THE EFFECT OF MORPHINE-LIDOCAINE-KETAMINE-DEXMEDETOMIDINE CO-INFUSION ON MINIMUM ALVEOLAR CONCENTRATION OF ISOFLURANE IN DOGS

Thesis

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By

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ABSTRACT

The purpose of this study was to determine the effects of an infusion of dexmedetomidine, a co-infusion of morphine-lidocaine-ketamine (MLK), and a co-infusion of dexmedetomidine-morphine-lidocaine-ketamine (alpha-MLK) on minimum alveolar concentration (MAC) of isoflurane in dogs. The MAC of an inhalant anesthetic required to prohibit purposeful movement is a measure of anesthetic potency (Eger et al 1965). Isoflurane is the most commonly used inhalant anesthetic in veterinary practice (Lozano et al 2009), but has potent vasodilatory effects and causes a dose-dependent decrease in mean arterial pressure in anesthetized dogs (Steffey and Howland 1977). Additional drugs are used during anesthesia to decrease the inhalant anesthetic requirement, a concept referred to as balanced anesthesia. Each of the drugs we infused has a different central nervous system receptor mechanism of action. Co-infusion of MLK, as well as the infusion of each drug separately, has been shown to reduce MAC in isoflurane-anesthetized dogs (Muir et al 2003). Dexmedetomidine, an alpha-2 agonist, has been shown to reduce isoflurane MAC in dogs (Pascoe et al 2006). Dexmedetomidine is a commonly used sedative with analgesic and muscle relaxant properties. The effect of a co-infusion of alpha-MLK on isoflurane anesthesia has not been critically evaluated in the dog. Our hypothesis was that infusion of alpha-MLK would significantly decrease isoflurane MAC (MAC-Iso) compared to dexmedetomidine or MLK infusions.
For this study, six dogs were anesthetized to determine MAC-Iso (baseline). Following MAC-Iso determination, each dog was anesthetized on three separate occasions with isoflurane, with a minimum of 7 days between anesthetic episodes. On each occasion, and in random order, dexmedetomidine, MLK, or alpha-MLK infusions were administered, and MAC was determined. Additionally, hemodynamic and metabolic parameters as well as bispectral index (BIS) were measured during each experiment. The investigator was blinded to the infusion given. Data were analyzed using ANOVA for repeated measures. Dunnett and Tukey posttests were performed to identify differences within and among groups, respectively, when differences were detected.

The MAC-Iso was 1.3 ± 0.15%. Dexmedetomidine, MLK, and alpha-MLK significantly lowered MAC-Iso by 30, 55, and 90%, respectively. Heart rate was significantly decreased from baseline in all infusion groups. Heart rate did not differ significantly between dexmedetomidine and alpha-MLK infusion groups. Mean arterial pressure for alpha-MLK increased significantly from baseline. Systemic vascular resistance was significantly increased for the dexmedetomidine and alpha-MLK infusion groups. The BIS values increased significantly compared to baseline for the MLK and alpha-MLK infusion groups. There was no significant difference for time to extubation and sternal recumbency among groups.

Infusions of dexmedetomidine, MLK, and alpha-MLK reduced MAC-Iso in dogs. Changes in cardiovascular parameters were within clinically acceptable ranges for
healthy dogs. BIS can be an effective monitoring tool for measuring the depth of anesthesia.
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<td>$\alpha_2$-agonist</td>
<td>alpha 2 adrenergic receptor agonist</td>
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<tr>
<td>alpha-MLK</td>
<td>dexmedetomidine-morphine-lidocaine-ketamine</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
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<td>BIS</td>
<td>bispectral index</td>
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<td>CaO$_2$</td>
<td>oxygen content of arterial blood</td>
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<td>CI</td>
<td>cardiac index</td>
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<td>CNS</td>
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<td>DO$_2$</td>
<td>oxygen delivery to tissue</td>
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<td>ECG</td>
<td>electrocardiogram</td>
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<td>EEG</td>
<td>electroencephalogram</td>
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<td>EMG</td>
<td>electromyography</td>
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<td>ETCO$_2$</td>
<td>end tidal carbon dioxide</td>
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LiDCO  lithium dilution cardiac output
LRS     lactated Ringer’s solution
MAC     minimum alveolar concentration
MAC-Iso minimum alveolar concentration of isoflurane
MAP     mean arterial pressure
MLK     morphine, lidocaine, ketamine
NMDA    N-methyl-D-aspartate
NSAIDs  non-steroidal anti-inflammatory drugs
SAP     systolic arterial pressure
SD      standard deviation
SpO₂    oxygen saturation of hemoglobin
SV      stroke volume
SVR     systemic vascular resistance
SVRI    systemic vascular resistance index
VO₂     oxygen consumption
Inhalant anesthetics are commonly used to produce general anesthesia in the clinical setting. Their main advantage in contrast to injectable agents for maintenance of general anesthesia is their ease of use, allowing depth of anesthesia to be altered rapidly. The anesthetic potency, also called the minimum alveolar concentration (MAC), which prevents gross purposeful movement in 50% of subjects in response to a noxious stimulus, was first determined for isoflurane in dogs and cats by Steffey and Howland (1977). Several groups of analgesic drugs, i.e. opioids, alpha$_2$ adrenergic receptor agonists ($\alpha_2$-agonists), local anesthetics and non-steroidal anti-inflammatory drugs (NSAIDs), have been shown to reduce MAC, thereby decreasing cardiorespiratory depression and providing a safer anesthetic experience (Himes et al 1979, Tranquilli et al 1984, Ilkiw et al 2002; Ko et al 2009). MAC reduction and multi-modal analgesia (i.e. co-administration of multiple analgesics, each targeting a distinct receptor mechanism) can effectively be provided by use of a continuous rate infusion (CRI) of morphine-lidocaine-ketamine (MLK) in dogs undergoing general anesthesia for procedures that result in moderate to severe pain (Muir et al 2003). The individual components of MLK
provide analgesia via a different mechanism of action and the reduction in MAC can lead to fewer undesired side effects of inhalant anesthesia.

Inhalant anesthetic agents cause dose-dependent cardiovascular depression (Pagel et al 1991). Depressed myocardial function with decreased contractility and reduced cardiac output (CO) is observed together with vasodilation and decreased peripheral vascular resistance. The combination of decreased CO and vasodilation can lead to hypotension and may ultimately reduce organ perfusion (Mazzaferro and Wagner 2001). Inhalant anesthetics also cause dose-dependent respiratory depression, so assisted ventilation may be required to maintain normocapnia when the concentration of inhalant required to maintain appropriate anesthetic depth is high (Boothe 2001). In a prospective study designed to determine the complications and mortality associated with small animal anesthesia, the most common complication in both dogs and cats was hypotension. Hypotension occurred in 7% (179 of 2,556) of anesthetized dogs, and 8% (58 of 683) of anesthetized cats (Gaynor et al 1999). In a retrospective report, hypotension represented 37.9% of complications, the second-highest behind hypoventilation at 63.4% (Redondo et al 2007). Hypotension under general anesthesia has been defined as a mean arterial pressure (MAP) less than 60 mmHg that corresponds to a systolic arterial pressure (SAP) less than 80 mmHg in dogs and cats (Waddell 2000). Arterial blood pressure is the main determinant of cerebral and coronary perfusion (Haskins 2007). When MAP is less than 60 mmHg, organs such as the kidney and brain cannot auto-regulate their blood supply, which leads to under-perfusion and the potential for short- or long-term central nervous system (CNS) or renal dysfunction (Gaynor et al 1999). Therefore, this common
anesthetic complication has the potential to lead to a decreased quality of life for an animal after the anesthetic episode. Also, persistent hypotension warrants administration of inotropic drugs, such as ephedrine and dopamine, to maintain blood pressure (Chen et al 2007). This can lead to increased cost and technical support staff needed during the anesthetic period. Therefore, a goal when using inhalant anesthetics should be to reduce the amount of gas needed to keep a patient anesthetized. The use of balanced anesthesia, combining opioid analgesic agents with decreased doses of inhaled anesthetics, can aid in maintaining adequate anesthetic depth without further depressing cardiovascular function (Mazzaferro et al 2001).

Analgesia and hypnosis are usually considered to be independent properties of anesthetic agents. Inhalant anesthetics, such as isoflurane, have minimal analgesic effects but do possess two qualities associated with general anesthesia: immobility in response to a noxious stimulus, and amnesia (Eger et al 2002). Noxious stimuli under conditions of inadequate analgesia, however, may cause arousal from the hypnotic state. Lowering the MAC of an inhalant, or MAC reduction, is a method used to determine whether a test substance supplements anesthetic-induced CNS depression, induces analgesia, or both (Muir et al 2003). Assessment of a surgical plane of anesthesia can be more accurately measured if the anesthetist has a more sensitive measurement of corticocerebral activation and arousal (March and Muir 2005). The bispectral index (BIS) is routinely used to monitor anesthetic depth during inhalant or injectable anesthesia in people and can be used to determine if the administration of a second drug produces analgesia (Telci
et al 2002). It is thought that drugs that lower the MAC while the BIS value increases during inhalant anesthesia are considered to have produced an analgesic effect.

Inhalant anesthetic-sparing drug protocols are widely used in human and veterinary medicine. Multimodal or balanced analgesia results from the administration of analgesic drugs in combination and acting at multiple sites that leads to an absence of pain by affecting different parts of the nociceptive pathway (Lamont 2008). This method allows for reduction of individual drug dosages, thereby decreasing the potential for adverse side effects. Pain is easy to overlook in animals and increased efforts to assess, prevent, and alleviate pain will improve the quality of care that animals receive. In 2003, the American Animal Hospital Association included pain as the fourth vital sign to be assessed during initial examination of veterinary patients (Hellyer et al 2007). A perioperative analgesic plan will impact the well-being of the patient and provides benefits beyond the immediate anesthetic period. It has been recognized in humans that adequate pain management results in quicker clinical recovery, shorter hospital visits, fewer readmissions, and improved quality of life (Phillips 2000). Pre-emptive analgesia has been shown to decrease subsequent analgesic requirements in the postoperative period (Campiglia et al 2010) and therefore the overall cost associated with their use.

A previous study (Muir et al 2003) showed that administration of additional drugs to reduce MAC did not cause adverse hemodynamic effects or significant changes in metabolic status. That study combined drugs known to be compatible and potentially synergistic, therefore lowering dosage requirement and minimizing known adverse effects. Our study was modeled on the Muir et al study with the addition of another drug,
dexmedetomidine, which may work synergistically when co-administered with opioids, lidocaine and ketamine. The $\alpha_2$-agonists produce significant synergy with opioids in mice (Fairbanks et al 2002). Such synergy may offer an opportunity to decrease opioid requirements, and therefore side effects, without sacrificing analgesia (Belgrade and Hall 2010). Both opioids and $\alpha_2$-agonists, but particularly $\alpha_2$-agonists, are frequently administered in conjunction with dissociative anesthetics (ketamine) to enhance sedation, analgesia and muscle relaxation (Ueyama et al 2008). There are several reports of medetomidine used in combination with ketamine (Moens and Fargetton 1990; Ko et al 2000). A study by Ko et al. (2001) evaluated premedication of dogs with medetomidine followed by induction with ketamine. Medetomidine induced lateral recumbency and facilitated a better anesthetic induction compared to isoflurane mask induction. Analgesia remained excellent and recovery quality from anesthesia was smooth. Ideally, the co-administration of drugs for MAC reduction should not have negative cardiorespiratory or metabolic effects. Therefore, care should be taken to ensure that, when selecting a multimodal drug combination that allows for the desired reduction of inhalation agents, unwanted side effects associated with each drug are minimized.

The goal of our study was to assess the inhalant anesthetic-sparing and hemodynamic effects of morphine, lidocaine, ketamine and dexmedetomidine co-administered as a CRI dogs. Each of these drugs works at a separate receptor and the use of a combination of drugs working at different receptors is referred to as multi-modal anesthesia (Figure 1.1). To our knowledge, the effect of dexmedetomidine on multi-modal analgesia when co-administered with MLK has not been evaluated.
Figure 1.1 – Multi-modal analgesia involving the µ opioid receptor, sodium channel, NMDA receptor, and α₂ receptor.
2.1 Inhalant Anesthesia in the Dog

2.1.1 Overview

The discovery of the first inhalant anesthetics - nitrous oxide, diethyl ether and chloroform - in the 1840s led to their use for anesthesia during surgery for nearly a century. The use of newer compounds in the 1920s – 1940s became popular because they led to a more rapid recovery from anesthesia. Modern inhalant anesthetics, which are partly halogenated with fluorine, have virtually replaced all previous agents due to safety reasons (Eger et al 2002). Isoflurane is widely used in veterinary practice today due to its relative ease of administration and rapid onset of action and recovery. Isoflurane is an economical choice compared to other inhalants. The adjunct use of analgesics, local anesthetics and sedatives with inhalant anesthesia has led to a more balanced approach to anesthesia. This balanced approach can provide the benefits of a smoother induction and recovery with decreased injectable drug requirements, while also allowing the amount of inhalant anesthetic requirements, and thus detrimental side
effects, to be decreased. This MAC-reduction potential has been the focus of numerous studies in the past two decades.

2.1.2 MAC

The minimum alveolar concentration, or MAC, of an inhalant anesthetic that produces immobility in 50% of animals in response to a supramaximal noxious stimulus has been determined in several species. MAC is typically used to compare inhalant anesthetic potency. A lower MAC value indicates a more potent anesthetic. MAC is determined in the laboratory setting in healthy animals in the absence of other drugs. The standard stimulus in dogs and other animals has been application of forceps to the base of the tail or dewclaw, but electrical stimulation applied beneath the oral mucosa has also been validated for MAC determination (Steffey and Mama 2007). In order to achieve a surgical plane of anesthesia, 1.2 to 1.5 times MAC is usually required (Ko 2007).

Several factors can affect MAC, either increasing or decreasing it. Factors that increase MAC include: increasing body temperature, drug-induced increase in CNS catecholamine levels, and hypernatremia. Factors that decrease MAC include: hypotension (<40 mm Hg), anemia (PCV < 13%), hypothermia, hyponatremia, extreme hypoxia (PaO₂ < 38 mmHg), increasing age (after 1 year of age), premedication and local anesthetics, pregnancy, and concurrent use of nitrous oxide. There are several factors that have no effect on MAC and these include: duration of anesthesia, gender, PaCO₂ between range of 14-95 mmHg, anesthetic metabolism, PaO₂ between 38-500 mmHg, arterial blood pressure > 40 mm Hg, thyroid gland dysfunction and potassium level (Ko 2007; Stoelting and Hiller 2006).
MAC is the highest in patients less than a year of age (Taylor and Lerman 1991) and decreases by nearly 50% in geriatric human patients (Gold et al 1993). A study on the effect of age in dogs showed that sevoflurane MAC for older dogs (8 to 10 years old) was significantly lower than for younger dogs (2 years old), 1.86 ± 0.29% vs. 2.25 ± 0.15%, respectively. (Yamashita et al 2009). MAC studies in dogs typically utilize dogs of a similar age group to decrease variability of MAC requirements.

2.1.3 Isoflurane

2.1.3.1 Pharmacology

Isoflurane is a halogenated methyl ether that exists as a clear, nonflammable liquid at room temperature. Its intermediate solubility in blood along with a high potency permits rapid onset and recovery from anesthesia. Less than 1% of isoflurane is metabolized by the cytochrome P-450 enzyme. Isoflurane is mainly exhaled unchanged by the lungs (Stoelting and Hillier 2006).

2.1.3.2 MAC

Several studies have shown the MAC-Iso in dogs to be between 1.31 to 1.50% (Eger et al 2002). In one study, the reported MAC-Iso was 1.28 ± 0.06% (Steffey et al 1977). Muir et al. (2003) reported a MAC-Iso value of 1.38 ± 0.08% in dogs.

2.2 Neural Mechanisms of Inhalant Anesthetics

Previously, inhalant anesthetics were thought to inhibit transmission or central interpretation by the nervous system (Eger 1974). Current thought is that inhalant anesthetics act primarily on the spinal cord to prevent movement and that a lesser
component of this immobility results from cerebral effects (Sonner et al 2003). Three studies describe the importance of the spinal cord as a site of anesthetic action. In a study by Rampil et al. (1993) the forebrains of rats anesthetized with isoflurane were removed. MAC was determined before and after the forebrain removal. There was no significant change in MAC after decerebration, suggesting that structures rostral to the transection were not important sites where isoflurane acts to inhibit movement. Another study looked at hypothermic spinal cords in rats transected at the thoracic level (Rampil 1994). MAC was determined in the forelimbs and hindlimbs before and after transection. MAC was unchanged below the level of the transection, suggesting that the movement response is generated from within the spinal cord without descending influences from the cerebrum in rats, and that isoflurane acts at the spinal cord to prevent movement. In another study (Antognini and Schwartz 1993) the brain and spinal cord in goats were perfused separately. Isoflurane MAC was 1.2% before bypassing the spinal cord, increasing to 2.9% during bypass when the brain was preferentially anesthetized, and decreasing to 1.3% after bypass. This indicates that the spinal cord is important in suppressing movement due to noxious stimuli under general anesthesia (Antognini and Carstens 2003).

Nevertheless, the brain does play an important role in the production of general anesthesia. Amnesia and unconsciousness are presumed to occur as a result of anesthetic action at this site. However, the concentration needed for these two states to occur is approximately 25-40% of that needed to suppress purposeful movement (Dwyer et al 1992). This likely indicates that areas of the brain associated with memory and
unconsciousness are more sensitive to inhalant anesthetics than are areas associated with purposeful movement (Antognini and Schwartz 1993).

2.3 Pain

Pain is a complex sensation that is defined as an unpleasant sensory and emotional perception associated with actual or potential tissue damage. It is a subjective experience for the individual that can be accompanied by fear, anxiety, and panic (Tranquilli et al 2007). Pain can further be classified as three distinct types: nociceptive, inflammatory and pathological. Nociceptive pain is a high-threshold pain only activated in the presence of intense stimuli and acts as a physiological protective mechanism. Inflammatory pain is caused by the activation of the immune system by tissue injury or infection. It is an adaptive and protective type of pain that causes increased sensitivity to the injured area, reducing the risk of further damage and allowing the body to heal. Pathological pain is a maladaptive form of pain resulting from abnormal function of the nervous system (Woolf 2010). Pain can be further quantified by whether it is acute or chronic. Acute pain is caused by trauma, surgery, or infection; it begins abruptly and is relatively short-lived. Chronic pain persists beyond the usual amount of time for an injury to heal, sometimes lasting months to years. It differs from acute pain in that it is seldom relieved by a single analgesic, serves no biological function, and is considered a disease itself instead of just a symptom of disease (Siddall and Cousins 2004).

2.3.1 Nociception

Nociception is the reception, conduction, and central processing of nerve impulses caused by stimulation of nociceptors. Nociception involves the physiologic processes of
transduction of a chemical, mechanical or thermal stimulus, transmission along afferent nerve fibers, modulation of the signal in the dorsal horn of the spinal cord, projection of the noxious stimulus to the brain, and perception of pain in the cerebral cortex (Figure 2.1). The mechanism of nociception, in which primary sensory neurons detect pain-producing stimuli, involves many molecules and signaling pathways. There are different nociceptors to detect different types of pain. The large-diameter, myelinated, rapidly-conducting Aβ primary sensor fibers usually detect non-harmful stimuli and therefore do not contribute to pain. Small- and medium-diameter cell bodies give rise to most of the nociceptors. These include the unmyelinated, slowly-conducting C fibers and the thinly-myelinated, more rapidly-conducting Aδ fibers (Figure 2.2). The two main ascending spinal nociceptive pathways are the spinocervicothalamic tract, which transmits superficial pain, and the spinoreticular tract, which primarily transmits deep pain and visceral sensations. Glutamate is the predominant excitatory neurotransmitter for nociceptors (Julius and Basbaum 2001).

2.3.2 Pain Modulation

The CNS can alter activity in the nociceptive pathways in the periphery, spinal cord, brain stem, and higher centers. Exposure to inflammatory mediators in the periphery, such as prostaglandins and leukotrienes, reduces the threshold of “silent nociceptors” that normally have a high threshold to noxious stimuli. Further stimulation leads to the release of peptides, such as substance P, histamine, bradykinin, serotonin, neurokinin A, and calcitonin gene-related peptide, from the peripheral nociceptive primary afferent fibers (Levine et al 1993). Results of this peptide release include
changed excitability of sensory and sympathetic nerve fibers, vasodilation, extravasation of plasma proteins, and inflammatory cell release of chemical mediators, contributing to the so-called ‘inflammatory soup’. This leads to a sensitization of high-threshold nociceptors and development of peripheral sensitization (Figure 2.3). Peripheral sensitization is associated with increased sensitivity to both mechanical and thermal stimuli at non-harmful (alldynia) and harmful (hyperalgesia) levels (Siddall et al 2004). Central sensitization is the extension of peripheral sensitization to the dorsal horn (Figure 2.4). This leads to spinal facilitation of pain, or windup, and this occurs with rapid, continuous firing of primary nociceptive afferents. This stimulates release of glutamate, substance P and brain-derived neurotrophic factor (BDNF) in the synaptic cleft of C fibers, which activates N-methyl-D-aspartate (NMDA) receptors on the postsynaptic membrane. Activation of NMDA receptors causes influx of calcium ions onto the postsynaptic neuron, ultimately resulting in the up-regulation of receptors. Substance P and BDNF bind with G-protein-coupled receptors and cause an intracellular signaling cascade that increases the membrane sensitivity to further stimulation (Tranquilli et al 2007). The final result of windup is an exaggerated response to subthreshold noxious stimuli following a primary injury and worsening of postoperative pain (Woolf and Thompson 1991). Although it is clear that central NMDA receptors are important in the induction and maintenance of neuronal hyperexcitability after a painful event, a growing body of evidence suggests peripheral sensitization and visceral pain may also be mediated by NMDA receptors (Petrenko et al 2003). Therefore, NMDA antagonists show potential as a therapy for preventing both central and peripheral hyperalgesia.
2.3.3 Response to Pain

Nociceptive stimulation leads to neuroendocrine and metabolic responses. These responses include increased sympathetic tone and catecholamine secretion, which leads to increased heart rate (HR), CO, arterial blood pressure, systemic vascular resistance (SVR), and myocardial oxygen consumption. These responses also include an increase in circulating free fatty acids, blood lactate, ketones and hyperglycemia. Anxiety can cause cortically-mediated increases in blood viscosity, clotting times, fibrinolysis, and platelet aggregation. These responses can be damaging if prolonged. Intense vasoconstriction and the release of antidiuretic hormone and aldosterone may cause renal compromise. In patients with severe trauma or postsurgical pain, these responses may be severe enough to cause shock. Attenuation of the stress response with adequate pain control and supportive therapy should improve patient outcome and promote healing (Wright and Woodson 1990; Tranquilli et al 2007).
Figure 2.1 – Sensory nervous system process of pain perception.
Figure 2.2 - The mechanism of nociception, in which primary sensory neurons detect pain-producing stimuli, involves many molecules and signaling pathways.
Figure 2.3 - Peripheral sensitization is caused by exposure to inflammatory mediators at the peripheral nerve terminal, leading to an increase in signaling molecules in the C fiber axon.
Figure 2.4 – Central sensitization occurs when peripheral sensitization extends to the dorsal horn neuron and leads to windup.
2.4 Drugs having analgesic properties that are used in the peri-anesthetic period

2.4.1 Dexmedetomidine

2.4.1.1 Pharmacology

Dexmedetomidine is an $\alpha_2$-agonist, a class of drugs used in veterinary medicine for their sedative, muscle relaxant and analgesic properties. This potent, selective, and specific $\alpha_2$-agonist exerts its effects by stimulating pre- and post-synaptic $\alpha_2$ adrenergic receptors in both the central and peripheral nervous system (Nguyen et al 1992). The synthetic $\alpha_2$-agonist, medetomidine, which is approved by the Food and Drug Administration for use as a sedative analgesic in dogs, contains two optical enantiomers, dexmedetomidine and levomedetomidine. Dexmedetomidine is the active D-isomer of medetomidine and is reported to provide more potent analgesia and have a shorter half-life compared with a similar dose of medetomidine (Kuusela et al 2000).

The $\alpha_2$-receptor is a transmembrane, G protein-coupled receptor (Khan et al 1999). Stimulation of the receptor results in the inhibition of adenyl cyclase, causing decreased formation of cAMP, an important regulator of cellular function (Aghajanian and VanderMaelen 1982). Stimulation ultimately results in inhibition of neurotransmitter (norepinephrine) release mediated by a decrease in calcium ion conductance, involving direct regulation of calcium influx by voltage-gated calcium ion channels (Lipscombe et al 1989).

2.4.1.2 Effect on MAC

Dexmedetomidine decreases MAC in dogs. A previous study in dogs has shown that epidural administration of dexmedetomidine resulted in dose-dependent decreases in
MAC-Iso and that effect decreased over time (Campagnol et al 2007). Another study evaluating the anesthetic and hemodynamic effects of dexmedetomidine during isoflurane anesthesia in dogs determined that a 20 µg kg\(^{-1}\) bolus of dexmedetomidine IV reduced MAC-Iso by 86% (Weitz et al 1991). The effect of three CRI's of dexmedetomidine (1, 2, or 3 µg kg\(^{-1}\) hour\(^{-1}\)) on overall tissue perfusion, isoflurane requirements, hemodynamics, and quality of recovery in canine surgical patients has been evaluated (Uilenreef et al 2008). Dexmedetomidine administered as a loading dose (5 µg kg\(^{-1}\)) and followed by a CRI as an adjunct to isoflurane anesthesia contributed to stable hemodynamic parameters and adequate tissue oxygen delivery. A dexmedetomidine CRI of 1 µg kg\(^{-1}\) hour\(^{-1}\) was judged to be preferable to the higher dose regimens based on evidence from cardiac rhythm, arterial blood gas measurements and recovery characteristics. This rate allowed end-tidal isoflurane (ET-Iso) to be lowered to 0.93 ± 0.08% during surgical stimulation, while a rate of 2 and 3 µg kg\(^{-1}\) hour\(^{-1}\) allowed ET-Iso to be lowered during surgery to 0.75 ± 0.09% and 0.81 ± 0.08%, respectively (Uilenreef et al 2008). Another study by Pascoe et al. (2006) demonstrated a significant decrease in MAC-Iso relative to control values for dexmedetomidine infusion at 0.5 µg kg\(^{-1}\) hour\(^{-1}\) and 3 µg kg\(^{-1}\) hour\(^{-1}\). The MAC was lowered from 1.51 ± 0.25% to 1.22 ± 0.27% and 0.62 ± 0.16%, respectively. This represents a MAC reduction of 18 ± 12% and 59 ± 7% compared to a saline control group for dexmedetomidine infusions of 0.5 µg kg\(^{-1}\) hour\(^{-1}\) and 3 µg kg\(^{-1}\) hour\(^{-1}\), respectively.

2.4.2 Morphine

2.4.2.1 Pharmacology
Morphine is a relatively specific mu opioid receptor agonist and has been used to provide analgesia during the perioperative period. Morphine can be synthetically produced, but is more easily derived from opium, which comes from the unripened seedpods of the poppy plant. Morphine is a phenanthrene alkaloid with a pH of 7.4, with the tertiary amine nitrogen being highly ionized, making the molecule water soluble. Morphine acts as an agonist at stereospecific opioid receptors at pre- and postsynaptic sites in the brainstem, spinal cord and in peripheral tissue. These opioid receptors are normally activated by three endogenous peptide opioid receptors: enkephalins, endorphins, and dynorphins. Morphine mimics the actions of these endogenous ligands by binding to opioid receptors and results in activation of antinociceptive systems (Stoelting and Hillier 2006).

The three main opioid receptors are classified as mu, delta, and kappa receptors (Atcheson and Lambert 1994). The main effect of opioid receptor activation is a decrease in neurotransmission. This occurs mostly by presynaptic inhibition of neurotransmitters, (e.g. acetylcholine, dopamine, norepinephrine, and substance P) and possibly by inhibition of postsynaptic activity. The biochemical events that occur after receptor activation include increased potassium conductance, leading to hyperpolarization, and calcium channel inactivation. This causes an immediate decrease in neurotransmitter release (Stoelting and Hillier 2006).

2.4.2.2 Effect on MAC

In a recent study, morphine alone or in combination with carprofen was shown to lower MAC-Iso in dogs. A 1 mg kg⁻¹ dose of IV morphine significantly decreased the
control MAC of 1.24 ± 0.15% to 0.81 ± 0.18% and when combined with 2.2 mg kg\(^{-1}\) carprofen the MAC-Iso was lowered to 0.68 ± 0.31% (Ko et al 2009). Another study evaluated morphine as a CRI of 3.3\(\mu\)g kg\(^{-1}\)min\(^{-1}\)and resulted in a decrease of the MAC-Iso from 1.38 ± 0.08% to 0.72 ± 0.12%, a 48% reduction (Muir et al 2003). Epidural morphine, at 0.1 mg kg\(^{-1}\), has been shown to decrease halothane MAC in dogs (Valverde et al 1989).

2.4.3 Lidocaine

2.4.3.1 Pharmacology

Lidocaine is an amide-type local anesthetic with anti-arrhythmic, anti-shock, sympatholytic, anesthetic-sparing and adjunctive analgesic effects that acts via blockade of sodium channels. Lidocaine is used subcutaneously and epidurally for peripheral nerve blocks and intravenously for the treatment of acute, chronic and neuropathic pain syndromes (Wu et al 2002). Local anesthetics are weak bases and have pK values slightly above physiologic pH. Local anesthetics with pKs nearest physiologic pH, such as lidocaine, have the most rapid onset of action. Factors such as tissue acidosis will result in poor quality of local anesthesia. Lidocaine is initially taken up in the lungs with distribution to highly-perfused tissues such as the brain, heart, and kidneys. Lipid solubility, tissue blood flow, and protein binding influence the absorption and distribution of lidocaine. The principal metabolic pathway of lidocaine is oxidative dealkylation in the liver (Stoelting and Hillier 2006).

Beneficial effects from lidocaine include analgesia when given intravenously as a low-dose infusion. Plasma concentrations of 1 to 2 \(\mu\)g mL\(^{-1}\) decreased the severity of
postoperative pain and decreased the requirements for opioids without leading to systemic toxicity (Cassuto et al. 1985). Cough suppression is obtained by plasma concentrations of > 2 µg mL⁻¹, which can be helpful during endotracheal intubation (Yukioka et al. 1985). In humans, local anesthetics modulate the inflammatory response and may be useful in the perioperative period to inhibit neutrophil accumulation and impair free radical and mediator release (Kiefer et al. 2003). Harmful side effects include CNS toxicity and cardiovascular system toxicity at higher plasma concentrations. One study reported signs of toxic doses of lidocaine (muscle tremors visualized) at approximately 11 mg kg⁻¹ (Lemo et al. 2007).

2.4.3.2 Effect on MAC

Himes et al. (1979) demonstrated lidocaine’s ability to lower the MAC of enflurane, a structural isomer of isoflurane, by 15 to 37% in dogs when arterial plasma lidocaine concentrations were 1 to 3.5 µg ml⁻¹. In a study evaluating the effect of lidocaine on MAC-Iso in dogs, lidocaine infusion was found to decrease MAC-Iso in a dose-dependent manner and did not induce clinically significant changes in HR or blood pressure. Two doses of lidocaine evaluated were 50 µg kg⁻¹ minute⁻¹ and 200 µg kg⁻¹ minute⁻¹. They resulted in an 18.7% and 43.3% reduction in MAC-Iso, respectively (Valverde et al. 2004). Muir (et al. 2003) demonstrated that a constant rate infusion of 50 µg kg⁻¹ minute⁻¹ of lidocaine resulted in a decrease of MAC-Iso to 0.97 ± 0.04%, a 29% reduction.

2.4.1 Ketamine

2.4.1.1 Pharmacology
Ketamine is a potent non-competitive NMDA antagonist that is capable of preventing CNS hypersensitivity and hyperalgesia (Stubhaug et al 1997). Ketamine has a low molecular weight, a \( \text{pK}_a \) near physiologic pH, and a high lipid solubility, which leads to a rapid onset of action (Anis et al 1983). There are two isomers of ketamine: S (+) ketamine and R (-) ketamine. The analgesic and anesthetic potency of S (+) ketamine is about threefold superior to R (-) ketamine (Adams 1998). Effects of this drug are mediated by NMDA, opioid and muscarinic receptors. Ketamine is a dissociative anesthetic that has a mechanism of action at the phencyclidine site of the NMDA receptor where it antagonizes glutamate, the excitatory neurotransmitter (Kohrs and Durieux 1998). Due to its high lipid solubility and low protein binding, ketamine is extensively distributed in the body. Ketamine is metabolized by hepatic microsomal enzymes. Ketamine is first demethylated to norketamine, which is then hydroxylated to dehydronorketamine (Kaka and Hayton 1980).

Ketamine causes increased intracranial pressure, bronchodilation and stimulation of the cardiovascular system. It is used for premedication, sedation, induction and maintenance of general anesthesia. Ketamine is an ideal anesthetic agent for trauma victims, patients with hypovolemic and septic shock, and patients with pulmonary disease (Van der Linden et al 1990). Subanesthetic doses of ketamine have analgesic effects, so ketamine is also recommended for postoperative analgesia. However, some studies show that ketamine is not always effective as an analgesic in treatment of chronic pain and that some side effects may limit its use in such patients (Radovanović and Pjević 2003).
2.4.1.2 Effect on MAC

Ketamine decreases the MAC of several inhalant anesthetics. In a study by Solano et al. (2006), six plasma ketamine concentrations were evaluated to determine the effect on MAC-Iso in dogs. The targeted plasma ketamine concentrations were 0.5, 1, 2, 5, 8, and 11 µg ml\(^{-1}\) and resulted in MAC-Iso reductions of approximately 11 to 40%, 27 to 44%, 25 to 85%, 44% to 78%, 70 to 92%, and 72 to 95%, respectively. The authors concluded that ketamine has a potential role in balanced anesthesia in dogs. Another study showed that ketamine lowered the sevoflurane MAC in dogs. Ketamine infusions of 50 and 100 µg kg\(^{-1}\) minute\(^{-1}\) reduced MAC by 40% and 44.7%, respectively (Wilson et al 2008). In another study, a constant rate infusion of 10 µg kg\(^{-1}\) minute\(^{-1}\) of ketamine caused a mean decrease in MAC-Iso from 1.38 ± 0.08% to 1.03 ± 0.07%, a 25% reduction (Muir et al 2003).

2.5 BIS

The BIS is a measure of consciousness in man and animals and is determined by measuring the bicoherence between various leads of an electroencephalogram (EEG) (Kissen 2000). BIS is inversely related to anesthetic depth. BIS values are unit-less and range from 0-100, with 100 indicating full consciousness and 0 indicating no brain activity (i.e. a flat line EEG) (Johansen and Sebel 2000). In humans, a BIS value below 60 has been accepted as being indicative of surgical anesthesia, while greater values suggest increasing levels of consciousness from deep sedation to total consciousness. The validation of BIS is more complete than other parameters and is better for determining
movement in dogs after painful stimuli (Muthuswamy and Sharma 1996). Morgaz et al. (2009) considered BIS a good parameter to establish the anesthetic depth in puppies anesthetized with sevoflurane. They also observed a correlation between BIS and both MAP and diastolic arterial pressure (DAP). Muir et al. (2003) found that BIS tended to increase as ET-Iso decreased in dogs, independent of the drug infusion administered. The mean BIS value for dogs at MAC-Iso was 61 ± 11. When the same dogs were given CRIs of morphine, lidocaine, ketamine, and MLK in addition to isoflurane the mean BIS values at equipotent anesthetic levels were 76 ± 12, 65 ± 13, 73 ± 6, and 67 ±10, respectively. These changes in BIS were significant compared to baseline for the morphine, lidocaine, and ketamine infusions. Green et al. (2003) studied the effect of medetomidine administration on BIS measurements in dogs anesthetized with isoflurane. They found that BIS significantly decreased with increasing MAC multiples of isoflurane over the range of 0.8 to 2.0 MAC. During isoflurane control (saline administration) anesthesia, mean BIS measurements at 0.8, 1.0, 1.5, and 2.0 MAC were 65 ± 8, 60± 7, 52 ±3, and 31 ± 28, respectively. During isoflurane-medetomidine anesthesia, mean BIS measurements at 0.8, 1.0, 1.5, and 2.0 MAC were 77 ± 4, 53 ± 7, 31 ±24, and 9 ± 20, respectively. Greene et al. concluded that BIS monitoring in dogs anesthetized with isoflurane has a predictive value in regard to the amount of CNS depression. Greene et al. (2002) also evaluated the relationship of BIS to multiples of sevoflurane MAC in dogs. They obtained mean BIS values at 0.8, 1.0, 1.5, and 2.0 MAC that were 77 ± 3, 73 ± 5, 57 ± 7, and 53 ± 7, respectively, for patch electrodes. They concluded BIS significantly decreased with increasing sevoflurane MAC multiples. A study evaluating
the effect of morphine on the BIS during isoflurane anesthesia in dogs concluded that intravenous (IV) administration of morphine (0.05 mg kg\(^{-1}\)) did not cause clinically significant changes in the BIS of unstimulated dogs at an ET-iso of 1.81%. Baseline BIS for the morphine and saline treatment groups were 63 ± 10 and 58 ± 9, respectively. The BIS in the morphine group was 4.8% lower at 20, 75, 90 and 105 minutes compared to the saline group. There were no differences in BIS between baseline and any other measurements for the two groups (Henao-Guerrero et al 2009).

2.6 Objectives

The goal of this study was to evaluate the effect of dexmedetomidine combined with morphine, lidocaine, and ketamine (alpha-MLK) on MAC-Iso in dogs. The BIS value was used as a measure of the level of consciousness or depth of anesthesia. The specific aims of the study were:

1) To determine MAC-Iso reduction with MLK infusion, alpha-MLK infusion, and dexmedetomidine infusion.

2) To compare the cardiovascular effects of isoflurane alone, isoflurane with MLK, isoflurane with dexmedetomidine, and isoflurane with alpha-MLK.

3) To evaluate the level of consciousness using BIS during isoflurane anesthesia and the administration of dexmedetomidine, MLK, and alpha-MLK in conjunction with isoflurane.
4) To evaluate recovery time and quality during isoflurane anesthesia and the administration of dexmedetomidine, MLK, and alpha-MLK in conjunction with isoflurane.

2.7 Hypothesis

Our hypotheses were:

1) Dexmedetomidine-morphine-lidocaine-ketamine infusion would reduce MAC-Iso more than dexmedetomidine or MLK infusion. A MAC-Iso reduction of at least 20-25% would be considered clinically significant for any treatment group, and we anticipated that this would occur in all groups.

2) Cardiovascular changes, such as reduced CO, HR and increased arterial blood pressure, would occur with the dexmedetomidine and alpha-MLK, but that these changes would be within clinically acceptable limits.
CHAPTER 3

MATERIALS AND METHODS

3.1 Dogs

The subjects of this study were six conditioned, mixed-breed, intact dogs (three males and three females) determined to be of normal health based on physical exam and thoracic auscultation, complete blood count, serum biochemistry, electrocardiogram (ECG) and fecal analysis for parasites. The dogs ranged in age from 10 to 12 months (11 ± 0.89) and weighed 6.5 to 21.2 kg (14.4 ± 5.8).

The study was approved by the Animal Care and Use Committee of The Ohio State University and was performed according to the Ethical Guidelines of the International Association for the Study of Pain.

3.2 Study Design

The study was performed in a prospective, randomized and blinded design. The MAC of six isoflurane anesthetized dogs administered MLK, dexmedetomidine or alpha-MLK was determined. The determination of MAC was performed according to a
standard bracketing technique. A power test was performed, which demonstrated that six dogs would be adequate to detect a difference in MAC-lowering effect if $\alpha = 0.05$.

Pilot studies were performed in two conscious dogs to assess alpha-MLK’s potential anesthetic effect. The dogs were given a 5 ml kg$^{-1}$ bolus (containing 3.3 µg kg$^{-1}$ morphine + 50 µg kg$^{-1}$ lidocaine + 10 µg kg$^{-1}$ ketamine + 0.5 µg kg$^{-1}$ dexmedetomidine) over 20 minutes, and then a continuous infusion of alpha-MLK was administered at 5 ml kg$^{-1}$ hr$^{-1}$ for 3 hours. A baseline sedation score was recorded, then the sedation score was repeated every 30 minutes until the infusion was discontinued. The sedation score assessed vocalization, posture, appearance, interactive behaviors, ability to restrain, and noise response. The dogs were given a numerical score from -3 to 3, according to preset criteria (Appendix). This sedation scoring system was adapted from a previous study evaluating the effects of hydromorphone and oxymorphone, with or without acepromazine, on preanesthetic sedation in dogs (Smith et al 2001). Adverse effects, such as vomiting, were recorded. Heart rate, respiratory rate and time to walking after infusion ended were also recorded. The dogs became laterally recumbent within 30 minutes of the bolus and remained quiet or softly whined, but quieted with a soothing touch. The dogs vomited multiple times during the bolus of alpha-MLK. The dogs appeared normal except for one dog having protruding nictitating membranes for about 30 minutes. The dogs remained conscious, had a brisk response and would lift their head when spoken to or touched and would lie on the floor with minimal restraint. The dogs walked within 20 minutes of ending the infusion. The administration of alpha-MLK in the absence of inhalant anesthetic did not cause general anesthesia in the pilot studies.
3.3 Instrumentation

The dogs were held off food, but not water, for approximately 12 hours prior to each experiment. A 20 gauge IV catheter\textsuperscript{a} for the administration of drugs and fluids was inserted into a cephalic vein 15 to 20 minutes after application of transdermal lidocaine cream\textsuperscript{b} to the shaved skin. Anesthesia was induced with propofol\textsuperscript{c} (6 mg kg\textsuperscript{-1} IV) and endotracheal intubation with auffed endotracheal tube\textsuperscript{d} was secured in place. A gas-sampling line was placed between the tube and the Y-piece. All dogs were positioned in left lateral recumbency.

Following induction of anesthesia, each dog was ventilated mechanically using an ascending-bellows, volume-cycled, pressure-regulated ventilator\textsuperscript{e} connected to a standard circle rebreathing anesthetic circuit. A tidal volume of 10 to 15 mL kg\textsuperscript{-1} was delivered at a rate of 6 to 12 breaths per minute to maintain an end tidal carbon dioxide (ETCO\textsubscript{2}) value between 35 and 45 mmHg. Isoflurane\textsuperscript{f} was delivered in 100% oxygen by an out-of-circle precision vaporizer\textsuperscript{g} set for a targeted ET-Iso concentration of 1.6% and allowed to equilibrate at this setting for at least 60 minutes following induction of anesthesia. The oxygen flow rate was set at 3 L min\textsuperscript{-1} until the palpebral reflex disappeared, then reduced to 1 L min\textsuperscript{-1} for the remainder of the study period. A temperature probe was inserted into the esophagus past the thoracic inlet to determine core body temperature. Body temperature was maintained between 37.5 and 38 °C by the use of a warm water
circulating blanket and a warm forced-air blanket. Inspired and expired gas samples were continuously monitored by use of an infrared gas analyzer. The anesthetic analyzer was calibrated to a known concentration of gases according to manufacturer configuration recommendations (Campagnol et al. 2007). A lead II ECG and pulse oximeter monitor were used to continuously monitor HR and rhythm and oxygen saturation (SpO₂). A direct arterial pressure monitor was used to measure systolic, diastolic, and mean arterial blood pressures via an arterial line inserted in the dorsal pedal artery.

Electroencephalogram activity was obtained using a 2-channel referential montage from platinum subdermal needle electrodes arranged in a bifrontal configuration with the reference electrode positioned on the midline of the head, a second electrode placed lateral to the first one and a third electrode placed next to the lateral canthus of the eye. The ground electrode was positioned on the neck at the transverse process level of the atlanto-occipital bone (Figure 3.1). The EEG activity and the BIS values were continuously obtained and recorded by use of a specifically designed BIS monitor with the high-frequency filter set at 70 Hz and the low-frequency filter set at 2 Hz. The BIS number was automatically calculated and digitally displayed every 5 seconds and represented the EEG activity during the past minute (Figure 3.2).

Cardiac output was obtained via the lithium dilution CO (LiDCO) method. A LiDCO CO computer was used to measure CO values (Figure 3.3). A previous study (Mason et al. 2001) describes the technique and set-up in detail and was followed for our protocol. Briefly, the sensor for the lithium chloride (LiCl) measurements was attached to the
side port of a 3-way stopcock that was connected to a seven-inch extension set attached to a peripheral arterial catheter. The sensor was prepared as described in the LiDCOplus operation manual. The inlet port for the sensor was attached to the arterial catheter and the outlet port was attached via tubing to a disposable blood collection bag. The tubing between the sensor and collection bag passed through a flow regulator pump that, when turned on, withdrew blood from the peripheral artery and forced the arterial blood across the sensor at a constant rate of 4 mL hr\(^{-1}\) into the collection bag (Figure 3.4). To measure CO by LiDCO technique, the input of several values including the sensor constant, injection dose of LiCl, SpO\(_2\), hemoglobin concentration and serum sodium concentration was required. Sodium and hemoglobin values used were obtained from the arterial blood gas sample taken at MAC (See MAC determination below).

The LiCl dose was calculated as follows:

\[
\frac{\text{body weight (kg) x 0.002 mmol kg}^{-1}}{\text{concentration of LiCl}}
\]

The concentration of LiCl used was 0.15 mmol ml\(^{-1}\). Prior to injection of LiCl, the IV fluids were stopped and the line was flushed with saline. The determined dose of LiCl was drawn up and attached to the IV line by a 3-way stopcock and 20 mL of saline was used to flush the LiCl into the patient. The ventilator was switched off during LiDCO measurement. The PulseCO\(^\text{®}\) was calibrated to LiDCO after the instrumentation process was completed.

The instrumentation process took approximately 30 minutes following induction of anesthesia (Figure 3.5).
Figure 3.1 – Bifrontal configuration of the EEG leads for BIS monitoring in the dog.

Figure 3.2 – BIS monitor taking EEG measurements on a dog under isoflurane anesthesia.
Figure 3.3 – The LiDCOplus cardiac output monitoring showing the PulseCO values that correlate to the direct blood pressure monitor after calibration to LiDCO values.

Figure 3.4 – The set-up for the direct arterial catheter with extension set connected to a 3-way stopcock attached to the LiDCO sensor and the flow regulator pump.
Figure 3.5 – Final instrumentation setup in the laboratory.
3.4 Study Design

The dogs were anesthetized on 4 separate occasions that involved one control and three different treatments. The MAC-Iso for each dog was determined during the first week of the study. Following initial MAC-Iso determination, the investigator was blinded to the particular infusion that had been assigned using a random order table prior to the beginning of the study. Anesthetic events for each dog were separated by a minimum of 7 days.

3.4.1 Isoflurane MAC Determination

The ET-Iso was set to 1.6% and allowed to equilibrate for 60 minutes prior to the first stimulation. For MAC determination a supramaximal electrical stimulus was delivered to the buccal mucosa of each dog. Two 24-gauge, 10-mm platinum subdermal needle electrodes were placed into the maxillary buccal oral mucosa caudal and dorsal to the incisors and approximately 1 cm apart. The electrodes were connected to the electrical stimulator that delivered a predetermined stimulus of 50 volts, 5 hertz, and 10 milliseconds duration for a period of 1 minute. The stimulus was discontinued if the dog showed gross purposeful movement before the end of the 1 minute stimulation. Gross purposeful movement was defined as lifting of the head and/or repeated movement of one or more limbs. The following were not considered to be gross purposeful movements (i.e. were recorded as a negative response): slight paw movements, back arching, blinking, nystagmus, chewing, or swallowing. If no response occurred, the ET-Iso concentration was decreased by 20% and allowed to equilibrate for 15 minutes. The
process was repeated until the dog responded with gross purposeful movement (positive response). Following a positive response the ET-Iso concentration was increased by 10% and allowed to equilibrate for 15 minutes before retesting. MAC was determined to be the average of the lowest ET-Iso concentration that prevented a response, and the ET-Iso at which a positive response occurred. MAC was calculated as the mean of multiple MAC determinations for each dog. Data collection included respiratory rate, SpO$_2$, ETCO$_2$, end-tidal oxygen concentration, ET-Iso, HR, core body temperature, SAP, MAP, and DAP and were recorded prior to stimulation for MAC determination. PulseCO values recorded included oxygen delivery to tissue (DO$_2$), CO, SVR, systemic vascular resistance index (SVRI), and stroke volume (SV). Eight total BIS values were recorded starting 1 minute before and 1 minute after buccal mucosal stimulation. The dog was allowed to equilibrate at MAC for at least 20 minutes before an arterial blood sample was obtained for immediate analysis. A LiDCO value was obtained, using recent values for sodium and hemoglobin from the arterial blood gas sample. Following these determinations, the vaporizer setting was increased to achieve an ET-Iso of 1.5 x MAC and allowed to equilibrate for 20 minutes, then data and LiDCO values were obtained (See Figure 3.6).
Induce Anesthesia and set vaporizer for ET-Iso at 1.6%

Instrumentation

Wait 60 minutes from start of inhalant

Pre-Stimulus Data

Stimulate

Post-Stimulus Data

Repeat until positive response

Negative Response
Decrease Inhalant by 20%
Equilibrate 15 minutes

Positive Response
Increase Inhalant by 10%
Equilibrate 15 minutes

MAC
Average ET-Iso of last positive and last negative response

Wait 20 minutes at MAC, then collect arterial blood gas sample, LiDCO measurements and data.

Key
Data = Heart rate; Respiratory rate; Oxygen saturation; Arterial blood pressure; End tidal oxygen concentration; End tidal carbon dioxide; End tidal isoflurane; Oxygen delivery to tissue; Cardiac output; Cardiac index; Systemic vascular resistance; Systemic vascular resistance index; Stroke volume; Bispectral index

Take ET-Iso to 1.5 x MAC and wait 20 minutes
Collect data and LidCO values

Figure 3.6: Timeline for Isoflurane MAC determination
Patient Preparation

Induce Anesthesia

ET-Iso set at MAC determined previously
Start treatment infusion

Instrumentation

Wait 120 minutes from start of infusion

Pre-Stimulus Data
Stimulate
Post-Stimulus Data

Repeat until positive response

Negative Response
Decrease Inhalant by 20%
Equilibrate 15 minutes

Positive Response
Increase Inhalant by 10%
Equilibrate 15 minutes

new MAC
Average ET-Iso of last positive and last negative response

Wait 20 minutes at new MAC
Collect arterial blood gas sample
Collect data

Key: Data = Heart rate; Respiratory rate; Oxygen saturation; Arterial blood pressure; End tidal oxygen concentration; End tidal carbon dioxide; End tidal isoflurane; Oxygen delivery to tissue; Cardiac output; Cardiac index; Systemic vascular resistance; Systemic vascular resistance index; Stroke volume; Bispectral index

Figure 3.7: Timeline for MAC determination for the infusion groups
3.4.2 Treatments

3.4.2.1 Infusion Preparation

Each infusion was prepared in a 500mL bag of lactated Ringer’s solution° (LRS) and labeled as follows: Infusion 1, Infusion 2, or Infusion 3. Infusion 1 (dexmedetomidine) contained 0.0001 mg of dexmedetomidine× mL⁻¹ delivered at 5 mL kg⁻¹ h⁻¹. The dexmedetomidine solution was prepared by adding 0.05mg (0.10 mL) of 0.05% dexmedetomidine into 500 mL of LRS. Infusion 2 (MLK) was delivered at 5 mL kg⁻¹ h⁻¹ and contained 0.04 mg of morphine sulfateν mL⁻¹, 0.6 mg of lidocaine hydrochlorideω mL⁻¹, and 0.12 mg of ketamine hydrochlorideaa mL⁻¹. The MLK solution was prepared by adding 24mg (1.6 mL) of morphine sulfate, 300mg (15 mL) of 2% lidocaine hydrochloride, and 60mg (0.6 mL) of ketamine hydrochloride into 500 mL LRS. Infusion 3 (alpha-MLK) was prepared by combining the drug volumes used in Infusion 1 and Infusion 2 and adding them to a 500 mL bag LRS. An equivalent volume of fluid was removed from the bag of fluids prior to addition of the drugs.

3.4.2.2 Infusion Administration

A volumetric infusion pumpbb was used to ensure accurate doses of infusions were administered. The pump was calibrated before the experiments began. No loading doses were given. The dogs were allowed to equilibrate at their predetermined individual MAC-Iso for 2 hours before stimulations were started. Prior studies have shown that each of the drugs used in our study has a MAC lowering potential (Muir et al 2003; Pascoe et al 2006), therefore, it was reasonable to expect no gross purposeful movement at our starting point for stimulation.
3.4.3 Data Collection

The MAC for each treatment was determined using the same bracketing technique as the MAC-Iso method (Figure 3.7). A total of 8 BIS values were obtained 1 minute before and 1 minute after stimulation for MAC determination. LiDCO values including CO and cardiac index (CI), respiratory rate, SpO₂, ETCO₂, end-tidal oxygen concentration, ET-Iso, HR, core body temperature, PulseCO values (DO₂, SVR, SVRI, SV) and SAP, MAP, DAP were recorded after MAC determination after the dog was allowed to equilibrate for 20 minutes at MAC.

3.5 Recovery

Following the last LiDCO determination, mechanical ventilation and delivery of the inhalant and infusion was stopped, and end time for anesthesia was recorded. The BIS monitoring electrodes and gas analyzer were left in place for continued monitoring during recovery, but the rest of the instrumentation was removed. The dog was allowed to breathe spontaneously and continued to breathe oxygen via the breathing system. When the dog began to swallow, four BIS readings were obtained prior to extubation. Following extubation, an additional four BIS reading were recorded, after which the electrodes were removed. The ET-Iso concentration was also recorded at extubation. The time to sternal position was recorded.

At the conclusion of each experiment, the dogs were closely monitored for any adverse reactions in recovery. If any of the subjects experienced dysphoria in the
recovery period, a rescue sedation protocol of acepromazine (0.02 mg kg\(^{-1}\) IV) was given. The dogs were returned to their cages when they were able to walk.

3.6 Statistical Analysis

A power analysis was determined by use of a computer program based on a power > 80% to detect a difference between means with a significance level (alpha) of 0.05, based on a predicted decrease in MAC-Iso of 20%.

Data were reported as mean ± standard deviation (SD), and the differences were considered significant at P < 0.05. The effect of MLK, dexmedetomidine and the alpha-MLK on MAC-Iso, cardiorespiratory variables, and metabolic parameters were determined by analysis of variance (ANOVA) for repeated measures. Recovery data were analyzed using ANOVA. The Dunnett and Tukey posttests were performed to identify differences within and among groups, respectively, when differences were detected. All data were analyzed using a computer based statistical program.
CHAPTER 4

RESULTS

4.1 MAC

All the mean ± SD recorded values are listed in Tables 4.1 and 4.2. The MAC-Iso was determined to be 1.3 ± 0.15%. Dogs that had gross purposeful movement during the MAC-Iso determination did so within the first minute of electrical stimulation. The most common positive response to the electrical stimulus was lifting of the head or leg movement. In comparison to baseline, the infusions of dexmedetomidine, MLK, and alpha-MLK significantly reduced MAC-Iso to 0.91 ± 0.2, 0.58 ± 0.2 and 0.13 ± 0.1%, respectively. MAC-Iso reduction achieved by dexmedetomidine, MLK and alpha-MLK infusions was 30 ± 7%, 55 ± 12% and 91 ± 10%, respectively. The MACs determined during the three treatment groups were also significantly different from each other (Figure 4.1).

4.2 Cardiovascular variables

The MAP showed an increasing trend with the three treatments, but only the alpha-MLK infusion group was significantly increased in comparison to MAC-Iso. The MAP taken at 1.5 x MAC-Iso was significantly decreased compared to the other treatment groups, but not from baseline MAP. The MAP at MAC-Iso was 64 ±7 mmHg
and the MAP for the alpha-MLK group was 76 ± 8 mmHg (Figure 4.2). The mean HR decreased significantly with all three treatments in comparison to baseline and there was a significant difference between all treatment groups except when comparing the dexmedetomidine infusion to the alpha-MLK infusion. The mean HR at MAC-Iso was 99 ± 11 beats per minute. The mean HR for the dexmedetomidine, MLK, and alpha-MLK groups were 53 ± 10, 78 ± 17, and 49 ± 6 beats per minute, respectively (Figure 4.3). There was a significant decrease in CO compared to baseline for the dexmedetomidine infusion group, but there was no difference from baseline for MLK or alpha-MLK infusion groups. The MAC-Iso CO was 2.3 ± 0.7 L min⁻¹ and the CO values for the dexmedetomidine, MLK, and alpha-MLK treatment groups were 1.3 ± 0.5, 1.8 ± 0.4, and 1.5 ± 0.9 L min⁻¹, respectively (Figure 4.4). Cardiac index for the treatment groups did not change significantly from baseline values. However, CI in the 1.5 x MAC-Iso group was significantly increased from the dexmedetomidine and alpha-MLK groups (Figure 4.5). Stroke volume had no significant differences between groups (Figure 4.6). Systemic vascular resistance was significantly increased in the dexmedetomidine and alpha-MLK groups compared to baseline. Also, the 1.5 x MAC-Iso SVR was significantly decreased compared to dexmedetomidine and alpha-MLK (Figure 4.7). Oxygen delivery to tissue was significantly lower in the dexmedetomidine group compared to baseline, 269 ± 89 ml O₂ min⁻¹ and 665 ± 464 ml O₂ min⁻¹, respectively.

4.3 BIS values

BIS values were significantly increased for MLK and alpha-MLK infusions compared to baseline. This BIS value at 1.5 x MAC-Iso was decreased compared to all
infusion groups. The BIS value was lower in the dexmedetomidine infusion compared to MLK and alpha-MLK (Figure 4.8). The BIS values at MAC-Iso, dexmedetomidine, MLK and alpha-MLK were 74 ± 7.3, 71 ± 7.6, 80 ± 5.5, and 82 ± 6, respectively.

4.4 Additional findings

The SpO₂ remained above 97% in all dogs and core body temperature remained between 37.5-38.0°C during MAC determination readings. The pH and blood gas values did not show any clinically significant changes.

4.5 Recovery data

There was no significant difference in the time to extubation and time to sternal recumbency for the treatment groups in comparison to baseline. During recovery for the isoflurane baseline, four dogs became dysphoric and received acepromazine and one dog regurgitated in the recovery period. In the dexmedetomidine infusion group, two dogs vomited in recovery. Another dog regurgitated in the recovery period in the MLK infusion group. No adverse events occurred in any of the dogs in the alpha-MLK recovery period.
Figure 4.1: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on MAC of isoflurane in dogs. * indicates significantly different from isoflurane MAC; ° indicates significantly different from other infusion groups.
Figure 4.2: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on mean arterial pressure in dogs. * indicates significantly different from isoflurane MAC; ° indicates significantly different from other infusion groups.
Figure 4.3: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on heart rate in dogs. * indicates significantly different from isoflurane MAC; a indicates significantly different from all infusion groups; b indicates significantly different from MLK infusion group; c indicates significantly different from dexmedetomidine and alpha-MLK infusion groups.
Figure 4.4: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on cardiac output in dogs. * indicates significantly different from isoflurane MAC; ° indicates significantly different from infusion groups.
Figure 4.5: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on cardiac index in dogs. ° indicates significantly different from dexmedetomidine and alpha-MLK infusion groups.
Figure 4.6: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on stroke volume in dogs.
Figure 4.7: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on systemic vascular resistance in dogs. * indicates significantly different from isoflurane MAC; a indicates significantly different from dexmedetomidine and alpha-MLK groups.
Figure 4.8: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on BIS values in dogs. * indicates significantly different from isoflurane MAC; º indicates significantly different from infusion groups; a indicates significantly different from MLK and alpha-MLK; b indicates significantly different from dexmedetomidine group.
Figure 4.9: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on time to extubation from the end of isoflurane anesthesia in dogs.
Figure 4.10: The effect of infusions of dexmedetomidine, MLK, and alpha-MLK on time to sternal recumbency in dogs.
Table 4.1 – Variables recorded in dogs (n = 6) anesthetized with isoflurane and given infusions of dexmedetomidine, morphine-lidocaine-ketamine, and dexmedetomidine-morphine-lidocaine-ketamine on four separate occasions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1.5xMAC</th>
<th>MAC-Iso</th>
<th>Dexmed</th>
<th>MLK</th>
<th>Alpha-MLK</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC (%)</td>
<td>1.9 ± 0.2†</td>
<td>1.3 ± 0.2</td>
<td>0.91 ± 0.2†</td>
<td>0.58 ± 0.2†</td>
<td>0.13 ± 0.1†</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>78 ± 16†</td>
<td>91 ± 10</td>
<td>115 ± 8*</td>
<td>119 ± 14*</td>
<td>117 ± 11*</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>54 ± 6†</td>
<td>64 ± 7</td>
<td>70 ± 7</td>
<td>73 ± 4</td>
<td>76 ± 8*</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>44 ± 4†</td>
<td>51 ± 6</td>
<td>56 ± 6</td>
<td>57 ± 5</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>97 ± 4†</td>
<td>99 ± 11</td>
<td>53 ± 10*</td>
<td>78 ± 17*</td>
<td>49 ± 6*</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>10 ± 2</td>
<td>9 ± 2</td>
<td>15 ± 5</td>
<td>11 ± 2</td>
<td>10 ± 2*</td>
</tr>
<tr>
<td>ETCO2 (mm Hg)</td>
<td>40 ± 2</td>
<td>39 ± 4</td>
<td>35 ± 3</td>
<td>36 ± 5</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>SpO2 (%)</td>
<td>99 ± 0.98</td>
<td>99 ± 0.98</td>
<td>99 ± 0.75</td>
<td>99 ± 1</td>
<td>99± 1</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>2.7 ± 1.0†</td>
<td>2.3 ± 0.7</td>
<td>1.3 ± 0.48*</td>
<td>1.8 ± 0.42</td>
<td>1.5 ± 0.86</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>4.86 ± 2.3cb</td>
<td>3.93 ± 0.9</td>
<td>2.18 ± 0.3</td>
<td>3.06 ± 0.6</td>
<td>2.39 ± 1.0</td>
</tr>
<tr>
<td>SVR (dyn/sec/cm⁻⁵)</td>
<td>2117±1002cb</td>
<td>1983±1023</td>
<td>4367±1494*</td>
<td>3200±692</td>
<td>4642±2012*</td>
</tr>
<tr>
<td>SV (mL/beat)</td>
<td>23.3 ± 11</td>
<td>31.3 ±21</td>
<td>25.8 ±10</td>
<td>23.8 ±6.7</td>
<td>28 ±12</td>
</tr>
<tr>
<td>DO₂ (mL O₂/min)</td>
<td>465 ± 268</td>
<td>665 ± 464</td>
<td>269 ± 89*</td>
<td>344 ± 37</td>
<td>300 ± 132</td>
</tr>
<tr>
<td>BIS</td>
<td>63 ± 8*†</td>
<td>74 ±7</td>
<td>71 ± 8†</td>
<td>80 ±6*</td>
<td>82 ± 6*</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.7 ±0.5</td>
<td>37.6 ±0.1</td>
<td>37.6 ±0.1</td>
<td>37.7 ±0.1</td>
<td>37.7 ±0.1</td>
</tr>
<tr>
<td>Rec ET-iso (%)</td>
<td>n/a</td>
<td>0.53 ± 0.15</td>
<td>0.40 ± 0.09b</td>
<td>0.28 ± 0.2</td>
<td>0.03± 0.1*</td>
</tr>
<tr>
<td>Recovery BIS</td>
<td>n/a</td>
<td>82 ± 8</td>
<td>85 ± 8ab</td>
<td>89 ± 5*</td>
<td>91 ± 6*</td>
</tr>
<tr>
<td>Time extub (min)</td>
<td>n/a</td>
<td>13.6 ± 8.8</td>
<td>15 ±8.6</td>
<td>7.6 ± 7</td>
<td>8.0 ± 5.1</td>
</tr>
<tr>
<td>Time sternal (min)</td>
<td>n/a</td>
<td>n/a</td>
<td>15.2 ± 7.9</td>
<td>16.7 ± 5.9</td>
<td>20.2 ± 11.4</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. *Significantly (P < 0.05) different from isoflurane MAC value. †Significantly differs from all treatment groups. a= significantly differs from MLK. b= significantly differs from alpha-MLK. c= significantly differs from dexmedetomidine. MAC-Iso = the isoflurane minimum alveolar concentration. 1.5xMAC = 1.5 times the isoflurane MAC. MLK = morphine-lidocaine-ketamine. Alpha-MLK = dexmedetomidine-morphine-lidocaine-ketamine. SAP = systolic arterial pressure. MAP = mean arterial pressure. DAP = diastolic arterial pressure. ETCO₂ = end tidal carbon dioxide. SpO₂ = oxygen saturation of hemoglobin. CO = cardiac output. CI = cardiac index. BIS = bispectral index. Rec ET-iso = isoflurane end-tidal concentration at extubation. BUN = blood urea nitrogen. SaO₂ = arterial oxygen saturation. PaCO₂ = arterial carbon dioxide concentration. Time extub = time to extubation after isoflurane stopped. n/a = data not collected at this time.
Table 4.2—Metabolic variables recorded from arterial blood gas in dogs (n = 6) anesthetized with isoflurane and given infusions of dexmedetomidine, morphine-lidocaine-ketamine, and dexmedetomidine- morphine-lidocaine-ketamine on four separate occasions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MAC-Iso</th>
<th>Dexmed</th>
<th>MLK</th>
<th>Alpha-MLK</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.41 ± .02</td>
<td>7.44 ± 0.02</td>
<td>7.43 ± 0.08</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>568 ± 31.4</td>
<td>578 ± 20.5</td>
<td>556 ± 33.3</td>
<td>556 ± 35.7</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>31.5 ± 2.3</td>
<td>32.3 ± 2.69</td>
<td>33.3 ± 5.41</td>
<td>35.8 ± 1.95</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>99.4 ± 0.2</td>
<td>99.8 ± 0.42</td>
<td>99.7 ± 0.22</td>
<td>99.6 ± 0.25</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>35 ± 3.4</td>
<td>38.3 ± 1.5*</td>
<td>38.8 ± 2.3*</td>
<td>37.5 ± 1.9</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.3 ± 1.6</td>
<td>13.5 ± 1.8</td>
<td>13.0 ±0.76</td>
<td>13.2 ± 1.7</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>142 ± 1.3</td>
<td>142.7 ± 1.4</td>
<td>140.5 ± 3.5</td>
<td>141.2 ± 1.8</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.9 ± 0.36</td>
<td>3.9 ± 0.22</td>
<td>3.5 ± 0.31</td>
<td>4.0 ± 0.29</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>113.6 ±2</td>
<td>113.6 ± 0.59</td>
<td>113.7 ± 3.5</td>
<td>112.8 ± 1.4</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>5.19 ± 0.2</td>
<td>5.32 ± 0.2</td>
<td>5.11 ±0.15</td>
<td>5.37 ± 0.13</td>
</tr>
<tr>
<td>Magnesium (mg/dL)</td>
<td>1.3 ± 0.06</td>
<td>1.19 ± 0.12</td>
<td>1.14 ± 0.12</td>
<td>1.25 ± 0.12</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.5 ± 1</td>
<td>1.0 ± 0.38</td>
<td>1.9 ± 0.99</td>
<td>0.87 ± 0.33</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>18.2 ± 5.3</td>
<td>14 ± 2.4</td>
<td>15.2 ± 4.4</td>
<td>15.8 ± 2.7</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.95 ± 0.2</td>
<td>0.98 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.83 ± 0.05</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>102 ± 6.4</td>
<td>110 ± 14.3</td>
<td>132 ± 20*</td>
<td>139 ± 23*</td>
</tr>
<tr>
<td>HCO3 (mmol/L)</td>
<td>20.2 ± 1</td>
<td>22.4 ± 1.8</td>
<td>22.3 ± 1.7</td>
<td>21.8 ± 1.4</td>
</tr>
<tr>
<td>BEecf (mmol/L)</td>
<td>-4.6 ± 1</td>
<td>-2.0 ± 1.9</td>
<td>-2.3 ± 2.5</td>
<td>-3.4 ± 1.6</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. *Significantly (P < 0.05) different from isoflurane MAC value. 

**a** = significantly differs from MLK. 

**b** = significantly differs from alpha-MLK. 

CHAPTER 5

DISCUSSION

This study determined that the intravenous co-administration of morphine, lidocaine, ketamine, and dexmedetomidine reduces MAC-Iso in healthy dogs by 90%. Morphine, lidocaine, and ketamine reduced MAC-Iso by 55%, and dexmedetomidine infusion resulted in a 30% decrease in MAC-Iso.

Our MAC-Iso was 1.3 ± 0.15%. This is similar to other studies that report MAC-Iso in dogs from 1.28 ± 0.06% (Steffey et al 1977) to 1.38 ± 0.08% (Muir et al 2003). Another study obtained a higher MAC-Iso of 1.43 ± 0.18% (Pypendop and Ilkiw 2005).

Several factors can increase or decrease MAC values. Increasing age has been shown to significantly decrease MAC (Yamashita et al 2009), so the use of particular age group of dogs in one study can influence the mean MAC value compared to another study that uses dogs in a different age group. Temperature is also another factor that affects MAC and can differ between studies. Previous studies have determined that hypothermia can decrease anesthetic requirements in a linear manner such that for each 1°C decrease in body temperature, MAC decreases by 5% to 10% (Antognini 1993; Regan and Eger 1967). MAC studies typically attempt to keep the subjects in a specific body temperature range to eliminate its influence on MAC.
Our results confirm and extend the results of other studies (Pascoe et al 2008; Uilenreef et al 2008) that evaluated dexmedetomidine infusions. However, our study demonstrated a greater mean MAC-Iso reduction than the Pascoe et al. study. Pascoe found that the same rate of dexmedetomidine infusion (0.5 µg kg⁻¹ hr⁻¹) caused an 18% ± 12% reduction in MAC-Iso, compared to our 30% ± 7% reduction in MAC-Iso.

Differences between Pascoe’s and our study include: 1. the use of a loading dose of 0.5 µg kg⁻¹ vs no loading dose 2. a waiting period of 60 minutes vs 120 minutes prior to baseline MAC determination 3. body temperature was maintained between 37.8 and 39.6 °C vs 37.5 to 38 °C 4. placement of the stimulating electrodes on the thoracic and pelvic limbs vs the buccal oral mucosa in our study. Another possible explanation for the differences between our study and previous studies is the variability in individual MAC for dogs.

Our results confirm the MAC-Iso lowering potential of MLK infusion in dogs shown by the Muir et al. (2003) study, but we found a greater MAC reduction (55% vs. 45%) The difference in MAC values for MLK between the Muir et al. study and our study may be explained by the range of MAC-Iso in dogs that is specific for an individual animal and is dependent on age, physical status, and the magnitude and duration of the noxious stimulus (Quasha et al 1980; Zbinden et al 1994). Another study evaluating the reduction of the MAC-Iso in dogs using a CRI of lidocaine-ketamine in combination with either morphine or fentanyl resulted in a 45% and 97% MAC reduction, respectively (Aguado et al 2010). The effects of MLK in this study are consistent with the Muir study. The main differences in the Aguado study compared to our study include: 1. the use of
premedication (acepromazine and midazolam) 2. dogs were undergoing ovariohysterectomy in the clinical setting 3. older dogs (mean age of 4.9 ± 45 years) 4. a loading dose of MLK was given over 5 minutes followed by a CRI for 30 minutes after induction and at least 15 minutes before a skin incision 5. and a baseline MAC-Iso of 0.7% determined by Dixon’s up-and-down method (Dixon 1965), which has been used in clinical patients to determine MAC-Iso. Another study by Wilson et al. (2008) evaluating the effects of IV lidocaine, ketamine, and the combination on the MAC of sevoflurane in dogs reported a baseline sevoflurane MAC of 1.9 ± 0.2%. The combination of ketamine (3 mg kg$^{-1}$ loading dose; 100 µg kg$^{-1}$ min$^{-1}$ CRI) and lidocaine (2 mg kg$^{-1}$ loading dose; 100 µg kg$^{-1}$ min$^{-1}$ CRI) significantly reduced baseline sevoflurane MAC by 62.8%. When the two drugs were given alone at the above rates, ketamine reduced sevoflurane MAC by 44.7% and lidocaine reduced sevoflurane MAC by 29%. The combination of ketamine and lidocaine at the dosages studied had a greater effect than the two drugs alone, but was about 10% less than needed to demonstrate an additive effect. Another study in goats demonstrated an additive effect of ketamine and lidocaine on reduction of MAC-Iso (Doherty et al 2007). The baseline MAC-Iso of 1.13 ± 0.03% was decreased by 18.3%, 49.6%, and 69.4% when lidocaine, ketamine, and lidocaine-ketamine, respectively, were infused.

We hypothesized that the alpha-MLK would cause a greater MAC-Iso reduction than MLK. The results of our study show that a 90% reduction in MAC-Iso is possible with the combination of morphine-lidocaine-ketamine-dexmedetomidine given as a CRI
in dogs. We are not aware of any previous studies that have examined the effect of this co-infusion on MAC-Iso in dogs.

The combination of drugs to achieve hypnosis and immobility, two critical clinical end-points of general anesthesia, can produce interactions that are either “synergistic,” “additive,” or “infra-additive” when their combined effect exceeds, equals, or is less than the sum of the effects of the drugs on an individual basis, respectively (Hendrickx et al 2008). Synergy of drugs can be useful because it allows for a smaller dose of each drug to be given and can potentially decrease side effects. Administration of drug combinations can, however, result in unforeseen adverse effects. Drug interactions may also provide a better understanding of the mechanism(s) of action. If drugs strictly have an additive interaction, then anesthetics are acting identically at a single site. This supports, but does not prove, a single site of action. A series of review papers (Shafer et al 2008; Hendrickx et al 2008) suggested that additivity is not a common finding for drugs acting at different sites of action, and IV anesthetics with receptor sites that are known to have differing effects usually, but not always, exhibit synergy in regards to producing immobility. In our study, the combination of dexmedetomidine and MLK was approximately additive, or slightly synergistic, if the MAC-Iso reduction for each is considered compared to alpha-MLK: 30% (dexmedetomidine) plus 55% (MLK) is 85%, which is comparable to 90% found with administration of alpha-MLK. In the Muir et al. (2003) study, infusion of morphine resulted in a 48% mean reduction of ET-Iso and the addition of lidocaine and ketamine (i.e. MLK infusion) resulted in a 45% reduction. They speculated that the likely cause for their inability to detect a significant difference in
reduction of MAC-Iso between morphine and MLK was the insensitivity of MAC
determination for quantitating subtle differences in mechanism of drug action and drug-
related analgesic effects.

BIS has been used in humans, validated in other species, and proven to be a
reliable tool for the monitoring of anesthetic depth (March and Muir 2005). Our study
incorporated both MAC determination and BIS in order to determine a clinically useful
assessment of analgesia (MAC) and depth of anesthesia (BIS). The results of our study
demonstrated that BIS may be a useful monitoring tool for assessing the level of
consciousness in isoflurane-anesthetized dogs.

In our study, a mean BIS value of 74 ± 7 was obtained at MAC-Iso. The mean
BIS value in the dogs went down significantly to 63 ± 8 at 1.5 x MAC-Iso. A study by
Campagnol et al. (2007) evaluated use of BIS to monitor depth of anesthesia in isoflurane
anesthetized dogs and obtained comparable values. Campagnol reported a mean BIS
value of 72 ± 6 at 1.5% ET-Iso with the BIS sensors positioned in the bifrontal
configuration, similar to our study value of 74 ± 7 at 1.3% ET-Iso. The Campagnol et al.
study evaluated two other BIS sensor positions, frontal-occipital and frontal-temporal,
and recorded mean BIS values of 66 ± 8 and 66 ± 6, respectively at 1.5% ET-Iso.
Overall, they found that BIS values derived from the 3 sensor positions did not differ.
They concluded that, in dogs, BIS values may not reflect changes in depth of isoflurane
anesthesia in the absence of noxious stimulation. They found that BIS and ET-Iso
concentration were poorly correlated and mean BIS values did not change significantly as
ET-Iso was increased. Our study was different than the Campagnol et al. study because we applied a supramaximal stimulus in order to determine MAC-Iso. A study by Greene et al. (2003) found that BIS during isoflurane anesthesia predictably decreased with increasing MAC multiples. Inversely, as MAC is decreased (in our study by the use of adjunct anesthetic drugs), the BIS may increase.

The infusion of dexmedetomidine, which allowed a 30% reduction of ET-Iso, produced a mean BIS value of 71 ± 8, which was not significantly lower than baseline. The α2-agonists have both potent sedative and analgesic properties and MAC reduction has been reported with their use (Pascoe et al 2006). Therefore, the potent sedative effects of dexmedetomidine may contribute to a decreased level of consciousness at the same time a decrease in isoflurane concentration occurs. Greater CNS depression is expected at a given MAC of inhaled anesthetics with co-administration of an α2-agonist compared with the same MAC of inhaled anesthetic alone. Greene et al. (2003) demonstrated this by finding that the addition of medetomidine (8 µg kg⁻¹, IM) to isoflurane anesthesia at 1.0 and 1.5 MAC was associated with a decrease in the BIS, compared to isoflurane-saline anesthesia alone. Greene had the same baseline MAC value compared to our study (1.3 ± 0.2%) and a lower mean BIS measurement of 60 ± 7 at 1.0 MAC-Iso compared to the mean value of 74 ± 7 at MAC-Iso found in our study. Greene determined a mean BIS measurement of 53 ± 7 at 1.0 MAC for isoflurane anesthesia with IM administration of medetomidine. This was lower than the mean BIS value (71 ± 8) for isoflurane with infusion of dexmedetomidine found in our study. This
difference could be attributed to the difference in potency and dosages between medetomidine and dexmedetomidine, in route of administration, and time of collection of the BIS value in relation to administration of medetomidine since the order of determining MAC multiples was randomized over a 2 hour period in the Greene study. Another study (Silva et al 2010) comparing the hemodynamics and BIS of dogs anesthetized with midazolam and ketamine in combination with either dexmedetomidine (20 µg kg\(^{-1}\) hr\(^{-1}\)) or medetomidine (30 µg kg\(^{-1}\) hr\(^{-1}\)) continuous infusion for ovariohysterectomy found significantly lower (P<0.05) BIS values after induction compared to the control in both groups. The dexmedetomidine group, however, had significantly lower BIS values compared to the medetomidine group after premedication and 10 minutes after induction of anesthesia. It is difficult to compare our study to the Silva study because other drugs (ketamine and midazolam) were being continuously infused in addition to the dexmedetomidine. It has been shown in humans that BIS values depend on the specific sedative used (Kasuya et al 2009). In a study by Campagnol et al. (2007) evaluating the effects of epidural administration of dexmedetomidine on the MAC-Iso in dogs, showed that, during all treatments with noxious stimulation, BIS increased. These changes in BIS were correlated with increases in electromyography (EMG) activity. The artifact generated by increased EMG activity may have contaminated the BIS calculations. High levels of awareness (cortical activity) can contribute to EMG noise. In order to eliminate EMG, a neuromuscular blocking agent could be given. A possible reason for our study not showing a significant change in BIS value for the dexmedetomidine infusion group compared to baseline group may be
that a MAC reduction of 30% was not enough to cause a significant increase cortical awareness and therefore an increase in the BIS value.

The MLK infusion at MAC significantly increased the mean BIS values to 80 ± 6, an increase of 8% compared to baseline. In the Muir et al. (2003) study, baseline BIS for MAC-Iso was 61 ± 11. In that study, infusion of MLK resulted in a mean BIS value of 67 ± 10 which was not statistically significant compared to baseline. Our mean BIS value of 80 ± 6 at MLK MAC-Iso is higher than the Muir study value of 67 ± 10 for MLK. This may be due to inherent differences in the dogs used in the two studies. However, we had a higher MAC reduction of 55% in our study compared to the 45% MAC reduction for MLK in the Muir study. The lower amount of ET-Iso in our study compared to the Muir study could explain the higher mean BIS values we obtained. The combination of drugs allowed the amount of isoflurane to be decreased significantly compared to baseline as well as the dexmedetomidine infusion group. Therefore the dogs were likely at a lighter plane of anesthesia (i.e. comparatively more conscious), but due to the antinociceptive effects of the infused drugs they did not respond to the noxious stimuli. A recent study (Henao-Guerrero et al 2009) concluded that IV administration of 0.5 mg kg$^{-1}$ morphine sulfate did not cause clinically significant changes in the BIS in unstimulated Beagle dogs anesthetized with a constant ET-Iso of 1.81%. Our ET-Iso of 1.9% at 1.5 X MAC resulted in a BIS value of 63 ± 8, which was significantly different from the mean BIS value of 74 ± 7 at MAC-Iso. Since ketamine is known to paradoxically increase BIS under sevoflurane anesthesia in humans (Hans et al 2005),
this could be a possible explanation for the significant increase in BIS during MLK infusion during our study.

The alpha-MLK infusion significantly increased the mean BIS values to $82 \pm 6$, an increase of 11% compared to baseline. This was a significant increase compared to the dexmedetomidine infusion, but not different from the MLK infusion. Therefore the MLK and alpha-MLK infusions had the same effect on BIS despite the difference in MAC reduction. It is surprising that the CNS depressant effects of dexmedetomidine fail to cause a difference in BIS values between these two groups, especially since the alpha-MLK co-infusion results in a higher reduction in MAC-Iso compared to MLK.

Hemodynamic variables recorded during our study included SAP, DAP, MAP, HR and rhythm, CO, CI, SV, and SVR. The rhythm detected by ECG was normal in all dogs given isoflurane alone. Infusion of dexmedetomidine and alpha-MLK resulted in a sinus arrhythmia in all dogs. Infusion of MLK resulted in sinus arrhythmia in 3 of the 6 dogs. The HR significantly decreased compared to baseline in all the infusion groups in our study. Mean HR decreased significantly in the dexmedetomidine and alpha-MLK groups to $53 \pm 10$ and $49 \pm 6$ bpm, respectively. The lowest recorded HR was 42 beats per minute in one dog in the alpha-MLK infusion group, but the direct arterial pressure was 110/69 (83) mmHg. Decreases in heart rate should be evaluated in relation to blood pressure, and ultimately CO and tissue perfusion if possible.

The mean HR for the dexmedetomidine infusion obtained in our study was comparable to the Uilenreef et al. (2007) study. Uilenreef found that, with a
dexmedetomidine CRI of 1 µg kg⁻¹ hr⁻¹, HR increased over time during surgical anesthesia to 68 ± 6 bpm, with a preincisional HR of 59 ± 4 bpm. This was similar to our study that found a mean HR of 53 ± 10 bpm. These changes in heart rate are expected based on previous studies of dexmedetomidine (Lin et al 2008; Murrell and Hellebrekers 2005; Pypendop and Verstegen 1998; Bloor et al 1992). Typical hemodynamic changes induced by α₂-agonists include vasoconstriction and subsequent baroreflex-mediated physiological decrease of HR and CI, which is perpetuated by reduced sympathetic tone (Sinclair 2003).

The mean HR for MLK infusion obtained in our study (78 ± 17 bpm) was similar to the mean HR for MLK infusion in the Muir et al. (2003) study (84 ± 17 bpm). In both studies the HR was significantly lower compared to baseline isoflurane alone. It is likely that the decrease in HR found in the MLK infusion group compared to baseline is due to the effects of morphine since opioids are known to cause bradycardia (Bednarski 1992). Muir also determined that infusion of ketamine resulted in a significant increase in HR compared to isoflurane baseline and that lidocaine infusion had no effect on HR compared to isoflurane baseline, so it is not likely that either ketamine or lidocaine were responsible for the decrease in HR we demonstrated for our MLK infusion.

During our study, the MAP increased significantly during alpha-MLK infusion. The SAP significantly increased compared to baseline in all three infusion groups. The DAP did not change significantly in any of the infusion groups. A comparison of our values for the dexmedetomidine infusion to the Uilenreef et al. (2007) study, which found
that MAP was stable within the 1 μg kg\(^{-1}\) hr\(^{-1}\) dexmedetomidine CRI group, with a value of 101 ± 4 mmHg preincision and a value of 99 ± 4 mmHg during surgery. This is higher than the value for MAP of 70 ± 8 mmHg seen in the 0.5 μg kg\(^{-1}\) hr\(^{-1}\) dexmedetomidine infusion group. This is not surprising since dexmedetomidine causes a dose-dependent increase in systemic vascular resistance (Flacke et al 1993), which is related to MAP by the following equation:

\[
\text{MAP} = \text{CO} \times \text{SVR}
\]

The MAP obtained at MLK MAC-Iso in the Muir et al. (2003) study was 83 ± 6 mmHg.

Our study showed MAP at MLK MAC-Iso of 73 ± 4 mmHg.

Our study demonstrated a significant decrease in CO compared to baseline isoflurane during dexmedetomidine infusion. There were no significant changes in CI among infusion groups compared to Iso-MAC. In our study, at MAC-Iso (ET-Iso of 1.3%) the mean CO was 2.3 ± 0.7 L min\(^{-1}\). In a study by Bernard et al. (1990), sevoflurane and isoflurane administration were associated with similar systemic vasodilation, tachycardia, dose-dependent decrease in aortic blood pressure, and SV. They demonstrated at 2 MAC (4.7% sevoflurane and 2.6% isoflurane) both inhalants produced a significant decrease in CO. At 1.2 MAC (or 1.6% isoflurane) the CO was 1.9 ± 0.1 L min\(^{-1}\). Our higher CO values of 2.3 ± 0.7 L min\(^{-1}\) at a lower ET-Iso of 1.3% may be due to increased venous return or decreased afterload in our patients since inhalants
are known to cause a dose-dependent cardiovascular depression (Page et al 1991; Mazzaferro and Wagner 2001). In one study, LiDCO was evaluated in healthy female dogs undergoing ovariohysterectomy in a clinical setting. The mean CI was $4.26 \pm 0.26$ L/min/m$^2$ and $3.40 \pm 0.26$ L/min/m$^2$ for spontaneously breathing and mechanically ventilated dogs, respectively (Miyake et al 2005). Our study had a lower value of $2.2 \pm 0.3$ L/min/m$^2$ for CI in mechanically ventilated dogs. Another study evaluated the accuracy of a commercial ultrasonographic CO in anesthetized dogs by comparison with thermodilution CO. Isoflurane concentrations of 0.5% to 1.5% resulted in thermodilution CO values of $2.07 \pm 0.84$ L min$^{-1}$ and ultrasonographic cardiac output values of $2.10 \pm 0.74$ and $1.83 \pm 0.82$ L min$^{-1}$ for the subxiphoid and thoracic-inlet positions, respectively, for spontaneously breathing dogs (Scansen et al 2009). These values were similar to our mean CO value ($2.3 \pm 0.7$ L min$^{-1}$) at Iso-MAC, but since CO does not factor in weight, like a CI value, it is difficult to directly compare these two studies.

In the dexmedetomidine infusion group, the mean CO was $1.3 \pm 0.5$ L min$^{-1}$, a significant decrease compared to baseline. A decrease in CO after administration of dexmedetomidine has been previously reported (Bloor et al 1992; Roekaerts et al 1996). Roekaerts determined that dexmedetomidine reduced primary determinants of myocardial oxygen consumption, such as HR. Inhalant anesthetics are known to reduce oxygen demand. In a study by Scheeren et al (1999), five commonly used inhalation anesthetics were characterized by a uniform relationship between CO and oxygen consumption ($VO_2$) with an almost linear relationship up to 2 MAC, regardless of the anesthetic in use. They determined metabolic regulation of blood flow functions during inhalant anesthesia.
up to 2 MAC and that decreases in VO₂ determines CO. This implies that CO alone provides limited information on the function of the circulation during inhalant anesthesia unless related to VO₂. Although there was no significant difference when comparing CO values between the dexmedetomidine and alpha-MLK infusion groups, it is interesting that the alpha-MLK did not cause a significant decrease in CO compared to baseline and dexmedetomidine infusion did. A possible explanation for this difference between the two infusions might be the addition of ketamine in the alpha-MLK infusion group. S-(+)-ketamine as a monoanesthetic has significant sympathomimetic properties and increased plasma levels of adrenaline and noradrenaline are observed. The increase in plasma catecholamines leads to increased arterial pressure and HR (Adams 1997). In rats, it has been determined that S-(+)/ketamine stereoselectively blocks dopamine uptake and therefore elevates synaptic dopamine levels (Nishimura and Sato 1999). The sympathomimetic properties of ketamine may have been enough to balance out the sympatholytic properties possessed by α₂-agonists (Maze and Tranquilli 1991).

In our study, SV and SVR were calculated during the three infusions groups. Stroke volume did not differ among infusion groups. The SVR increased significantly in the dexmedetomidine and alpha-MLK infusion groups. Another study evaluated arterial pressure waveform analysis with LiDCO in anesthetized dogs (1.5% to 1.8% ET-Iso) and demonstrated SVR values of 1,754 ± 551 (dyn/sec/cm⁻⁵) at baseline (Chen et al 2005). This value was lower than our value for SVR at MAC-Iso (1.3% ET-Iso) of 1983 ± 1023 (dyn/sec/cm⁻⁵).
The LiDCO™ plus hemodynamic monitor was utilized in our study. LiDCO was developed for use in critically ill people and for estimating CO in animals (Linton et al 1997; Mason et al 2001). The LidCO monitor works by running two proprietary algorithms: a continuous arterial waveform analysis system (PulseCO™) coupled to a single point lithium indicator dilution calibration system (LiDCO™). Studies have validated the use of LiDCO in healthy dogs under anesthesia (Miyake et al 2005). LiDCO monitoring is a less invasive method of measuring CO than previously used methods such as indocyanine green dye dilution and thermodilution because LiDCO only requires catheterization of a peripheral artery and vein to estimate CO. The other methods require cardiac catheterization which is associated with a higher risk of complications and expense, making them less ideal for CO monitoring in clinical patients (Miyake et al 2005). Multiple cardiac catheterizations were outside the scope of this study.

Cardiac output in liters per minute is a product of SV multiplied by HR. Stroke volume is affected by preload, contractility, and afterload. Stroke volume can be calculated by the following formula:

\[ SV \ (ml/beat) = 1000 \times \frac{CO}{HR} \]

Cardiac index relates CO to body surface area, therefore allowing for a more accurate comparison of CO among subjects with differing body weights. Assessment of tissue perfusion or oxygen delivery to the cell (DO₂) is the ultimate goal of measuring cardiopulmonary function. This is difficult to assess accurately in the clinical situation. DO₂ is affected by CO and the oxygen content of arterial blood (CaO₂):
\[ \text{DO}_2 = \text{CO} \times ((\text{hemoglobin concentration} \times 1.34 \times \text{SaO}_2) + (\text{PaO}_2 \times 0.0031)) \]

The calculated CaO\(_2\) values based on our recorded variables were 18.19, 19.77, 19.05, and 19.39 (mL O\(_2\) dL\(^{-1}\)) for MAC-Iso, dexmedetomidine, MLK, and alpha-MLK, respectively. In our study, DO\(_2\) decreased with administration of dexmedetomidine compared to MAC-Iso. At MAC-Iso the DO\(_2\) was 665 ± 464 (mL O\(_2\) min\(^{-1}\)) and at MAC for the dexmedetomidine infusion DO\(_2\) decreased to 269 ± 89 (mL O\(_2\) min\(^{-1}\)). Decreased DO\(_2\) has been shown to occur with administration of dexmedetomidine in dogs. But adequate oxygenation was maintained above critical oxygen delivery levels during constant rate dexmedetomidine infusion while dogs were under isoflurane anesthesia (Lin et al 2008).

Muir et al. (2003) demonstrated that infusion of the MLK combination was well tolerated in healthy young dogs and caused no unsafe cardiovascular changes. Similarly, the cardiovascular changes that occurred during the three infusions in our study were well tolerated in a similar group of dogs.

The mean times to extubation and sternal recumbency did not differ significantly among treatment groups in our study. Isoflurane alone resulted in extubation times of 13.6 ± 8.8 minutes. Treatment with dexmedetomidine, MLK and alpha-MLK infusions resulted in extubation times of 13.8 ± 8, 7.6 ± 7, and 8 ± 5 minutes, respectively. The dogs attained sternal recumbency within 15 to 20 minutes after turning the inhalant and infusion off for all treatment groups. The most noticeable difference between baseline and the treatment groups was the occurrence of dysphoria and vomiting and/or regurgitation. Four dogs in the baseline isoflurane group experienced dysphoria and were
treated with acepromazine. The dysphoria was most likely attributed to the lack of sedative drugs given to the patient in the anesthetic period. In the dexmedetomidine and MLK infusion groups, 2 dogs vomited and one dog regurgitated, respectively. In the alpha-MLK infusion group, no adverse effects were noted in the recovery period. This can be attributed to the combined sedative effects of the dexmedetomidine, morphine, lidocaine and ketamine given during the anesthetic period, resulting in the dogs recovering quietly.

Our results are comparable to a study by Lopez et al. (2009) that compared recovery from anesthesia with isoflurane, sevoflurane, or desflurane in healthy dogs. The dogs in that study were induced with propofol and received 2.0% isoflurane for 120 minutes. In that study mean ± SD time to extubation was 13.0 ± 5.4 minutes and time to sternal was 22.2 ± 8.8 minutes for isoflurane. Our study obtained a comparable time to sternal recumbency of 15 ± 11.4 minutes. In the Lopez study, the ET-Iso was 0.4 ± 0.1% at the time of extubation which is comparable to our mean ET-Iso value of 0.53 ± 0.15%. The mean ET-Iso at the time of extubation was 0.03 ± 0.1% in the alpha-MLK infusion group in our study. This value was significantly decreased compared to isoflurane alone and the dexmedetomidine infusion group, with mean ET-Iso values of 0.53 ± 0.15% and 0.40 ±0.09%, respectively. This decreased amount of isoflurane could have played a role in absence of dysphoria at the time of recovery for some of the infusion groups.

Our study design had inherent limitations. Since this was not a clinical trial, all our dogs were healthy and free of any pre-existing pain. Both ketamine and lidocaine best produce their analgesic effects in the presence of chronic or acute severe pain in
humans (Noppers et al 2010; Kroenke et al 2009), so it is possible that their full anesthetic sparing effect was not elicited in these experiments. Further studies using clinical patients with pre-existing pain conditions may yield a different outcome. The number of dogs used in the study was determined by a power calculation in order to demonstrate statistical significance, assuming that each infusion would cause a decrease in MAC-Iso of at least 20%. However, incorporating more dogs in this study would have allowed for a greater range of MAC values for each treatment group and give a better idea of the true mean statistical value for each group.

The end-tidal agent monitor fluctuated between 0.2% and 0% when dogs received very low amounts of isoflurane during MAC determination in the alpha-MLK group, as well as during collection of recovery data after the inhalant anesthesia was turned off. This likely limited accuracy at these times, however we believe that this had a minimal impact on our overall results.

Another limitation to our study was that the three components of the MLK infusion – morphine, lidocaine, and ketamine- were not assessed separately. Our decision to not perform these individual assessments was due to the fact that MAC reduction of morphine, lidocaine and ketamine infusions has been assessed individually in previous studies in this laboratory for isoflurane (Muir et al 2003) and in another laboratory for sevoflurane (Wilson et al 2008). We recognize that determining MAC values for the individual drugs would have provided a more accurate evaluation of each drug’s contribution to MAC reduction.
Intravenous co-infusions of dexmedetomidine, morphine-lidocaine-ketamine, and dexmedetomidine-morphine-lidocaine-ketamine, significantly reduce the MAC-Iso in healthy dogs. The BIS may be a useful monitoring tool for assessing level of consciousness in isoflurane-anesthetized dogs receiving MLK or alpha-MLK infusions. Overall, the alpha-MLK infusion allowed for a significant decrease in the amount of isoflurane required, and cardiovascular changes, when they occurred, were well-tolerated. Recovery following infusion of alpha-MLK was smooth with no adverse effects.

Future directions based on this research include a clinical trial involving healthy patients undergoing elective surgery (for example, orthopedic correction of torn anterior cruciate ligament). This would allow for the assessment of alpha-MLK in the clinical setting, including its impact on post-anesthetic analgesic requirements. Additionally, assessment of the pharmacokinetics of the alpha-MLK infusion would be appropriate.
Footnotes

a. Surflo® I.V. Catheter, Terumo Medical Corp., Elkton, MD.

b. Lidocaine and Prilocaine Cream, 2.5%/2.5%, E. Fouger & Co., Melville, NY.

c. Propofol (PropoFlo™), Abbott Laboratories, North Chicago, IL.

d. Endotracheal Tube- Cuffed, Murphy, Tyco Healthcare Group LP, Pleasanton, CA.

e. Hallowell EMC Model 2KIE Veterinary Anesthesia Ventilator, Hallowell Engineering and Manufacturing Corp., Pittsfield, MA.

f. Isothesia™ Isoflurane, USP, Butler Animal Health Supply, Dublin, OH.

g. Ohmeda Isotec 3, GE Health Care, Pataskala, OH.

h. Gaymar® T/pump®, Gaymar Industries, Inc., Orchard Park, NY.


j. Datascope® Gas Module SE™, Datascope Corp., Mahwah, NJ.

k. Datascope®, Passport 2, Datascope Corp., Mahwah, NJ.

l. SpaceLabs Medical, SpaceLabs Medical, Inc., Redmond WA.

m. Genuine Grass Platinum Subdermal Needle Electrodes, Astro-Med, Inc., West Warwick, RI.

n. BIS VISTA™ Monitoring System, Aspect Medical Systems, Inc., Norwood, MA.

o. LiDCOplus Hemodynamic Monitor, LiDCO™ Limited, London, UK.

q. Lithium Chloride Injection 0.15mmol/ml Sterile Solution, LiDCO Ltd., London, UK.

r. Extension Set with Female Luer Lock Connector and Spin-Lock® Connector, Braun Medical Inc., Bethlehem, PA.


t. Flow Regulator, LiDCO Ltd., London, UK.

u. Grass SD9 Stimulator, Grass Medical Instruments, Quincy, MA.

v. Stat Profile® Critical Care Xpress, Nova Biomedical, Flintshire, UK.

w. Lactated Ringer’s Injection USP, Baxter Healthcare Corp., Deerfield, IL.


y. Morphine Sulfate, Baxter Healthcare Corp., Deerfield, IL.

z. Lidocaine Injectable, Sparhawk Laboratories, Inc., Lenexa, KS.


bb. Heska™ Vet/IV™ 2.2, Heska Corporation, USA.


dd. Power and Precision, Biostat™, Englewood, NJ.

ee. GraphPad Prism® (Version 5), GraphPad Software, La Jolla, CA.


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Appendix

Sedation Scoring System for Pilot Study

Sedation scoring system used to evaluate effects of morphine, lidocaine, ketamine and dexmedetomidine given as a bolus then as a continuous infusion in dogs for the pilot study. Maximum sedation score = 14. Not sedated/agitated = -10.

<table>
<thead>
<tr>
<th>Observation</th>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vocalization</td>
<td>0</td>
<td>Quiet</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>Whining softly but quiets with soothing touch</td>
</tr>
<tr>
<td></td>
<td>-2</td>
<td>Whining continuously</td>
</tr>
<tr>
<td></td>
<td>-3</td>
<td>Barking continuously</td>
</tr>
<tr>
<td>Posture</td>
<td>3</td>
<td>Lateral recumbency</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Sternal recumbency</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Sitting or ataxic</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Standing</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>Continuous movement</td>
</tr>
<tr>
<td>Appearance</td>
<td>3</td>
<td>Eyes sunken, glazed, unfocused, ventromedial rotation</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Eyes glazed but follow movement</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Nictitating membrane protruded; normal visual response</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Normal appearance</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>Pupils dilated; abnormal facial expression</td>
</tr>
<tr>
<td>Interactive</td>
<td>3</td>
<td>Recumbent; no response to voice or touch</td>
</tr>
<tr>
<td>Behaviors</td>
<td>2</td>
<td>Recumbent; lifts head in response to voice or touch</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Recumbent but rises in response to voice or touch</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Standing or sitting up; normal response to voice or touch</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>Moves away from voice or touch (“jumpy”)</td>
</tr>
<tr>
<td></td>
<td>-2</td>
<td>Growls/hisses when approached or touched</td>
</tr>
<tr>
<td></td>
<td>-3</td>
<td>Bites/swats when approached</td>
</tr>
<tr>
<td>Restrainability</td>
<td>2</td>
<td>Lies on floor with minimal restraint needed</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Lies of floor with light restraint of head/neck</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Sits up on floor; attempts to jump despite restraint</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>Struggles against restraint continuously</td>
</tr>
<tr>
<td></td>
<td>-2</td>
<td>Cannot be restrained for &gt; 20 seconds</td>
</tr>
<tr>
<td>Noise response</td>
<td>3</td>
<td>No response to a hand clap near the head</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Minimal response to a hand clap near head</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Slow/moderate response to a hand clap near head</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>Brisk response to a hand clap; raises head, eyes open</td>
</tr>
</tbody>
</table>