical nociception (Akopian et al. 1999) and cold nociception (Zimmermann et al. 2007). $Na_v1.9$ plays a role in setting resting membrane potential as well as contributing to sub-threshold electrogenesis in small dorsal root ganglion (DRG) neurons (Cummins et al. 1999; Herzog et al. 2001). It also has a role in modulating neurotransmitter release in the dorsal horn of the spinal cord at the first synapse of the pain-signaling pathway (Dib-Hajj et al. 2015). $Na_v1.9$ is a key determinant in cold nociception (Lolignier et al. 2015).

1.2.2.2 Voltage-gated calcium channels (VGCCs)

VGCCs role in chronic and neuropathic pain have been well established, particularly the N- and T-type channels (Doan 2010). The N- ($Ca_v2.2$) and T- ($Ca_v3.1$-$Ca_v3.3$) type calcium channels appear to have the most critical role in nociceptive signaling (Bourinet et al. 2014). N-($Ca_v2.2$) type calcium channels are located on synaptic nerve terminals in lamina I and II of the dorsal horn, and are activated by membrane depolarization. Their opening results in the release of neurotransmitters such as glutamate, substance P and calcitonin gene-related peptide (CGRP) (Zamponi et al. 2009). Therefore, N-type calcium channels play a major role in controlling synaptic transmission in pain processing (Park & Luo 2010) and changes in sensory excitability (Doan 2010). Differently from N-type channels, which are activated by strong membrane depolarizations, T-type channels activate at low-voltage, more negative membrane potentials. T-($Ca_v3.1$-$Ca_v3.3$) type calcium channels can lower the threshold for action potentials, facilitating bursting activity and synaptic excitation (Zamponi et al. 2009; Doan 2010). While they can be found throughout the body (cardiac, smooth muscle and neuronal tissue), their presence in the

DRG cell bodies and free nerve endings is of significant importance as channel inhibitors have shown positive effects in neuropathic pain (Doan 2010).

1.2.2.3 Potassium channels (K^+ channels)

Upon activation, K^+ channels facilitate a rapid transmembrane K^+ efflux, resulting in repolarization (or even hyperpolarization) of the neuronal membrane, therefore limiting action potential (AP) generation and firing rate (Tsantoulas & McMahon 2014). In sensory neurons, K^+ channel conduction inhibits peripheral excitability by several mechanisms including counteracting AP initiation at peripheral nerve terminals, reducing conduction fidelity across the axon or limiting neurotransmitter release at central terminals (Tsantoulas & McMahon 2014). K^+ channels consist of 4 distinct types, namely voltage-gated (K_v), calcium-activated (K_{Ca}), inward rectifier (K_{ir}) and two-pore domain (K_{2P}) potassium channels (Ocaña et al. 2004; Nishizawa et al. 2009; Du & Gamper 2013). Voltage-gated (K_v) potassium channels play an important role in setting the resting membrane potential, controlling repolarization of the AP and modulating the frequency of firing (Chi & Nicol 2007). Loss of functional K_v1.1 (Clark & Tempel 1998; Chi & Nicol 2007) results in increased sensitivity to noxious stimuli (Clark & Tempel 1998) and reduced firing threshold (Chi & Nicol 2007). The depletion of K_v 3.4 expression in nociceptive neuron results in mechanical hypersensitivity (Chien et al. 2007). K_v 7 channels play an essential role in setting the resting membrane potential, controlling the neuronal excitability (Brown & Passmore 2009; Wulff et al. 2009) and regulating the AP firing (Brown & Passmore 2009). Reduced functional K_v 7.2 and K_v 7.3 channels results in an increase in neuronal excitability as well as mechanical allodynia (Zheng et al. 2013). Calcium-activated (K_{Ca}) potassium channels are important determinants of the after-hyperpolarization following an action potential, neuronal firing pattern (Waxman & Zamponi 2014) and synaptic transmission at nerve terminals (Ocaña

et al. 2004). Inward rectifier (K_{ir}) potassium channels conduct atypical inward K^+ currents at depolarized membrane potentials to offset extracellular K^+ accumulation during neuronal firing (Tsantoulas & McMahon 2014). Two-pore domain (K_{2P}) potassium channels (also known as KCNK channels) are constitutively open (Du & Gamper 2013), thus encode background or leak K^+ currents, which are essential for the regulation of the resting membrane potential and excitability of many excitable cells (Busserolles et al. 2016).

1.2.2.4 Glutamate receptors

Glutamate receptors are broadly classified as ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs) (Osikowicz et al. 2013), which are ligand-gated ion channels and G protein-coupled receptors, respectively (Pereira & Goudet 2019). iGluRs comprise 3 receptor types, which are named based on their selective pharmacological ligands, as α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor, Kainate (KA) receptor and N-methyl-D-aspartate (NMDA) receptor (Bourinet et al. 2014; Guida et al. 2017). iGluRs are involved in the mechanisms of neurotransmitter release and the transmission of nociceptive stimuli (Osikowicz et al. 2013). AMPA receptors located on the central terminal of primary afferent fibers mediate primary afferent depolarization and modulate glutamate release (Bardoni 2013). If the impulse is short and acute in nature (both high and low threshold stimuli), glutamate released from primary afferents acts on AMPA receptors on second-order neurons (Voscopoulos & Lema 2010; McKune et al. 2015; Wiese & Yaksh 2015) resulting in a prominent but transient depolarization (Wiese & Yaksh 2015). AMPA receptors are also involved in the rapid excitatory synaptic transmission associated with glutamatergic signaling within the ascending nociceptive pathway, including inputs from sensory neurons in the spinal

cord to the brainstem and thalamus, as well as from thalamic neurons to sensory cortices (Bleakman et al. 2006). KA receptors are expressed on presynaptic primary afferent terminals and modulate glutamate release in the dorsal horn (Bardoni 2013). KA receptors on the postsynaptic membrane of excitatory neurons have an excitatory role (Bleakman et al. 2006) contributing to the sensory transmission occurring at high threshold Aδ and C fibers (Zhuo 2017). NMDA receptors have high calcium permeability (Bourinet et al. 2014) and are blocked by Mg^{2+} at physiological resting membrane potential (Meintjes 2012; Bourinet et al. 2014; Wiese & Yaksh 2015), thereby preventing its activation (Wiese & Yaksh 2015). Under repetitive and high-frequency stimulus from C fibers, the Mg^{2+} blockade is removed and the NMDA receptor can be activated (Voscopoulos & Lema 2010). The activation of NMDA receptors requires the binding of co-agonists glycine and glutamate (Bleakman et al. 2006; Meintjes 2012; Bourinet et al. 2014). NMDA receptor activation is responsible for central sensitization (Bardoni 2013; Wiese & Yaksh 2015).

mGluRs comprise 8 subtypes (mGlu1-mGlu8), which are further divided into 3 groups (group I-group III) based on sequence homology, intracellular pathways and pharmacological profile (Goudet et al. 2009; Guida et al. 2017). Except for mGlu6, all other subtypes are expressed within the nociceptive pathway where they modulate pain transmission (Pereira & Goudet 2019). Group I mGluRs (mGlu1 and mGlu5) are mainly coupled to G_q proteins resulting in the activation of the phospholipase C pathway and generation of intracellular calcium signals (Goudet et al. 2009). Group I mGluRs are mainly expressed in postsynaptic element (Goudet et al. 2009; Palazzo et al. 2014). Activation of spinal group I mGluRs usually results in pro-nociceptive effects (Goudet et al. 2009; Chiechio & Nicoletti 2012), therefore spinal group I mGluRs are critical for spinal central sensitization (Goudet et al. 2009). Group II (mGlu2 and

mGlu3) and group III (mGlu4, mGlu6, mGlu7 and mGlu8) mGluRs are coupled to $G_{i/o}$ proteins, therefore inhibiting adenylyl cyclase (Goudet et al. 2009). Group II and Group III mGluRs also regulate neuronal excitability and synaptic transmission through $G_{\beta\gamma}$ subunits, which prominently inhibit voltage-sensitive calcium channels and activate potassium channels (Pereira & Goudet 2019). Receptors of both groups are located in presynaptic element (Goudet et al. 2009; Pereira & Goudet 2019). Activation of group II and group III mGluRs results in depression of nociceptive transmission (Chiechio & Nicoletti 2012).

1.2.2.5 Neurokinin-1 receptors (NK-1 receptors)

NK-1 receptors are G protein-coupled receptors (Garcia-Recio & Gascon 2015; Gautam et al. 2016; Chang et al. 2019) with preferentially binding affinity to substance P (Garcia-Recio & Gascon 2015; Chang et al. 2019). The receptor is present throughout the dorsal horn with the highest density in lamina I (Todd 2009; Shilo & Pascoe 2014) and the lowest density in lamina II (Todd 2009). The NS projection neurons in lamina I express the NK-1 receptors and contribute to the nociceptive transmission to the brain (Braz et al. 2014; Riegelhaupt & Angst 2019). Activation of NK-1 receptors produce a decrease in K^+ currents and increase in Ca^{2+} currents, resulting in the excitatory effects on the nociceptive transmission (Shilo & Pascoe 2014).

Examples of drugs that are effective at pain transmission include local anesthetics, α_2-agonists (Epstein 2018; Grubb 2018), ketamine (Bell & Kalso 2018) and opioids (Grubb 2018).

1.2.3 Modulation

The process of inhibition or enhancement of signal, which occurs at the level of the

spinal cord is known as modulation (Tranquilli et al. 2004). Modulation of pain occurs from the action of spinal interneurons (Fan 2014; Grubb 2018) and the descending control from the brainstem (Westlund 2014; Grubb 2018).

1.2.3.1 Interneurons

Interneurons comprise nearly all the neurons in lamina II and most of those in lamina I and III (Todd 2010) and can be divided into two main classes as excitatory and inhibitory interneurons (Todd 2010; Todd 2015). They modulate sensory throughput at the dorsal horn of the spinal cord in response to signaling received from both peripheral afferent fibers and central descending axons (Short III & Vetter 2012).

Excitatory interneurons use glutamate as their neurotransmitter (Riegelhaupt & Angst 2019). They account for 60-70 % of the neuronal population in lamina I-III (Todd 2015). They can increase the response of NS neurons and WDR neurons (D'Mello & Dickenson 2008), thus providing an excitatory increase to spinal nociceptive processing (Westlund 2014).

Inhibitory interneurons use gamma-aminobutyric acid (GABA) and glycine as their main neurotransmitters (Braz et al. 2014; Riegelhaupt & Angst 2019). GABA and glycine act on $GABA_A$/$GABA_B$ receptors and glycine receptors, respectively. Activation of $GABA_A$ and glycine receptors results in increase Cl^- conductance, leading to moderate membrane hyperpolarization and an increase in shunting current. These effects reduce the ability of an excitatory input to depolarize the cell (Yaksh 2014), impairing the propagation of excitatory postsynaptic potentials along the dendrite or neurons (Zeilhofer 2005). $GABA_B$ receptors are G protein-coupled receptors that are also inhibitory (Yaksh 2014). GABA and glycine receptors are located on both primary afferent (presynaptic) and second-order neurons (postsynaptic),

therefore GABA and glycine exert their effect on both presynaptic and postsynaptic inhibition (Yaksh 2014; Riegelhaupt & Angst 2019). The reduction of spinal GABAergic tone resulting from the loss of GABAergic interneurons due to nerve injury may underlie the mechanical allodynia and thermal hyperalgesia that are observed in neuropathic pain conditions (Braz et al. 2014). The inhibitory interneurons play a major role in the gate control theory (Braz et al. 2014), which was proposed by Melzack and Wall (1965). The theory suggests that the inhibitory interneurons located in the substantia gelatinosa (lamina II, V) play a critical role in controlling incoming sensory signal before transmitting the signal to the brain through the ascending pathway. The activity of inhibitory interneurons in the substantia gelatinosa can be either activated by input from large myelinated peripheral nerves (e.g., Aß fibers) or inhibited by inputs from small fibers (e.g., C fibers), resulting in the inhibition or exaggeration of the arriving signals that were transmitted by projection neurons to the brain. The gate control theory can be used to explain the observation that non-noxious tactile stimulation (e.g., massage and vibration) can reduce the perception of pain (Hellyer et al. 2007).

1.2.3.2 Descending modulation

Descending control pathways from brainstem regions project into the dorsal horn of the spinal cord to modulate the transmission of the nociceptive signal, which they can either facilitate (i.e. enhancing the pain) or inhibit (i.e. reducing the pain) (Bell 2018). The pathways that play a major role in pain modulation include the endogenous opioid system (further discussed in chapter 2), descending noradrenergic and descending serotonergic systems (Gupta 2014).

Descending noradrenergic projections to the spinal dorsal horn, which are the only source of spinally released noradrenaline (NA) (Riegelhaupt & Angst 2019), arise mainly from the noradrenergic cell groups A5, A6 (locus coeruleus) and A7 in the pons (Howorth et al. 2009; Ossipov et al. 2014; Shilo & Pascoe 2014). NA can exert pronociceptive or antinociceptive effects depending on expressed adrenergic receptor subtype. Activation of spinal α_1 – adrenergic receptors augment pronociceptive signaling, whereas activation of spinal α_2 – adrenergic receptors produce antinociceptive effects (Riegelhaupt & Angst 2019). The antinociceptive effect of NA can be mediated through both presynaptic (binding of NA to α_{2A} – adrenergic receptors that are expressed on the central terminals of substance P- containing C fibers) and postsynaptic inhibition (binding of NA to α_{2C} – adrenergic receptors that are expressed on the axons of spinal projection neurons) (Bannister et al. 2009). The descending noradrenergic projections make only scarce direct contact with axon terminals in the spinal cord; therefore, volume transmission is likely to play a major role in the spread of NA from descending noradrenergic axon terminals to the site of α_2 – adrenergic action within the spinal cord (Pertovaara & Almeida 2006). After transducing their specific signals, NA molecules are either uptaken by the presynaptic NA transporters to be recycled or are degraded by enzymes in the synaptic cleft and at the nerve terminal (Llorca-Torralba et al. 2016). Noradrenergic systems have little influence on baseline pain sensitivity (Pertovaara 2006: Pertovaara & Almeida 2006). However, this pathway is recruited when a more persistent injury occurs (as in acute inflammatory conditions) and is responsible for attenuating the sensation of pain (Llorca-Torralba et al. 2016).

Descending serotonergic projections originate mainly from nucleus raphe magnus (NRM), the major nucleus of rostral ventromedial medulla (RVM) (Ren & Dubner 2008; Todd

2010; Todd 2015). The serotonergic projections from the brainstem are the only source of spinally released serotonin (Riegelhaupt & Angst 2019). Serotonin (5-hydroxytryptamine, 5-HT) plays a role in both descending inhibition and facilitation depending on the receptor subtypes activated (DeFelice & Ossipov 2016; Schliessbach & Maurer 2017; Riegelhaupt & Angst 2019). Both 5-TH_{2A} and 5-HT_3 receptors facilitate nociception, whereas $5HT_{1A}$, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_7 receptors exert inhibitory effects (Riegelhaupt & Angst 2019). The antinociceptive effects of descending serotonin are largely mediated through spinal 5-HT_1 receptors (Bannister et al. 2009). Activation of postsynaptic $5HT_{1A}$ receptors inhibits excitability of spinothalamic neurons and excitatory interneurons, while activation of presynaptic 5-HT_{1B} and 5-HT_{1D} receptors inhibit neurotransmitter release from primary nociceptive afferents (Benarroch 2008). The pronociceptive effects of this descending control are predominantly mediated through 5-HT3 receptors (Bannister et al. 2009). The activation of presynaptic and postsynaptic 5-HT3 receptors will increase neurotransmitter release from primary nociceptive afferents and excitability of spinothalamic neurons, respectively (Benarroch 2008).

Examples of drugs that are effective at pain modulation include local anesthetics, opioids, NSAIDs, NMDA-receptor antagonists (ketamine, amantadine), α_2-agonists, tricyclic antidepressants (amitriptyline), NK-1 receptor antagonists (maropitant), anticonvulsants (gabapentin and pregabalin), norepinephrine reuptake inhibitors (duloxetine) and serotonin reuptake inhibitors (fluoxetine) (Epstein 2018; Grubb 2018).

1.2.4 Perception

Nociception is the physiologic process of neural pathway activation after noxious stimulation (Ringkamp et al. 2018). Pain requires the noxious stimuli to reach the cortical level

and be perceived as pain (Hellyer et al. 2007). Pain is a conscious subjective experience (Baliki & Apkarian 2015; Garcia-Larrea & Bastuji 2018) and therefore consciousness is required for pain to be perceived. Conscious perception of noxious stimuli is generally considered pain. Third-order neurons transmit information from the thalamus to the higher (cortical) brain centers. The ventral posterior nuclei (VPN) and the posterior ventral medial nucleus (VMpo) of the lateral thalamus project to the somatosensory cortex and the insula cortex, respectively, while the nuclei of the medial thalamus projects to the anterior cingulate cortex (Riegelhaupt & Angst 2019). The cerebral cortex is considered the target for noxious stimuli. At this level, the animals will perceive pain (Posner 2008). Several parts of cerebral cortex are designated to be responsible for each dimension of pain. The somatosensory cortex processes the sensory-discriminative (Bourne et al. 2014; Westlund 2014; Johnson 2018) and the anterior cingulate cortex processes the affective-motivational dimension of pain (Bourne et al. 2014; Johnson 2018), while the insula cortex appears to have an important role in both dimensions of pain perception (Johnson 2018).

Example of drugs that are effective at perception: injectable and inhalant anesthetics, opioids, α_2-agonists (Epstein 2018; Grubb 2018), NMDA-receptor antagonists (ketamine, amantadine), tricyclic antidepressant, anticonvulsants (gabapentin, pregabalin), norepinephrine and serotonin reuptake inhibitors (Grubb 2018), benzodiazepines and phenothiazines (Epstein 2018).

1.3 Perioperative pharmacologic interventions

Various pharmacologic interventions can be performed to inhibit nociception through distinct mechanisms along the nociceptive pathway. The pharmacologic interventions used in

the management of pain can be divided into traditional analgesics (i.e., opioids, nonsteroidal anti-inflammatory drugs and local anesthetics) and analgesic adjuvants. Analgesic adjuvants are primarily indicated to use in conditions other than pain, but have shown analgesic properties in certain painful conditions (Lamont & Mathews 2007; Lamont 2008) or synergistic effects when combined with traditional analgesics, such as opioids or NSAIDs (Haldane & Storay 2012) and include α_2-adrenergic receptor agonists, NMDA antagonists and anticonvulsants (gabapentin, pregabalin) among others. This chapter will discuss all traditional analgesics (except for opioids, which are discussed separately in chapter 2) and selected analgesic adjuvants frequently used in the perioperative period.

1.3.1 Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs play a role in both pain transduction (Hellyer et al. 2007; Epstein 2018; Grubb 2018; van Rensburg & Reuter 2019) and modulation (Epstein 2018; Grubb 2018). NSAIDs exert their analgesic and anti-inflammatory effects via the blockage of prostaglandin (PG) synthesis at both the periphery and central nervous system (CNS) (Brogan et al. 2019) by occupying the hydrophobic channel of cyclooxygenase (COX) enzymes, thus precluding amino acid entry to its active site, leading to inhibition of pro-inflammatory mediators (e.g., PGE_2 and PGI_2) (Lees 2018). Therefore, NSAIDs are able to restrict the initiation of both peripheral and central sensitizations (Lemke & Creighton 2010). The two main COX enzymes comprise COX-1 and COX-2 (Haldane & Storay 2012; Lees 2018). Both enzymes participate in regulatory and homeostatic functions as well as in pain and inflammation processes (Papich & Messenger 2015). COX-1 is fundamentally expressed in several types of cells and is involved in the preservation of various normal physiologic functions, for example blood clotting (Lees 2018),

vascular homeostasis (thromboxane and prostacyclin production), gastric protection (Haldane & Storay 2012; Monteiro & Steagall 2019) and maintenance of renal perfusion in face of hypotension (Monteiro & Steagall 2019). A COX-1 splicing variant, COX-3, was identified in the brain of rats (Kis et al. 2003), dogs (Chandrasekharan et al. 2002) and humans (Qin et al. 2005) and seems to play an important role in the synthesis of prostanoid mediators associated with pain and fever. While COX-2 is constitutively expressed in a variety of organs and has an important role in the healing of gastric mucosa and normal homeostasis of the brain, kidney and reproductive systems (Haldane & Storay 2012; Lees 2018; Monteiro & Steagall 2019), COX-2 is mainly released after tissue injury. It is responsible for the synthesis of pro-inflammatory (e.g., PGE_2) and anti-inflammatory prostaglandins (e.g., $15dPGJ_2$) in the early phases of the acute inflammatory response and in the resolution phase, respectively (Lees 2018).

NSAIDs can be classified based on selectivity to COX enzyme, which is often expressed as the COX-1: COX-2 inhibitory ratio. The higher the value above 1.0, the greater the inhibitory action of the drug is for COX-2 compared to COX-1 (Papich & Messenger 2015). They can be classified as preferential or selective COX-1 inhibitors (e.g., aspirin, tepoxalin), nonselective COX inhibitors (e.g., flunixin, ketoprofen, phenylbutazone), slightly or moderately selective COX-2 inhibitors (e.g., carprofen, deracoxib, etodolac, etoricoxib, mavacoxib) and highly or very highly selective COX inhibitors (e.g., cimicoxib, firocoxib, robenacoxib, valdecoxib) (Lees 2018). COX-3 inhibition is thought to be involved in the analgesic and antipyretic effects of paracetamol (acetaminophen) (Chandrasekharan et al. 2002; Sharma & Mehta 2014). The mechanism of action of paracetamol is not well understood (Anderson 2008; Kam & So 2009; Smith 2009; van Rensburg & Reuter 2019), but is thought to involve centrally inhibition of COX-2 (Anderson 2008; Smith 2009) and COX-3 (Chandrasekharan et al. 2002; Sharma &

Mehta 2014) as well as activation of endogenous serotonergic (Anderson 2008; Smith 2009; Sharma & Mehta 2014) and opioid systems (Smith 2009). The efficacy of different classes of NSAIDs is similar (Gurney 2012; Monteiro & Steagall 2019), however the safety profile may differ (Monteiro & Steagall 2019). Potential adverse effects of NSAIDs administration are associated with prostaglandin inhibition (Monteiro & Steagall 2019) and include protein-losing enteropathy, gastrointestinal irritation, renal damage, prolonged bleeding time (Lees 2018; Monteiro & Steagall 2019), delayed parturition, delayed soft tissue and fracture healing (Lees 2018). The most frequent and clinically significant side effects of NSAIDs are related to the gastrointestinal tract (Papich & Messenger 2015; Lees 2018).

1.3.2 Local anesthetics

Local anesthetics are the only drug class that provide complete blockade of the nociceptive input to cerebral cortex, abolishing patient perception of a nociceptive stimulus (Barletta & Reed 2019). They inhibit the nociceptive impulses by blocking the action potentials in neurons through inhibition of voltage-gated sodium channels. Local anesthetics can abolish transduction (local infiltration, intrapleural and intraperitoneal administration) and transmission (perineural blockade, epidural administration and intrathecal administration) (Hellyer et al. 2007). Local anesthetics are classified based on their structure into aminoamides or aminoesters (Garcia 2015; Barletta & Reed 2019). Most aminoester local anesthetics (e.g., procaine, tetracaine) are metabolized by cholinesterase enzymes in the plasma and liver (Martin-Flores 2013; Campoy & Read 2015; Garcia 2015). Aminoamide local anesthetics (e.g., lidocaine, mepivacaine, bupivacaine, ropivacaine) are highly protein bound (Lemke & Creighton 2010) and are metabolized mainly by the liver (Lemke & Creighton 2010; Campoy & Read 2015;

Garcia 2015). Potential toxicity of systemic local anesthetics is associated with high plasma drug concentrations and includes neurotoxicity and cardiotoxicity (Barletta & Reed 2019). High plasma drug concentrations are typically a result of overdose, unintentional intravascular administration or relative overdose due to slower biotransformation and/or elimination (as in hepatic or renal compromised patients) (Martin-Flores 2013; Campoy & Read 2015). Albeit uncommon, direct nerve toxicity due to intraneural injection of local anesthetics has been described (Campoy & Read 2015).

Lidocaine is also frequently used as an analgesic adjunct by continuous intravenous infusion during the perioperative period (Eipe et al. 2016). The analgesic, anti-hyperalgesic and anti-inflammatory mechanisms of systemic lidocaine are not fully understood (Eipe et al. 2016; Gabriel et al. 2019). Studies suggest that lidocaine prevents depolarization of the neuronal membranes of damaged nerves and can reduce proliferation and spontaneous firing of active sodium channels in injured tissues, which may explain its beneficial effects in neuropathic pain. Intravenous lidocaine also seems to reduce central sensitization by decreasing the activity and sensitivity of spinal cord neurons. In addition, NMDA receptor inhibition, as well as a decrease in inflammatory mediators, has also been shown in patients receiving systemic lidocaine (Eipe et al. 2016; van der Wal et al. 2016).

1.3.3 α_2-adrenergic receptor agonists

The α_2-adrenergic receptor agonists (i.e. α_2-agonists) provide sedation, analgesia, muscle relaxation (Seddighi 2014; Rankin 2015), anxiolysis and anesthetic sparing effects (Sinclair 2003). Although α_2-agonists are not considered first-line analgesics as are opioids and NSAIDs, their use as analgesic adjuvants is growing in popularity (Pypendop 2015a). α_2-agonists reduce

opioid induced tolerance and act synergistically with opioids (Posner & Burns 2009). The α_2-adrenergic receptor agonists exert their effects by binding to the α-adrenergic receptors (both α_1 and α_2). The α_2-adrenergic receptors are G protein-coupled receptors that are linked to the cyclic adenosine monophosphate (cAMP) second messenger system. Receptor activation inhibits adenylate cyclase resulting in the reduction of cAMP. Clinically, the magnitude of sedative and analgesic effects of α_2-agonists is dependent on the location, type and density of α_2-adrenergic receptors in the animal as well as the specificity and affinity of the drug to the α_1 and α_2 receptors (Sinclair 2003). Drugs with a high α_2:α_1 selectivity ratio are more specific for the desired effects of sedation and analgesia (Posner & Burns 2009). Antinociceptive mechanisms of α_2-agonists include inhibition of neurotransmitter release from the primary afferent fibers to second-order neurons, pre- and postsynaptic modulation of nociceptive inputs occurring segmentally in the dorsal horn of the spinal cord as well as alteration in both descending modulation from the brainstem and ascending modulation of nociceptive inputs at the level of diencephalon and limbic areas (Murrell & Hellebrekers 2005). Therefore, α_2-agonists exert their analgesia through different points along the pain pathway including transmission, modulation and perception (Epstein 2018; Grubb 2018). The analgesia produced by α_2-agonists is of shorter duration compared to the sedation (Sinclair 2003; Berry 2015). Cardiovascular effects include bradycardia and bradyarrhythmias, a decrease in cardiac output and an initial increase in peripheral vascular resistance (Seddighi 2014). Other effects include reduction in the respiratory rate without changes in minute ventilation (Pypendop 2015b), reduction in gastrointestinal motility (Pawson 2008; Rankin 2015) and body temperature (Sinclair 2003; Posner & Burns 2009), hyperglycemia (Pawson 2008) and diuresis (Pawson 2008; Perkowski 2015). Hence,

these drugs should only be used in healthy and young to middle-aged animals (Pypendop 2015a).

1.3.4 N-Methyl-D-Aspartate antagonists

Ketamine, at subanesthetic doses, is an analgesic adjunct recommended as part of a multi-modal approach to treat severe pain (Mathews & Grubb 2018). It blocks NMDA receptors in the dorsal horn of the spinal cord (Lemke & Creighton 2010) by binding to a phencyclidine receptor inside the NMDA receptor, thus NMDA receptor must be already opened and active so that ketamine can exert its effect (Epstein 2014). After binding to the receptor, ketamine reduces time and frequency of channel opening, leading to the reduction of both Ca^{2+} influx and secondary intracellular signaling cascade (Epstein 2014). The blockade of NMDA receptors by ketamine may prevent spinal facilitation of pain (wind up) and the initiation of central sensitization (Lemke & Creighton 2010). Ketamine exerts its analgesic effects during modulation (Epstein 2018; Grubb 2018) at the pain pathway. Subanesthetic doses of ketamine are typically administered as an intravenous infusion to avert or manage the allodynia and hyperalgesia that usually occurs in association with central sensitization during major surgery procedures (Ruel & Steagall 2019). It is commonly administered in combination with an opioid during intraoperative and postoperative period in patients undergoing major surgical procedures or having preexisting central sensitization (Lemke & Creighton 2010). The combination of low dose ketamine and fentanyl infusions provided superior postoperative analgesia than fentanyl infusions alone in dogs subjected to forelimb amputation (Wagner et al. 2002). Ketamine should be used with caution in patients with hepatic dysfunction (Mathews & Grubb 2018) and/or renal impairment (Ruel & Steagall 2019).

1.3.5 Anticonvulsant

Although gabapentinoids (gabapentin and pregabalin) have a structure that is similar to the inhibitory neurotransmitter γ-aminobutyric acid (GABA), they do not interact with GABA receptors (Schmidt et al. 2013; Pang 2015; Brogan et al. 2019) nor are converted metabolically into GABA or GABA agonists (Brogan et al. 2019). The main analgesic mechanism of gabapentinoids is by binding to the $\alpha_2\delta$-1 subunits of the presynaptic voltage-gated calcium channels (VGCCs) in the dorsal horn of the spinal cord (Schmidt et al. 2013; Answine 2018). The $\alpha_2\delta$-1 subunits are critical for the stabilization of the VGCCs at the cell membrane surface. The uncoupling of the $\alpha_2\delta$-1 subunits by the binding of gabapentinoids leads to internalization (Helander et al. 2017; Answine 2018) and degradation of the VGCCs, resulting in the reduction of VGCCs expressions at the presynaptic terminals, which leads to the reduction of calcium movement into the cell, thus the reduction of neurotransmitter release (Answine 2018). In nerve injury, the $\alpha_2\delta$-1 subunits are upregulated and contribute to the enhancement of glutamate release in the dorsal horn of spinal cord leading to increase in postsynaptic nociceptive neuron activation, resulting in central sensitization and secondary hyperalgesia seen in neuropathic pain (Answine 2018; Chincholkar 2018). The upregulation of $\alpha_2\delta$-1 subunits are seen in nerve damage and are the target for gabapentinoids to effectively treat neuropathic pain (Answine 2018; Chan et al. 2018; Chincholkar 2018). However, the potential effectiveness of gabapentinoids in treating acute pain is not clear as there is no upregulation of $\alpha_2\delta$-1 subunits in acute tissue injury (Answine 2018). The potential benefits of gabapentin administration include opioid synergism in terms of sedation and analgesia (Berry 2015), alleviation of established hyperalgesia (Slingsby 2008) and prevention of central sensitization when administered pre-operatively by reducing the hyper-excitability of secondary nociceptive neurons in the dorsal

horn of spinal cord (Mathews & Grubb 2018). Possible adverse effects of gabapentin in dogs are sedation, ataxia (Ruel & Steagall 2019) and muscle weakness (Berry 2015). Administration of gabapentin as an adjunct analgesic agent in two prospective clinical trials did not show significant reduction in postoperative pain in dogs subjected to forelimb amputation (Wagner et al. 2010) and in dogs subjected to intervertebral disc surgery (Aghighi et al. 2012). However, in a recent prospective clinical trial, administration of pregabalin as an adjunctive agent resulted in significant reduction in postoperative pain when compared to placebo in dogs undergoing surgical treatment of intervertebral disc disease (Schmierer et al. 2020).

Understanding the pain pathway and where commonly used medications are most likely to have their effects provides the basis for evaluation of newer compounds. Chapter two will further detail opioid pharmacology and application to pain management.

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CHAPTER 2: OPIOIDS

2.1 Introduction

Opioids are one of the most important analgesic agents used for pain management in human and veterinary medicine. They interact with opioid receptors (KuKanich & Wiese 2015) and can be exogenous or endogenous in origin (Laycock & Bantel 2019). Exogenous opioids are classified into 3 groups: naturally occurring compounds, semi-synthetic compounds and synthetic compounds (Laycock & Bantel 2019; James & Williams 2020). Naturally occurring compounds are opium alkaloids that are derived from the poppy (Papaver somniferum) (McDonald & Lambert 2016; Laycock & Bantel 2019) and include morphine, codeine, papaverine and thebaine (James & Williams 2020). Semi-synthetic compounds are originated from chemical modifications of opium alkaloids (James & Williams 2020) and include diamorphine, oxycodone, buprenorphine (Laycock & Bantel 2019; James & Williams 2020), nalbuphine and naloxone (James & Williams 2020). Synthetic compounds are designed in a laboratory (Drug Enforcement Administration, Department of Justice 2020) and are not structurally related to morphine (McDonald & Lambert 2016; Laycock & Bantel 2019). Examples of drugs in this group include butorphanol, methadone, pethidine and fentanyl (James & Williams 2020).

2.2 Opioid receptor

The opioid receptor is a G protein-coupled receptor (GPCR), which consists of seven hydrophobic trans-membrane domains connected to each other by short loops, an intracellular C terminus and an extracellular N terminus (Dickenson & Kieffer 2013). Opioid receptors have 4

types, namely μ, σ, κ opioid receptors and a nociceptin/orphanin FQ (N/OFQ) peptide receptor (NOP) (McDonald & Lambert 2016; Corder et al. 2018). The first 3 types are considered classical opioid receptors and the last one is considered a non-opioid branch of the opioid receptor family due to its lack of sensitivity to naloxone (McDonald & Lambert 2016). In addition, the μ, σ, κ opioid receptors have several subtypes but the significance of these is not well defined (Smith & Lee 2003). Activation of opioid receptors leads to the pertussis toxin-sensitive G protein (Gi or Go or both) activation (Al-Hasani & Bruchas 2011; Fukuda 2015), which will cause a cell signaling cascade resulting in the closure of voltage-sensitive calcium channels, stimulation of potassium efflux and reduction in cyclic adenosine monophosphate production. These actions lead to reduction in neuronal excitability via cellular hyperpolarization and inhibition of neurotransmitters release (e.g., acetylcholine, dopamine, norepinephrine, substance P and GABA) (Duke-Novakovski 2014). Activated opioid receptors are desensitized by the coordinated actions of G protein-coupled receptor (GPCR)-regulated kinases (GRKs) that phosphorylate receptors and β-arrestins that internalize the phosphorylated receptors (Corder et al. 2018; Machelska & Celik 2018; Grim et al. 2020; Manglik 2020). Opioid receptors are expressed throughout the body (spinal cord, brain, chemoreceptor trigger zone, gastrointestinal tract, synovium, urinary tract, leukocytes and uterus) and therefore, the effects of systemic opioids are widespread (KuKanich & Wiese 2015). The μ receptor plays the most important role in regards to analgesia and most clinically efficacious opioid analgesics work by activating the μ receptor. The κ receptor also mediates analgesia, both in the central and peripheral nervous system, while the σ receptor seems to not have a direct effect and drugs that activate this receptor seem to be poor analgesics. The NOP receptor seems to have a pronociceptive effect (Smith & Moran 2001).

2.3 Endogenous opioid peptides

Endogenous opioid peptides are naturally expressed in the central nervous system (CNS) as well as in peripheral tissues. They consist of 4 major families, including β-endorphin, enkephalin, dynorphins and nociceptin/orphanin FQ (Corder et al. 2018). Endogenous opioid peptides are produced in the CNS as well as in the adrenal and pituitary glands (Janecka et al. 2004). In the nervous system, they are synthesized and secreted by nerve cells and exert their effects as neurotransmitters and neuromodulators of other neurotransmitters within the brain and the spinal cord (Froehlich 1997; Janecka et al. 2004). Based on prevalence of receptor affinity, the endogenous agonists for the classical opioid receptors μ, σ, and κ, are ß-endorphin, enkephalin and dynorphin, respectively (Fukuda 2015). Under stressful, painful or traumatic situations, endogenous opioid peptides are released and help lessen the sensitivity to noxious stimuli by generating analgesia and euphoria (Froehlich 1997). However, the duration of endogenous opioid analgesia is fairly short (Roques et al. 2012). Differently from the other endogenous opioid peptides, nociceptin only binds to the NOP receptor and instead of analgesia it has a pronociceptive effect (Smith & Moran 2001).

2.4 Exogenous opioids

The exogenous opioids used clinically can be functionally classified into 4 groups based on their activity on the receptors: full μ-agonists, partial μ-agonists, agonist-antagonists and antagonists (Schug 2013; Epstein 2015). Full or pure μ-opioid agonists are able to elicit maximal receptor activation upon binding to the receptor, resulting in maximal analgesia (Lamont & Mathews 2007). Drugs in this class include morphine, hydromorphone, fentanyl and methadone. Partial μ-agonists do not fully stimulate the μ-receptor, resulting in less profound

analgesia compared to full μ agonists (Hammond et al. 2008). Buprenorphine is partial μ-agonist (and will be discussed in more depth later in the chapter). Agonist-antagonists have competitive antagonistic activities at the μ receptors, but produce analgesia by functioning as an agonist at κ receptors (Lamont & Mathews 2007). Drugs in this class include butorphanol and nalbuphine. Antagonists bind to opioid receptors but do not activate them. Antagonists are capable of displacing the opioid agonists from μ and κ receptors (Lamont & Mathews 2007), resulting in a reversal of their effects. Examples of drugs in this class are naloxone, nalmefene and naltrexone.

2.5 Analgesic effect of opioids

Opioid analgesics are the main drug class used to treat moderate (Dickenson 2001) to severe pain (Dickenson 2001; Pasternak & Pan 2013) in clinical settings. Opioids exert analgesia via two main mechanisms: direct inhibition of ascending nociceptive transmission from the dorsal horn of the spinal cord and activation of descending inhibitory pathways (from midbrain through rostral ventromedial medulla to the dorsal horn of the spinal cord) (Fukuda 2015). Most opioid analgesics available in the clinical setting act through the μ opioid receptor (Schaefer et al. 2017; Lueptow et al. 2018). Full μ agonists (e.g., morphine, hydromorphone, fentanyl, remifentanil, alfentanil and methadone) produce the most profound analgesic affects. Partial μ agonists (i.e., buprenorphine) produce less analgesia than a full μ agonist, but also seem to be associated with less adverse effects. They are typically used to manage mild to moderate pain. However, some studies in cats (Slingsby & Waterman-Pearson 1998; Dobbins et al. 2002; Stanway et al. 2002; Robertson et al. 2003) and dogs (Linton et al. 2012) suggest that buprenorphine may provide comparable analgesia to that of a full μ agonist for some types of pain with fewer adverse effects. Opioid receptors are located along the nociceptive circuitry as

well as at numerous sites that are responsible for nociceptive inhibition, and therefore exert analgesia at the supraspinal (e.g, periaqueductal gray, brain stem and cortex), spinal (dorsal root ganglion, DRG) and peripheral (nociceptor) levels (Walwyn et al. 2010).

2.5.1 Opioid mediated supraspinal analgesia

The brain regions that are most important in opioid centrally mediated analgesia are the periaqueductal gray (PAG) and the rostroventral medulla (RVM) (Ossipov et al. 2004; Dickenson & Kieffer 2013; Lau & Vaughan 2014). Microinjection of morphine into either the PAG (Park et al. 2010; Kim et al. 2018) or RVM (Hathway et al. 2012; Li et al. 2015; Gomtsian et al. 2018) produces analgesia by increasing activity of the descending inhibitory pathway to the spinal cord (Ossipov et al. 2004; Vanegas et al. 2010). Supraspinal opioid analgesia is thought to be due to the inhibition of active GABAergic neurons which project to the descendant inhibitory neurons of the brainstem at the level of PAG (Lau et al. 2020) and RVM (Li et al. 2015), resulting in increased descending inhibitory signal to the spinal cord (Lau & Vaughan 2014). In addition, opioids also act on the brain regions that are responsible for the affective component of pain (e.g., subcortical and cortical sites) (Corder et al. 2018) resulting in reduction of aversive qualities of pain (affective component of pain) (Yaksh 1997; Corder et al. 2018; Gomtsian et al. 2018; Bannister & Dickenson 2020).

2.5.2 Opioid mediated spinal analgesia

The opioid receptors at the spinal cord are predominantly of the μ and σ types and are located in the C fiber termination zone (the substantia gelatinosa) in the superficial dorsal horn (Dickenson 2001). The main mechanism of spinal opioid analgesia for both endogenous and

exogenous opioids is via activation of presynaptic opioid receptors, which will selectively decrease the release of neurotransmitters from nociceptive afferents and thus decrease nociceptive transmission without affecting the innocuous evoked activity (Dickenson & Kieffer 2013). Examples of neurotransmitters inhibited by opioid receptor activation at the level of the spinal cord include glutamate, substance P and nitric oxide (Ghelardini et al. 2015). Using intrathecal or epidural administration to target spinal opioid receptors have the advantage of minimizing the side effects mediated by opioid receptors in the brain and periphery (Dickenson 2001). The duration of analgesia will depend on each individual drug characteristics. Opioids with a high lipid solubility have low spinal bioavailability (Bujedo 2013), while opioids that have low lipid solubility (i.e. morphine) have higher spinal bioavailability and therefore a longer duration of action. Spinal opioid administration shows enhanced potency in the presence of inflammation, which may occur from an increase in either the amount or the affinity of spinal opioid receptors (Dickenson & Kieffer 2013).

2.5.3 Opioid mediated peripheral analgesia

Opioid receptors are also expressed in peripheral sensory neurons (nociceptors). They are synthetized in the nociceptor cell bodies in the trigeminal and dorsal root ganglia and are then transported to the peripheral terminals in the tissues (skin, joints, viscera) (Machelska & Celik 2018). Opioid mediated peripheral analgesia is minimal in normal conditions (Dickenson & Kieffer 2013). However, under an inflammatory process, the opioid receptors at the peripheral sensory nerve endings increase in expression and coupling, thereby increasing opioid mediated peripheral analgesia (Dickenson & Kieffer 2013; Machelska & Celik 2018).

2.6 Side-effects

Apart from producing analgesia, opioid agonists (especially μ agonists) have several other effects in the body, as is expected based on the broad distribution of opioid receptors. Some can be beneficial in certain circumstances, while others may have significant negative implications. Opioids typically cause dose-dependent sedation, but excitation and/or increased locomotor activity can be seen at high doses or rapid intravenous administration, especially in certain species as cats, horses and ruminants (KuKanich & Wiese 2015). Similarly, opioids's anesthetic sparing effects vary between species, type of opioid and dose (KuKanich & Wiese 2015). Respiratory depression is dose-dependent, but not as significant in healthy domestic species (when used at clinically relevant doses) as it is in human beings and non-human primates (Campbell et al. 2003; Grimm et al. 2005; Maiante et al. 2009; Wunsch et al. 2010). However, significant hypoventilation may occur when opioids are used in combination with anesthetic agents and support of ventilation is often necessary during anesthesia. Opioids cause minimal cardiovascular depression, maintaining cardiac output and arterial blood pressure. Bradycardia is the main cardiovascular effect seen with the administration of opioids but is easily corrected with the administration of anticholinergics (Grimm et al. 2005; Guedes et al. 2007). A few opioids, particularly morphine and meperidine, may cause histamine release after rapid intravenous administration, which can lead to significant hypotension. Opioid effects in the gastrointestinal system may include initial vomiting and defecation (more likely to occur after intramuscular or subcutaneous than intravenous administration) (Robertson et al. 2009), alterations in the gastrointestinal tract contraction (i.e., decreased propulsive and increased non-propulsive contractions) and secretions (i.e., decreased fluid secretion) (KuKanich & Wiese 2015), which may predispose to ileus and constipation. Opioids can cause urine retention,

especially with neuraxial administration (Malinovsky et al. 1998; Baldini et al. 2009), which may require manual expression or catheterization of the bladder until effects subside. Opioids also affect the thermoregulatory center and may cause either hypothermia or hyperthermia depending on the species (Posner et al.2007).

2.7 Opioid of interest

Our studies, which will be presented in the following chapters, focus on the evaluation of recent formulations of buprenorphine in dogs, which would allow for longer periods of analgesia after a single dose administration. Therefore, a brief review of buprenorphine and available formulations is provided.

2.7.1 Buprenorphine

Buprenorphine is a highly protein bound and highly lipophilic semisynthetic opioid analgesic (Duke-Novakovski 2014; KuKanich & Allen 2014), that has been shown to be effective for the treatment of mild to moderate pain, with mild adverse effects in dogs (Slingsby et al. 2006; Shih et al. 2008; Ko et al. 2011; Slingsby et al. 2011; Linton et al. 2012) and cats (Giordano et al. 2010; Taylor et al. 2010; Catbagan et al. 2011). Buprenorphine's pharmacology is quite complex, but it has been traditionally considered to be a partial μ opioid agonist. However, its ceiling effect for analgesia has been questioned in humans (Dahan et al. 2006) and some studies suggested that its analgesic effects were similar to a full μ agonist in human clinical practice (Pergolizzi et al. 2010; Davis 2012; Raffa et al. 2014).

Conflicting information is also noted on the veterinary literature, and much of the variability observed seem to be influenced by the type of pain, dose and route of drug

administration. Intramuscular buprenorphine was reported to provide comparable analgesia to a long-acting transdermal fentanyl solution in dogs undergoing soft tissue and orthopedic surgeries in a clinical setting (Linton et al. 2012), while other studies indicated that intramuscular buprenorphine provided less analgesia compared to methadone in dogs undergoing orthopedic surgeries (Hunt et al. 2013) or ovariohysterectomy (Shah et al. 2018). In cats, intramuscular buprenorphine was reported to provide better postoperative analgesia than oxymorphone after onychectomy and neutering (Dobbins et al. 2002), and then morphine after various surgical or invasive diagnostic procedures (Stanway et al. 2002). Intramuscular administration of buprenorphine seems to provide comparable effects in increasing thermal threshold (but with longer duration) as morphine administered via the same route (Robertson et al. 2003), but subcutaneous administration has shown inferior ability in increasing thermal and pressure threshold compared to those of either morphine or methadone administered via the same route (Steagall et al. 2006).

Buprenorphine has a greater safety profile compared to full μ-opioid receptor agonists, particularly with respect to respiratory depression in humans (Johnson et al. 2005). Studies in dogs have demonstrated a ceiling effect for both analgesia and adverse effects in dogs administered buprenorphine and subjected to ovariohysterectomy (OVH) (Slingby et al. 2011). Buprenorphine's therapeutic dose usually does not exceed 0.04 mg/kg while the lethal dose 50 (LD_{50}) of buprenorphine in dogs is 79 mg/kg (KuKanich & Papich 2018), indicating a great safety margin. Buprenorphine has a high binding affinity to the μ-opioid receptor (1000 times that of morphine) and may competitively displace full μ-opioid agonists from binding to the receptor. This characteristic allows buprenorphine to be used to reverse adverse effects of full μ-opioid agonists, like morphine or fentanyl, while still maintaining analgesia (Hammond et al.

2008; Duke-Novakovski 2014). The dissociation half-life of buprenorphine from the μ-opioid receptor is reported to be approximately 166 minutes, resulting in a longer duration of action (Duke-Novakovski 2014).

Buprenorphine can be administered via intravenous (IV), subcutaneous (SC), intramuscular (IM) or oral transmucosal (OTM) routes (KuKanich & Wiese 2015), with the IV and IM routes preferred in the perioperative setting (Giordano et al. 2010). At the standard dose of 20 μg/kg administered to dogs intramuscularly (Slingsby et al. 2006: Shih et al. 2008; Slingsby et al. 2011; Linton et al. 2012) or intravenously (Ko et al. 2011; Pieper et al. 2011), buprenorphine requires dosing at 6-8 h intervals. While buprenorphine has a longer dosing interval compared to most other μ agonists, it still requires hospitalization of clinical patients or intermittent handling of research animals. Buprenorphine formulations that provide longer-acting analgesia have become available in recent years.

2.7.2 Long-acting buprenorphine formulations

The benefits of long-acting buprenorphine formulations include less frequent dosing, more constant drug plasma concentrations, reduction in stress associated with repetitive restraint and drug administration, better compliance in pain management and the ability to provide better analgesia for outpatients (particularly when non-steroidal anti-inflammatory agents are contraindicated).

2.7.2.1 Transdermal buprenorphine patch

The transdermal buprenorphine (TDB) patch uses a new matrix technology, in which the

drug is embedded homogenously into a solid polymer from which it is released continuously into the circulation (Murrell et al. 2007). The TDB patch has been available for several years and is approved by the FDA for the treatment of moderate to severe pain in humans. The patch is available in 3 sizes that are designed to deliver 35, 52.5, or 70 µg/h of buprenorphine (Smith 2014).

An initial study in cats, using the 35 µg/h TDB patch (equivalent to a 5-15 µg/kg dose), applied to shaved skin, produced measurable serum drug concentrations starting at 6 h after patch application and beyond the 72 h time point when the patch was removed. In fact, drug concentrations remained above the limit of quantification for an additional 24 h after patch removal. However, there was significant inter-individual variability and the patch failed to increase thermal thresholds throughout the testing period (96 h after patch application) despite peak plasma concentrations being similar to plasma levels shown to increase thermal thresholds after systemic buprenorphine administration (Murrell et al. 2007).

A study in dogs, using a 52.5 µg/h TDB patch (equivalent to a 4 µg/kg/h dose), showed a significant increase in thermal threshold starting at 6 h after patch application and lasting until patch removal (72 h after patch application) (Pieper et al. 2011). Another study, which evaluated the effects of a 70 µg/h patch in dogs undergoing ovariohysterectomy, showed comparable analgesia to 20 µg/kg buprenorphine administered subcutaneously 30 min prior to anesthesia and at every 6 h for 38 h after surgery. However, analgesia was reported to be incomplete with both treatments. The 70 µg/h TDB patch was applied 48 h prior to surgery and kept in place for 86 h (Moll et al. 2011) based on findings of a preliminary study that showed a continuous increase in drug plasma concentrations over the first 36 h when it achieved steady-state and

maintained plasma drug concentrations between 0.7 and 1.0 ng/ml until 108 h (Andaluz at al. 2009).

Some disadvantages noted with this delivery technique include significant inter-individual variability in drug plasma concentrations and analgesic efficacy, the slow increase in drug plasma concentrations (requiring the patch be placed several hours in advance or following systemic buprenorphine administration) and concerns with frequent displacement of the patches (particularly in cats) (Murrell et al. 2007; Andaluz at al. 2009; Moll et al. 2011; Pieper et al. 2011).

2.7.2.2 Sustained-release buprenorphine formulations

In recent years, sustained-release formulations of buprenorphine have been introduced, with the proposed benefit of providing long lasting analgesia after a single subcutaneous injection. Some early formulations were associated with skin lesions and abscesses at the site of injection, likely due to characteristics of the formulation matrix, but new versions seem to have minimized or eliminated this adverse reaction.

One of these newer formulations is an extended-release buprenorphine (ER-buprenorphine) in which lipid-bound buprenorphine is suspended in medium chain fatty acid triglyceride (MCT) oil. The buprenorphine in this formulation is encapsulated by lipid, which makes it be released slowly and allows for a high dose to be administered without risk of overdose or toxicity (Barletta et al. 2018). Administration of ER-buprenorphine (a single 0.2 mg/kg, SC) provided drug plasma concentrations around 1.0 ng/ml and antinociception (increased thermal threshold) in dogs for 72 h, with only transient, mild clinical signs typically

associated with opioid administration, such as sedation, bradycardia, hypothermia and inappetence (Barletta et al. 2018).

A different formulation, and the one we chose to evaluate as part of this dissertation is a patented injectable solution of a sustained-release polymer system (Buprenorphine SR™) from ZooPharm™. This sustained-release buprenorphine formulation was designed to provide continuous buprenorphine release for 72 h after a single subcutaneous injection. The manufacturer's recommended doses for cats and dogs are 0.12 mg/kg and 0.03-0.06 mg/kg, respectively. These doses are expected to provide buprenorphine plasma levels greater than 1 ng/ml. (Buprenorphine SR information sheet from ZooPharm™, 2013).

A study evaluating a single subcutaneous dose of Buprenorphine SR (0.12 mg/kg, prior to surgery) in cats undergoing OVH, showed comparable analgesia to an equivalent dose of regular buprenorphine (0.02 mg/kg OTM at premedication and at 12 h intervals for 60 h). All cats also received a single post-operative dose of meloxicam (Catbagan et al. 2011). A similar study was performed in dogs undergoing OVH, where the analgesic efficacy of buprenorphine SR (0.2 mg/kg SC, single dose) and regular buprenorphine (0.02 mg/kg SC, every 12 h for 4 days), both combined with meloxicam (0.1 mg/kg once a day for 4 days), were compared (Nunamaker et al. 2014). The study indicated both treatments to be equally efficacious, with comparable side effects. The single dose of Buprenorphine SR provided plasma buprenorphine concentrations above 0.6 ng/ml for about 136.0 ± 11.3 h (Nunamaker et al. 2014).

2.7.2.3 High concentration buprenorphine

A high-concentration buprenorphine formulation (Simbadol™) has been approved by the FDA for once daily subcutaneous administration in cats for up to 3 days (KuKanich & Wiese

2015). Unlike the sustained release formulations, this is a simple aqueous buprenorphine formulation in a very concentrated form (1.8 mg/ml) delivered at high doses, without any special matrix. The recommended dosage from the manufacturer is 0.24 mg/kg SC once daily, for 3 days, with the first dose being administered about 1 h before surgery (Simbadol™ prescribing information from Zoetis 2014). Buprenorphine has been shown to not be well absorbed after subcutaneous administration in cats (Steagall et al 2013). However, when a very high dose is administered, as is the case with this formulation, the slow uptake works favorably, providing adequate plasma concentrations and prolonged analgesia (Taylor et al. 2016).

A study in cats comparing high doses of the high-concentration buprenorphine formulation showed that both 0.12 mg/kg and 0.24 mg/kg (single dose, SC) provided effective antinociception (thermal threshold) for at least 24 h with no adverse effects (Taylor et al. 2016). The same two high doses of high concentration buprenorphine were evaluated in cats undergoing OVH and were shown to provide equal effective analgesia with minimal side effects (Leedham et al. 2019). Simbadol™ has been shown to be safe and well tolerated in young cats (4 months) after being administered at 0.24 mg/kg (the proposed dose), 0.72 mg/kg (3 times the proposed dose), or 1.2 mg/kg (5 times the proposed dose), subcutaneously, once daily for 9 consecutive days. Only 2 cats were reported to have developed drug-related adverse events, which were described as dilated pupils and behavioral changes (hyperactivity, agitation and disorientation) (Sramek et al. 2015).

While the use of Simbadol™ at traditional doses (0.02 mg/kg) has been evaluated in dogs (Watanabe et al. 2018), there are no studies evaluating the use of high dose Simbadol™ in dogs similar to what is recommended for cats.

With the goal of providing veterinary clinicians more options for adequate and practical

pain management in dogs, this dissertation focused on expanding our knowledge regarding the use of two different long-lasting buprenorphine formulations in dogs. One study evaluated the pharmacokinetics and selected behavioral, physiological and antinociceptive effects of 2 different doses of a sustained released buprenorphine formulation (Buprenorphine SR™) in dogs (Chapter 3), and the other study evaluated the analgesic efficacy of 2 high doses of a high concentrated buprenorphine formulation (Simbadol™) in dogs undergoing neutering (Chapter 4).

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CHAPTER 3: PHARMACOKINETICS AND PHARMACODYNAMICS OF SUSTAINED-RELEASE BUPRENORPHINE IN DOGS

3.1 Introduction

Buprenorphine is a highly lipophilic and highly protein bound semisynthetic opioid analgesic (Duke-Novakovski 2014; KuKanich & Allen 2014) that has been shown to be effective in the treatment of mild to moderate pain with minimal adverse effects in dogs (Slingsby et al. 2006; Shih et al. 2008; Ko et al. 2011; Slingsby et al. 2011; Linton et al. 2012) and cats (Giordano et al. 2010; Taylor et al. 2010; Catbagan et al. 2011). While buprenorphine's pharmacology is quite complex, it has been traditionally considered to be a partial μ opioid receptor agonist, and a ceiling effect, in terms of both analgesia and adverse effects, has been shown in dogs undergoing ovariohysterectomy (OVH) (Slingsby et al. 2011). However, its ceiling effect for analgesia has been questioned in humans (Dahan et al. 2006) and some studies suggested that its analgesic effects were similar to a full μ agonist in human clinical practice (Pergolizzi et al. 2010; Davis 2012; Raffa et al. 2014). Some variability in the analgesic response of buprenorphine has been reported in veterinary studies as well, but type of pain, dose and route of administration seem to play a significant role.

While buprenorphine has a longer dosing interval compared to most other μ agonists, it still requires hospitalization of clinical patients or intermittent handling of research animals. At the standard dose of 20 μg/kg administered to dogs either intramuscularly (IM) (Slingsby et al. 2006: Shih et al. 2008; Slingsby et al. 2011; Linton et al. 2012) or intravenously (IV) (Ko et al. 2011; Pieper et al. 2011), buprenorphine typically requires redosing between 6 and 12 hours.

In recent years, new formulations of buprenorphine have been introduced and have the potential to provide longer duration of analgesia in dogs (Nunamaker et al. 2014; Tomas et al.

2015; Barletta et al. 2018). One of these formulations consists of Buprenorphine HCL in a proprietary sustained release biodegradable liquid polymer matrix (Buprenorphine SR, ZooPharm, Laramie, WY,), which claims to provide 72 h of analgesia after a single (0.03-0.06 mg/kg) subcutaneous injection in dogs. Initial studies have reported favorable results in several species, including dogs (Nunamaker et al. 2014), cats (Catbagan et al. 2011) and various laboratory species (Foley et al. 2011; Carbone et al. 2012; Chum et al. 2014; Walkowiak & Graham 2015; Kendall et al. 2016; Seymour et al. 2016; Smith et al. 2016).

The combination of Buprenorphine SR and meloxicam was shown to provide effective analgesia for at least 72 hours after ovariohysterectomy in dogs (0.2 mg/kg SC, single dose) (Nunamaker et al. 2014) and in cats (0.12 mg/kg SC, single dose) (Catbagan et al. 2011). A different extended release formulation, a lipid-encapsulated buprenorphine (ER-buprenorphine), has also been evaluated and demonstrated antinociceptive effects in dogs for 72 hours (0.2 mg/kg SC, single dose) using a thermal threshold testing device (Barletta et al. 2018), and for 24 to 72 hours after stifle arthrotomy based on ground reaction forces (GRFs) (Tomas et al. 2015). Reported side effects in dogs included soft stool, diarrhea and appetite suppression (Nunamaker et al. 2014) as well as hypothermia, mild bradycardia and appetite suppression (Barletta et al. 2018).

The aim of this study was to evaluate the plasma concentrations and selected behavioral, physiologic and antinociceptive effects of two different doses of a sustained-release buprenorphine formulation (Buprenorphine SR) in dogs. We also aimed to record the occurrence of any adverse effects associated with this formulation at the doses evaluated.

3.2 Material and Methods

3.2.1 Animals

The study protocol was approved by the Institutional Animal Care and Use Committee of Colorado State University. Six healthy beagles (3 males and 3 females) aged 18.8 ± 1.6 months and weighing 11.3 ± 0.9 kg were included in the study. They were considered healthy based on a thorough physical examination, hematological and biochemical analyses. All dogs were housed at the University's Laboratory Animals Resources (LAR) facilities within the Veterinary Teaching Hospital and were provided with fresh water and commercially dry dog food ad libitum. They were routinely socialized through LAR's socialization program. The dogs were also allowed a 3-week period for acclimation, where they got familiarized with study personnel, environment, and a mechanical nociceptive threshold testing device (C-clamp) (Self-built C-clamp; Colorado State University, CO, USA) prior to the beginning of the study.

3.2.2 Instrumentation

In the morning of the study, dogs were weighed and the jugular area was clipped and aseptically prepared for jugular catheter placement under manual restraint and local anesthetic infiltration. While it was attempted to familiarize the dogs with the restrain, draping and neck manipulation prior to study day, 3 of the dogs were considered too energetic and unamenable to jugular catheter placement under manual restraint. These 3 dogs were lightly sedated with propofol to facilitate jugular catheter placement 24 h prior to the start of the study. A 14 gauge jugular catheter (MILACATH Long term, MILA International, Inc.) was aseptically placed percutaneously by Seldinger technique, after local infiltration with 2% lidocaine. The jugular catheter was sutured in place and covered with soft bandage, elastikon and bite free cervical

collar (KVP International, Inc.). The jugular catheter was kept in place for 120 hours to facilitate blood sample collection.

3.2.3 Study design

Dogs were randomly assigned to receive two doses (0.06 mg/kg and 0.18 mg/kg, subcutaneously) of sustained release buprenorphine (Buprenorphine HCl SR, ZooPharm, Laramie, WY; 3 mg/mL) in a balanced crossover design, with a 2-week washout period between treatments. The doses were chosen based on manufacturer recommendation (0.06 mg/kg - low dose group, LD) and previous data from the investigators (0.18 mg/kg - high dose group, HD). The LD is the high end of the manufacturer recommendation and is equivalent to 10 μg/kg every 12 hours for 72 hours, while the HD is equivalent to 30 μg/kg every 12 hours for 72 hours. Both doses were administered as a single subcutaneous injection. Blood collection, behavioral, physiological and antinociceptive assessments were performed by the same investigator, who was blinded to the dose administered. Blood samples were collected after behavioral and physiological assessments but prior to antinociceptive assessment.

3.2.4 Buprenorphine plasma concentrations and pharmacokinetic analysis

Venous blood samples (2-3 mL) were collected from a preplaced jugular catheter at baseline and at 30 minutes, 1, 2, 4, 6, 12, 18, 24, 36, 48, 60, 72, 96 and 120 hours after drug administration for drug plasma concentration determination. Packed cell volume (PCV) and total protein (TP) were evaluated at baseline and at 1, 6, 24, 48, 72, 96 and 120 hours. The total amount of blood withdrawn did not exceed 10% of each dog's total blood volume. Blood samples were centrifuged (2500 rpm, 5^{o}C, 10 minutes) and plasma harvested and stored at -80^{o}C

until analyzed. Plasma buprenorphine concentrations were determined using liquid chromatography-tandem mass spectrometry by the CSU Pharmacology Laboratory. Standard dilutions of buprenorphine were prepared in acetonitrile and added to control plasma. Samples were prepared by using 50 µL plasma. Each sample was spiked with 5 µL acetonitrile or 5 µL of the buprenorphine standard and 5 µL of 10 µg/mL naringenin as an internal standard. Samples were vortexed briefly, and then 100 µL acetonitrile was added for protein precipitation. Samples were vortexed continuously for 10 min followed by centrifugation for 10 min at 20,800 × *g* at 4 °C; 150 µL of each supernatant was collected and transferred to HPLC vials with inserts for analysis. Positive-ion electrospray ionization mass spectra were obtained by using a triple quadrupole mass spectrometer (MDS Sciex 3200 Q-TRAP, Applied Biosystems, Foster City, CA) with a turbo ionspray source interfaced with an HPLC system (model LC-20AD. Shimadzu, Kyoto, Japan). All samples were chromatographed by using a 2.5 µm, 4.6 × 50 mm column (XBridge Phenyl, Waters, Milford, MA) protected by a C18 guard cartridge (4.0 × 2.0 mm, Phenomenex, Torrance, CA). Gradient elution was used for all compounds. Mobile phase A consisted of 10 mM ammonium acetate in Milli-Q–purified water, and mobile phase B consisted of 100% acetonitrile. Chromatographic resolution was achieved by linearly holding the B solvent at 25% for 1 min. The solvent mixture then was altered by increasing mobile phase B linearly from 25% to 98% between 1 and 2 min, maintaining at 98% between 2 and 4.5 min, and then decreasing linearly from 98% to 25% between 4.5 and 4.75 min, followed by reequilibration of the column at 25% mobile phase B from 4.75 to 6 min. The flow rate was 1000 µL/min and the sample injection volume was 40 µL. The mass spectrometer settings were as follows: turbo ionspray temperature 550 °C; ion spray voltage 5500 V; curtain gas N_2:30 units; collision gas N_2:5 units; nebulizer gas N_2:60 units; and auxiliary gas N_2:60 units. Samples

were quantified in the multiple-reaction monitoring mode by monitoring the relevant ion transitions and then summing the counts for each transition. The dwell time for each ion transition was 100 ms. Q_1 and Q_2 were both operated in unit resolution mode. Chromatographic conditions were optimized for peak shape. Quantitation was based on linear standard curves. The lower limit of quantification (LLOQ) was 0.010 ng/mL. A Non-Compartmental Pharmacokinetic analysis was performed using Phoenix WinNonlin software (Pharsight, Cary, NC).

3.2.5 Behavioral assessment

Behavioral assessment included level of sedation when undisturbed (sedation score 0-3, **Table 3.1**) and during interactive moments: response to voice and noise (voice and noise score 0-4, **Table 3.2**) as well as their ability to walk over a pole (gait score 1-5, **Table 3.3**). All behavioral assessments as well as the presence of salivation, vomit, urine and feces was recorded prior to drug administration (baseline) and at 30 minutes, 1, 2, 4, 6, 12, 18, 24, 30, 36, 48, 54, 60, 72, 84, 96, 108 and 120 hours after drug administration.

Table 3.1 Sedation score

Score	Description
0	No sedation
1	Mild sedation: less alert but still active
2	Moderate sedation: drowsy, recumbent but can walk
3	Profound sedation: very drowsy, unable to walk

Table 3.2 Score for response to voice and noise

Score	Description
0	Dramatic or Exuberant response (e.g., jumping at kennel door, spinning excitedly)
1	Moderate response (e.g., rises, come to see what is going on)
2	Mild response (e.g., head lift, listening, tail wag)
3	Slight or just perceptible response (e.g., slight head lift or half a tail wag)
4	No response

Table 3.3 Gait score

Score	Description
1	Jumps the pole or run over it
2	Clears pole with all four feet at a walk
3	Hits pole with one or two feet
4	Hits pole with three or four feet
5	Unable to get to move to walk over pole

3.2.6 Physiological assessment

Selected physiological variables (heart rate, respiratory rate and rectal temperature) were recorded at baseline and at 30 minutes, 1, 2, 4, 6, 12, 18, 24, 36, 48, 60, 72, 96 and 120 hours after drug administration. Heart rate (HR) was measured by auscultation and respiratory rate (RR) by observation of thoracic excursions, both of which were measured over 1 minute. Rectal temperature (RT) was measured after HR and RR to avoid the potential influences of thermometer insertion on HR and RR measurements.

3.2.7 Antinociceptive assessment

Antinociceptive efficacy was assessed using a mechanical nociceptive threshold testing device (C-clamp) that was developed at CSU and has been validated in previous studies (Niyom et al. 2012; Niyom et al. 2015). This device is a manually applied C-clamp which is 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 responds (pull the leg back). The C-clamp was applied in a dorsopalmar manner just distal to the large foot pad over the metacarpal bone. At each measurement time point, 2 to 3 measurements were taken to obtain at least 2 measurement values within 10% of each other, and the average of these values was recorded as the mechanical threshold at each time point. Values were then converted from lb/cm^2 to Newtons. A cut off at 20 lb/cm^2 was used to minimize tissue damage. The C-clamp device was calibrated daily using known weights.

All dogs were familiarized with the C-clamp device for one week prior to the study. During the acclimation period, the C-clamp was applied to dogs at the same time points as during the study period: baseline and at 1, 2, 4, 6, 12, 18, 24, 30, 36, 48, 54, 60, 72, 84, 96, 108 and 120 hours after the first C-clamp application to obtain the mechanical nociceptive thresholds for a negative control and to observe the effects of learning and anticipation on the mechanical threshold.

During the study period, the C-clamp was applied to dogs at baseline and at 30 minutes, 1, 2, 4, 6, 12, 18, 24, 30, 36, 48, 54, 60, 72, 84, 96, 108 and 120 hours after drug administration. In order to minimize variability, the same investigator applied the C-clamp during both acclimation and throughout the study period.

3.2.8 Statistical Analysis

Statistical analyses were performed using the statistical software SAS 9.4 (SAS/STAT software, version 9.4, SAS Institute Inc., Cary, NC.). Behavioral variables (undisturbed sedation, response to voice and noise and gait scores) were presented as median (min and max) for each dose and time point. Wilcoxon's signed rank test was used to compare the two doses at each time point separately for each response variable. McNemar's test was then used to compare proportion responding at each time point. Physical variables (heart rate, respiratory rate and rectal temperature) and antinociceptive responses (mechanical nociceptive threshold) were presented as mean and standard deviation. A repeated measure analysis (ANOVA) was performed for each response variable separately using SAS Proc Mixed (SAS Institute Inc., Cary, NC.). Specifically, dose, time, and dose*time interactions were treated as fixed effects. In order to account for the crossover design, dog and dog*dose were included in the model as random effects. For each dose, subsequent time points were compared to baseline using Dunnett's method. For each time point, the two doses were compared. Due to the large number of time points a Benjamini-Hochberg adjustment was used to account for multiple testing. Significance for all statistical tests was established at $p < 0.05$.

3.3 Results

3.3.1 Buprenorphine plasma concentrations and pharmacokinetic analysis

The average plasma buprenorphine concentrations over a 120 h period, following a single subcutaneous dose of sustained release buprenorphine at 0.06 mg/kg (LD) or 0.18 mg/kg (HD), are shown in **Table 3.4** and **Figure 3.1**. Plasma buprenorphine concentrations were detectable at all time points up to 120 h (LLOQ 0.01 ng/mL), but didn't reach the previously

suggested therapeutic level of 0.6 ng/mL (Ko et al. 2011) until 12 h in the LD group and 2 h in the HD group. The average plasma concentration in the HD group at 30 min was higher than 0.6 ng/mL, however that is due to one individual dog presenting particularly elevated plasma concentrations (2.95 ng/mL) at that time point (the average plasma concentration of the other 5 dogs was 0.22 ± 0.12 ng/mL). Plasma buprenorphine concentrations peaked at 18 h (0.64 ± 0.42 and 1.33 ± 0.86 ng/mL in the LD and HD groups, respectively) for both treatments and decreased below 0.6 ng/mL by 24 and 60 h in the LD and HD groups, respectively. The pharmacokinetic parameters (mean ± SD) for both doses of sustained release buprenorphine are shown in **Table 3.5**.

Blood samples from one dog in the LD group could not be used for analysis at 1 h, 36 h and 48 h due to hyperlipidemia. One dog in the HD group developed a fever at 72 hours after drug administration, which was likely associated with the jugular catheter. The catheter was immediately removed and the fever subsided. Blood samples at the last 2 time points were collected by direct venipuncture (jugular vein) and plasma buprenorphine concentrations were included in the study, but the dog was excluded from further behavioral, physiological and antinociceptive assessments.

Table 3.4 Plasma buprenorphine concentrations (mean ± SD) of 6 beagle dogs after a single subcutaneous dose of 0.06 mg/kg Buprenorphine-SR (LD) or 0.18 mg/kg Buprenorphine-SR (HD) at fixed time points over a period of 120 h (n = 6, except were noted).

Time (h)	Buprenorphine-SR (LD) (ng/mL)	Buprenorphine-SR (HD) (ng/mL)
0	< LLOQ	< LLOQ
0.5	0.34 ± 0.26	0.67 ± 1.12
1	0.42 ± 0.33 (n=5)	0.46 ± 0.40
2	0.42 ± 0.36	0.61 ± 0.60
4	0.34 ± 0.19	0.73 ± 0.46
6	0.41 ± 0.23	1.04 ± 0.73
12	0.63 ± 0.25	0.95 ± 0.64
18	0.64 ± 0.42	1.33 ± 0.86
24	0.53 ± 0.34	0.88 ± 0.62
36	0.29 ± 0.19 (n=5)	0.62 ± 0.34
48	0.24 ± 0.10 (n=5)	0.67 ± 0.32
60	0.23 ± 0.16	0.47 ± 0.19
72	0.17 ± 0.16	0.32 ± 0.12
96	0.14 ± 0.10	0.19 ± 0.09
120	0.09 ± 0.06	0.13 ± 0.07

Table 3.5 Pharmacokinetic parameters (mean ± SD) for plasma buprenorphine concentrations of 6 beagles after a single subcutaneous dose of sustained-release buprenorphine at 0.06 mg/kg Bup-SR (LD) or 0.18 mg/kg Bup-SR (HD).

Pharmacokinetic parameters	Bup-SR (LD)	Bup-SR (HD)
C_{max} (ng/mL)	0.78 ± 0.42	1.5 ± 0.99
T_{max} (h)	14.3 ± 7.5	16.1 ± 17.2
$AUC_{0\text{-}120}$ (ng x h/mL)	34.53 ± 18.47	61.83 ± 26
AUC/D (ng x h/mL per mg/kg)	744 ± 336	402 ± 155
C_{max}/D (ng/mL per mg/kg)	13.01 ± 6.97	8.36 ± 5.48
$T_{1/2}$	35.3	32.9

C_{max}, maximal plasma concentration; T_{max}, time when C_{max} is reached; $AUC_{0\text{-}120}$, area under the curve from time 0 to time 120; D, total dose received; $T_{1/2}$, elimination half-life.

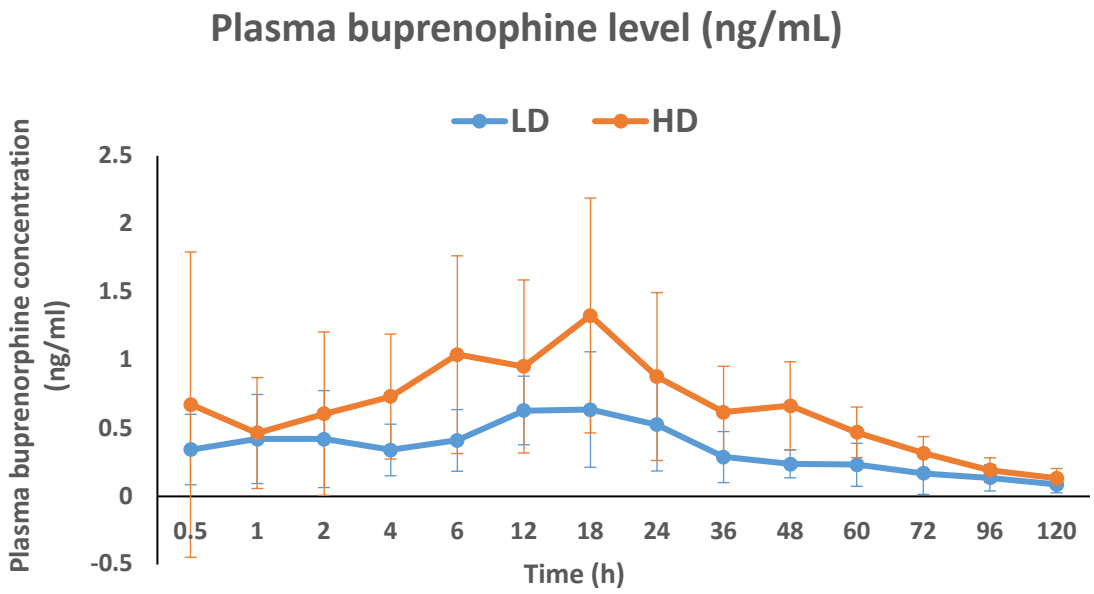

Figure 3.1: Plasma buprenorphine concentrations (mean ± SD [error bars]) of 6 beagles after a single subcutaneous dose of sustained-release buprenorphine at 0.06 mg/kg (LD) or 0.18 mg/kg (HD) at fixed time points over a period of 120 h.

3.3.2 Behavioral responses

There were no significant differences in any of the behavioral responses (sedation when undisturbed, response to voice, response to noise and gait scores) between the two treatment groups. Sedation (mild to moderate) was only observed within the first 24 hours after drug administration for both treatments. Moderate sedation (sedation score 2), was the highest score recorded and was noted in 3 dogs in the LD group and 2 dogs in the HD group. The response to voice and noise (kibble in the can) scores ranged between 0 and 1 (dramatic or exuberant to moderate response) for the LD group and 0 and 2 (dramatic or exuberant to mild response) for the HD group. The maximum gait score observed was 2 (clears pole with all four feet at a walk) for both treatments. All dogs were fully capable of walking throughout the study period. Adverse effects noted included nausea (3/6 dogs in each group), vomiting (1/6 and 2/6 dogs in

LD and HD group, respectively), soft stool (3/6 dogs in each group) and diarrhea (1/6 and 2/6 dogs in LD and HD group, respectively).

3.3.3 Physiologic responses

All physiological parameters remained within normal limits throughout the study period. There were no significant differences in PCV, TP, heart rate, respiratory rate and rectal temperature between treatments. PCV and TP were not significantly different from baseline in the LD group. In the HD group, PCV was significantly higher than baseline at 120 h and TP at 24 h (**Table 3.6)**. When compared to baseline, heart rate and respiratory rate did not change significantly within groups. Rectal temperature was significantly lower than baseline at 4 h, 6 h, 12 h, 18 h and 24 h in the HD group and at 12 h in the LD group (**Table 3.7)**.

Table 3.6 Hematocrit and total plasma protein values (Mean ± SD) in 6 beagles after a single dose of sustained-release buprenorphine at 0.06 mg/kg Bup-SR (LD) and 0.18 mg/kg Bup-SR (HD) at fixed time points over a period of 120 h.

Time (h)	Bup-SR (LD)		Bup-SR (HD)	
	Hematocrit (%)	Total protein (g/dl)	Hematocrit (%)	Total protein (g/dl)
0	49 ± 2	5.8 ± 0.3	47 ± 4	5.9 ± 0.2
1	46 ± 2	5.7 ± 0.3	47 ± 5	5.8 ± 0.2
6	49 ± 4	6.0 ± 0.3	50 ± 4	6.1 ± 0.2
24	50 ± 4	6.0 ± 0.3	49 ± 4	6.2 ± 0.4*
48	51 ± 4	6.0 ± 0.3	50 ± 5	6.1 ± 0.2
72	50 ± 3	5.9 ± 0.2	50 ± 4	6.0 ± 0.3
96	52 ± 4	6.1 ± 0.2	52 ± 5	6.1 ± 0.4
120	51 ± 3	6.0 ± 0.2	52 ± 4*	6.1 ± 0.2

*Value differs significantly ($p < 0.05$) from respective baseline value; n=6

Table 3.7 Selected physiological variables (Mean ± SD) of 6 healthy beagles after a single subcutaneous dose of sustained-release buprenorphine at 0.06 mg/kg Bup-SR (LD) or 0.18 mg/kg Bup-SR (HD) at fixed time points over a period of 120 h.

Time (h)	Heart rate (beats/minute)		Respiratory rate (breaths/minnute)		Rectal Temperature (°C)	
	Bup-SR (LD)	Bup-SR (HD)	Bup-SR (LD)	Bup-SR (HD)	Bup-SR (LD)	Bup-SR (HD)
0	100 ± 12	100 ± 15	21 ± 3	18 ± 3	38.7 ± 0.1	38.8 ± 0.1
0.5	98 ± 10	95 ± 16	21 ± 4	19 ± 5	38.7 ± 0.3	38.9 ± 0.3
1	93 ± 13	93 ± 9	19 ± 3	19 ± 4	38.7 ± 0.1	38.8 ± 0.5
2	98 ± 11	91 ± 14	22 ± 4	19 ± 5	38.8 ± 0.5	38.5 ± 0.2
4	96 ± 14	95 ± 8	19 ± 4	19 ± 3	38.6 ± 0.6	38.3 ± 0.5*
6	102 ± 14	95 ± 13	18 ± 5	17 ± 4	38.4 ± 0.4	38.1 ± 0.5*
12	86 ± 16	91 ± 19	19 ± 5	17 ± 3	38.2 ± 0.2*	38.1 ± 0.4*
18	91 ± 14	82 ± 12	17 ± 3	19 ± 6	38.3 ± 0.4	38.1 ± 0.3*
24	96 ± 18	93 ± 11	18 ± 4	18 ± 5	38.5 ± 0.3	38.1 ± 0.6*
30	97 ± 14	91 ± 6	21 ± 5	17 ± 3	38.6 ± 0.3	38.5 ± 0.2
36	96 ± 10	92 ± 10	23 ± 2	19 ± 4	38.8 ± 0.3	38.4 ± 0.3
48	104 ± 11	100 ± 12	19 ± 4	19 ± 5	38.8 ± 0.2	38.7 ± 0.3
54	103 ± 14	102 ± 8	18 ± 3	19 ± 2	38.8 ± 0.3	38.6 ± 0.3
60	95 ± 5	99 ± 5	20 ± 3	18 ± 3	38.8 ± 0.3	38.7 ± 0.3
72	102 ± 6	113 ± 13	20 ± 5	22 ± 6	39.0 ± 0.4	39.2 ± 0.4
84	106 ± 10	104 ± 6	21 ± 3	23 ± 4	39.0 ± 0.3	39.0 ± 0.2
96	105 ± 15	108 ± 9	22 ± 5	20 ± 4	39.0 ± 0.1	38.9 ± 0.1
108	108 ± 10	106 ± 4	21 ± 3	21 ± 1	38.8 ± 0.2	38.9 ± 0.2
120	108 ± 13	103 ± 6	22 ± 4	20 ± 3	39.0 ± 0.3	38.9 ± 0.3

*Value differs significantly from baseline ($p < 0.05$). (n = 6 except in HD group for times 72 to 120 h where n = 5).

3.3.4 Antinociceptive response

Mechanical nociceptive threshold was not significantly different between treatments. When compared to baseline, a significant increase in mechanical threshold was observed between 1 and 84 hours after drug administration for both treatments. The mechanical nociceptive threshold obtained during the acclimation period (negative control), was significantly lower than baseline from 12 to 120 hours after the first C clamp application. The mechanical nociceptive threshold for both treatments was significantly higher than the negative control from 2 to 120 hours after drug administration (**Table 3.8**. and **Figure 3.2**).

Table 3.8 Mean ± SD values for mechanical nociceptive threshold in 6 healthy beagles during the acclimation period (Neg Ctrl) and after a single subcutaneous dose of sustained-release buprenorphine at 0.06 mg/kg Bup-SR (LD) or 0.18 mg/kg Bup-SR (HD) at fixed time points over a period of 120 hours.

Time (h)	Neg Ctrl (Newtons)	Bup-SR (LD) (Newtons)	Bup-SR (HD) (Newtons)
0	32.1 ± 6.6	24.9 ± 8.6	25.6 ± 3.0
0.5	N/A	30.5 ± 3.0	31.9 ± 6.3
1	32.5 ± 5	38.6 ± 5.0*	36.6 ± 9.5*
2	31.0 ± 6.5	40.1 ± 3.1*[a]	40.0 ± 8.7*[a]
4	28.8 ± 3.4	43.2 ± 3.0*[a]	42.9 ± 10.2*[a]
6	27.4 ± 1.6	43.8 ± 4.2*[a]	41.2 ± 7.6*[a]
12	22.2 ± 2.1*	45.0 ± 5.9*[a]	40.9 ± 9.9*[a]
18	17.7 ± 2.4*	43.7 ± 6.1*[a]	41.5 ± 7.8*[a]
24	17.3 ± 2.9*	46.7 ± 3.8*[a]	42.7 ± 5.3*[a]
30	16.1 ± 2.7*	47.6 ± 2.3*[a]	40.1 ± 9.0*[a]
36	13.8 ± 1.3*	43.8 ± 3.3*[a]	40.8 ± 10.0*[a]
48	14.1 ± 2.4*	46.5 ± 3.6*[a]	38.5 ± 10.8*[a]
54	14.4 ± 1.7*	41.6 ± 5.6*[a]	41.0 ± 12.6*[a]
60	15.1 ± 1.2*	43.7 ± 2.3*[a]	37.5 ± 11.7*[a]
72	15.3 ± 2.5*	39.6 ± 10.4*[a]	42.6 ± 4.9*[a]
84	14.2 ± 3.0*	34.3 ± 10.5*[a]	37.8 ± 5.9*[a]
96	13.9 ± 2.0*	31.5 ± 8.4[a]	35.8 ± 4.2[a]
109	14.8 ± 2.2*	31.9 ± 7.6[a]	32.8 ± 7.4[a]
120	14.8 ± 2.8*	29.1 ± 6.9[a]	32.1 ± 3.5[a]

*Value differs significantly from baseline ($p < 0.05$). [a]Value differs significantly from the acclimation period (Neg Ctrl) at the same time points ($p < 0.05$). $n = 6$ except for HD group between 72 and 120 h where $n = 5$.

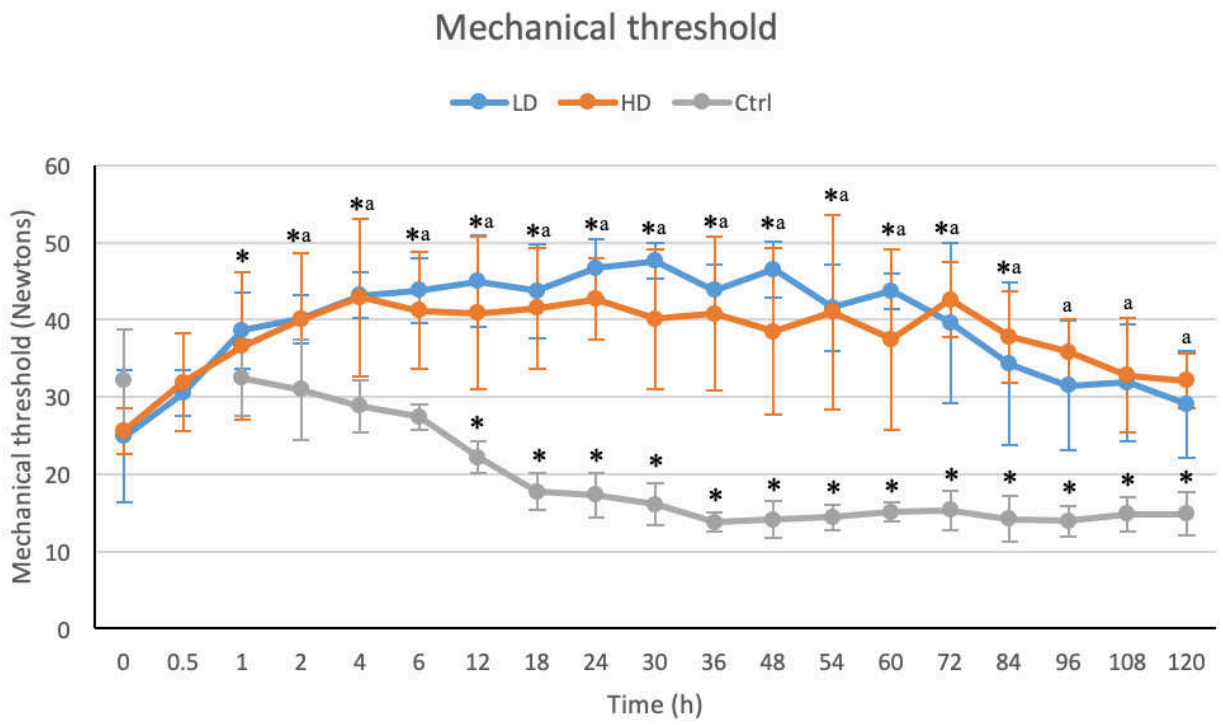

Figure 3.2 Mechanical nociceptive threshold (mean ± SD [error bars]) of 6 beagles obtained during negative control (Ctrl) and after a single subcutaneous dose of sustained-release buprenorphine at 0.06 mg/kg (LD) or 0.18 mg/kg (HD) at fixed time points over a period of 120 h. *Value differs significantly from baseline within the same treatment ($p < 0.05$). [a]Value differs significantly from the acclimation period (Neg Ctrl) at the same time points ($p < 0.05$). n = 6 except for HD group between 72 and 120 h where n = 5.

3.4 Discussion

Both doses of sustained-release buprenorphine provided similar behavioral, physiological and antinociceptive effects with mild adverse effects. Plasma buprenorphine concentrations were detected in all dogs for both treatment groups from 30 minutes to 120 hours post drug administration, which suggests that the sustained-release formulation used in this study can provide reliable drug delivery and systemic absorption of buprenorphine in dogs. The pharmacokinetic parameters were proportional for the doses of sustained-release buprenorphine used in the study. The maximal plasma concentration (HD C_{max}) reported in this study was much lower (1.50 ± 0.99 vs 5.6 ± 3.0 ng/mL) when compared to a previous study using a similar

buprenorphine formulation at a similar dose (0.2 mg/kg) in dogs undergoing OVH (Nunamaker et al. 2014). Dogs in Nunamaker et al. (2014) were anesthetized, which may have influenced drug absorption and elimination. The rate of absorption can be influenced by the amount of blood flow in the area of drug administration. The increase in temperature from contact with a heating pad or the vasodilating effects of anesthetics may increase blood flow to the area and increase the rate of drug absorption, which has been shown with fentanyl patches (Frölich et al. 2001; Weaver 2014). In addition, anesthesia has been shown to reduce buprenorphine clearance (Bullingham et al. 1980), which may have influenced both C_{max} and the elimination half-life, which was also much longer (32.9 vs 64.5h) in Nunamaker et al. (2014) than in the current study.

Average plasma buprenorphine concentrations were only above the proposed therapeutic concentration (0.6 ng/mL) for dogs (Ko et al. 2011) for approximately 6 hours (from 12 - 18 h after drug administration) in the LD group and 46 hours (from 2 - 48 h after drug administration) in the HD group. Ko et al. (2011), suggested that plasma concentrations above 0.6 ng/mL were needed to provide adequate analgesia after OVH, based on the plasma concentrations of dogs requiring rescue analgesia. A later study, which evaluated the concurrent use of meloxicam and the same sustained-release buprenorphine formulation used in this study, noted that buprenorphine plasma levels did not seem to correlate well with analgesia after OVH in dogs (Nunamaker et al. 2014). In that study, some dogs that required rescue analgesia had buprenorphine plasma concentrations as high as 2 ng/mL while other dogs that seemed comfortable had plasma concentrations of less than 0.1 ng/mL, suggesting that buprenorphine plasma concentrations may not be a reliable indicator for analgesia. This lack of correlation was also observed in the current study. The mechanical threshold (MT) values were higher than

baseline between 1 and 84 hours for both treatments, while buprenorphine plasma levels only reached or exceeded the suggested therapeutic concentration between 12 and 18 hours in the LD group and 2 and 48 hours in the HD group. It could be speculated that plasma levels of buprenorphine do not reflect drug concentrations at the receptor site. Buprenorphine has strong affinity and slow dissociation half-life from the μ-opioid receptor as well as slow diffusion out of the central nervous system (Duke-Novakovski 2014; KuKanich & Papich 2018), suggesting that significant amounts of buprenorphine may be present at the receptor site, despite decreasing plasma levels.

The lack of difference in the antinociceptive response between treatments seem to support the analgesic ceiling effect described for partial μ-opioid receptor agonists, and previously shown in dogs undergoing OVH (Slingsby et al. 2011) and during the evaluation of minimum alveolar concentrations of isoflurane in dogs receiving buprenorphine (Queiroz-Williams et al. 2014). It could also be argued that the nociceptive stimuli might not have been significant enough to demonstrate a difference between doses, and further clinical studies with dogs undergoing surgical procedures are recommended.

The mechanical threshold values obtained during the acclimation period (Neg Ctrl) were significantly lower from 12 to 120 hours after the first C-clamp application. This is likely the result of the dogs learning and anticipating the mechanical threshold device. When those values are compared to the two treatment groups, dogs in both treatments showed significantly higher mechanical threshold values from 2 hours to 120 hours after drug administration, further supporting the ability of sustained-release buprenorphine in providing prolonged antinociceptive effect.

There were no behavioral or physiological differences between treatments, which may

also be a result of buprenorphine's ceiling effect (Slingsby et al. 2011). While sedation is a common side effect of buprenorphine (Abbo et al. 2008), only mild to moderate sedation was observed in the current study for both treatments, and it was limited to the first 24 hours. Similar results were noted by Nunamaker et al. (2014), which used the same sustained-release buprenorphine formulation at a similar dose. Bradycardia and hypothermia were reported in a study evaluating a lipid-encapsulated formulation of extended release buprenorphine in dogs (Barletta et al. 2018), but were not observed in the current study. A decrease in rectal temperature was observed after buprenorphine administration in the HD group, but it was not clinically significant and did not characterized hypothermia. All physiological parameters remained within normal limits throughout the study period. Adverse effects (nausea, vomiting and diarrhea) were mild and similar to the ones reported by Nunamaker et al. (2014).

In summary, sustained-release formulations are a practical alternative for pain management in animals, and have the advantage of providing long-lasting analgesia with stable plasma concentrations, without repeated handling and stress for the animals. In addition, with the heightened concerns regarding opioid abuse, it provides the ability of adequate pain management without the need to prescribe a controlled substance to an outpatient. The results of this study showed that both doses of the sustained-release buprenorphine formulation (0.06 mg/kg and 0.18 mg/kg) evaluated were able to provide comparable behavioral, physiological and antinociceptive effects using a mechanical threshold testing device in dogs. The antinociceptive effects of the sustained-release buprenorphine were present even when plasma concentrations were below previously postulated therapeutic levels. Side effects were minimal and included nausea, vomiting and diarrhea. Further studies investigating the analgesic efficacy of these doses in a clinical setting, with naturally occurring pain, are recommended.

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CHAPTER 4: EVALUATION OF THE ANALGESIC EFFICACY AND ADVERSE EFFECTS OF TWO HIGH DOSES OF A HIGH-CONCENTRATION BUPRENORPHINE FORMULATION IN DOGS BEING NEUTERED

4.1 Introduction

Buprenorphine is a partial μ opioid receptor agonist (Duke-Novakovski 2014; KuKanich & Allen 2014), and its usefulness in the treatment of mild to moderate pain is well established in dogs (Slingsby et al. 2006; Shih et al. 2008; Ko et al. 2011; Slingsby et al. 2011; Linton et al. 2012) and cats (Giordano et al. 2010; Taylor et al. 2010; Catbagan et al. 2011). A ceiling effect for analgesia and adverse effects was demonstrated in dogs administered buprenorphine and subjected to ovariohysterectomy (OVH) (Slingby et al. 2011). Compared to full μ-opioid receptor agonists, buprenorphine has a better safety profile (Johnson et al. 2005), and its high lethal dose 50 (LD_{50}) indicates a wide safety margin (KuKanich & Papich 2018). Buprenorphine can be administered through various routes in dogs and cats (KuKanich & Wiese 2015) and at the standard dose of 20 μg/kg IM (Slingsby et al. 2006; Shih et al. 2008; Slingsby et al. 2011; Linton et al. 2012) or IV (Ko et al. 2011; Pieper et al. 2011), requires redosing every 6 to 8 hours. While buprenorphine has a longer dosing interval compared to most other μ agonists, frequent dosing is still required.

A high-concentration buprenorphine (1.8 mg/mL) formulation (Simbadol™) has been approved by the FDA for use in cats at 0.24 mg/kg, SC, once daily, for 3 days, to provide postoperative analgesia (Kukanich & Wiese 2015). At the manufacturer's recommended therapeutic dose, which is much higher than traditionally used buprenorphine doses, Simbadol™ is expected to provide analgesia for 24 hours, after a single subcutaneous injection in cats. This high-concentration formulation has been shown to be safe and well tolerated in young cats (4

months) after being administered at 0.24 mg/kg (the proposed therapeutic dose), 0.72 mg/kg, or 1.2 mg/kg, subcutaneously, once daily for 9 consecutive days. Only 2 cats were reported to have developed drug-related adverse events, which were described as dilated pupils and behavioral changes (hyperactivity, agitation and disorientation) (Sramek et al. 2015).

The antinociceptive effect of this high-concentration buprenorphine formulation has been evaluated in conscious cats using a thermal threshold testing device, and was shown to provide thermal antinociception for at least 24 hours (Taylor et al. 2016; Doodnaught et al. 2017). A recent study, comparing the high-concentration buprenorphine (0.24 mg/kg, SC, every 24 hours for 3 days) and the regular buprenorphine formulation (0.02 mg/kg, IM every 8 hours for 3 days), both in combination with meloxicam, reported comparable post-operative analgesia in cats undergoing dental extractions (Watanabe et al. 2020). While the high-concentration buprenorphine formulation has been evaluated in dogs at traditional doses (20 µg/kg, IM) (Watanabe et al. 2018), there are no studies evaluating the use of a high dose Simbadol™ in dogs similar to what is recommended for cats.

The aim of this study was to evaluate the analgesic efficacy and potential side-effects of two high doses of a highly concentrated buprenorphine formulation (Simbadol™) in dogs undergoing neutering.

4.2 Material and methods

4.2.1 Animals and study allocation

The study protocol was approved by the Institutional Animal Care and Use Committee and the Clinical Review Board of Colorado State University. The study was divided in 2 phases:

Phase I (Preliminary study) and phase II (Clinical study).

In Phase I, twelve healthy beagles (6 males and 6 females), aged 48 ± 31 months and weighing 10.9 ± 2.7 kg, undergoing surgical neutering were used. All dogs were housed at the University's Laboratory Animals Resources (LAR) facilities within the Veterinary Teaching Hospital and were provided with fresh water and commercially dry dog food ad libitum. They were routinely socialized through LAR's socialization program. The 6 males and 6 females were randomly divided into 2 groups (P-LD and P-HD), so that each group included 3 males and 3 females. Dogs in P-LD group received a single dose of high-concentration buprenorphine (Simbadol ™), subcutaneously, at 0.12 mg/kg, which is the anecdotally suggested dose for dogs. Dogs in P-HD group received a single dose of high-concentration buprenorphine (Simbadol ™), subcutaneously, at 0.24 mg/kg, which is the recommended dose by the manufacturer for cats. The goal of phase I was to ensure analgesic efficacy and safety of the proposed doses being studied before adopting the doses in the clinical study (Phase II).

In phase II (Clinical study), twenty-four healthy female dogs, aged 17 ± 13 months and weighing 17.6 ± 5.3 kg, undergoing ovariohysterectomy (OVH) through Colorado State University's Community Practice were enrolled. Informed consent was provided by the shelter institutions responsible for the dogs. Dog breeds included Pit bull (4), Pit bull mix (2), Siberian husky mix (3), Shepherd mix (3), Silky terrier mix (1), Heeler mix (3), Catahoula mix (1), Labrador Retriever (1), Labrador Retriever mix (1), Bull terrier mix (1), Pointer mix (1), English Staffordshire (1), and mixed breed (2). Dogs in Phase II were randomly assigned to receive a single dose of high-concentration buprenorphine (Simbadol ™), subcutaneously, at 0.12 mg/kg (C-LD group) or 0.24 mg/kg (C-HD group).

4.2.2 Anesthetic and surgical procedures

All dogs (Phase I and II) were anesthetized following a standardized anesthetic protocol. In addition to their group-specific buprenorphine treatment (0.12 mg/kg or 0.24 mg/kg, SC) dogs were administered acepromazine (0.02 mg/kg) and atropine (0.02 mg/kg) subcutaneously as part of the anesthetic premedication, approximately one hour prior to catheter placement and induction of anesthesia. Propofol (6 mg/kg) was intravenously administered, slowly to effect, to induce anesthesia and allow orotracheal intubation. Anesthesia was maintained with isoflurane in 100% oxygen delivered via a circle system.

Heart rate and rhythm (ECG), respiratory rate, end-tidal CO_2, arterial hemoglobin oxygen saturation (SpO_2), blood pressure (Oscillometric or Doppler technique) and body temperature (esophageal temperature probe) were monitored continuously during anesthesia using a multiparametric monitor. Lactated ringers solution (LRS) was intravenously administered at 5-10 mL/kg/h. In case of hypotension, a dopamine CRI was started at 5 mcg/kg/min and adjusted as needed to maintain a mean arterial pressure (MAP) > 65 mmHg or a systolic arterial pressure (SAP) > 90 mmHg. Atropine was administered as needed to treat bradycardia. Anesthesia time (vaporizer on to vaporizer off), surgery time (skin incision to closure) and time to extubation (vaporizer off to endotracheal extubation) were recorded. Intraoperative complications were also recorded for Phase II.

In the preliminary study (Phase I), the surgical procedures (ovariohysterectomy and castration) were performed in all dogs by the same Board Certified Surgeon, in order to standardize tissue handling. During the clinical study (Phase II), the surgical procedures (ovariohysterectomy) were performed by senior veterinary students under the direct supervision of a Community Practice Veterinarian.

4.2.3 Behavioral, physiological and pain evaluations

Behavioral assessments included the level of sedation when undisturbed (sedation score 0-3, **Table 4.1**) and during interactive moments: response to voice (voice score 0-4, **Table 4.2**). All behavioral assessments as well as any adverse effects (nausea, vomit, diarrhea) were recorded at baseline (prior to drug administration), immediately prior to catheter placement (approximately 1 h after drug administration), at 15, 30 and 60 min after extubation (15 m pEx, 30 m pEx and 60 m pEx) and at 4, 6, 8, 12, 18 and 24 h after drug administration (4 h pD, 6 h pD, 8 h pD, 12 h pD, 18 h pD and 24 h pD). In Phase II, sedation quality at catheter placement, as well as induction and recovery qualities were subjectively scored (good, fair or poor) and recorded.

Physiological assessments were performed after the behavioral evaluation (at the same time points) and included heart rate, respiratory rate, and rectal temperature. Heart rate (HR) was measured by auscultation and respiratory rate (RR) by observation of thoracic excursions, both of which were measured over 1 minute. Rectal temperature (RT) was measured after HR and RR to avoid the potential influences of thermometer insertion on HR and RR measurements.

Table 4.1 Sedation score (undisturbed)

Score	Description
0	No sedation
1	Mild sedation: less alert but still active
2	Moderate sedation: drowsy, recumbent but can walk
3	Profound sedation: very drowsy, unable to walk

Table 4.2 Sedation score for response to voice (interactive)

Score	Description
0	Dramatic or Exuberant response (e.g., jumping at kennel door, spinning excitedly)
1	Moderate response (e.g., rises, come to see what is going on)
2	Mild response (e.g., head lift, listening, tail wag)
3	Slight or just perceptible response (e.g., slight head lift or half a tail wag)
4	No response

Pain evaluation was performed last, at the same time points as for behavioral and physiological assessments. The Visual Analogue Scale (VAS), the CSU pain scale (CSUPS) and the Glasgow Composite Pain Scale – Short Form (GCPS-SF) were used to assess pain. If rescue analgesia was deemed necessary (VAS score > 4, CSUPS > 2.5, GCPS-SF > 5/20, 6/24), regular buprenorphine was administered at 20 mcg/kg, IV and pain evaluation repeated 1 h after rescue drug administration. If additional analgesia was still deemed necessary, carprofen at 2.2 mg/kg was administered subcutaneously.

All evaluations (behavioral, physiological and pain scoring) were performed by the same investigator, who was blinded to the treatment administered.

4.2.4 Blood sampling

Blood samples for buprenorphine plasma concentration analysis were collected during the preliminary study (Phase I). Venous blood samples (2-3 mL) were collected at catheter placement (approximately 1 h after drug administration), at the end of surgery and at 12 and 24 h after drug administration. For the time points that coincided with behavioral, physiological and pain assessments, blood samples were collected after the behavioral and physiological

assessments, but before pain evaluation. If at any point in the study rescue analgesia was deemed necessary, an additional blood sample was collected immediately prior to the rescue analgesic administration. Blood samples were centrifuged (2500 rpm, 5ºC, 10 minutes) and plasma harvested to store at -80 ºC until analyzed.

4.2.5 Statistical Analysis

Statistical analyses were performed using the statistical software SAS 9.4 (SAS/STAT software, version 9.4, SAS Institute Inc., Cary, NC.). Summary statistics (mean, SD, min, median, max) were calculated for each variable, treatment and time point. Residual diagnostic plots were used to evaluate model assumptions for normality and equal variance. For signalment (age, body weight), PCV and total protein, and time (surgery time, anesthesia time, and time to extubation), comparisons were made between treatments using two-sample t-test. Physiologic parameters (heart rate, respiratory rate, rectal temperature) were analyzed using a mixed model. Specifically, treatment (P-LD vs P-HD and C-LD vs C-HD) and time and treatment*time interaction were treated as fixed effect. For each time point, comparisons were made between treatments. For each treatment, comparisons between downstream time points versus baseline were performed using Dunnett's method. For sedation and pain scores, comparisons were made between treatments at predetermined time points using Wilcoxon rank-sum test. For complications, comparisons were made between treatments using Fisher's Exact Test (FET). Significance for all statistical tests was established at $p < 0.05$.

4.3 Results

Age, body weight, preoperative packed cell volume and total protein, anesthesia time,

surgery time and time to extubation for the preliminary study (Phase I) and for the clinical study (Phase II) are presented in **Table 4.3** and **Table 4.4**, respectively. There were no significant differences in any of the aforementioned parameters between treatment groups for both Phase I and Phase II.

Table 4.3 Age, body weight, preoperative packed cell volume and total protein, surgery and anesthesia times and time to extubation (mean ± SD) of 12 beagles undergoing neutering and enrolled in the preliminary study (Phase I). Dogs were randomly allocated to either treatment P-LD (high-concentration buprenorphine, 0.12 mg/kg, SC) or treatment P-HD (high-concentration buprenorphine, 0.24 mg/kg, SC).

Variable	P-LD (*n=6*)	P-HD (*n=6*)	*p* value
Age (months)	51 ± 36	46 ± 38	0.85
Body weight (kg)	11.3 ± 3.3	10.6 ± 2.4	0.66
PCV (%)	48 ± 2	48 ± 2	0.90
Total protein (g/dL)	6.0 ± 0.5	6.0 ± 0.4	0.95
Surgery time (min)	23 ± 24	25 ± 21	0.93
Anesthesia time (min)	49 ± 32	50 ± 25	0.93
Time to extubation (min)	13 ± 4	10 ± 3	0.24

Table 4.4 Age, body weight, preoperative packed cell volume and total protein, surgery and anesthesia times and time to extubation (mean ± SD) of 24 beagles undergoing ovariohysterectomy and enrolled in the clinical study (Phase II). Dogs were randomly allocated to either treatment C-LD (high-concentration buprenorphine, 0.12 mg/kg, SC) or treatment C-HD (high-concentration buprenorphine, 0.24 mg/kg, SC).

Variable	C-LD (*n=12*)	C-HD (*n=12*)	*p* value
Age (months)	14 ± 9	20 ± 16	0.31
Body weight (kg)	17.0 ± 4.6	18.3 ± 6.1	0.56
PCV (%)	48 ± 6	45 ± 4	0.12
Total protein (g/dL)	6.4 ± 0.8	6.5 ± 0.4	0.93
Surgery time (min)	145 ± 51	127 ± 39	0.34
Anesthesia time (min)	192 ± 55	181 ± 41	0.60
Time to extubation	20 ± 16	18 ± 10	0.62

4.3.1 Quality of sedation at catheter placement, quality of induction and recovery, and anesthetic complications (Phase II)

In Phase II, quality of sedation at intravenous catheter placement was considered good for all dogs in both treatments, with only one dog showing profound sedation (C-LD). Quality of induction was rated excellent or good for the majority of dogs, with only 1 dog in C-LD and 2 dogs in C-HD having a fair induction. Similarly, recovery quality was rated good or excellent for most dogs, with only 3 dogs in C-LD having a fair recovery. While mild hypotension was noted during anesthesia in 75% (9/12) of dogs in each treatment, other anesthetic complications were rare and included hypothermia (1/12 C-LD; 4/12 C-HD), hypoventilation and bradycardia (1/12 C-LD) and regurgitation (2/12 C-LD).

4.3.2 Behavioral assessment

There were no significant differences in sedation scores (undisturbed and interactive) at any time points between the two treatment groups in Phase I (**Table 4.5**). Similar results were observed in Phase II, with no significant differences in sedation scores (undisturbed and interactive) between the two treatment groups, except at 18 h pD, where dogs in the C-LD treatment had significantly higher sedation scores than dogs in the C-HD group ($p = 0.01$) (**Table 4.6**).

Nausea was the only adverse effect noted in Phase II and it was observed with both treatments (5/12 and 7/12 dogs in C-LD and C-HD group, respectively), predominantly during the post-extubation period.

Table 4.5 Sedation scores (Median [min, max]) of 12 healthy beagles (6 females and 6 males) after premedication with acepromazine (0.02 mg/kg, SC), atropine (0.02 mg/kg, SC) and a single subcutaneous dose of either 0.12 mg/kg high-concentration buprenorphine (P-LD) or 0.24 mg/kg high-concentration buprenorphine (P-HD) – Phase I (preliminary study).

Time	Undisturbed sedation scores (0-3)		Interactive sedation scores (0-4)	
	P-LD	P-HD	P-LD	P-HD
1 h pD	2 (1, 2)	1 (1, 2)	1 (1, 2)	1 (1, 1)
15 m pEx	1.5 (1, 3)	1 (1, 2)	1 (1, 3)	1 (1, 2)
30 m pEx	1.5 (1, 3)	1 (1, 2)	1 (1, 3)	1 (1, 2)
60 m pEx	1 (0, 3)	1.5 (1, 2)	1 (0, 3)	1 (1, 2)
4 h pD	1 (0, 3)	1 (1, 2)	1 (0, 3)	1 (1, 2)
6 h pD	1 (0, 2)	1 (0, 1)	1 (0, 2)	1 (0, 1)
8 h pD	1 (0, 2)	1 (0, 1)	1 (0, 2)	1 (0, 1)
12 h pD	0 (0, 1)	0 (0, 1)	0 (0, 1)	0 (0, 1)
18 h pD	0 (0, 1)	0 (0, 0)	0 (0, 1)	0 (0, 0)
24 h pD	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

Dogs were induced with propofol and anesthesia was maintained with isoflurane in 100% oxygen, to perform castration or ovariohysterectomy. (n=6 [3 males and 3 females] for each treatment group). pD, post drug administration; pEx, post-extubation.

Table 4.6 Sedation scores (Median [min, max]) of 24 healthy female dogs after premedication with acepromazine (0.02 mg/kg, SC), atropine (0.02 mg/kg, SC) and a single subcutaneous dose of either 0.12 mg/kg high-concentration buprenorphine (C-LD) or 0.24 mg/kg high-concentration buprenorphine (C-HD) – Phase II (clinical study).

Time	Undisturbed sedation scores (0-3)		Interactive sedation scores (0-4)	
	C-LD	C-HD	C-LD	C-HD
1 h pD	2 (1, 2)	2 (2, 2)	2 (1, 3)	2 (1, 3)
15 m pEx	3 (2, 3)	3 (2, 3)	3 (1, 3)	3 (2, 3)
30 m pEx	3 (2, 3)	3 (2, 3)	3 (1, 3)	3 (1, 3)
60 m pEx	3 (2, 3)	3 (2, 3)	3 (1, 3)	3 (1, 3)
6 h pD	3 (2, 3)	3 (1, 3)	3 (1, 3)	3 (1, 3)
8 h pD	2 (0, 3)	2 (1, 3)	2 (0, 3)	2 (1, 3)
12 h pD	2 (0, 2)	1 (0, 2)	1.5 (0, 2)	1 (0, 2)
18 h pD	0.5 (0, 1)[a]	0 (0, 0)[b]	0.5 (0, 1)[a]	0 (0, 0)[b]
24 h pD	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

Dogs were induced with propofol and anesthesia was maintained with isoflurane in 100% oxygen, to perform ovariohysterectomy. (n=12 for each treatment group). pD, post drug administration; pEx, post-extubation.
[a,b] Value differs significantly between treatments at the same time point ($p < 0.05$).

4.3.3 Physiological assessment

All selected physiological variables (heart rate, respiratory rate and rectal temperature) are presented for Phase I (**Table 4.7**) and Phase II (**Table 4.8**).

There were no significant differences in physiological variables between treatment groups during Phase I. Heart rate was significantly lower than baseline at 1 h pD, 30 m pEx and from 4 h to 24 h pD in the P-LD treatment and from 6 to 24 h pD in the P-HD treatment. Respiratory rate was significantly lower than baseline at 1 h pD, from 15 to 60 m pEx and from 6 to 18 h pD in the P-LD treatment, and at 60 m pEx, and from 6 to 18 h pD in the P-HD treatment. Rectal temperature was significantly lower than baseline from 1 to 24 h pD for both treatments.

There were no significant differences in heart rate and respiratory rate between treatment groups during Phase II. Rectal temperature was also not significantly different between treatment groups except at 24 h pD where the rectal temperature in the C-LD treatment was significantly higher than in the C-HD treatment. Heart rate was significantly higher than baseline at 15 m pEx in the C-LD treatment, but there were no differences from baseline in the C-HD treatment. Respiratory rate was significantly lower than baseline at every time point throughout the study period in both treatment groups, except for 18h pD in the C-HD treatment. Rectal temperature was significantly lower than baseline at all time points up to 8h pD in both treatments. In treatment C-HD, rectal temperature was also lower than baseline from 18-24 h pD.

Table 4.7 Physiological variables (Mean ± SD) of 12 healthy beagles (6 females and 6 males) after premedication with acepromazine (0.02 mg/kg, SC), atropine (0.02 mg/kg, SC) and a single subcutaneous dose of either 0.12 mg/kg high-concentration buprenorphine (P-LD) or 0.24 mg/kg high-concentration buprenorphine (P-HD) – Phase I (preliminary study).

Time (h)	Heart rate (beats/min)		Respiratory rate (breaths/min)		Rectal temperature (°C)	
	P-LD	P-HD	P-LD	P-HD	P-LD	P-HD
0	115 ± 19	115 ± 9	32 ± 10	26 ± 7	38.4 ± 0.3	38.6 ± 0.1
1h pD	84 ± 10*	99 ± 20	18 ± 2*	21 ± 4	36.5 ± 0.2*	36.8 ± 0.2*
15 m pEx	116 ± 25	128 ± 24	21 ± 5*	21 ± 5	35.9 ± 0.5*	35.8 ± 0.2*
30 m pEx	92 ± 16*	108 ± 27	20 ± 3 *	22 ± 6	36.1 ± 0.9*	36.5 ± 0.6*
60 m pEx	94 ± 14	100 ± 17	20 ± 5*	18 ± 3*	36.5 ± 0.7*	36.5 ± 0.6*
4 h pD	89 ± 10*	94 ± 9	25 ± 6	21 ± 4	36.7 ± 0.4*	36.8 ± 0.7*
6 h pD	80 ± 16*	86 ± 12*	20 ± 3*	19 ± 2*	36.8 ± 0.5*	37.2 ± 0.4*
8 h pD	75 ± 9*	79 ± 10*	20 ± 3*	18 ± 4*	37.2 ± 0.5*	37.2 ± 0.7*
12 h pD	85 ± 10*	88 ± 6*	21 ± 3*	19 ± 3*	37.4 ± 0.3*	37.5 ± 0.3*
18 h pD	83 ± 9*	83 ± 4*	20 ± 2*	20 ± 2*	37.3 ± 0.3*	37.4 ± 0.3*
24 h pD	87 ± 15*	84 ± 9*	23 ± 4	22 ± 2	37.6 ± 0.5*	37.7 ± 0.5*

*Value differs significantly from baseline ($p < 0.05$). Dogs were induced with propofol and anesthesia was maintained with isoflurane in 100% oxygen, to perform castration or ovariohysterectomy. (n=6 [3 males and 3 females] for each treatment group). pD, post drug administration; pEx, post-extubation.

Table 4.8 Physiological variables (Mean ± SD) of 24 healthy female dogs after premedication with acepromazine (0.02 mg/kg, SC), atropine (0.02 mg/kg, SC) and a single subcutaneous dose of either 0.12 mg/kg high-concentration buprenorphine (C-LD) or 0.24 mg/kg high-concentration buprenorphine (C-HD) – Phase II (clinical study).

Time (h)	Heart rate (beats/min)		Respiratory rate (breaths/min)		Rectal temperature (°C)	
	C-LD	C-HD	C-LD	C-HD	C-LD	C-HD
0	103 ± 16	104 ± 18	26 ± 10	26 ± 9	38.3 ± 0.7	38.4 ± 0.5
1h pD	100 ± 14	113 ± 27	17 ± 5*	17 ± 3*	37.2 ± 0.7*	37.1 ± 0.5*
15 m pEx	119 ± 25*	119 ± 15	17 ± 6*	20 ± 5*	37.5 ± 0.4*	37.2 ± 0.5*
30 m pEx	114 ± 22	113 ± 20	18 ± 5 *	20 ± 5*	37.5 ± 0.4*	37.2 ± 0.4*
60 m pEx	103 ± 20	109 ± 17	17 ± 4*	20 ± 5*	37.5 ± 0.3*	37.2 ± 0.5*
6 h pD	103 ± 25	106 ± 15	16 ± 3*	21 ± 6*	37.4 ± 0.5*	37.4 ± 0.5*
8h pD	96 ± 17	101 ± 13	17 ± 4*	20 ± 9*	37.5 ± 0.4*	37.7 ± 0.7*
12 h pD	97 ± 16	101 ± 17	20 ± 6*	19 ± 6*	38.2 ± 0.8	38.0 ± 0.5
18 h pD	101 ± 19	103 ± 19	19 ± 6*	21 ± 6	38.1 ± 0.6	37.8 ± 0.3*
24 h pD	103 ± 18	100 ± 11	19 ± 3*	19 ± 5*	38.3 ± 0.5[a]	37.8 ±0.4*,[b]

*Value differs significantly from baseline ($p < 0.05$). [a,b]Value differs significantly between treatments at the same time point ($p < 0.05$). Dogs were induced with propofol and anesthesia was maintained with isoflurane in 100% oxygen, to perform ovariohysterectomy. (n=12 for each treatment group). pD, post drug administration; pEx, post-extubation.

4.3.4 Postoperative pain assessment

There were no significant differences in post-operative pain scores (VAS, CSUPS and GCPS-SF) at any time point between the two treatment groups in Phase I (**Table 4.9**), and none of the dogs required rescue analgesia. Postoperative pain scores (median) ranged from 0 to 1 (VAS), 0.25 to 0.38 (CSUPS), and 1 to 1.5 (GCPS-SF) in the P-LD treatment and from 0 to 1 (VAS), 0.25 to 0.5 (CSUPS), and 1 to 2 (GCPS-SF) in the P-HD treatment. The highest VAS score recorded in the P-LD treatment was 2 and was noted in 1/6 dog from 30 m pEx to 4 h pD;

while in the P-HD treatment, the highest VAS score recorded was 1 and was noted in 6/6 dogs with the highest prevalence from 12 to 24 h pD. The highest CSUPS and GCPS-SF scores recorded in the P-LD treatment were 0.75 and 3, respectively and were noted in 1/6 dogs at 4 h pD. In the P-HD treatment, the highest CSUPS score recorded was 1 and was noted in 1/6 dogs at 12 and 18 h pD, while the highest GCPS-SF score recorded was 2 and was noted in 5/6 dogs with highest prevalence at 60 m pEx.

Similarly, to Phase I, there were no significant differences in post-operative pain scores (VAS, CSUPS and GCPS-SF) at any time points between the two treatment groups in Phase II (**Table 4.10**), and none of the dogs required rescue analgesia. Post-operative pain scores (median) for the C-LD treatment were at 2 (VAS), ranged from 0.25 to 0.63 (CSUPS) and remained at 1 (GCPS-SF), while for the C-HD treatment scores were at 2 (VAS), ranged from 0.25 to 0.75 (CSUPS) and 1 to 2 (GCPS-SF). The highest VAS score recorded was 3, noted in 4/12 dogs in C-LD and 6/12 dogs in C-HD group. The time point that had the highest amount of dogs with a VAS score of 3 was 12 h pD in C-LD group and 18 h pD in C-HD group. For the CSUPS pain score, the highest score recorded was 1.75 and was noted in 2/12 dogs at 12 h pD in C-LD group and 1/12 dog at 8 h pD in C-HD group. For GCPS-SF pain score, the highest score recorded was 4 and was noted in 2/12 dogs (one dog at 12 h pD and another at 18 h pD) in the C-LD group and 1/12 dog at 8 h pD in C-HD group.

Table 4.9 Pain scores (Median [min, max]) of 12 healthy beagles (6 females and 6 males) after premedication with acepromazine (0.02 mg/kg, SC), atropine (0.02 mg/kg, SC) and a single subcutaneous dose of either 0.12 mg/kg high-concentration buprenorphine (P-LD) or 0.24 mg/kg high-concentration buprenorphine (P-HD) – Phase I (preliminary study).

Time (h)	VAS		CSUPS		GCPS-SF	
	P-LD	P-HD	P-LD	P-HD	P-LD	P-HD
0	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
1h pD	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
15 m pEx	1 (0, 1)	0 (0, 1)	0.25 (0, 0.25)	0.25 (0, 0.25)	1.5 (1, 2)	1 (1, 2)
30 m pEx	1 (0, 2)	0.5 (0, 1)	0.25 (0, 0.5)	0.25 (0, 0.5)	1.5 (1, 3)	1 (1, 2)
60 m pEx	1 (0, 2)	1 (0, 1)	0.25 (0, 0.75)	0.25 (0, 0.75)	1 (1, 3)	2 (1, 2)
6 h pD	1 (0, 1)	1 (0, 1)	0.38 (0, 0.75)	0.25 (0, 0.75)	1 (1, 2)	1 (1, 2)
8h pD	1 (0, 1)	1 (0, 1)	0.5 (0, 0.75)	0.25 (0, 0.75)	1 (1, 1)	1 (1, 1)
12 h pD	1 (0, 1)	1 (1, 1)	0.25 (0, 0.75)	0.5 (0.25, 1)	1 (1, 1)	1 (1, 2)
18 h pD	1 (1, 1)	1 (1, 1)	0.38 (0.25, 0.75)	0.5 (0.25, 1)	1 (1, 1)	1 (1, 2)
24 h pD	1 (1, 1)	1 (1, 1)	0.25 (0.25, 0.75)	0.5 (0.25, 0.75)	1 (1, 2)	1.5 (1, 2)

Dogs were induced with propofol and anesthesia was maintained with isoflurane in 100% oxygen, to perform castration or ovariohysterectomy. (n=6 [3 males and 3 females] for each treatment group). VAS, Visual Analogue Scale; CSUPS, Colorado State University Pain Scale; GCPS-SF, Glasgow Composite Pain Scale – Short Form. pD, post drug administration; pEx, post-extubation.

Table 4.10 Pain scores (Median [min, max]) of 24 healthy female dogs after premedication with acepromazine (0.02 mg/kg, SC), atropine (0.02 mg/kg, SC) and a single subcutaneous dose of either 0.12 mg/kg high-concentration buprenorphine (C-LD) or 0.24 mg/kg high-concentration buprenorphine (C-HD) – Phase II (clinical study).

Time (h)	VAS		CSUPS		GCPS-SF	
	C-LD	C-HD	C-LD	C-HD	C-LD	C-HD
0	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
1h pD	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
15 m pEx	2 (1, 3)	2 (1, 3)	0.25 (0.25, 0.25)	0.25 (0.25, 0.25)	1 (1, 2)	1 (1, 2)
30 m pEx	2 (1, 3)	2 (1, 3)	0.38 (0.25, 0.5)	0.25 (0.25, 0.25)	1 (1, 2)	1 (1, 3)
60 m pEx	2 (1, 3)	2 (1, 3)	0.25 (0.25, 0.5)	0.25 (0.25, 0.25)	1 (1, 3)	1.5 (1, 2)
6 h pD	2 (1, 2)	2 (1, 3)	0.25 (0.25, 0.5)	0.25 (0.25, 0.5)	1 (1, 2)	2 (1, 2)
8h pD	2 (1, 3)	2 (1, 3)	0.25 (0.25, 0.75)	0.5 (0.25, 1.75)	1 (1, 2)	1 (1, 4)
12 h pD	2 (1, 3)	2 (1, 3)	0.5 (0.25, 1.75)	0.63 (0.25, 1.25)	1 (1, 4)	1.5 (1, 3)
18 h pD	2 (2, 3)	2 (2, 3)	0.5 (0.25, 1.75)	0.75 (0.25, 1.25)	1 (1, 4)	2 (1, 3)
24 h pD	2 (2, 2)	2 (1, 3)	0.63 (0.5, 1.25)	0.75 (0.25, 1.25)	1 (1, 2)	1 (1, 2)

Dogs were induced with propofol and anesthesia was maintained with isoflurane in 100% oxygen, to perform ovariohysterectomy. (n=12 for each treatment group). VAS, Visual Analogue Scale; CSUPS, Colorado State University Pain Scale; GCPS-SF, Glasgow Composite Pain Scale – Short Form. pD, post drug administration; pEx, post-extubation.

4.3.5 Plasma buprenorphine concentrations

No results were reported for plasma buprenorphine concentrations, due to persistent logistical issues at the CSU Pharmacology Laboratory in 2020, which prevented sample analysis. The samples remain stored until analysis can be performed.

4.4 Discussion

The results of the current study suggest that a single subcutaneous administration of either 0.12 mg/kg or 0.24 mg/kg high-concentration buprenorphine provides effective postoperative analgesia for up to 24 h in dogs undergoing neutering (Castration or OVH; preliminary study) or ovariohysterectomy (clinical study) with minimal side effects.

This study was set to evaluate the use of 2 different doses of a high-concentration formulation of buprenorphine (Simbadol ^{TM}) in dogs, in a similar manner as it is approved for use in cats. The doses selected represent the manufacturer recommended dose for cats (0.24 mg/kg) and the dose that is anecdotally reported to be effective in dogs (0.12 mg/kg). The study was conducted in 2 phases, a preliminary study (Phase I) with a smaller group of research beagles, undergoing either castration or OVH, where surgeries were performed by same board-certified surgeon. The goal of Phase I was to ensure that the proposed doses were acceptable for the procedures being performed (in terms of both analgesia and side-effects) prior to implementing them in the larger clinical study (Phase II). Even though there were not enough numbers to statistically compare between spays and neuters in Phase I, the fact that analgesia

was deemed appropriate for all animals in both treatment groups (without the need of rescue analgesia) and with minimal side effects supported the use of both doses in Phase II. Since analgesia was deemed appropriate for all dogs undergoing spay (a more invasive intervention compared to castration), it was considered that evaluating only dogs undergoing OVH in Phase II would be more meaningful. In Phase II, OVH were performed by senior veterinary students (under the direct supervision of a Community Practice Veterinarian), which added more variability in terms of tissue handling and longer duration of surgery and anesthesia.

Only mild anesthetic complications were noted during anesthesia in the clinical study (Phase II) and included the ones typically associated with anesthesia (hypotension, hypothermia, hypoventilation, bradycardia and regurgitation) (Brodbelt et al. 2015; Cummings & Wetmore 2016; Grubb et al. 2020).

Sedation scores were not different between treatment groups in both Phase I or Phase II. During Phase II, sedation scores were only different between treatments at 18 h post drug administration (undisturbed sedation). While dogs in treatment C-LD had a statistically significant higher sedation score than dogs in C-HD group at that timepoint ($p = 0.01$), the median sedation scores were very similar (0.5 vs. 0) and statistical significance occurred because 6/12 dogs in C-LD had mild sedation (score 1), while all dogs in C-HD group had no sedation (score 0). This difference is likely not clinically relevant as dogs with mild sedation (score 1) were less alert but still active. During Phase I, overall sedation was mostly mild, while in Phase II profound sedation was noted in the post-extubation period and up to 8 h post drug administration, which may be explained due to the differences in anesthesia time (< 1 h *vs.* 3 h, for Phase I and II, respectively). Since acepromazine was used as part of the premedication protocol, it is likely that it had some influence on the level of sedation observed in the initial

hours post-extubation (Lemke 2007), but since the same premedication protocol was used in both study phases it does not explain the variation in sedation levels between Phase I and II.

Physiological variables (heart rate, respiratory rate and rectal temperature) did not differ significantly between treatments in both Phase I and II. Heart rate, respiratory rate and rectal temperature values were lower than baseline for the majority of the post-operative period for both treatments in Phase I. In Phase II, only respiratory rate and rectal temperatures were lower than baseline. Mild decreases in heart rate have been described after buprenorphine and buprenorphine-acepromazine administration in dogs (Martinez et al. 1997; Watanabe et al. 2018) and may have contributed to the decrease in HR seen in Phase I. However, heart rate and respiratory rate remained within the normal range for dogs throughout the study and the observed reductions were not clinically relevant. Periods of mild hypothermia (35.6ºC – 36.7ºC) (Pachtinger 2013) were noted in the post-operative period for both treatments in Phase I. While rectal temperatures were slightly lower than baseline for both treatments in Phase II, hypothermia did not occur. Hypothermia is commonly observed after general anesthesia (Brodbelt et al. 2015; Cummings & Wetmore 2016; Grubb et al. 2020) and therefore the mild hypothermia observed during the post-operative period in Phase I is not unexpected. Buprenorphine likely contributed to the observed decreases in rectal temperatures, as opioids are known to affect the hypothalamic thermoregulatory system, and tend to cause a decrease in body temperature, particularly when associated with other CNS depressants drugs (Lamont & Matthews 2007). Acepromazine also interferes with the thermoregulatory control, and its use in association with buprenorphine likely also influenced the observed decrease in body temperature (Lemke 2007).

Pain scores were not significantly different between treatments in the current study, and

both study doses (0.12 and 0.24 mg/kg) seemed to provide adequate post-operative analgesia over a 24 h period. This is not unexpected, as similar results have been described in cats (Taylor et al. 2016; Leedham et al. 2019). Taylor et al. (2016) evaluated multiple doses of a high concentration buprenorphine in cats using a thermal threshold device, and showed that doses of 0.12 mg/kg or above (0.24 mg/kg) were efficacious in increasing the thermal threshold up to at least 24 h post drug administration. Leedham et al. (2019) evaluated similar doses of high concentration buprenorphine in a large clinical study in cats undergoing OVH, and no differences between treatments or requirement for rescue analgesia were identified. The lack of differences in regards to analgesic efficacy (as well as behavioral and physiological variables) between treatments in the current study, seem to support the analgesic ceiling effect described for partial μ-opioid receptor agonists, and previously shown in dogs undergoing OVH and treated with different doses of buprenorphine (Slingsby et al. 2011).

In summary, both doses (0.12 mg/kg and 0.24 mg/kg, SC, single dose) of a high-concentration buprenorphine were shown to provide effective and comparable analgesia up to 24 h in dogs undergoing neutering, without the need of rescue analgesia and with minimal side effects. These findings suggest that the use of high doses of buprenorphine at a high-concentration, as previously approved for use in cats, is an effective alternative for longer-lasting pain management in dogs.

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CONCLUSIONS

Pain is a distressing experience that is associated with actual or potential tissue damage with significant sensory, emotional, cognitive and social components. It is perceived as an unpleasant experience, and untreated pain has significant long-term detrimental effects. Pain is a very complex process and understanding its pathophysiology, as well as the pharmacology of the analgesic drugs, is essential to find the analgesic drugs and adjuncts that will most effectively and safely alleviate pain.

Opioids are one of the most important analgesic agents used for pain management in human and veterinary medicine. The μ opioid receptor agonists are considered the most effective to treat moderate to severe pain, however they are also associated with more side effects. Partial μ opioid agonists, such as buprenorphine, while not typically as effective as full μ receptor agonists, have shown to provide adequate analgesia for moderate pain with fewer side effects. Buprenorphine has been shown to be effective in the treatment of mild to moderate pain with minimal adverse effects in dogs, cats and several other species.

Traditional presentations of μ opioid receptor agonists or partial agonists usually require frequent dosing, which can lead to significant stress due to frequent animal handling, variability in the level of analgesia and can be labor intensive and time consuming for veterinary personal. There are also additional limitations and concerns in prescribing opioids for pain management of outpatients, due to the potential for abuse.

In recent years, new formulations of buprenorphine have been introduced and have the potential to provide longer lasting analgesia after a single subcutaneous injection. This dissertation presented two studies evaluating the analgesic properties and potential side effects

of two different long-lasting buprenorphine formulations in dogs. The first study aimed to evaluate a sustained-release buprenorphine formulation (buprenorphine HCL in a proprietary sustained release biodegradable liquid polymer matrix). The pharmacokinetics and selected behavioral, physiologic and antinociceptive effects of two doses of this sustained-release formulation were evaluated in dogs using a mechanical nociceptive threshold testing device. The second study aimed to evaluate high doses of a high-concentration buprenorphine formulation, which is FDA approved for use in cats. The analgesic efficacy, as well as selected behavioral and physiological variables, of two doses of this high-concentration formulation were evaluated in dogs undergoing neutering using three different pain scoring system (VAS - Visual Analogue Scale, CSUPS - Colorado State University Pain Scale, GCPS-SF – Glasgow Composite Pain Scale Short form).

The sustained-release buprenorphine formulation, at the two doses evaluated (0.06 mg/kg and 0.18 mg/kg), demonstrated comparable behavioral, physiological and antinociceptive effects (mechanical threshold) in dogs, with minimal side effects. The antinociceptive effects of the sustained release buprenorphine were observed up to 84 hours and were present even when plasma concentrations were below previously suggested therapeutic levels.

The high-concentration buprenorphine formulation, at the high doses evaluated (0.12 mg/kg and 0.24 mg/kg, SC, single dose) demonstrated effective and comparable analgesia up to 24 h in dogs undergoing neutering, without the need of rescue analgesia and with minimal side effects. These findings suggest that the use of high doses of buprenorphine at a high concentration (as approved for use in cats) is also an adequate alternative for pain management in dogs.

These findings suggest that both formulations have the potential to be effective and practical alternatives in providing safe longer-lasting analgesia in dogs.