

**Assessment of the Effect of Prior Noxious Stimulation on Minimum
Alveolar Concentration Determinations in the Dog and Rabbit**

by

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ABSTRACT

Assessment of the Effect of Prior Noxious Stimulation on Minimum Alveolar Concentration Determinations in the Dog and Rabbit

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This thesis determines and compares the sparing effect of ketamine on the minimum alveolar concentration (MAC) of isoflurane in rabbits and dogs using two methods in a crossover random design. One method determined the MAC of isoflurane and ketamine combined after previous determination of the MAC of isoflurane during the same day. The second method determined the MAC for the combination without prior determination of the MAC of isoflurane. The concentration of ketamine and norketamine in plasma were also determined in dogs.

For the first method, the MAC of isoflurane in rabbits was $2.15 \pm 0.09\%$ (mean \pm SD) and decreased to $1.63 \pm 0.07\%$ during ketamine administration (1 mg/kg bolus and a constant rate infusion [CRI] of 40 $\mu\text{g}/\text{kg}/\text{min}$). In dogs, the same ketamine dose decreased the MAC of isoflurane from $1.18 \pm 0.14\%$ to $0.88 \pm 0.14\%$. Re-determination of MAC of isoflurane performed after stopping the CRI yielded a MAC value of $2.04 \pm 0.11\%$ in rabbits and of $1.09 \pm 0.16\%$ in dogs.

MAC values of the isoflurane and ketamine combination determined for the second method were $1.53 \pm 0.22\%$ in rabbits and $0.79 \pm 0.11\%$ in dogs. MAC values of

isoflurane after stopping the CRI of ketamine were $1.94 \pm 0.25\%$ in rabbits and $1.10 \pm 0.17\%$ in dogs.

The MAC value obtained in dogs for the isoflurane and ketamine combination with the first method was significantly higher with respect to the MAC value obtained with the second method (0.88 ± 0.14 versus $0.79 \pm 0.11\%$); whereas in rabbits, MAC values were similar.

During ketamine administration in dogs, plasma concentrations of ketamine at MAC values were similar for the first and second method (824 ± 195.7 ng/mL and 729 ± 133.4 ng/mL, respectively). After stopping the CRI, plasma concentrations during isoflurane MAC were also similar for the two methods (407 ± 176.2 ng/mL and 347 ± 81.2 ng/mL, respectively).

MAC values for the isoflurane and ketamine combination obtained with both methods were statistically different in dogs but not in rabbits, however these findings may be the result of the design of the study and tolerable experimental error derived from MAC studies rather than true species differences.

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Finally I would like to dedicate this work to my wife Dr. Leontine Benedicenti who not only was by my side each step of the way, but also followed me around the world in order to do so.

"Ubi major minor cessat" (anonymous)

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DECLARATION OF WORK PERFORMED

I declare that, with the exception of the listed items, all work reported in this thesis was performed by me.

The research proposal providing funding for this study was written and submitted by Dr. Alexander Valverde. Anesthesia of the dogs and rabbits during the experiments and data collection was performed with the assistance of Dr. Valverde, Dr. Eva Rioja and Amanda Hathaway. Dr. Ron Johnson and the laboratory technician Yu Gu performed the high performance liquid chromatography determinations for the plasma ketamine concentrations and provided the description of the method.

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CHAPTER I

GENERAL LITERATURE REVIEW

INTRODUCTION

Since the introduction over half a century ago of the concept of minimum alveolar concentration (MAC) of an inhalant anesthetic that prevents movement in response to a supramaximal noxious stimulus in 50% of a population, MAC studies have proven to be the standard method of comparison of anesthetic potency of inhalant anesthetics. Knowledge of the specific MAC value for any inhalant anesthetic in any species also provides a guideline for the patient's requirements and clinical anesthetic management whether the inhalant anesthetic is used alone or in combination with other injectable anesthetic drug(s).

In general, injectable anesthetic drugs administered in the perioperative period can decrease the requirements for inhalant anesthetics, known as a MAC sparing effect. Inhalant anesthetics cause dose-dependent cardiorespiratory depression, which can be theoretically lessened by combining them with injectable anesthetics that induce the MAC sparing effect. However, this may not always hold true if the injectable anesthetic has the potential of inducing cardiorespiratory changes that may be additive or synergistic with those of the inhalant anesthetic. For this reason, MAC studies usually involve the determination of the precise MAC sparing effect of injectable anesthetics on a particular inhalant anesthetic and the combined cardiorespiratory effects compared to those of the inhalant anesthetic alone.

In regards to determining the sparing effects of injectable anesthetics, the veterinary and human literature have an abundance of publications, often describing the same combination of injectable anesthetic and inhalant anesthetic drugs but different results. These differences may be due to accepted variations between animals (usually 10-20%) even within the same species, but also due to differences in the methodology used to determine MAC (Quasha et al. 1980). Factors such as route of administration of the drug, single injection versus constant rate infusion, time to achieve MAC determinations, type and intensity of the nociceptive stimulus used, altitude of the location where the study was completed, characteristics of the population being studied, and the number of experiments used to determine MAC, are not always standardized between investigators and laboratories that carry out this type of research and can potentially lead to different results.

This investigation attempts to determine if the two reported methods of MAC determination are interchangeable. MAC for an inhalant anesthetic can be determined in a very predictable way, but introducing a second drug such as an injectable anesthetic may result in different results according to the method used to determine MAC for the combination of the two drugs. One method involves determining MAC for the inhalant anesthetic and subsequently determining MAC for the combination of the inhalant and injectable anesthetic, all during the same experiment day. The other method involves determining the MAC for the inhalant anesthetic on a single experiment and then the MAC for the combination on a separate day. It is not known if both methodologies are equivalent.

The scope of this literature review is to introduce and develop the intertwined relationship of the disciplines involved in MAC studies and to have an in depth view of factors that may influence results from the two reported methods of MAC determination.

NOCICEPTION AND PAIN

Pain has been studied by scientists and philosophers dating as far back as ancient Greece and the Roman Empire, subsequently in the Middle Ages and later on during the scientific revolution in the 1600's. However, it was not until the mid-1800's that a more scientific approach associated harmful or potentially harmful insult to tissues with physiological mechanisms (Sherrington 1907). One century later, the gate control theory of pain was introduced by Ronald Melzack and Patrick Wall and proposed the physiologic interaction of sensation (touch) with pain, mediated by inhibitory pathways at the level of the dorsal horn of the spinal cord that were able to modulate pain signals before they reached the brain (Wall 1978). Unfortunately this theory lacked the scientific depth to fully explain the complex and plastic mechanisms that occur with chronic pain.

Definition of pain and nociception

The most widely recognized definition of pain comes from the International Association for the Study of Pain (IASP) and states that pain is a subjective unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Anon 1979).

Nociception is defined as the event of sensing mechanical, chemical and thermal stimuli, which have the ability or the potential ability to cause damage to the tissues, by

neuronal structures known as nociceptors. Nociceptors are free nerve endings of the first order neurons (Sosnowski et al. 1992) that have the ability to encode physical insults into electrical signals and transduce them to the dorsal horn of the spinal cord (Lamont et al. 2000). In specific situations, nociceptors can lower their activation threshold following repeated stimulation, which results in peripheral sensitization. Conversely, nociceptors can also be desensitized as a consequence of excessive stimulation and activation (Lamont et al. 2000).

Nociception is the physiologic process of sensing, encoding, and transducing a noxious stimulus, while pain is the global endpoint of the whole process that involves all structures of the central and peripheral nervous system and results in awareness of the sensation (Lamont et al. 2000).

Pain is classified in two major categories: the first one is defined as physiologic pain and follows normal physiologic pathways, the second, known as pathologic pain, is the consequence of pathology to nociceptors and conducting pathways (Lamont et al. 2000).

Pain transmission

Typically, the perception of pain travels through three orders of neurons from the periphery to the brain cortex (Angevine & Cotman 1981; Fu et al. 2011). First-order neurons carry information from the nociceptors to the macula densa in the dorsal horn of the spinal cord; second-order neurons relay this information from the spinal cord to the thalamus; and third-order neurons transmit the information from the thalamus to the primary sensory cortex, where the information is processed.

First order neurons

There are three classes of nociceptive afferent neurons in the periphery that provide input via the spinal cord to higher centers of the central nervous system (Angevine & Cotman 1981; Fu et al. 2011).

1. Mechano-thermal afferents- A δ fibers (Thermal and mechanical stimuli);
2. Polymodal afferent C fibers (Thermal, mechanical and chemical stimuli); and
3. High threshold mechanoreceptive afferents (A δ fibers that respond to intense mechanical stimuli).

Second order neurons

Three classes of neurons relay the information received from the first order neurons at the dorsal horn of the spinal cord via specific tracts to higher centers in the brain (Angevine & Cotman 1981; Fu et al. 2011).

1. Low threshold mechanosensitive neuron (LT) (for light touch, pressure, proprioception);
2. Nociceptive specific neuron (NS) (for noxious stimuli); and
3. Wide dynamic range neuron (WDRN) (Respond to a wide range of stimulus intensities from non-noxious to noxious).

Third order neurons

These neurons relay information received from second order neurons in the thalamus to the somatosensory cortex (Angevine & Cotman 1981; Fu et al. 2011).

Tracts

The spinal tracts that project to the brain (second order neurons) are the neospinothalamic, paleospinothalamic, and archispinothalamic tracts (Angevine & Cotman 1981; Fu et al. 2011).

Neospinothalamic tract

A δ fibers from the periphery (first order neurons) terminate mostly in lamina I (also lamina II) of the dorsal horn. Second order neurons that compose the neospinothalamic tract include long fibres which cross the midline through the anterior commissure and pass upwards in the contralateral anterolateral columns of the spinal cord to ascend towards the ventral area of the thalamus. This is the classic lateral spinothalamic tract arrangement. Third order neurons travel from the thalamus and communicate with the somatic sensory cortex (Angevine & Cotman 1981; Fu et al. 2011). This type of conduction is used for fast pain and is readily localized.

For the face, head, and intraoral structures, conduction happens through the trigeminal tract, which is also a neospinothalamic tract (Fu et al. 2011). The noxious stimulus is transmitted to the trigeminal ganglion from where trigeminal fibers enter the pons, descend to the medulla and synapse in the spinal trigeminal nucleus before crossing the midline and ascending as a trigeminothalamic tract. Typically, A δ fibers go to the ventral thalamus and from there to the somatosensory cortex, providing awareness of the exact location of pain; whereas C fibers terminate in the parafasciculus and centromedian thalamus (known as intralaminar thalamic nuclei [IL]) (Fu et al. 2011).

Paleospinothalamic tract

This tract includes several tracts that synapse in different locations of the brain. The spinoreticular tract synapses in the mesencephalic reticular formation (MRF) and in the periaqueductal gray (PAG); the spinotectal or spinomedullary tract synapses in the tectum; and the spinothalamic tract synapses in the parafasciculus and centromedian thalamus (IL) (Angevine & Cotman 1981; Fu et al. 2011).

For the paleospinothalamic tract, first order neurons synapse at laminae II (also lamina III). Laminae II is known as the substantia gelatinosa. Second order neurons synapse in laminae IV-VIII (V is the most relevant) after leaving laminae II and III. The innervation of the paleospinothalamic tracts is bilateral because some of the ascending fibers do not cross to the opposite side of the cord and continue to run ipsilaterally (Angevine & Cotman 1981; Fu et al. 2011). Third order neurons run from the parafasciculus and centromedian thalamus complex (IL) and synapse bilaterally in the somatosensory cortex. They elicit the emotional and visceral response to pain but also activate the brain stem nuclei to allow descending pain suppression pathways that regulate noxious input at the spinal cord level.

Archispinothalamic tract

This is a multisynaptic diffuse tract in which first order neurons synapse at laminae II (substantia gelatinosa), second order neurons ascend to laminae IV-VII and synapse at the mesencephalic reticular formation (MRF) and the periaqueductal gray (PAG), and third order neurons ascend to the parafasciculus and centromedian thalamus complex (IL) areas of the thalamus and also send collaterals to the hypothalamus and to the limbic

system nuclei to mediate visceral, emotional and autonomic reactions to pain (Angevine & Cotman 1981).

The structural organization of the different tracts determines that the neospinothalamic tract is used for fast pain conduction (pricking pain) and the paleospinothalamic and archispinothalamic tract for slow pain conduction (burning pain), including visceral pain.

Descending monoaminergic pathways that either inhibit or facilitate transmission of nociceptive information at the level of the dorsal horn of the spinal cord also largely modulate nociception (Basbaum & Fields 1984). These monoamines (serotonin, norepinephrine and dopamine) with their direct action on specific receptors exert an intricate modulation of neurotransmitter release from nociceptive afferents and excitability of dorsal horn neurons. This descending modulatory circuit mostly involves the periaqueductal gray (PAG), the rostral ventromedial medulla (RVM) and the spinal cord (Ren & Dubner 2002).

The rostral ventromedial medulla (RVM) includes the nucleus raphe magnus and adjacent reticular formation, which play a major role in descending modulation of spinal nociceptive processes, including opioid analgesia. Three types of cells are present in the RVM and based on their roles on nociceptive modulation, two types of cells are reflex-modulating neurons known as ON and OFF cells and have direct actions on nocifensor reflexes, whereas the third type, called neutral cells appear to have no actions on nociception. ON cells are active during nociceptive processes, allowing a permissive effect of nocifensor reflexes, whereas OFF cells are active during nociceptive processes

in inhibiting nocifensor reflexes (Heinricher et al. 1999). ON and OFF cells project from the nucleus raphe magnus and nucleus reticularis magnocellularis of the RVM to the spinal cord, where they facilitate (ON cells) or suppress (OFF cells) movement in response to noxious stimuli (Fields & Heinricher 1989; Fields et al. 1995; Heinricher et al. 1999; Jinks et al. 2004).

Opioids actions on the RVM induce anti-nociception through a direct mechanism of ON cells inhibition and by two mechanism that involve OFF cells; the first mechanism is recruitment of OFF cells within the RVM in response to systemic opioid administration through increased input from excitatory amino acids (glutamate, aspartate) that act on receptors located in OFF cells, and the second mechanism involves indirect activation of OFF cells through disinhibition that involves inhibition of GABAergic inhibitory input on OFF cells; therefore, ON cells are hyperpolarized by opioids, resulting in suppressed firing and prevention of their pronociceptive role, whereas OFF cells become active in response to systemic opioid administration, facilitating the antinociceptive effects of opioids (Heinricher et al. 1994; Heinricher et al. 1999).

Central sensitization and wind up

The nervous system is capable of undergoing structural and functional changes in response to stimuli from the environment. This ability to change is known as plasticity and it is important to facilitate processes of learning and memory. However, it can also result in abnormal phenomenon such as hyperalgesia from high frequency stimulation that elicits long term potentiation of pre- and post-synaptic changes between 1st and 2nd order neurons at the level of the spinal cord (Cooke & Bliss 2006). At the molecular

level, it involves the expression of neurokinin 1 receptor (NK1) that binds substance P and also the activation of the N-methyl-D-aspartate (NMDA) glutamate receptor and a rise in free intracellular Ca^{2+} (Cooke & Bliss 2006). The NMDA receptor is a ligand-gated and voltage dependent ion channel (Petrenko et al. 2003) primarily activated by endogenous glutamate and to a lesser extent by endogenous aspartate, and requires co-activation with glycine to be fully activated. This receptor plays a key role in the molecular mechanism of central nervous system plasticity in response to pain and learning (Muir 2010). The NMDA receptors are composed of different combinations of NR-1, NR-2 and NR-3 subunits and to be functional it requires at least one combination of the NR-1 and NR-2 subunits (Petrenko et al. 2003).

All three orders of neurons (first, second and third) undergo plastic changes during central sensitization. It has been shown in flexor motor neurons, lamina I and V neurons of the dorsal horn of the spinal cord (A. Cook et al. 1986; Dougherty & Willis 1992), the thalamus (Dostrovsky & Guilbaud 1990) and the anterior cingulate cortex (Wei & Zhuo 2001). With the aid of functional magnetic resonance, researchers have demonstrated, in human subjects, changes during central sensitization also in the periaqueductal gray zone (PAG) (Peyron et al. 2000).

Central sensitization is characterized by a reduction in the stimulation threshold, an expansion of cutaneous receptive fields, and an increase in background activity of spinal neurons. It is usually a consequence of injury to peripheral tissues, but it can also be triggered by windup-inducing repetitive electrical stimulation (Li et al. 1999). Numerous features characterize central sensitization: adaptation of nociceptive-specific neurons to wide dynamic range neurons that now respond to low threshold innocuous and

high threshold noxious stimuli, cumulative increases in the responses generated by a normal series of repeated innocuous stimuli, a wider spatial range of their input, and by changes that continue even after the termination of the triggering event (Ji et al. 2003).

Windup is defined as a progressive, frequency dependent facilitation of the responses of a neuron after the application of repetitive (usually electrical) stimuli of constant intensity (Herrero et al. 2000). Dorsal horn neurons are more easily excitable after the application of a windup-evoking stimulus (Cook et al. 1987). Windup occurs only if stimulation of the nerve or tissues is of sufficient intensity to activate C-fibers and delivered at frequencies greater than 0.3 Hz (Li et al. 1999) and this phenomenon can be inhibited by blockade of the NMDA receptors (Davies & Lodge 1987).

Windup and central sensitization are not equivalent; while windup may be sufficient to cause central sensitization, it is not necessary, meaning that the presence or absence of windup cannot by itself be a marker of the presence of central sensitization (Woolf 1996).

MINIMUM ALVEOLAR CONCENTRATION (MAC)

MAC has been considered since the early 1960s as the standard for assessing and comparing inhaled anesthetic potency. MAC is defined as the end-tidal concentration, expressed as a percentage, of an inhaled anesthetic that prevents movement in response to a supramaximal noxious stimulus in 50% of the studied individuals (Eger & Saidman 1965).

MAC is considered a valuable measurement, especially in recent years, for studying the effects of parenteral anesthetic drugs on reducing requirements of inhalant

anesthetic. Since cardiovascular depression is dose-dependent for inhaled anesthetics, decreasing the required amount with the concomitant use of a parenteral anesthetic benefits in most instances hemodynamic function.

MAC can be determined using the quantal design, which is historically most commonly used in human MAC studies (Sonner et al. 2000). Each individual is exposed to a single inhalant anesthetic concentration and a single noxious stimulus, most commonly skin incision to determine movement (positive) or lack of movement (negative) response. This procedure is repeated in consecutive individuals by increasing or decreasing the delivered anesthetic concentration with respect to the previous individual and the presence of a positive or negative response, respectively. MAC is the mathematical average of values halfway between positive and negative responses. With the application of the quantal study design MAC can be determined for a specific population but not for each individual (Sonner et al. 2000).

A second MAC design is the bracketing technique, which is the most commonly used method in animals and allows MAC determination for each individual. An animal is exposed to an anesthetic concentration that prevents or allows movement in response to a noxious stimulus, and subsequently the opposite response is reached by decreasing or increasing the inhalant anesthetic concentration by 0.1 to 0.2 vol %, respectively (Valverde et al. 2003). MAC for the population is the average of the MAC values for each individual (Sonner et al. 2000).

The effect of an injectable anesthetic drug on the inhalant MAC sparing effect has been determined using two methods. The first method involves determining MAC for the inhalant anesthetic alone, followed by the administration of the injectable anesthetic of

interest to determine its sparing effect, which is referred to from now on as same day determination. The second method known consists of obtaining the MAC value for the inhalant anesthetic alone and the MAC that represents the sparing effect of the injectable anesthetic drug of interest on two separate experiments done on different days, referred to as separate day determination. There is no clarity if both methods are interchangeable.

Many factors can potentially affect MAC values obtained for a species. They include: circadian rhythm, age, gender, type of noxious stimulus, hypothermia, depressed cardiovascular function, hypercarbia ($\text{PaCO}_2 > 95$ mm of Hg), extreme hypoxemia ($\text{PaO}_2 < 38$ mm of Hg), pregnancy and metabolic acidosis. In addition, the type of noxious stimulation can affect MAC values (Eger & Saidman 1965; Quasha et al. 1980; Valverde et al. 2003). Skin incision is considered a supramaximal noxious stimulus in human and compares to electrical stimulation (Quasha et al. 1980); however in animals skin incision cannot be considered as a supramaximal stimulus (Valverde et al. 2003) and clamping of the extremities or electrical stimulation are commonly used (Eger & Saidman 1965; Eger et al. 1988; Valverde et al. 2003). Supramaximal noxious stimuli suitable for MAC determinations in animals include: clamping the extremities or tail with a non-traumatic hemostat delivered until the response (positive or negative) is established or use of electrical stimulation (30 to 50V at 50 cycles/s for 10 milliseconds) through needles inserted in the cheek, limbs or sensitive mucosa (Valverde et al. 2003).

MAC and pain

All mechanisms involved in pain transmission and modulation play a pivotal role on the outcome of MAC studies since nociception is the major endpoint of MAC, despite

the fact that anesthetic drugs without analgesic actions also can cause reductions in MAC through central nervous system depression. In spite of the widespread use of this technique in human and animal studies it is not fully understood how inhalant anesthetics suppress purposeful movement (You et al. 2004). Ligand-gated ion channels, including GABA(A) receptors, glycine receptors, neuronal nicotinic acetylcholine receptors, and glutamate receptors, as well as voltage gated ion channels, including calcium channels, potassium channels, and sodium channels, are all molecular targets for various general anesthetics that contribute to the observed effects of inhalant and injectable anesthetics and influence the observed response during MAC determinations (You et al. 2004). All clinically used inhalant anesthetics inhibit currents conducted by the alpha subunit of the sodium 1.4 voltage gated sodium channel, this is consistent with the role of sodium channels in the mechanism of anesthetic immobilization.

MAC values, represented by lack of movement in response to a supramaximal noxious stimulus, are dependent on spinal and supraspinal interactions (Jinks et al. 2004; Jinks et al. 2010). Facilitation or inhibition activity occurring in the brainstem and spinal cord account for movement or no movement, respectively, in response to supramaximal noxious stimulation. At the spinal level, the proposed mechanism involves activation of primary afferent nociceptors, which in turn activate nociceptive dorsal horn neurons, followed by activation of inhibitory and excitatory neurons in the ventral horn of the spinal cord, until rhythmic motoneuron discharges are elicited, leading to organized movement. At 1 MAC concentration or higher, the activity of ventral horn neurons is inhibited, preventing movement. At the brainstem level, peri-MAC concentration inhibition of movement in response to supramaximal noxious stimulation is the result of

reduced facilitation activity of the mesencephalic locomotor region on the ventral horn neurons (motoneuron) responsible for organized movement (Jinks et al. 2010). Descending modulation from the rostral ventromedial medulla (RVM) on the dorsal horn also contributes to preventing or facilitating movement through the actions of ON and OFF cells (Fields & Heinricher 1989; Fields et al. 1995; Heinricher et al. 1999; Jinks et al. 2004). Supramaximal noxious stimulation enhances the activity of ON cells and depresses the activity of OFF cells, facilitating the occurrence of reflex movement (Fields & Heinricher 1989). Contrary to what occurs near peri-MAC concentrations, at which descending modulation of nociceptive processing is inhibited, at sub-MAC isoflurane concentrations descending modulation remains functionally intact and permits movement in response to supramaximal noxious stimulation (Jinks et al. 2003; Jinks et al. 2004). Administration of injectable anesthetic drugs, such as morphine, can modify this response by depressing ON cells and facilitating OFF cells (Barbaro et al. 1986; Heinricher et al. 1999), so that sub-MAC inhalant anesthetic concentrations achieve 1 MAC concentrations from the interaction of the inhalant and injectable anesthetic, therefore preventing movement. This is known as the MAC sparing effect of injectable anesthetics.

KETAMINE

Ketamine is a phencyclidine anesthetic that exerts a non-competitive antagonist of the ionotropic glutamate NMDA receptor (Thomson et al. 1985). Historically ketamine has been used in various species as a short acting general anesthetic and at low doses it is currently used for its analgesic properties as an adjuvant during general anesthesia and in

the conscious patient (Pedraz et al. 1985). The clinical use of ketamine was first reported in 1966 (Domino et al. 1966) and its use as a single anesthetic drug resulted in unpleasant emerge reactions, inadequate relaxation, and cardiovascular stimulant properties, which limited its clinical use. The anesthetic state produced by ketamine is described as a functional and electrophysiological dissociation between the thalamocortical and limbic systems. Ketamine as the sole anesthetic produces a cataleptic state with nystagmus and intact corneal and light reflexes (Reich & Silvay 1989).

Effects on pain, CNS, cardiovascular

The analgesic actions of ketamine can be attenuated by the opioid receptor antagonist naloxone, which suggests the involvement of opioid receptors, alongside with the NMDA antagonism, in the mechanism of ketamine-induced analgesia (Sarton et al. 2001). It has been suggested that ketamine is an antagonist at the μ -receptor and agonist at the κ -receptor (Smith et al. 1980; Hustveit et al. 1995). This may also explain the mild respiratory depression associated with ketamine (Bourke et al. 1987).

Ketamine also has a local anesthetic effect, especially at higher doses or when administered intrathecally or epidurally, more likely through the same mechanism of sodium channel blockade action of local anesthetics (Dowdy et al. 1973). Sub-anesthetic doses of ketamine are analgesic (Nielsen et al. 1992; Arendt-Nielsen et al. 1995; Schmid et al. 1999; Wagner et al. 2002). Low doses of ketamine can inhibit central temporal summation by blocking of a previously opened NMDA receptor through nociceptive stimulation (Arendt-Nielsen et al. 1995). These findings stress the current theory that NMDA receptors are not significantly involved in the response to acute nociception

evoked by stimulation of normal somatic pain pathways and is part of the reason as to why ketamine seems to be effective in chronic pain but fails to be effective as a preemptive analgesic if used alone without prior noxious stimulation (Chang et al. 2011).

Sub-anesthetic doses of ketamine used as an adjunct to general anesthesia reduce pain and opioid requirements in the postoperative period (Himmelseher & Durieux 2005). The analgesic effects of ketamine are less evident when other analgesic techniques are also used, such as epidural anesthesia that masks the benefits of ketamine, and also when low single bolus doses are used without a constant rate infusion, which may not result in a steady state of plasma concentrations (Ilkjaer et al. 1998; Dahl et al. 2000).

Ketamine is considered to have a different effect on somatic and visceral pain (Olivar & Laird 1999). Visceral acute pain models using colorectal, esophageal, and ureter or urinary bladder distention (McRoberts et al. 2001; Castroman & Ness 2002; Strigo et al. 2005) have shown that the involvement of the NMDA receptor appears to be more relevant in the transmission of acute nociceptive visceral pain than acute nociceptive somatic pain, especially in animals (Castroman & Ness 2002; McRoberts et al. 2001). It has been suggested that ketamine's actions on visceral pain are mediated through the periaqueductal gray descending inhibitory system, whereas somatic pain involves inflammation and hyperalgesia and its mediated through NMDA blockade (Olivar & Laird 1999; Kawamata et al. 2000; Strigo et al. 2005). It is possible that ketamine activates the monoaminergic descending inhibitory system by interaction with opioid receptors at a supraspinal level (Kawamata et al. 2000).

Ketamine's ability to stimulate the cardiovascular system is attributed to several factors, including its effects on increasing PaCO₂ and/or decreasing PaO₂ since the

increases in heart rate, systemic arterial blood pressure and vascular resistance have been demonstrated in studies without controlling ventilation. In vitro studies have demonstrated a direct inhibition of the contractile force of the myocardium, that is usually masked by an increase in sympathetic drive in individuals with normal cardiovascular function (Saegusa et al. 1986). The current theory is that ketamine causes an increase in central catecholamine levels mainly by inhibiting their reuptake (Lundy et al. 1986). Additionally, ketamine is an antagonist at the acetylcholine receptors; acetylcholinesterase inhibitors reduce the duration of ketamine anesthesia without affecting its metabolism or clearance (Aronstam et al. 1982).

Ketamine administration as a general anesthetic or an adjunct to inhalant anesthesia can maintain stable hemodynamic function due to sympathetic stimulation in patients with acute hypovolemic shock (Idvall 1981; Hemmingsen & Nielsen 1991; Duque et al. 2005). Heart rate, blood pressure, and cardiac output are minimally affected or even increase when ketamine is administered to hypovolemic patients (Hemmingsen & Nielsen 1991; Duque et al. 2005). However in one study, cardiac output decreased more during hemorrhage and hypovolemia in dogs anesthetized with ketamine than in those anesthetized with isoflurane. Higher lactate concentrations were also detected in the dogs anesthetized with ketamine since oxygen demands were probably not met with this protocol (Weiskopf et al. 1981).

Pharmacokinetics

Commercially available ketamine is a chiral compound. It is the racemic mixture of the two optical isomers (R)-ketamine and (S)-ketamine, the (S)-ketamine enantiomer is

considered to have a greater affinity for the NMDA receptor and to be more potent (Muir 2010). These two enantiomers are N-demethylated into R- and S-norketamine by the liver by cytochrome P-450 enzymes. The percentage of this metabolism varies between species (Muir 2010). The pharmacokinetics of ketamine have been reported for numerous species. In rabbits it has been reported for healthy rabbits and rabbits with impaired renal function. The plasma half-life of ketamine in healthy rabbits is 44 minutes and increases dramatically when renal function is impaired. The volume of distribution for the central compartment in rabbits is very high (4.75 L/kg) but this probably does not really reflect the physiological distribution because the plasma protein binding is affecting it (Pedraz et al. 1985). In dogs the plasma terminal half-life is 94 minutes, while the volume of distribution and the clearance are 371.3 mL/kg and 58.2 mL/kg/min, respectively (Pypendop & Ilkiw 2005).

The relationship between ketamine blood concentrations and MAC in dogs has been reported in three independent studies using either enflurane, isoflurane or sevoflurane (Schwieger et al. 1991; Pypendop et al. 2007; Love et al. 2011). In two of the studies, traditional MAC (prevention of movement) was the endpoint (Schwieger et al. 1991; Pypendop et al. 2007); while MAC to block autonomic responses (MAC-BAR) was used in the other study (Love et al. 2011). In the studies that involved MAC-movement, ketamine decreased in a dose-dependent manner according to plasma concentrations (Schwieger et al. 1991; Pypendop et al. 2007). Fifty percent of the maximal MAC reduction induced by ketamine is expected to be reached at a plasma ketamine concentration of approximately 3,000 ng/mL (Pypendop et al. 2007). For MAC-BAR, clinical doses of ketamine (12.5, 25 and 50 µg/kg/min) decreased MAC-

BAR of sevoflurane not in a dose dependent or linear fashion, since ketamine may have altered arterial blood pressure and heart rate due to its cardiovascular stimulant properties and made it difficult to assess the increases in heart rate and blood pressure associated with noxious stimulation (Love et al. 2011).

RATIONALE, HYPOTHESIS AND OBJECTIVES

MAC studies that determine the effect of an injectable anesthetic drug on the MAC value for a specific inhalational anesthetic have been completed using two methods, as described previously in this review. The comparison of the two methodologies has not been reported in a controlled study and there is no knowledge if both methods are interchangeable. The objective of this study was therefore two-fold; first, to determine the sparing effect of ketamine, on the MAC of isoflurane in rabbits and dogs, and second to determine if the analgesic characteristics of ketamine yields different results when both MAC methods of same day or separate day determinations are used. Our hypotheses were that ketamine causes a significant decrease in the MAC of isoflurane in rabbits and dogs and that both methods of MAC determination yield similar results.

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CHAPTER II

COMPARISON OF THE SPARING EFFECT OF KETAMINE ON THE ISOFLURANE MINIMUM ALVEOLAR CONCENTRATION (MAC) DURING SAME DAY OR SEPARATE DAY EXPERIMENTS IN RABBITS

SUMMARY

Objective- To compare the sparing effect of ketamine on the MAC of isoflurane when administered after or without MAC determination of isoflurane alone in rabbits.

Study Design- Prospective randomized crossover study.

Animals- Eight adult female New Zealand rabbits.

Methods- Rabbits were randomly allocated to two treatment groups, 4-6 days apart. General anesthesia was induced using isoflurane in oxygen via mask. Group 1 (same day determination) had the MAC sparing effect of ketamine (1 mg/kg bolus followed by a CRI of 40 µg/kg/min; IV) determined after previous MAC determination of isoflurane alone. A third MAC determination was started 30 min after stopping the CRI. Group 2 (separate day determination) had the MAC sparing effect of ketamine determined without previous MAC determination of isoflurane alone. A second MAC determination was started 30 min after stopping the CRI.

Results- In group 1 the MAC of isoflurane ($2.15 \pm 0.09\%$) was significantly reduced by ketamine ($1.63 \pm 0.07\%$). The MAC after stopping the CRI was significantly lower ($2.04 \pm 0.11\%$) than the MAC of isoflurane and significantly higher than the MAC during the CRI. In group 2, ketamine reduced isoflurane MAC ($1.53 \pm 0.22\%$), which increased significantly ($1.94 \pm 0.25\%$) after stopping the CRI. There was no significant difference

between groups for MAC values during ketamine administration or after stopping ketamine.

Conclusion and clinical relevance- Prior MAC determination of isoflurane alone did not affect the MAC sparing effect of ketamine. Both methods for MAC determination do not appear to affect results.

INTRODUCTION

MAC is defined as the end-tidal concentration of an inhalant anesthetic that prevents purposeful movement in 50% of the study population in response to a supramaximal noxious stimulus and is considered the standard method to compare potency of inhalant anesthetics since its introduction in the 1960's (Eger & Saidman 1965). The MAC method is a useful tool to quantify the inhalant sparing properties of injectable anesthetic drugs that possess analgesic and/or sedative properties.

The noxious stimulus consists of clamping of the tail or limbs with a pressure device or includes the use of electrical current delivered to the oral mucosa or subcutaneous tissue of the limbs (Valverde et al. 2003). The application of the noxious stimulus is repetitive during MAC determinations and it is possible that the type of noxious stimulus and its intensity can cause local tissue trauma and associated activation or inactivation of specific pain conduction mechanisms (Laster et al. 1993). A short interval between application of the noxious stimulus or an increase in the intensity of the stimulus can generate movement by inducing temporal summation (Dutton et al. 2003) and affect values obtained during MAC studies since purposeful movement is the endpoint during MAC determinations. Therefore, the timing and intensity of stimulation during MAC determinations should be considered.

Historically, MAC studies that investigate the sparing effect of injectable anesthetic drugs have been designed using one of two distinct methods. The first method (same day determination) (Golder et al. 1998; Valverde et al. 2004; Pascoe et al. 2007) consists of determining MAC for the inhalant anesthetic alone, followed immediately by administration of the injectable anesthetic or analgesic drug of interest to determine its

sparing effect. The second method (separate day determination) (Pypendop et al. 2006; Seddighi et al. 2009; Ferreira et al. 2009) consists of obtaining the MAC value for the inhalant and the inhalant/injectable anesthetic combination during different experiments on separate days; a variation of this method is to use a population mean MAC that has already been determined for that specific population in a separate study (Pypendop et al. 2007). There is no scientific evidence supporting the hypothesis that these two methods are equivalent. On the contrary there is conflicting information; for example, cats that received epidural morphine in two independent studies yielded different results when inhalant and inhalant/epidural morphine MAC values were determined during the same day or separate days. There was a significant reduction in isoflurane requirements after administration of epidural morphine if the MAC determinations were completed on the same day (Golder et al. 1998) whereas no difference if each MAC was determined individually on different days (Pypendop et al. 2006). Despite some differences in the design of those two studies, it was also suggested that a plausible justification for these results may be that in order to obtain a MAC sparing effect of an analgesic drug, such as morphine, it is necessary to induce prior activation of pain pathways from repetitive noxious stimulation (Fields 2004), and this may have occurred in the study that determined the MAC of isoflurane alone.

The MAC sparing effect of ketamine has been reported in several animal species, including dogs (Solano et al. 2006; Love et al. 2011), cats (Pascoe et al. 2007), and horses (Muir & Sams 1992), but not in rabbits. The comparison of the two methodologies used to determine the sparing effect of injectable anesthetics has not been reported in a controlled study. The objective of this study was therefore two-fold; first, to determine

the sparing effect of ketamine, on the MAC of isoflurane in rabbits, and second to determine if the analgesic characteristics of ketamine yields different results when both MAC methods of same day or separate day determinations are used. Our hypotheses were that ketamine causes a significant decrease in the MAC of isoflurane in rabbits and that both methods of MAC determination yield similar results.

MATERIALS AND METHODS

Animals

Eight female New Zealand rabbits, approximately one year of age and weighing 4.1 to 4.7 kg were used in this study. All animals had normal physical examination findings before inclusion in the study but no other diagnostic test was done to confirm their normal health status. Rabbits were randomly allocated in two treatment groups using a cross over design with 4 to 6 days between treatments. Food and water were available ad libitum prior to anesthesia. The Institutional Animal Care and Use Committee of the University of Guelph approved the study.

Anesthesia and instrumentation

Anesthesia was induced via a face mask with isoflurane (Forane, Baxter Corporation, Mississauga, Ontario, Canada) in 100% oxygen using a coaxial non-rebreathing system (Bain Circuit) with an oxygen flow rate of 200 to 300 mL/kg/min. Once the rabbit was unconscious it was positioned in right lateral recumbency and blindly intubated with a low pressure high volume cuffed endotracheal tube. Intermittent positive pressure ventilation was immediately established using an electronically controlled, time-cycled, pressure limited ventilator (EMC 2000, Hallowell, Massachusetts, USA), with a rate of 10 to 12 breaths/min and a tidal volume of 10 to 15 mL/kg in order to maintain end-tidal CO₂ between 30 and 40 mmHg. Rabbits were instrumented for monitoring during the first 30 min of anesthesia while at approximately 2.5% end-tidal isoflurane concentration. Monitoring included end-tidal concentrations of isoflurane and CO₂ using a side-stream infrared gas analyzer (Datex-Ohmeda S/5™

Anesthesia monitor, GE Healthcare Finland, Helsinki, Finland) attached between the endotracheal tube and the breathing system, with a sampling rate of 200 mL/min. The anesthesia monitor was calibrated each morning using a calibration gas specifically designed for this purpose (DOT-34 NRC 300/375M1014, Datex-Ohmeda Division, Helsinki, Finland). A digital thermometer was used to monitor rectal temperature every 10 min and maintained between 37 and 38.8 °C with the use of a fan heater device (Lancaster, Trileaf Distribution, Toronto, Ontario, Canada). Cardiovascular parameters including direct arterial blood pressure in some rabbits (n=2/group) or indirect systolic arterial blood pressure (Doppler) in all rabbits, electrocardiogram and heart rate were monitored (Datex-Ohmeda S/5™ Anesthesia monitor, GE Healthcare Finland, Helsinki, Finland) throughout the duration of the experiment and recorded before and after each MAC determination.

The left auricular vein and in some rabbits also the left auricular artery were catheterized with a 22-SWG (1.00 IN) catheter (BD Insyte-W, BD Infusion Therapy Systems Inc. Sandy, Utah USA) and a 24-SWG (0.75 IN) catheter (BD Insyte-W, BD Infusion Therapy Systems Inc. Sandy, Utah USA), respectively. The venous access was used to administer the treatment drugs and a balanced electrolyte solution (Plasmalyte A- Baxter Corporation, Mississauga, Ontario, Canada), mixed in the same syringe, at 3 mL/kg/hour with the use of a syringe pump (Graseby 3500 Anesthesia Pump, Smiths Medical International Ltd, Watford, Herts, UK); the arterial access was used to measure direct blood pressure and verify accuracy of the Doppler readings, with the use of an electronic pressure transducer (Becton Dickinson Infusion Therapy Systems, Sandy, Utah, USA) zeroed at the level of the sternum and interfaced with the anesthesia monitor.

The arterial catheter was also used for periodical sampling into heparinised syringes (Gastlyte, Marquest Medical Products, Englewood, Colorado, USA) for blood gas determination (CCX, Nova Biomedical, Waltham, Massachusetts, USA) to verify the end-tidal CO₂ readings.

MAC determinations

MAC was determined using a previous published technique (Valverde et al. 2003). The noxious stimulation consisted of 50 Volts at 50 cycles/sec for 10 milliseconds (S48 Stimulator, Astro-Medical Inc, West Warwick, Rhode Island, USA) applied SC by inserting two 25-SWG hypodermic polypropylene hub; 0.75 IN needles (Tyco Healthcare Group LP, Mansfield, Massachusetts, USA) 3 cm apart applied to the left forelimb and at the level of the ulna. The sequence of noxious stimulation consisted of applying 2 single stimuli followed by 2 continuous stimuli applied for 2 to 3 seconds, with 5-second intervals between all 4 stimuli. If gross purposeful movement, which consisted of jerking or twisting motion of the head or motion of the extremities (running motion and/or motion of a non-stimulated limb), was elicited at any time during the cycle the stimulation was immediately suspended and the event was recorded as a positive response. Subsequently the end-tidal isoflurane concentration was increased by 0.1%; conversely, if a negative response was obtained initially, then the end-tidal concentration was decreased by 0.1%. The noxious stimulation was repeated after 15 to 20 min of equilibration at the new concentration and if the opposite response to the previous stimulation was obtained then the end-tidal concentration was returned to the initial value, following a 15 to 20 min equilibration process, to verify that the first response was

identical to the one obtained previously at that concentration (duplicate MAC measurement). This procedure was repeated until opposite responses between immediate end-tidal concentrations of isoflurane were obtained in duplicate. The value midway between the lowest end-tidal concentration that prevented purposeful movement and the highest end-tidal concentration that allowed it was recorded as the MAC value.

Experimental design

After the instrumentation phase, rabbits assigned to group 1 (same day MAC determinations) had the end-tidal isoflurane concentration decreased to approximately 2.0%, which corresponds to the MAC value reported for rabbits (Drummond 1985; Sobair et al. 1993; Valverde et al. 2003) and maintained for at least 30 min to establish equilibration before starting MAC determinations while receiving a bolus and CRI of a balanced electrolyte solution at the same volume rate as the ketamine infusion to be used later on. Rabbits assigned to group 2 (separate day MAC determination) had the end-tidal isoflurane concentration decreased to approximately 1.7% for the same time duration (30 min) after receiving a bolus of ketamine (Vetalar, Bioniche Animal Health Canada Inc, Belleville, Ontario, Canada) of 1 mg/kg IV followed immediately by a CRI at 40 µg/kg/min using a syringe pump (Graseby 3500 Anesthesia Pump, Smiths Medical International Ltd, Watford, Herts, UK). The CRI infusion was maintained until the MAC determination was completed.

After the first MAC determinations were completed in each group, rabbits in group 1 were administered the ketamine bolus and CRI, as described above, and the end-tidal isoflurane concentration decreased to 1.7% and after 30 min of the infusion the

MAC redetermined. Rabbits in group 2 had the CRI of ketamine stopped, the end-tidal isoflurane concentration was increased to 2.0% during this time and the MAC determinations started once again 30 min later while receiving an infusion of a balanced electrolyte solution at the same volume and rate as the previous ketamine infusion. Rabbits in group 2 were allowed to recover from anesthesia once the second MAC determination was completed. Rabbits in group 1 had the CRI of ketamine stopped and replaced by an infusion of a balanced electrolyte solution at the same volume and rate as the previous ketamine infusion after the second MAC determination. The end-tidal isoflurane concentration was increased to 2.0% during this time and the MAC determinations started once again 30 min later for a third time and then allowed to recover from anesthesia. All rabbits were administered meloxicam (Metacam, Boehringer Ingelheim, Burlington, Ontario, Canada) 0.2 mg/kg, IV during recovery.

The time required to complete each MAC determination was recorded as the time elapsed from the beginning of the equilibration phase until the MAC value was determined. Total anesthesia time corresponded to the time from connecting the rabbit to the anesthetic machine until completion of the last MAC determination.

Data analysis

The MAC values were calculated by use of mathematical averaging of two subsequent concentrations of inhalant anesthetic at which gross purposeful movement and no gross purposeful movement were observed. Statistical comparison between the two groups and within groups were carried out for MAC values induced by ketamine administration, MAC values after stopping ketamine's administration and time of MAC

determinations using an ANOVA with Bonferroni correction, after verification of normality of the data by use of the D'Agostino-Pearson and Kolmogorov-Smirnov test (MedCalc Software; version 11.2.1, Mariakerke, Belgium). The MAC values and time of determination are reported as mean \pm SD. Indirect systolic blood pressure, end-tidal CO₂ concentrations, heart rate and rectal temperature throughout the study were compared between groups using an independent samples t test. Values of $P < 0.05$ were considered significant.

RESULTS

All rabbits had cardiorespiratory parameters within acceptable limits for MAC determinations and recovered uneventfully from the experiments. End-tidal CO₂, heart rate, indirect systolic blood pressure, and rectal temperature values were 34 ± 2.8 mmHg, 295 ± 29 beats/min, 78 ± 12 mmHg, and 38.8 ± 0.6 °C, respectively for group 1 and 34 ± 3.7 mmHg, 292 ± 39 beats/min, 74 ± 8 mmHg and 38.5 ± 0.7 °C, respectively for group 2, throughout the experiment.

The MAC for isoflurane was $2.15 \pm 0.09\%$ (group 1). In both groups, ketamine administration resulted in a lower MAC value than the baseline MAC value for isoflurane obtained in group 1 (Table 2.1). In both groups the MAC value after discontinuation of ketamine was lower than the MAC for isoflurane obtained in group 1, although not significantly different, but significantly higher within each group than the MAC value obtained during the ketamine CRI (Table 2.1). There was no difference between groups 1 and 2 in MAC values after ketamine administration or in MAC isoflurane values after discontinuation of ketamine.

There were no significant differences in times required for MAC determinations between the two groups. The time of ketamine CRI administration corresponds to the same time as for MAC determination of the isoflurane and ketamine combination (Table 2.1).

DISCUSSION

The results of this study determined that ketamine has a sparing effect on isoflurane MAC values in rabbits. The two methods, same day determination and separate day determination did not yield different results in the isoflurane MAC value obtained with the administration of a ketamine CRI. This is in contrast to two independent studies in which epidural morphine had a sparing effect after prior determination on the same day of the MAC of isoflurane alone (Golder et al. 1998) but no effect when the MAC of isoflurane was determined on a separate day from the MAC of isoflurane combined with epidural morphine (Pypendop et al. 2006).

Several factors may contribute to the variability in MAC values obtained in different studies and their sometimes contradictory results, including methodology used for MAC determinations and repeatability of MAC determinations. Plasticity of pain pathways (Woolf 2007) from repetitive noxious stimulation is likely and often not considered as a process that could affect MAC determinations. In addition, individual variation due to genetics has also been determined to influence MAC values (Sonner et al. 2000; Barter et al. 2004), which is rarely considered in studies. Using 15 different mouse strains with different genotypes, variations in MAC values for desflurane, isoflurane and halothane were 39%, 44%, and 55%, respectively (Sonner et al. 2000); however such variations have not been determined in other species.

Few studies have determined MAC values for isoflurane in rabbits, but a variation of up to 22% is noted (2.04-2.08% (Valverde et al. 2003), 2.49% (Turner et al. 2006), 2.05% (Sobair et al. 1993), and 2.07% (Drummond 1985) despite expected intra- and interspecies variations of 10-20% (Eger 1974; Quasha et al. 1980). This variation is also

present with halothane, up to 42% for rabbits (0.82% to 1.42%) (Davis et al. 1974; Drummond 1985; Sobair et al. 1993; Valverde et al. 2003). It is also common to other species with different inhalant anesthetics, up to 22% for halothane in dogs (0.81% to 1.04%) (Eger & Saidman 1965; Davis et al. 1974; Himes et al. 1977; Valverde et al. 1989; Quandt et al. 1994; Valverde et al. 2003) and up to 42% for isoflurane (1.27% to 1.80%) (Steffey 1977; Troncy et al. 1996; Hellyer et al. 2001; Valverde et al. 2003; Kushiro et al. 2007; Ueyama et al. 2009), whereas in cats variations of up to 56% for isoflurane are reported (1.24% to 1.94%) (Steffey 1977; Ilkiw et al. 2002; Barter et al. 2004; Brosnam et al. 2009; Ferreira et al. 2009).

In our study, variability was minimized by using a crossover design that involved the same animals acting as their own controls; therefore factors such as age, gender and variations in the interpretation of movement in response to noxious stimulation can be excluded, whereas other factors that may affect MAC determinations such as type of noxious stimulation, body temperature, cardiovascular status and blood gases were maintained constant throughout the study (Eger & Saidman 1965; Quasha et al. 1980; Valverde et al. 2003). Indirect blood pressure (Doppler) values were considered acceptable for rabbits, although lower than for other species. Average mean blood pressures in conscious rabbits correspond to 80 mmHg (van den Buuse & Malpas 1997) and are expected to be significantly lower in the anesthetized rabbit. Doppler blood pressures tend to be lower than direct readings in cats (Klevans et al. 1979; Grandy et al. 1992) and it is likely that due to the size of the rabbits similar findings are expected and values are therefore underestimated. In fact, direct blood pressure readings obtained simultaneously to Doppler pressures in this study were slightly higher, but not used in the

analysis due to the low number of rabbits that had arterial catheters (2/group). Other studies, using cats have also recorded relatively low systolic blood pressures (71 ± 8 mmHg) during MAC studies (Barter et al. 2004), which are most likely the result of underestimation of Doppler readings.

The MAC method used in this study consisted of bracketing inhalant concentrations up-and-down by 0.1 to 0.2%. We used 0.1% consistently to avoid variability since MAC values reported often use combinations (Credie et al. 2010), or 0.1% (Valverde et al. 2003), or 0.2% (Yamashita et al. 2009). The use of single, duplicate or even triplicate measurements during MAC determinations is also optional and not clearly described by researchers in their methodology; therefore values derived from single measurements that are not corroborated within the study may not be equivalent to those confirmed from more than one response.

Differences in MAC values are also related to the type and intensity of noxious stimulation that may result in desensitization of nerve fibers or in changes in plasticity that lead to sensitization. Both sensitization and desensitization have been associated with trauma from repetitive noxious stimulation either by enhanced tissue sensitivity and plasticity of nociceptive mechanisms or by damage and perineural inflammation that prevents impulse conduction (Sobair et al. 1993). Increasing the voltage (10, 15, 20 and 40 Volts) during electrical stimulation results in desensitization after fewer attempts with higher voltages so that a positive response becomes negative sooner (Dutton et al. 2003), despite MAC values being slightly higher for the higher voltage (Laster et al. 1993). In the original description of MAC methodology, 10 Volts was considered a submaximal

noxious stimulus but 30 and 50 Volts were both supramaximal and equivalent (Eger & Saidman 1965).

The method of MAC determination used in this study is a variation of the original described method in which the electrical stimulus of 50 Volts at 50 cycles/sec for 10 milliseconds was applied (Eger & Saidman 1965; Quasha et al. 1980); however the duration and mode of stimulation was altered according to a validation study in which the stimulus is applied using a sequence of 2 single stimuli followed by 2 continuous stimuli applied for 2 to 3 seconds, with 5-second intervals between all 4 stimuli (Valverde et al. 2003), instead of applying one single stimulus for up to 60 sec or less if a positive response is observed. This variation was chosen since it compared well to clamping techniques and minimizes trauma to the tissue (Valverde et al. 2003), which may avoid plasticity changes from repetitive stimulation.

Another important factor to take into account in MAC studies is the likelihood of developing sensitization from frequent stimulation of single synapses. This can eventually result in temporal summation, which contributes in part to the movement produced in response to noxious stimulation. Under normal circumstances, lack of movement may result from suppression of mechanisms underlying the summation process itself since blockade of temporal summation decreases the isoflurane concentration required to suppress movement (Dutton et al. 2003). Sensitization originates from intense stimulation during temporal summation to multiple fibers that synapse in the same post-synapses, which results in spatial summation. Both temporal and spatial summation mechanisms can lead to “wind-up” and “central sensitization” (You et al. 2004), and influence the results obtained in MAC studies. Temporal

summation occurs by shortening the interval between stimuli and/or the intensity of the stimulus and since temporal summation leads to movement, which constitutes the endpoint for assessment during MAC studies, it is feasible that values can be overestimated during those studies where conditions for sensitization have occurred. Theoretically, sensitization is more likely to occur when using submaximal MAC levels since isoflurane produces immobility in part by disrupting mechanisms underlying temporal summation, which implies that an insufficient amount of isoflurane will facilitate a response and movement (Dutton et al. 2003).

The choice to use of ketamine to test our hypothesis in this study may have influenced the results, since wind-up and eventually central sensitization depend on activation of NMDA receptors (Schaible et al. 1991; Svendsen 1999). Ketamine, as an NMDA antagonist, has the ability to block these receptors and prevent sensitization from repeated stimulation. In addition, ketamine through its glutamate receptor blockade can prevent ON-cell activity from the rostral ventromedial medulla and inhibit descending locomotor responses to repeated noxious stimuli (Jinks et al. 2007) despite the associated increases in withdrawal force at the site of stimulation with stimulus intensity and ON-cell activity (Jinks et al. 2004).

The variability in MAC values is also present for reported values on the sparing effect of injectable anesthetic or analgesic drugs. Route and mode of administration of injectable drugs are major factors that influence MAC results due to specific pharmacokinetic properties of absorption, plasma concentrations and half-life of each drug (Toutain & Bousquet-Melou 2004a; Toutain & Bousquet-Melou 2004b). MAC determinations, especially when done in duplicates or triplicates, take a considerable

amount of time and depending on the route and mode of administration of the injectable anesthetic, MAC may not be completed at plasma concentrations that reflect a steady-state. In this regard, constant rate infusions should be preferred over single or repeated boluses during MAC studies; however a majority of the published information on MAC sparing effect of injectable drugs has been derived from single dose injections and it is likely that variations in MAC sparing values are due to different plasma and tissue concentrations. The half-life of ketamine in conscious rabbits is 0.74 h (Pedraz et al. 1985) but because anesthesia may influence the disposition (clearance) of ketamine it is not known from this study if plasma concentrations for ketamine were at steady-state when MAC determinations were completed during ketamine administration (75 and 103 min in groups 1 and 2, respectively). The MAC values determined at approximately 80 min in both groups after discontinuing the CRI of ketamine were also lower than the initial MAC value for isoflurane in group 1, although not statistically. It is possible that not sufficient time was allowed to lower plasma ketamine concentrations to levels that had no influence on MAC or that MAC decreases overtime in rabbits as it has demonstrated for other species (Petersen-Felix et al. 1993; Barter et al. 2006; Stratmann et al. 2009).

In conclusion, ketamine had a significant sparing effect on the MAC of isoflurane in rabbits and it does not appear that the MAC methodology used to determine such effect (same day versus separate days) affects the results.

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Table 2.1 MAC values and time for MAC determinations after ketamine administration (1 mg/kg bolus IV followed immediately by a CRI of 40 µg/kg/min) in 8 rabbits with same day prior isoflurane MAC determination (Group 1) or without same day prior isoflurane MAC determination (Group 2).

	Isoflurane baseline	Isoflurane + ketamine	After stopping ketamine	Total anesthesia time (min)
Group 1				
(Same day)				
MAC (%)	2.15 ± 0.09	1.63 ± 0.07 * (P < 0.0001)	2.04 ± 0.11 † (P < 0.0001)	
Time (min)	80 ± 26	75 ± 16	80 ± 14	249 ± 15
Group 2				
(Separate days)				
MAC (%)	ND	1.53 ± 0.22 * (P < 0.0001)	1.94 ± 0.25 † (P < 0.0001)	
Time (min)	ND	103 ± 37	81 ± 20	205 ± 56

Values expressed as mean ± SD. ND = not determined. * Significant difference with isoflurane baseline. † Significant difference with isoflurane + ketamine.

CHAPTER III

COMPARISON OF THE SPARING EFFECT OF KETAMINE ON THE ISOFLURANE MINIMUM ALVEOLAR CONCENTRATION (MAC) DURING SAME DAY OR SEPARATE DAY EXPERIMENTS AND CORRELATION WITH PLASMA KETAMINE CONCENTRATIONS IN DOGS

SUMMARY

Objective- To compare the sparing effect of ketamine on the MAC of isoflurane when administered before or after MAC determination of isoflurane alone in dogs and to correlate it with plasma ketamine concentrations.

Study Design- Prospective randomized crossover study.

Animals- Three male and 5 female adult mixed-breed dogs.

Methods- Dogs were randomly allocated to two treatment groups 5 days apart. General anesthesia was induced using isoflurane in oxygen via mask. Group 1 (same day determination) had the MAC sparing effect of ketamine (1 mg/kg bolus followed by a CRI of 40 µg/kg/min; IV) determined after previous MAC determination of isoflurane alone. A third MAC determination was started 30 min after stopping the CRI. Group 2 (separate day determination) had the MAC sparing effect of ketamine determined without previous MAC determination of isoflurane alone. A second MAC determination was started 30 min after stopping the CRI. Plasma ketamine concentrations were determined in both groups before ketamine administration and at each MAC endpoint determined during the ketamine CRI and after stopping the CRI.

Results- Group 1 MAC of isoflurane ($1.18 \pm 0.14\%$) was significantly reduced by ketamine ($0.88 \pm 0.14\%$). The MAC after stopping the CRI was significantly lower ($1.09 \pm 0.16\%$) than the MAC of isoflurane and significantly higher than the MAC during the CRI. In group 2, ketamine reduced isoflurane MAC ($0.79 \pm 0.11\%$), which increased significantly ($1.10 \pm 0.17\%$) after stopping the CRI. There was a significant difference between groups for MAC values during ketamine administration but none after stopping it. Ketamine concentrations (ng/mL) were similar for both groups at MAC values determined during ketamine administration (823.9 ± 195.7 and 728 ± 133.4 , respectively) and after stopping the CRI (406.6 ± 176.2 and 346.7 ± 81.2 , respectively). Correlation between MAC and plasma concentrations was $r = 0.55$.

Conclusion and clinical relevance- Prior noxious stimulation affected differently MAC values obtained on same or separate days after ketamine and this effect should be considered when designing MAC studies.

INTRODUCTION

MAC is defined as the end-tidal concentration of an inhalant anesthetic that prevents purposeful movement in 50% of the study population in response to a supramaximal noxious stimulus and is considered the standard method to compare potency of inhalant anesthetics since its introduction in the 1960's (Eger & Saidman 1965).

The MAC sparing effect of ketamine has been reported in several animal species, including dogs (Solano et al. 2006; Love et al. 2011), cats (Pascoe et al. 2007), and horses (Muir & Sams 1992). In Chapter II of this thesis, it was demonstrated that ketamine also has a sparing effect on isoflurane requirements in rabbits. In that study, the sparing effect of ketamine using the same day method for MAC determination, consisting of determining the MAC of isoflurane alone first and then administering the ketamine throughout the determination period, was similar to that one obtained using the separate day method, consisting of determining the MAC of the combination of isoflurane and ketamine without prior MAC of isoflurane determination.

MAC values reported by different laboratories, which carry out this type of investigation, can vary between them. The variation in many instances is due to different methodology used by investigators, but it also obeys differences between species. For example, MAC values for isoflurane in dogs and rabbits determined by the same laboratory using electrical stimulation were $1.35 \pm 0.04\%$ (mean \pm SEM) and $2.04 \pm 0.02\%$, respectively (Valverde et al. 2003); representing 51% higher MAC value in rabbits. In this same study, the difference in isoflurane MAC values for a submaximal

noxious stimulus (skin incision) was only 12% between both species, with higher MAC values in dogs at $1.01 \pm 0.07\%$ than in rabbits at $0.90 \pm 0.02\%$ (Valverde et al. 2003). This difference also indicates that despite obtaining purposeful movement for different species in MAC studies, the values for submaximal and supramaximal noxious stimuli in those species may elicit different mechanisms of activating pain and motor actions that facilitate movement in response to the noxious stimuli, since values for MAC movement in response to supramaximal and submaximal stimuli varied by 127% in rabbits and by only 34% in dogs (Valverde et al. 2003).

Several studies have demonstrated dose-dependent effects between plasma concentrations of a given injectable anesthetic drug and its influence on MAC for different inhalant anesthetics. Administration of the injectable drug as a constant rate infusion (CRI), after elevating plasma concentrations with a loading dose, also facilitates a steady concentration of the drug in plasma. By increasing or decreasing the dose of the CRI of the injectable anesthetic, plasma concentrations can fluctuate in the same direction and influence the degree of MAC reduction. Good correlations have been established this way between plasma concentrations of remifentanyl or lidocaine or ketamine and the dose-dependent effect on MAC for enflurane or isoflurane in dogs (Michelsen et al. 1996; Valverde et al. 2004; Solano et al. 2006; Pypendop et al. 2007; Monteiro et al. 2010). In Chapter II of this thesis, ketamine plasma concentrations were not measured in rabbits and may have influenced the results obtained during the isoflurane MAC determination after discontinuation of the ketamine CRI.

The objective of this study was two-fold; first, to determine the sparing effect of ketamine in dogs by using two MAC determination methods, same day and separate day,

to allow comparisons with the results obtained in Chapter II of this thesis. The second objective was to measure plasma concentrations of ketamine during the determination of MAC and after discontinuation of the CRI to determine if there is a dose-dependent effect.

MATERIALS AND METHODS

Animals

Eight mixed-breed dogs (5 females and 3 males) weighing 17.3 to 27 kg and 1 to 4 years of age were used in this study. All animals had normal physical examination findings as well as CBC determination and serum biochemical analysis before inclusion in the study to confirm their health status. Dogs were randomly allocated in two treatment groups using a cross over design with at least 5 days between treatments. Food was withheld 12 hours prior to anesthesia but water was available ad libitum. The Institutional Animal Care and Use Committee of the University of Guelph approved the study.

Anesthesia and instrumentation

Anesthesia was induced via a face mask with isoflurane (Forane, Baxter Corporation, Mississauga, Ontario, Canada) in 100% oxygen using a coaxial rebreathing system (Universal F-circuit) with an oxygen flow rate of 3 to 4 L/min. Once the dog was unconscious and endotracheally intubated with a low pressure high volume cuffed endotracheal tube, it was positioned in right lateral recumbency and the oxygen flow rate decreased to 2 L/min. Intermittent positive pressure ventilation was immediately established using an electronically controlled, time-cycled, pressure limited ventilator (EMC 2000; Hallowell, Massachusetts, USA), with a rate of 8 to 12 breaths/min and a tidal volume of 10 to 15 mL/kg in order to maintain end-tidal CO₂ between 30 and 40 mmHg. Dogs were instrumented for monitoring during the first 30 min of anesthesia while at approximately 1.6% end-tidal isoflurane concentration. Monitoring included

end-tidal concentrations of isoflurane and CO₂ using a side-stream infrared gas analyzer (S/5; Datex-Ohmeda Division, Helsinki, Finland) attached between the endotracheal tube and the breathing system. The anesthesia monitor was calibrated each morning using a calibration gas specifically designed for this purpose (DOT-34 NRC 300/375M1014; Datex-Ohmeda Division, Helsinki, Finland). A digital thermometer was used to monitor rectal temperature that was maintained between 37 and 38.5 °C with the use of a hot air heating device. Cardiovascular parameters including direct arterial blood pressure, electrocardiogram and heart rate were monitored (Datex-Ohmeda S/5™ Anesthesia monitor, GE Healthcare Finland Oy, Helsinki, Finland) throughout the duration of the experiment and recorded before and after each MAC determination.

The left cephalic vein and the right dorsal pedal artery were catheterized with a 20-SWG (1.88 IN) IV catheter (BD Insite-W, BD Infusion Therapy Systems Inc. Sandy, Utah USA). The venous access was used to administer the treatment drugs, with the use of a syringe pump (Graseby 3500 Anesthesia Pump, Smiths Medical International Ltd, Watford, Herts, UK), and a balanced electrolyte solution (Plasmalyte A- Baxter Corporation, Mississauga, Ontario, Canada) at 3 mL/kg/hour; the arterial access was used to measure direct blood pressure, with the use of an electronic pressure transducer (Becton Dickinson Infusion Therapy Systems, Sandy, Utah, USA) zeroed at the level of the sternum and interfaced with the anesthesia monitor. The arterial catheter was also used for periodical sampling into heparinised syringes (Gastlyte, Marquest Medical Products, Englewood, Colorado) of blood gases (CCX, Nova Biomedical, Waltham, Massachusetts, USA) to verify the end-tidal CO₂ readings. From the arterial catheter

blood was also collected for the determination of the ketamine plasma concentration at each MAC endpoint.

MAC determinations

MAC was determined using a previous published technique (Valverde et al. 2003). The noxious stimulation consisted of 50 Volts at 50 cycles/sec for 10 milliseconds (S48 Stimulator, Astro-Medical, Inc., West Warwick, Rhode Island) applied SC by inserting two 25-SWG; 0.75 IN needles 20 cm apart applied to the left forelimb and at the level of the ulna. In brief, the sequence of noxious stimulation consisted of applying 2 single stimuli followed by 2 continuous stimuli applied for 2 to 3 seconds, with 5-second intervals between all 4 stimuli. If gross purposeful movement, which consisted of jerking or twisting motion of the head or running motion of the extremities, was elicited at any time during the cycle the stimulation was immediately suspended and the event was recorded as a positive response. Subsequently the end-tidal isoflurane concentration was increased by 0.1%; conversely, if a negative response was obtained initially, then the end-tidal concentration was decreased by 0.1%. The noxious stimulation was repeated after 15 to 20 min of equilibration at the new concentration and if the opposite response to the previous stimulation was obtained then the end-tidal concentration was returned to the initial value using the 15 to 20 min equilibration process to verify the first response (duplicate MAC measurement). This procedure was repeated until opposite responses between immediate end-tidal concentrations of isoflurane were obtained in duplicate. The value midway between the lowest end-tidal concentration that prevented purposeful

movement and the highest end-tidal concentration that allowed it was recorded as the MAC value.

Experimental design

After the instrumentation phase, dogs assigned to group 1 (same day MAC determinations) had the end-tidal isoflurane concentration decreased to 1.2%, which corresponds to the approximate MAC value reported for dogs (Valverde et al. 2003) and maintained for at least 30 min to establish equilibration before starting MAC determinations while receiving a bolus and CRI of a balanced electrolyte solution at the same volume rate as the ketamine infusion to be used later on. Dogs assigned to group 2 (separate day MAC determination) had the end-tidal isoflurane concentration decreased to approximately 0.9% for the same time duration (30 min) after receiving a bolus of ketamine (Vetalar, Bioniche Animal Health Canada Inc, Belleville, Ontario, Canada) of 1 mg/kg IV followed immediately by a CRI at 40 µg/kg/min using a syringe pump (Graseby 3500 Anesthesia Pump, Smiths Medical International Ltd, Watford, Herts, UK). The CRI infusion was maintained until the MAC determination was completed.

After the first MAC determinations were completed in each group, dogs in group 1 were administered the ketamine bolus and CRI, as described above, and the end-tidal isoflurane concentration decreased to 0.9% and after 30 min of the infusion the MAC redetermined. Dogs in group 2 had the CRI of ketamine stopped, the end-tidal isoflurane concentration was increased to 1.2% during this time and the MAC determinations started once again 30 min later while receiving an infusion of a balanced electrolyte solution at the same volume and rate as the previous ketamine infusion. Dogs in group 2 were

allowed to recover from anesthesia once the second MAC determination was completed. Dogs in group 1 had the CRI of ketamine stopped and replaced by an infusion of a balanced electrolyte solution at the same volume and rate as the previous ketamine infusion after the second MAC determination. The end-tidal isoflurane concentration was increased to 1.2% during this time and the MAC determinations started once again 30 min later for a third time and then allowed to recover from anesthesia. Two blood samples (5 mL) were collected into sodium heparin tubes (BD Vacutainer, sodium heparin (NH) 68 USP units, BD, Franklin Lakes, NJ, USA) at the time of MAC determination for analysis of ketamine concentrations. One sample corresponded to the end-tidal isoflurane concentration at which the animal responded to the noxious stimulus and the other to the end-tidal isoflurane concentration at which it did not respond. The plasma was harvested and stored at -80 °C until further analysis. The arterial blood was immediately centrifuged at 2000 rpm for 10 minutes (IEC MB Centrifuges, Damon/IEC Division, Needham Heights, Massachusetts, USA) and the plasma stored at -80 °C until further analysis. All dogs were administered meloxicam 0.1 mg/kg, IV (Metacam, Boehringer Ingelheim, Burlington, Ontario, Canada) before recovery.

The time required to complete each MAC determination was recorded as the time elapsed from the beginning of the equilibration phase until the MAC value was determined. Total anesthesia time corresponded to the time from connecting the dog to the anesthetic machine until completion of the last MAC determination.

HPLC determination of plasma ketamine and nor-ketamine

Determination of ketamine and norketamine concentrations in plasma was performed using a Waters Alliance 2695 HPLC separation system (Mississauga, Ontario, Canada) which included a Waters 2996 photodiode array detector. The system was connected to a PC with Empower 2 software (Waters, Mississauga, Ontario, Canada) for data collection and processing. The analytical column used was an XBridge C18 column (100 mm x 2.1 mm I.D., 3.5 μm) (Waters, Ireland), connected with an XBridge C18 guard column (2.1 mm x 10.0 mm I.D., 3.5 μm). Canine plasma samples were purified using Oasis MCX cartridges (Waters Milford, Massachusetts, USA). An isocratic chromatographic separation was performed using a mobile phase containing acetonitrile-methanol-10mM ammonium acetate (15:30:55 v/v/v) (Sigma-Aldrich-Fluka, Oakville, Ontario, Canada) (Caledon Laboratories Ltd., Georgetown, Ontario, Canada), at a flow rate of 0.25 mL/min. A 50 μL sample injection volume was used and the eluent was monitored at 222.0 nm. Under these conditions the retention times observed for ketamine, norketamine, and MK-801 were 8.9 minutes, 5.8 minutes and 11.3 minutes, respectively (Bolze & Boulieu 1998; Sevensson & Gustafsson 1996; Parkin et al. 2008).

Stock solutions of ketamine and norketamine (Cerilliant Corp, Round Rock, Texas, USA) and MK-801 (Sigma-Aldrich-Fluka, Oakville, Ontario, Canada) were prepared in methanol (Caledon Laboratories Ltd., Georgetown, Ontario, Canada) at a concentration of 500 $\mu\text{g}/\text{mL}$. Calibration curve (reference) standards were prepared by spiking 10 μL of norketamine and ketamine working solutions (10, 20, 50, 100 and 250 $\mu\text{g}/\text{mL}$) and 10 μL of MK-801 internal standard solution (50 $\mu\text{g}/\text{mL}$) into 500 μL blank (control) canine plasma samples. The quality control (QC) samples used in the validation

of the assay and during assay runs of unknown samples were prepared in the same way as reference standards, at the concentrations of 300 and 4000 ng/mL.

Ketamine and norketamine were extracted and purified using Oasis MCX (Mixed-mode cation exchange) cartridges. Ten μL of internal standard solution (50 $\mu\text{g}/\text{mL}$ MK 801 in methanol) were added to 500 μL aliquots of dog unknown plasma samples. The plasma samples were acidified with 500 μL of 4% phosphoric acid, solutions vortexed for 30s, and centrifuged at 3,000rpm for 3 min. The supernatants were used for SPE purification. Oasis MCX cartridges were washed and conditioned by passing 1 mL of methanol and 1 mL of water. The 1 mL acidified plasma samples were loaded; the cartridges washed with 1 mL of 0.1 N hydrochloric acid and 1 mL of methanol, then eluted with 1 mL of 5% ammonium in methanol. The elutes containing ketamine, norketamine and the internal standard MK 801 were evaporated under a constant flow of nitrogen. The residues were reconstituted with 100 μL of the mobile phase (acetonitrile-methanol-10mM ammonium acetate 15:30:55, v/v/v) and 50 μL of it was injected into the analytical column (Pypendop & Ilkiw 2005; Pypendop et al. 2007).

For validation of the assay method, canine unknown plasma samples were quantified using the ratios of the peak area of ketamine and norketamine to that of the MK 801 internal standard as the assay parameter. Peak area ratios were plotted against ketamine and norketamine concentrations and standard curves, in the form of $y = A + Bx$, calculated using weighted ($1/x^2$) least squares linear regression. A calibration curve of 5 points was plotted and showed to be linear and reproducible in the concentration range from 200 ng/mL to 5000 ng/mL for norketamine and ketamine, with the correlation coefficient (r^2) > 0.99 for all calibration curves. For assay specificity to ketamine and

norketamine, no interfering peaks were observed in blank plasma samples at retention times corresponding to the drug and internal standard.

The limits of detection (LOD) for the assay were 50 ng/mL for norketamine and 100 ng/mL for ketamine (based on 3 times the signal to noise ratio at the time of elution of the analytes). The limits of quantitation (LOQ) for norketamine and ketamine were both 200 ng/mL. The limit of quantitation was determined by spiking blank canine plasma samples at various concentrations of ketamine and norketamine with resulting values having a coefficient of variation of no greater than 20%.

The intra-day precision was assessed by triplicated analysis of 5 reference standards (200, 400, 1000, 2000, and 5000 ng/mL). The intra-day coefficients of variation for norketamine and ketamine were 10.13% and 5.64% respectively. The true value for each calibration standard was within 15% of the actual value except at LOQ (200 ng/mL), where it deviated by less than 20%.

The recovery rate was determined by comparing the peak areas of plasma blank samples spiked with different amounts of drug with the peak areas of the same standards prepared in the mobile phase. Average recoveries performed at 5 reference standard levels were 86.81% for norketamine and 100.02% for ketamine.

Data analysis

The MAC values were calculated by use of mathematical averaging of two subsequent concentrations of inhalant anesthetic at which gross purposeful movement and no gross purposeful movement were observed. Statistical comparison between the two groups and within groups were carried out for MAC values induced by ketamine

administration, MAC values after stopping ketamine's administration and time of MAC determinations using an ANOVA with Bonferroni correction, after verification of normality of the data by use of the D'Agostino-Pearson and Kolmogorov-Smirnov test (MedCalc Software; version 11.2.1, Mariakerke, Belgium). The MAC values and time of determination are reported as mean \pm SD. The MAC values and time of determination are reported as mean \pm SD. Direct arterial blood pressure, end-tidal CO₂ concentrations, heart rate and rectal temperature throughout the study were compared between groups using an independent samples t test. The correlation between the MAC values and plasma ketamine concentrations were assessed using linear regression analysis (MedCalc Software; version 11.2.1, Mariakerke, Belgium). Values of $P < 0.05$ were considered significant.

RESULTS

All dogs had cardiorespiratory parameters within acceptable limits for MAC determinations and recovered uneventfully from the experiments. End-tidal CO₂, heart rate, systolic-, mean- and diastolic arterial pressure, and rectal temperature values were 37 ± 2.1 mmHg, 87 ± 15 beats/min, 120 ± 13 mmHg, 74 ± 10 mmHg, 60 ± 8 mmHg, and 37.4 ± 0.6 °C, respectively for group 1, and 36 ± 2.9 mmHg, 98 ± 27 beats/min, 124 ± 24 mmHg, 78 ± 17 mmHg, 65 ± 14 mmHg, and 37.6 ± 0.5 °C, respectively for group 2, throughout the experiment.

The MAC for isoflurane was $1.18 \pm 0.14\%$ (group 1). In both groups, ketamine administration resulted in a lower MAC value than the baseline MAC value for isoflurane obtained in group 1 (Table 3.1). In both groups the MAC value after discontinuation of ketamine was lower than the MAC for isoflurane obtained in group 1, although not significantly different but significantly higher within each group than the MAC value obtained during the ketamine CRI (Table 3.1). There was no difference between groups in MAC isoflurane values after discontinuation of ketamine; however the MAC value for ketamine-isoflurane was significantly lower in group 2 than in group 1.

Ketamine concentrations (ng/mL; mean \pm SD) during the CRI and after discontinuation of the CRI were similar at the time of MAC values determinations (Table 3.2) for same and separate day groups. The correlation between MAC and ketamine plasma concentrations was $r = 0.55$ (Figure 3.1). Ketamine concentrations (ng/mL; mean \pm SD) during the CRI and after discontinuation of the CRI were similar at the time of MAC values determinations (Table 3.2) for same and separate day groups.

The norketamine plasma concentrations could only be determined on 9/32 samples collected after ketamine administration for group 1 and in 2/32 for group 2, because the concentrations during MAC determinations were below the LOQ of the assay (200 ng/mL). Norketamine concentrations between 205-294 ng/mL were measured in 5 of the 8 dogs in group 1 and of 228 and 259 ng/mL in 1 dog each of the 8 dogs in group 2. The correlation between MAC and ketamine plasma concentrations was $r = 0.55$ (Figure 3.1).

There were no significant differences in times required for MAC determinations between the two groups (Table 3.1). The time of ketamine CRI administration corresponds to the same time as for MAC determination of the isoflurane and ketamine combination (Table 3.1).

DISCUSSION

Ketamine had a sparing effect on the MAC value for isoflurane. The decrease in baseline MAC (group 1) with the ketamine CRI corresponded to a 25% reduction, similar to reported values reported by other authors in dogs using a loading dose of 0.6 mg/kg and a CRI of 10 $\mu\text{g}/\text{kg}/\text{min}$ (Muir et al. 2003). The reduction in sevoflurane MAC of a loading dose of 3 mg/kg and a CRI of 50 and 100 $\mu\text{g}/\text{kg}/\text{min}$ was 40% and 45%, respectively (Wilson et al. 2008). In Chapter II of this thesis, the reduction induced by ketamine in the MAC of isoflurane in rabbits was also similar (24%) to that one obtained with dogs in this study.

The MAC values obtained for the isoflurane and ketamine combination in dogs were statistically different for the two methods, same day determination and separate day determination. The value obtained during separate day determination was 10% lower than that one obtained during the same day, unlike the results obtained in rabbits in Chapter II of this thesis in which MAC values were similar despite a 6% lower value obtained during the separate day experiment with respect to the same day value. Due to the small difference in variation between the two species, it is likely that the differences can be attributed to the power of this study and that using a higher number of animals would yield similar results between both species. However, it is unclear until then if the two methods would yield similar or different results between them.

The factors that contribute to variability in MAC determinations have been discussed in Chapter II of this thesis and apply to findings from this study in dogs. Worth mentioning and expanding on is the potential influence of the use of ketamine. Ketamine inhibits the N-methyl-D-aspartate (NMDA) glutamate receptor. NMDA receptors

constitute one glutamate receptor subtype, and they require the co-agonists glycine and glutamate for activation (Johnson & Ascher, 1987). NMDA antagonism has a MAC sparing effect via supraspinal (Masaki et al. 2001) and spinal mechanisms (McFarlane et al. 1995; Ishizaki et al. 1996), which can be obtained from intracerebroventricular, intrathecal, or intravenous administration. Inhaled anesthetics also inhibit NMDA receptors, however nitrous oxide and xenon have greater *in vitro* effects than potent inhaled anesthetics (Yamakura & Harris 2000; deSousa et al. 2000). The actions of inhalant anesthetics on NMDA receptors contribute to their immobilizing capacity, but they seem to be less obvious for isoflurane since temporal summation appears to persist during isoflurane but not during xenon anesthesia and NMDA blockade with an antagonist eliminates temporal summation for isoflurane (Sonner et al. 2003).

Repeated stimuli delivered at close intervals lead to temporal summation and are more likely to provoke a response (movement). *In vitro*, blocking NMDA receptors blocks temporal summation, indicating that NMDA receptors lie in the pathway that mediates temporal summation (Woolf & Thompson 1991). Studies with isoflurane suggest that approximately 40% of the generation of movement evoked by noxious stimulation (MAC) depends on the interstimulus interval (Dutton et al. 2003), suggesting the persistence of temporal summation and transmission via NMDA pathways due to incomplete block of NMDA receptors. The administration of an NMDA antagonist abolishes summation (Dutton et al. 2003) and therefore the use of ketamine in this study should have decreased the influence of temporal summation. The fact that a statistical difference was observed between the two methods cannot be explained on the basis of temporal summation since the difference in the MAC values is clinically small (10%) and

not very different from that observed in rabbits (6%) in Chapter II of this thesis, which was not statistically different.

Plasma ketamine concentrations measured at the time of MAC determinations are consistent with those reported in other studies for the same degree of MAC reduction. In this study, the corresponding plasma ketamine concentration at which the MAC value was obtained was approximately 824 ng/mL for group 1 and 729 ng/mL for group 2. In other studies, at target plasma ketamine concentrations of 1,000 ng/mL after administration of a loading dose of 3 mg/kg and a CRI based on the individual dogs' pharmacokinetic rate constants to achieve a pseudo-steady-state at the desired concentration, the MAC of isoflurane was reduced by approximately 11 to 39% (Solano et al, 2006). In other study using sevoflurane and infusing ketamine at 50 or 100 $\mu\text{g}/\text{kg}/\text{min}$ after a loading dose of 3 mg/kg, plasma ketamine concentrations of 1057 ng/mL and 2191 ng/mL were obtained respectively, which resulted in higher MAC reductions of approximately 40-45% (Wilson et al. 2008). It is estimated that MAC is reduced by 50% at plasma ketamine concentrations of approximately 3,000 ng/mL (Pypendop et al. 2007).

Plasma ketamine concentrations have been correlated with the degree of sparing effect on the MAC of isoflurane (Pypendop et al. 2007). The relationship is described by an inverse sigmoid curve with the steepest section of the curve between approximately 1,000 ng/mL and 8,000 ng/mL, where it appears to be linear and in inverse relationship. The maximal MAC reduction has been estimated at 92% (Pypendop et al. 2007). Below or above those concentrations, the contribution to the MAC sparing effect is less, consequently the correlation tends to be lower as it occurred in this study ($r = 0.55$).

Dogs administered a loading dose of 26 mg/kg over 20 min and a CRI of 300 µg/kg/min during enflurane anesthesia achieved plasma concentrations of 22,000 ng/mL and only a 73% MAC reduction (Schwieger et al. 1991). It is also recognized that there is high variability in the disposition of ketamine in dogs under isoflurane anesthesia (Pypendop & Ilkiw 2005). The elimination half-life for dogs under isoflurane anesthesia is reported as 94 ± 37 min (Pypendop & Ilkiw 2005), whereas for dogs under enflurane anesthesia is 122 ± 9 min, after receiving a single bolus of 10 mg/kg, and of 141 ± 40 min after receiving a loading dose of 26 mg/kg and a CRI of 300 µg/kg/min. (Schwieger et al. 1991). These differences between the half-life under isoflurane and enflurane may be due to less effective clearance under enflurane (14-18 mL/kg/min) than under isoflurane (58 mL/kg/min) (Schwieger et al. 1991; Pypendop & Ilkiw 2005). In conscious dogs administered ketamine, the half-life of 15 mg/kg IV was 61 min (range of 44-77 min) (Kaka & Hayton 1980).

Norketamine (N-demethylketamine) is considered the most important product of hepatic metabolism of ketamine (Woolf & Adams 1987). Approximately 62% of ketamine is biotransformed to norketamine (Kaka & Hayton 1980). Pharmacokinetic studies done in dogs have shown that norketamine appears very rapidly in plasma, and maximum concentrations are achieved 6.5 ± 4.8 min after ketamine administration and have a terminal half-life of 64 ± 24 min (Pypendop & Ilkiw 2005). The decline in concentrations is parallel to that of ketamine (Kaka & Hayton 1980; Pypendop & Ilkiw 2005). The plasma concentrations of norketamine in this study were below the LOQ of the assay (200 ng/mL) for the majority of dogs. This differs from previous reports where plasma norketamine concentrations after single IV bolus of ketamine (3 or 15 mg/kg)

were above the LOQ of this study for at least 30-60 min (Kaka & Hayton 1980; Pypendop & Ilkiw 2005). It is possible that the lower loading dose of 1 mg/kg used in this study was not enough to raise the initial ketamine concentration (C_{max}) to levels that would result in significant amounts of norketamine. Using a loading dose of 3 mg/kg, a C_{max} of approximately 10,000 ng/mL was measured (Pypendop & Ilkiw 2005), whereas a dose of 15 mg/kg increased the concentration to approximately 15,000-25,000 ng/mL (Kaka & Hayton 1980). The low and inconsistent norketamine concentrations achieved in this study did not influence the results since norketamine only has approximately 10% of the anesthetic, analgesic and anti-inflammatory properties of ketamine (Muir 2010).

The MAC values for isoflurane obtained in both groups after stopping the CRI of ketamine were lower, although not statistically, by approximately 7% than the baseline value obtained in group 1. The determinations for those MAC values were completed 65 min (group 1) and 76 min (group 2) after stopping the CRI of ketamine. Based on the half-life of ketamine and the ketamine concentrations still measured during those MAC determinations, 407 ng/mL and 347 ng/mL, respectively, it is clear that not enough time was allowed for the clearance of ketamine to allow the return of MAC to baseline. In addition, this finding is consistent with the minimal effect on MAC (less than 10%) at plasma concentrations significantly below 1000 ng/mL (Pypendop et al. 2007).

In Chapter II of this thesis plasma ketamine concentrations were not measured in rabbits and it was hypothesized that the lower MAC values than baseline isoflurane MAC, obtained after stopping the ketamine CRI, were probably due to persistent plasma ketamine concentrations, which appears to be the most likely explanation.

In conclusion, ketamine had a significant sparing effect on the MAC of isoflurane in dogs and it appears that the MAC methodology used to determine such effect (same day versus separate days) affects the results.

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Table 3.1 MAC values and time for MAC determinations after ketamine administration (1 mg/kg bolus IV followed by 40 µg/kg/min) in 8 dogs with same day prior isoflurane MAC determination (Group 1) or without same day prior isoflurane MAC determination (Group 2).

	Isoflurane baseline	Isoflurane + ketamine	After stopping ketamine	Total anesthesia time (min)
Group 1				
(Same day)				
MAC (%)	1.18 ± 0.14	0.88 ± 0.14 * (P =0.0001)	1.09 ± 0.16 † (P =0.0079)	
Time (min)	48 ± 5.1	75 ± 16.4	65 ± 10.4	202 ± 22.6
Group 2				
(Separate days)				
MAC (%)	ND	0.79 ± 0.11 * (P =0.0001) § (P =0.028)	1.10 ± 0.17 † (P =0.0003)	
Time (min)	ND	74 ± 13.2	76 ± 20.2	170 ± 30.1

Values expressed as mean ± SD. ND = not determined. * Significant difference with isoflurane baseline. † Significant difference with isoflurane + ketamine. § Significant difference with isoflurane + ketamine from Group 1.

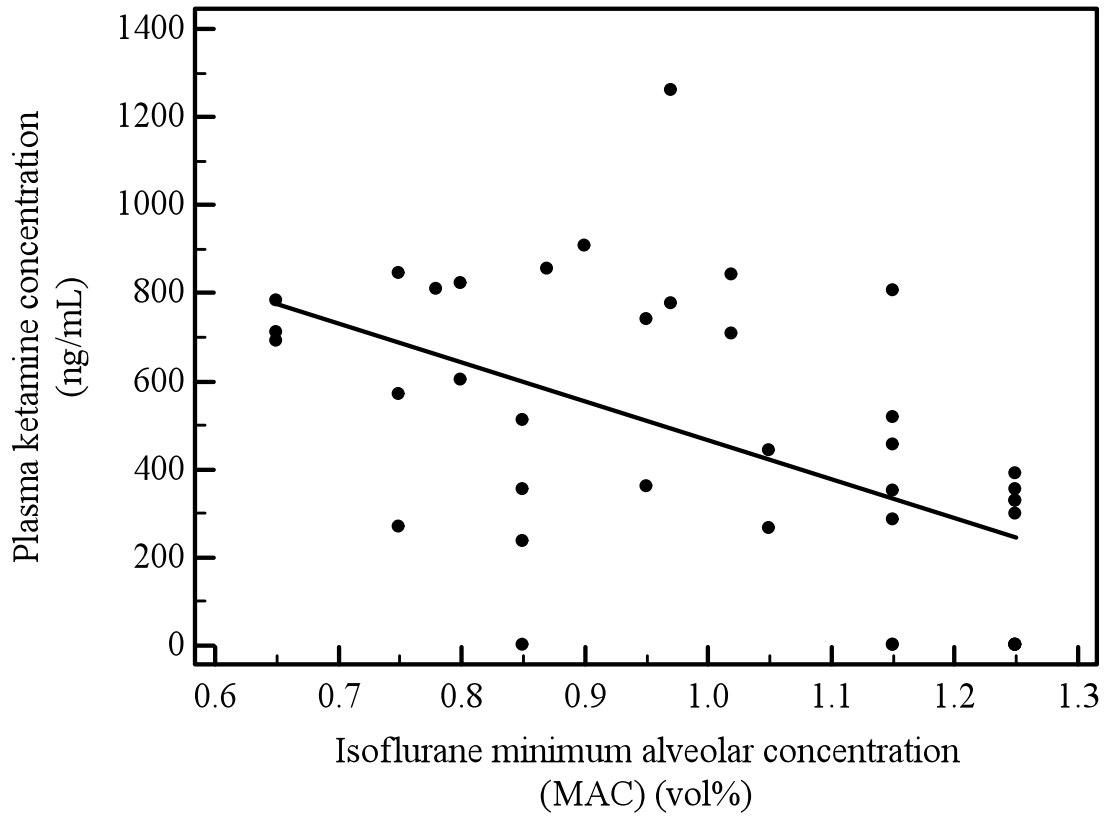
Table 3.2 Plasma ketamine concentration values and time at MAC determinations before, during and after stopping ketamine administration (1 mg/kg bolus IV followed by 40 µg/kg/min) in 8 dogs with same day prior isoflurane MAC determination (Group 1) or without same day prior isoflurane MAC determination (Group 2).

	Isoflurane	Isoflurane + ketamine	After stopping ketamine
Group 1			
(Same day)			
Ketamine plasma concentration (ng/mL)	0.00 ± 0.00	824 ± 196*	407 ± 176*
Time (min)	48 ± 5.	75 ± 16	65 ± 10
Group 2			
(Separate days)			
Ketamine plasma concentration (ng/mL)	0.00 ± 0.00	729 ± 133*§	347 ± 81*§
Time (min)	ND	74 ± 13	76 ± 20

Plasma concentrations are based on two arterial blood samples obtained from each dog during the corresponding bracketing of MAC (positive and negative response). Values expressed as mean ± SD. ND = not determined. * MAC not determined just ketamine

plasma concentration for baseline. * Significant difference with isoflurane value. § Non significant difference with group 1.

Figure 3.1 Minimum alveolar concentration (MAC) of isoflurane versus plasma concentration of ketamine (ng/mL); $r = 0.55$; $p < 0.001$).



CHAPTER IV

GENERAL DISCUSSION AND CONCLUSIONS

The application of repetitive noxious stimulation during MAC studies may result in neuroplastic changes that affect pain transmission in any of the recognized pain pathways along the spinal cord and CNS. We investigated if the repetitive noxious stimulation necessary for completing MAC studies affects the results obtained in such studies by altering the response to the stimulus either by sensitizing or desensitizing and increasing or decreasing MAC values, respectively. In this study we evaluated and compared the isoflurane MAC sparing effect of ketamine in rabbits and dogs when ketamine was administered with or without prior determination of the MAC of isoflurane alone. In dogs we also looked at the correlation between plasma concentrations of ketamine and the metabolite norketamine with the degree of MAC sparing effect. The study was conducted on two different species because the literature supports variability in MAC values within and between species as well as in the MAC sparing effect properties of injectable anesthetic drugs across species. By using a crossover design with animals acting as their own controls and the same laboratory conditions for the two MAC methods assessed, we eliminated common variables such as individual variation and discrepancies between researchers in methods used in MAC determinations and subjectivity in the assessment of movement as the end-point of MAC studies.

In this study ketamine had a sparing effect on the MAC of isoflurane in rabbits and dogs. However, in dogs the MAC value obtained from the combination of isoflurane and ketamine after having previously determined the MAC of isoflurane alone the same

day differed from the MAC value obtained for the combination when the MAC of isoflurane was not determined previously that same day. In rabbits both MAC values for the isoflurane and ketamine combination were similar despite methodology.

It is possible that noxious stimulation associated with prior MAC determination of isoflurane alone influences the results of subsequent MAC determinations when an injectable drug with analgesic properties is administered. It is not clear why there were differences in the results obtained between the two species studied, dogs and rabbits. However, possible explanations include that MAC values for isoflurane and halothane are higher in the rabbit than the dog; therefore there are already inherent differences between species. These differences have also been shown for responses to non-supramaximal noxious stimulation, where the isoflurane MAC to skin incision in rabbits was lower than in dogs, despite the fact that the isoflurane MAC to electrical stimulation (supramaximal stimulation) is higher in rabbits than in dogs (Valverde et al. 2003).

The sample size ($n = 8$) used in the study could have contributed to a low power that prevented the demonstration of significant differences. In both species the isoflurane MAC sparing effect of ketamine obtained in this study was approximately 24% when ketamine was administered after the determination of the MAC of isoflurane alone. The MAC value obtained for the combination of ketamine and isoflurane without prior determination of the MAC of isoflurane alone was 90% of the same value obtained with prior determination of MAC of isoflurane in dogs and 94% in rabbits. In the planning of this study it was initially predicted a ketamine sparing effect of 30%, based on published information for other species. The anticipated standard deviations were assumed not to exceed 0.25 of the mean, which resulted in a recommended sample size of 8 animals to

provide at least 95% confidence for a Type I error (α value) at 0.05 and 80% power to detect a difference of MAC values for the expected degree of MAC reduction. The slightly lower MAC sparing effect of ketamine demonstrated in this study may have influenced the no significance in rabbits. In addition, MAC values for the ketamine isoflurane combination in rabbits differed by 6% between the two methods, but there was a wider variation in the standard deviation (1.63 ± 0.07 versus 1.53 ± 0.22), whereas in dogs the values differed by 10% and had tighter standard deviations (0.88 ± 0.14 versus 0.79 ± 0.11), which contributed to the differences detected in this study.

The detection and interpretation of movement as the end-point of MAC studies is also subjective and difficult to standardize between studies completed at different laboratories. One additional confounding factor is the difference that may exist between species and how they move in response to noxious stimulation, which makes interpretation more difficult to analyze and differentiate from purposeful movement or simple reflexes (Love et al. 2011). Blinding the investigators to the treatment is one way of producing more consistent and reliable data than in non-blinded assessments (Jadad et al. 1996). In the pilot part of this study it was not possible to blind the investigator assessing the effects of ketamine on the MAC of isoflurane due to obvious signs of change in the depth of anesthesia and lack of resistance (bucking) to mechanical ventilation when the loading bolus was administered, which defeated the purpose of blinding. It is common to not blind the investigators in MAC studies (Valverde et al. 2003; Ferreira et al. 2009; Seddighi et al. 2011) as long as the assessment of purposeful movement is consistent.

The effect of ketamine on pain pathways and possible differences between the two

species used in this study are also not well defined and could have influenced the results. Ketamine has a different effect on acute somatic pain and acute visceral pain and the effects also vary with the species (Castroman & Ness 2002; Strigo et al. 2005).

One distinct difference in the methodology used between rabbits and dogs included the use of a coaxial non-rebreathing system (Bain system) in rabbits and a coaxial rebreathing system (F-circuit) in dogs. Oxygen flow rates of 200-300 mL/kg/min were used in rabbits and of 100 mL/kg/min in dogs. The higher oxygen flow rates used in rabbits may have interfered with the gas sampling technique by diluting the expired gases and affecting the end-tidal values. However, even if such interference existed, the effect was standardized and consistent in all rabbits throughout the experiment. End-tidal concentrations measured in the same way as in this study, between the endotracheal tube and the breathing circuit, and compared to concentrations measured by introducing the sample line into the lumen of the endotracheal tube in rabbits yielded similar concentrations between the two sites of inhalational anesthetics in a previous experiment, although those values were not reported (Valverde, personal communication).

Plasma ketamine concentrations were correlated with MAC values in dogs during both methodologies used for MAC (overall $r = 0.55$). Plasma concentrations for norketamine could only be determined on 9/32 samples collected after ketamine administration for group 1 and in 2/32 for group 2; this is due to the fact that the values obtained were very close to the LOQ of the assay used. MAC values for isoflurane did not return to the baseline value after discontinuation of the ketamine constant rate infusion since plasma ketamine concentrations were still present despite the provided washout period.

In conclusion, this investigation demonstrated the following results:

1. Ketamine significantly reduces the isoflurane MAC in dogs and rabbits.
2. Prior noxious stimulation influences the MAC values for the isoflurane and ketamine combination in dogs but not in rabbits; however these findings may be the result of the design of the study and tolerable experimental error derived from MAC studies rather than true species differences.
3. There is a linear correlation between MAC of isoflurane and the plasma ketamine concentrations in dogs.

FUTURE AREA OF RESEARCH

Ketamine's actions on antagonizing the NMDA receptor may have played a major role in the results obtained in this investigation. The use of other drugs with different mechanisms of actions will be useful to determine if plasticity is more likely to occur under these same conditions.

The recent introduction of the concept MAC no movement, as oppose to the current concept of MAC (50% probability of movement or no movement) and its implementation in a study like this investigation would also be important to help eliminate the subjectivity in telling apart purposeful movement from a reflex movement (Love et al. 2011).

The use of other species is also important to elucidate possible differences between them. With the results obtained in this study it will be easier to determine the number of animals needed to complete the study and avoid ambiguous results due to lack of power.

The assay used to detect norketamine had a LOQ that was too high for the plasma concentrations measured in this study. Using a different assay, such as mass spectrometry high pressure liquid chromatography that allows for a lower LOQ would allow for better and more accurate results to establish possible correlations of norketamine with MAC values.

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