ผลของเด็กซ์เมทดีโตมิดีนร่วมกับเพทิดีนต่อความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอด
สำหรับระงับปวดในสุนัข

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THE EFFECT OF DEXMEDETOMIDINE IN COMBINATION WITH PETHIDINE ON ISOFLURANE MINIMUM ALVEOLAR CONCENTRATION FOR CANINE ANTINOCICEPTION

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Thesis Title	THE EFFECT OF DEXMEDETOMIDINE IN COMBINATION WITH PETHIDINE ON ISOFLURANE MINIMUM ALVEOLAR CONCENTRATION FOR CANINE ANTINOCICEPTION
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กาญจนา วิญญูนันทกุล : ผลของเด็กซ์เมทดีโตมิดีนร่วมกับเพททิดีนต่อความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอดสำหรับระงับปวดในสุนัข (THE EFFECT OF DEXMEDETOMIDINE IN COMBINATION WITH PETHIDINE ON ISOFLURANE MINIMUM ALVEOLAR CONCENTRATION FOR CANINE ANTINOCICEPTION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. น.สพ. ดร.สุมิตร ดุรงค์พงษ์ธร, 44 หน้า.

การศึกษานี้ประเมินผลของเด็กซ์เมทดีโตมิดีนร่วมกับเพททิดีนต่อความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอด และต่อระบบการหายใจและไหลเวียนโลหิตในสุนัขเพศผู้ที่โตเต็มวัยและมีสุขภาพแข็งแรง 30 ตัว ที่มารับการผ่าตัดทำหมัน ที่โรงพยาบาลสัตว์เล็ก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย แบ่งสุนัขออกเป็น 3 กลุ่ม ได้แก่ กลุ่มที่ 1 จำนวน 6 ตัว เป็นกลุ่มควบคุมแบบลบซึ่งได้รับน้ำกลั่นสำหรับผสมยาเป็นยาหลอกฉีดเข้ากล้ามเนื้อ กลุ่มที่ 2 จำนวน 12 ตัว เป็นกลุ่มควบคุมแบบบวกซึ่งได้รับเด็กซ์เมทดีโตมิดีนขนาด 5 มคก.ต่อ กก.ฉีดเข้ากล้ามเนื้อ และกลุ่มที่ 3 จำนวน 12 ตัวได้รับเด็กซ์เมทดีโตมิดีนขนาด 5 มคก.ต่อ กก. ร่วมกับเพททิดีนขนาด 5 มก.ต่อ กก.ฉีดเข้ากล้ามเนื้อ ประเมินความซึมและทดสอบความเจ็บปวดต่อการถูกหนีบนิ้วที่ 3 หรือ 4 ของขาหลังของสุนัขในทุกกลุ่มภายหลังฉีดยา 15 นาที หลังจากนั้นเหนี่ยวนำการสลบโดยการสูดดมยาดมสลบไอโซฟลูเรนความเข้มข้น 4 เปอร์เซ็นต์ ในออกซิเจน 4 ลิตรต่อนาที ผ่านหน้ากากหายใจ เริ่มโดยตั้งค่าความเข้มข้นของไอโซฟลูเรนปลายลมหายใจออกที่ 1 เปอร์เซ็นต์ เป็นเวลาอย่างน้อย 15 นาที เพื่อให้มีสมดุลย์ยาดมสลบในระบบไหลเวียนก่อนที่จะทำการกระตุ้นความเจ็บปวด โดยการหนีบนิ้วที่ 3 หรือ 4 ของเท้าในขาหลังด้วยคีมห้ามเลือดขนาด 20 เซนติเมตร ดูการขยับหรือกระตุกของหัวหรือขาที่กำหนดเป็นการตอบสนองที่เป็นบวกต่อความเจ็บปวด หากมีการตอบสนองเป็นบวกจะทำการเพิ่มความเข้มข้นของไอโซฟลูเรนปลายลมหายใจออกขึ้น 0.1- 0.2 เปอร์เซ็นต์ และลดความเข้มข้นลง 0.1- 0.2 ปอร์เซ็นต์เมื่อมีการตอบสนองเป็นลบ หลังจากปรับความเข้มข้นของไอโซฟลูเรนปลายลมหายใจออกแล้ว รออย่างน้อย 15 นาทีเพื่อปรับสมดุลย์ยาดมสลบก่อนที่จะเริ่มกระตุ้นความเจ็บปวดใหม่ ความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอดนั้น คือค่ากึ่งกลางระหว่างค่าความเข้มข้นสูงสุดของไอโซฟลูเรนปลายลมหายใจออกที่พบการตอบสนองเป็นบวก และค่าความเข้มข้นต่ำสุดของไอโซฟลูเรนปลายลมหายใจออกที่ไม่พบการตอบสนองเป็นบวก หลังจากได้ค่าความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอดแล้ว สัตว์ได้รับการผ่าตัดทำหมันเพศผู้ อัตราการหายใจ อัตราการเต้นของหัวใจ ความดันโลหิตซีสโตลิค และค่าแก๊สต่างๆ ในหลอดเลือดดำได้รับการเฝ้าระวังและบันทึกก่อนและภายหลังได้รับยา ผลการทดลองพบว่า คะแนนความซึมของสุนัขกลุ่มที่ 2 และ 3 ต่ำกว่าของสุนัขกลุ่มที่ 1 อย่างมีนัยสำคัญทางสถิติ (p <0.05) และของสุนัขกลุ่มที่ 3 ต่ำกว่าของสุนัขกลุ่มที่ 2 อย่างมีนัยสำคัญทางสถิติ สุนัขทุกตัวมีการตอบสนองต่อการกระตุ้นความเจ็บปวดก่อนการเหนี่ยวนำสลบ ความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอดในสุนัขกลุ่มที่ 2 และ 3 ต่ำกว่าในสุนัขกลุ่มที่ 1 อย่างมีนัยสำคัญทางสถิติ และความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอดในสุนัขกลุ่มที่ 3 ต่ำกว่าในสุนัขกลุ่มที่ 2 อย่างมีนัยสำคัญทางสถิติอีกด้วย ค่าต่างๆของระบบการหายใจและไหลเวียนโลหิตยกเว้นอัตราการเต้นของหัวใจอยู่ในเกณฑ์ที่ยอมรับได้ทางคลินิก สรุป การให้เด็กซ์เมทดีโตมิดีน ร่วมกับเพททิดีนสามารถเพิ่มประสิทธิภาพในการทำให้ซึม และลดค่าความเข้มข้นต่ำสุดของไอโซฟลูเรนในถุงลมปอดในสุนัขได้ดีกว่าการให้เด็กซ์เมทดีโตมิดีนเพียงอย่างเดียว

ภาควิชา ศัลยศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา ศัลยศาสตร์ทางสัตวแพทย์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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5875303731 : MAJOR VETERINARY SURGERY

KEYWORDS: ANTINOCICEPTION / CANINE / DEXMEDETOMIDINE / ISOFLURANE MAC / PETHIDINE

KANJANA VINYUNANTAKUL: THE EFFECT OF DEXMEDETOMIDINE IN COMBINATION WITH PETHIDINE ON ISOFLURANE MINIMUM ALVEOLAR CONCENTRATION FOR CANINE ANTINOCICEPTION. ADVISOR: ASST. PROF. SUMIT DURONGPHONGTORN, D.V.M., D.V.Sc., D.T.B.V.S., 44 pp.

The effects of dexmedetomidine combined with pethidine on the minimum alveolar concentration (MAC) of isoflurane and cardiorespiratory variables in dogs were evaluated in thirty client-owned, healthy adult male dogs scheduled for castration at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The dogs were allocated into Group 1 (n=6) receiving sterilized water for injections (placebo) as a negative control group, Group 2 (n=12) receiving 5 μg kg^{-1} of dexmedetomidine IM as a positive control group, and, Group 3 (n=12) receiving 5 μg kg^{-1} of dexmedetomidine and 5 mg kg^{-1} of pethidine IM. At 15 minutes after injection of the tested drug, sedation and pain response to clamping of the 3rd or 4th digit of the hindimb were evaluated. Then, anesthesia was induced via a face mask with 4% isoflurane in 4 L min^{-1} of oxygen. The ET isoflurane had been initially set at 1% for at least 15 min for anesthetic equilibration before the noxious stimulation was carried out by clamping the 3rd or 4th digit of the hind limb using a 20-cm hemostat. A positive response was considered when there was gross purposeful movement of the head or extremities. Once the response was positive or negative, the ET isoflurane concentration was increased or decreased by 0.1 - 0.2%, respectively. The new ET concentration was maintained for at least 15 min for anesthetic equilibration before the noxious stimulation was repeated. The isoflurane MAC was the average concentration of isoflurane between the highest ET concentration at which the purposeful movement was detected and the lowest ET concentration at which the movement was not detected. After determining the isoflurane MAC, the animal was castrated. Respiratory rate, heart rate, systolic arterial pressure, and venous blood gas variables were monitored before and after injection of the tested drug. Sedation scores after dexmedetomidine given alone and in combination with pethidine were significantly higher than that after the placebo injection, and the score after the combination was significantly greater than that after dexmedetomidine given alone. All dogs responded to the noxious stimulation before anesthesia induction. The isoflurane MAC of Groups 2 and 3 were significantly less than that of Group 1 (p <0.05), and the isoflurane MAC of Group 3 was significantly less than that of Group 2. Cardiorespiratory variables except the heart rate were within the clinically acceptable limits. In conclusion, dexmedetomidine given with pethidine provided sedation and sparing effect on the isoflurane MAC significantly greater than dexmedetomidine given alone.

Department:	Veterinary Surgery	Student's Signature	.....
Field of Study:	Veterinary Surgery	Advisor's Signature	.....
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CHAPTER I
INTRODUCTION

1.1 Importance and Rationale

Pain or nociception is defined as an unpleasant sense or emotion associated with actual or potential tissue damage (Tranquilli et al., 2007). It is a complex experience involving both sensory and affected components (Robertson, 2005). Pain pathway is comprised of 4 consequences: transduction, transmission, modulation, and perception. The pain transmission begins when a noxious stimulus (chemical, mechanical, or thermal) activates nociceptors which transduce the stimulus into an electrical signal. Nociceptors are primary sensory neurons that detect and encode noxious stimuli then relay these signals to the CNS.

Unrelieved pain is a main stressor and results in decreased quality of life and cardiovascular function, increased metabolic and hormonal responses, delayed wound healing, catabolism and immunosuppression (Taylor and Robertson, 2004). Pain can affect physiological, neuroendocrine and metabolic parameters, such as heart rate, respiratory rate, blood pressure, capillary perfusion, ventilation, gastrointestinal motility, urinary functions, muscle contractility and nervous activity, protein and lipid metabolism, water-electrolyte balance, serum concentrations of glucose, cortisol, β-endorphin, catecholamine, glucagon, ADH, and insulin (Grant, 2006; Ledowski et al., 2012).

Pain recognition and assessment are important parts of veterinary clinical practice and would greatly promote pain management. Pain assessment in animals is challenging (Lucas et al., 2001; Anil et al., 2002; Hellyer, 2002; Members et al., 2007). Pain is an individual and unique experience. Animal responses to pain vary with species as well as with each individual's disposition (Tranquilli et al., 2007). Animals are unable to verbally express their comfort level. Many studies have tried to devise a pain scoring systems for animal use (Lucas et al., 2001; Anil et al., 2002; Hellyer, 2002; Members et al., 2007). Objective and subjective components of pain assessment are used to develop pain scoring systems. The systems combining behavior observation and interaction with the animal are considered most reliable. Rapid respiration, increased heart rate, increased blood pressure, dilated pupil, and salivation are clinical signs usually seen in pain animals. Body posture, vocalization and general appearance can also be used to assess pain.

Pain management is one of the most important ethical concerns in veterinary practice. Therefore, various aspects of analgesia have been searched, and many new techniques have

been proposed to improve pain management (KuKanich, 2013; Madden et al., 2014; Crociolli et al., 2015; Norkus et al., 2015). One of the main concern of inhalation anesthesia is cardiovascular depression related to the high anesthetic concentration. Analgesic drugs can reduce minimum alveolar concentration (MAC) and cardiopulmonary depressive effects of inhalation anesthetics (Muir et al., 2003; Aguado et al., 2011). Analgesia can be induced by various techniques. One of them is multimodal analgesia referred as the administration of more than one drug type or technique. Each of which acts via different receptors or mechanism to optimize pain control and to minimize side effects associated with relatively large doses of a single drug. The multimodal use can increase the efficacy of analgesia. The preferred protocol is to combine alpha-2 adrenergic agonists with opioids.

1.2 Objectives of Study

To assess the effect of dexmedetomidine when combined with pethidine on isoflurane minimum alveolar concentration in dogs.

1.3 Research Questions

Will dexmedetomidine combined with pethidine reduce the minimum alveolar concentration of isoflurane and have any effects on cardiorespiratory parameters in dogs?

1.4 Hypothesis

The combination of dexmedetomidine and pethidine would decrease isoflurane minimum alveolar concentration in dogs when compared with the use of dexmedetomidine alone.

Keywords: Antinociception, Canine, Dexmedetomidine, Isoflurane MAC, Pethidine

1.5 Advantages of Study

The combination of pethidine with dexmedetomidine could increase the efficacy of intraoperative analgesia in dogs. Therefore, the required concentration of isoflurane will be decreased resulting in less anesthetic risk and cost.

CHAPTER II
LITERATURE REVIEW

2.1 Antinociception

Antinociception or analgesia is defined as a loss of sensitivity to pain. Analgesic drugs can be administered either pre-operatively, intra-operatively, or post-operatively. Administering analgesic drugs to improve surgical outcomes would be better achieved if performed pre-emptively before pain actually occurs (Tranquilli et al., 2007). Analgesic drugs can reduce minimum alveolar concentration (MAC) and cardiopulmonary depressive effects of inhalation anesthetics (Muir et al., 2003; Aguado et al., 2011). Analgesic drugs available nowadays are NSAIDs, opioids, α2-agonists, local anesthetic drugs (lidocaine, bupivacaine and ropivacaine), and NMDA receptor antagonists (ketamine).

Analgesia can be induced by various techniques. One of them is multimodal analgesia referred as the administration of more than one drug type or technique. Each of which acts via different receptors or mechanism to optimize pain control and to minimize side effects associated with relatively large doses of a single drug. Multimodal analgesia has become the preferred method of pain control for invasive surgical procedures (Tranquilli et al., 2007). Numerous human and veterinary literatures reported lower post-operative analgesic requirements, fewer side effects, faster wound healing, and earlier hospital discharge in patients receiving multimodal analgesia compared to those receiving only a single medication or one route of administration (Pascoe, 2000; Anil et al., 2002; Hellyer, 2002; Members et al., 2007; Tranquilli et al., 2007; Young and Buvanendran, 2012). This technique can decrease dose of all agents and side-effects, and increase overall analgesic effectiveness. Opioids are often used in conjunction with NSAIDs, ketamine, or alpha-2 agonists. It is important that two agents acting at the same receptor, such as buprenorphine and morphine, should not be used otherwise overdose occurs (Stepien et al., 1995; Martinez et al., 1997).

Multimodal regimen can increase the efficacy of analgesia. The preferred protocol is to combine alpha-2 adrenergic agonists with opioids. Xylazine, medetomidine and dexmedetomidine are alpha-2 agonists licensed for small animal use. These agents are thought primarily as sedatives but they are analgesic as well. They act at the alpha–2 receptors in the periphery, spinal cord and brain. These agents are not used primarily for analgesia but for a multimodal regimen. The alpha-2 adrenergic agonists are potent sedative agents used in several species of animals. However, they have signifcant cardiovascular effects, such as vasoconstriction, bradycardia and decreased cardiac output (Dart, 1999).

2.2 Dexmedetomidine

Dexmedetomidine is an alpha-2 adrenoreceptor agonist which has the sedative and analgesic effects synergistic with opioids (Salmenpera et al., 1994; Slingsby et al., 2010). It provides sedation, anxiolysis and analgesia in small animals (Uilenreef et al., 2008). Bolus, CRI and epidural administrations of dexmedetomidine reduces the anesthetic requirement for the induction and maintenance of general anesthesia in dogs (Pascoe et al., 2006; Campagnol et al., 2007; Uilenreef et al., 2008). Common side effects of dexmedetomidine include decreased cardiac output and initial hypertension (Bloor et al., 1992). An advantage of dexmedetomidine is that its sedative effect can be reversed by an antagonist, atipamezole, for rapid recovery. The intravenous administration of 20 mcg/kg of dexmedetomidine in dogs reduced 50% of heart rate and more than 70% of cardiac output, and increased the mean arterial pressure and vascular resistance, lasting for 4 hours (Bloor et al., 1992). Intravenous administration of 10 and 20 mcg/kg of dexmedetomidine induced dose-dependent sedative effect (Kuusela et al., 2001).

To keep or potentiate the sedative effects of alpha-2 adrenergic agonists and to reduce the cardiovascular effects, the combination of these agents with other drugs have been proposed (Leppanen et al., 2006; Monteiro et al., 2008). Among them, opioid analgesics are mostly used because they potentiate the sedative and analgesic effects with minimal cardiovascular effects (Dart, 1999). Leppanen et al. (2006) combined dexmedetomidine with butorphanol, buprenorphine or diazepam in dogs scheduled for hip radiography and found that the combination potentiated the sedative effects without significant cardiorespiratory alterations. Dexmedetomidine used with morphine, methadone, pethidine or tramadol has been reported (Grint et al., 2009; Cardoso et al., 2014).

2.3 Opioids

Opioids are the gold standard in analgesia because of their highly effective and reasonably safe for treating acute and chronic pain. Opioids may be administered pre-operatively without the risk of renal problems or bleeding disorders associated with the use of non-steroidal anti-inflammatory drugs (Mathews et al., 1996). The primary analgesic action is via mu opioid receptors. Opioids exert action over the transduction, transmission, modulation and perception of the pain pathway (Hall, 2010). Opioid receptors localize in the brain, dorsal horn of the spinal cord, and periphery (Tranquilli et al., 2007). Opioids may be given via a variety of routes such as orally, parenterally, neuro-axially, and either as bolus administration or constant rate infusion (Barnhart et al., 2000; Lucas et al., 2001). Opioids are relatively safe analgesics with much wider safety margin than NSAIDs. Opioids are

respiratory depressants. They also exert effects on the vagal nuclei in the medulla which in turn causes bradycardia (Stoelting and Hillier, 2006). Opioids may cause sedation prior to analgesia, cough suppression, nausea, vomiting, cutaneous vasodilation, altered thermoregulation, and decreased gastrointestinal motility in most species. Side effects vary and depend on the chemical structure of the particular opioid, as well as the route by which the drug is administered, and the species. Full agonists tend to have more side effects than partial agonists. However, these infrequent and discreet effects in dogs and cats can be reversed by a pure opioid antagonist such as naloxone (Fox et al., 1994).

Pethidine is a mu opioid receptor agonist which has been used as an analgesic in dogs (Taylor and Herrtage, 1986; Grint et al., 2009). Intravenous administration of pethidine might be hazardous in animals, due to histamine release from mast cell degranulation (Clutton, 1987; Akcasu et al., 2009). Therefore, it is better given intramuscularly or subcutaneously. Pethidine has short duration of action, produces good analgesia for 1–2 h, and rarely causes vomiting. Pethidine has vagolytic effect (Stoelting, 1999), if administered with dexmedetomidine, it could prevent the bradycardia seen with the use of dexmedetomidine alone. The effect of dexmedetomidine when combined with pethidine has been studied only on sedation and cardiovascular system (Grint et al., 2009), but not on the antinociception and isoflurane sparing effects. This study aimed to evaluate the latter two effects of dexmedetomidine when combined with pethidine in dogs.

2.4 Minimum Alveolar Concentration (MAC)

Minimum alveolar concentration (MAC) is the alveolar concentration of a volatile anesthetic that inhibits a response to a supramaximal noxious stimulation in 50% of the tested animals. This measurement is done after there is an equilibration between gases in the alveoli, the blood, and the brain (Tranquilli et al., 2007). MAC is an assessment of volatile anesthetic potency and is fairly constant among species and under varying conditions (Tranquilli et al., 2007). Various drugs and some physical status may reduce or increase the inhalant requirement, causing a MAC sparing or MAC increasing effect, respectively. Analgesic drugs usually have MAC sparing effects by means of their analgesic, sedative, or muscle-relaxant effects. To determine the MAC of a volatile anesthetic, the animals must be subjected to noxious stimulation. Techniques used for such noxious stimulation in dogs include applying an electrical current to the buccal mucosa (Hellyer et al., 2001; Muir et al., 2003; Wilson et al., 2006; Ueyama et al., 2009), or to the front and hind limbs (Pascoe et al., 2006), clamping the tail or the third or fourth digit of the hind limb using forceps with rubber- or plastic-covered jaws to prevent tissue damage (Valverde et al., 2004; Matsubara et al., 2009; Acevedo-Arcique et al., 2014), and

pulling the ovary and ovarian ligament with a suture loop endoscopically placed around the ligament (Congdon et al., 2011).

2.5 Noxious Stimulation Techniques

2.5.1 Mechanical Stimulation

Valverde et al. (2004) reported the mechanical stimulation involved clamping the third and fourth digits of the hind-limb using sponge forceps. Once the presence or absence of purposeful movement was present, the end-tidal isoflurane was increased or decreased by 0.1%, and the new end-tidal concentration was kept for no less than 20 minutes before the noxious stimulation was repeated. The purposeful response is jerking or twisting of the head or a running motion of the extremities. Shivering, stiffening, or changing of respiration are not considered the purposeful response. The isoflurane MAC is the average concentration of isoflurane between the highest end-tidal concentration at which the purposeful movement is detected and the lowest end-tidal concentration at which the movement is not detected.

Congdon et al. (2011) determined the sevoflurane MAC by pulling ovary and ovarian ligament with force through a suture loop endoscopically placed around the ligament. The response considered positive or negative when the dog showed or did not show a purposeful movement, respectively, during 1 minute of ovarian pulling stimulation. Based on the response, the end-tidal sevoflurane was increased or decreased by 10% for the next stimulation and 15 minutes were allowed between the stimulation for anesthetic equilibration.

Acevedo-Arcique et al. (2014) applied noxious stimulation by clamping a paw at the fourth digit with 24-cm sponge forceps. When a positive response was elicited, the end tidal concentration of isoflurane was increased by 10% and maintained for at least 20 min before the noxious stimulation was repeated. When a negative response was detected, the end tidal isoflurane was decreased by 10% and maintained for at least 20 minutes before repeating the noxious stimulation.

2.5.2 Electrical Stimulation

Hellyer et al. (2001) stimulated nociception by applying an electrical stimulus (50 volts, 5 Hz, 10 milliseconds) to the buccal mucosa for a period up to 1 minute. Isoflurane concentration was reduced (after a negative response) or increased (after a positive response) by 10 to 20% and maintained for at least 20 minutes to allow for equilibration before repeating the noxious stimulation.

By Muir et al. (2003), two 24-gauge, 10 mm insulated stimulating electrodes were inserted 1 cm apart into the buccal mucosa at a location dorsal and caudal to the incisors. The opposite ends of the electrodes were connected to an electrical stimulating device that delivered a supramaximal

stimulus for determination of isoflurane MAC. The MAC for isoflurane was determined by delivering a predetermined stimulus of 50 V at 5 Hz for 10 milliseconds to the preplaced buccal mucosa electrodes for a maximum period of 1 minute. The stimulus was discontinued immediately when the dog had gross purposeful movement. If there was a negative response, the isoflurane concentration was decreased by 20% until the dog responded with gross purposeful movement. If there was a positive response, the inhalant anesthetic concentration was then increased by 10% of the preceding end tidal concentration of isoflurane. The dog was allowed a minimum of 15 minutes for anesthetic equilibration at each new anesthetic concentration before retesting.

By Valverde et al. (2004), the electrical stimulation involved the application of 50 V at 50 Hz for 10 milliseconds through two 25-SWG (2.5 cm) needles inserted subcutaneously 5 cm apart, at the level of the ulna. Once the presence or absence of purposeful movement was determined, the end tidal isoflurane was increased or decreased by 0.1%, and kept constant for at least 20 minutes before the noxious stimulus procedure was repeated.

Pascoe et al. (2006) determined MAC for isoflurane in triplicate using a supramaximal stimulus. The dog was stimulated with a subcutaneous electrical current, 20 mA at 50 cycles/second with 1 millisecond pulse duration for 60 seconds. Electrodes were placed on the pelvic limb (laterally over the gastrocnemius) and the thoracic limb (laterally over the antebrachium). These sites were stimulated separately by random allocation. Five minutes were allowed between stimulations. If there was no response to the stimulation, end-tidal isoflurane concentration was decreased by 10%, and if there was a positive response, the end tidal isoflurane was increased by 10%. The end-tidal isoflurane concentration was held constant for at least 20 minutes before each stimulation.

By Wilson et al. (2006), two 22-gauge needle electrodes inserted 4 cm apart in the buccal mucosa of the lower jaw were connected to an electrical stimulator. Electrical stimulation (6.5-millisecond duration) was delivered at 50 cycles/s and 40 V. If no movement occurred after stimulation, inhalant concentration was decreased by 20% and held constant for 15 minutes to allow inhalant equilibration, before the stimulation was repeated. Once movement was elicited, the inhalant concentration was increased by 10% and held constant for 15 minutes to allow inhalant equilibration. Stimulation was then repeated until no movement was detected.

Campagnol et al. (2007) reported the supramaximal nociceptive stimulation for isoflurane MAC determination in dogs using an electrical current (50 V at 50 cycles/s for 10 milliseconds) administered to one of the hind limbs. An electrical stimulator was connected to 2 subcutaneous 25-gauge stainless- steel needle electrodes placed at 5 cm from each other on the medial aspect of the middle third portion of the tibia. If a negative motor response was initially detected, the end tidal isoflurane concentration was reduced by 0.2% and the nociceptive stimulation was repeated after a

new equilibration period of 15 minutes. This procedure was repeated until a positive motor response was evident. After this, the end tidal isoflurane concentration was altered by 0.1% increments until the motor response to the nociceptive stimulation could be inhibited. In dogs that had an initial positive response, the end tidal isoflurane concentration adjustments were performed in a reverse order.

By Ueyama et al. (2009), MAC was determined by delivering a supramaximal electrical stimulus to the buccal mucosa of a dog. Two 24-gauge, 10-mm insulated stimulating electrodes were inserted 1 cm apart into the buccal mucosa at a location dorsal and caudal to the incisors. The opposite ends of the electrodes were connected to an electrical stimulator that delivered a predetermined stimulus (50 V at 5 Hz for 10-millisecond duration). Stimulation continued for 1 minute unless the dog had signs of gross purposeful movement (lifting of the head and repeated movement of the limbs) before completion of the 1-minute stimulation. When there was a negative response to the electrical stimulus, the end tidal isoflurane was decreased by 20% and allowed to equilibrate for at least 15 minutes before the stimulus was reapplied. This process was continued until the dog responded with gross purposeful movement. The end tidal isoflurane was then increased in increments of 10% until the dog failed to have signs of gross purposeful movement.

By Figueiro et al. (2016), the 10 mA, 30 mA and 50 mA electrical stimuli (square wave and 50 Hz) were delivered by a constant- current electrical stimulator connected to a pair of stainless steel needles, located 3 cm apart and inserted subcutaneously on the ventral base of the tail, as well as on the cranial aspects of the carpus and tarsus. Each stimulus was applied for a maximum of 60 seconds or stopped as soon as a positive response was elicited. Any obvious movement of mainly the head or limbs occurring during the noxious stimulation was considered to indicate a positive response. The end tidal concentration of isoflurane was modified by approximately 10%, but never by more than 20%, and maintained for 15 minutes. The end tidal concentration of isoflurane was increased or decreased according to the responses observed. If the majority of responses were positive, the concentration was increased. If the majority of responses were negative, it was decreased. Each MAC was determined as the mean between two consecutive end tidal concentration of isoflurane values eliciting a negative and a positive response independently of the order.

2.6 Effects of Dexmedetomidine and Opioids on the MAC of Volatile Anesthetics

MAC of volatile anesthetics after giving opioids, sedatives, tranquilizers or local anesthetics in dogs have been reported (Muir et al., 2003; Valverde et al., 2004; Pascoe et al., 2006). One of the main concern of volatile anesthesia is cardiovascular depression related to the high anesthetic concentration. Several studies have documented a MAC sparing effect of many opioids in multiple

species (Machado et al., 2006; Ko et al., 2009; Abreu et al., 2012). The effect varies with type of opioid used, dosage, and route of administration. Pure mu opioid agonists have the MAC sparing effect greater than agonist-antagonists (Ko et al., 2009). Muir et al. (2003) found morphine infusion peri-operatively reduced MAC by 48% and the combination of morphine, lidocaine and ketamine (MLK) reduced MAC by 45%. The latter study also found a reduction of MAC after lidocaine or ketamine alone, but not to the same degree as when an opioid was included in the infusion. Lidocaine and dexmedetomidine decrease the MAC of volatile anesthetics and the volatile anesthetic requirements, resulting in less risk of cardiopulmonary depression during general anesthesia (Steagall et al., 2006; Uilenreef et al., 2008; Matsubara et al., 2009). The combination of the two agents having different pharmacological mechanism of action provides greater analgesia and volatile-sparing effect (Muir et al., 2003; Doherty et al., 2007; Wilson et al., 2008; Aguado et al., 2011; Gutierrez-Blanco et al., 2013).

CHAPTER III
MATERIALS AND METHODS

3.1 Experimental Animals

Thirty healthy mature male dogs, 1-8 years of age and 2-10 kilograms of weight were used in this study. There was no breed predilection. Healthy was based on physical examination and hematology and chemistry evaluation. Pre-anesthetic physical examination and blood sampling for blood gas analysis were performed on the day of drug testing. Presence of systemic diseases and coagulopathy were the exclusion criteria. Each dog was assigned by alternate sequence into one of the three groups. Group 1 (n = 6) received distilled water as placebo IM at the same quantity as if dexmedetomidine given. Group 2 (n = 12) received 5 microgram/kg of dexmedetomidine IM. Group 3 (n = 12) received 5 microgram/kg of dexmedetomidine combined with 5 mg/kg of pethidine IM.

Dogs were withheld food for 12 h and water for 6 h prior to anesthesia. Heart rate (HR), respiratory rate (RR) and systolic arterial pressure (SAP) were measured before tested drug administration (T0), 15 min after tested drug administration (before mask induction of anesthesia with isoflurane in oxygen, T15), during anesthesia and post-anesthesia.

3.2 Experimental Procedures

After IM administration of placebo, dexmedetomidine or dexmedetomidine and pethidine, an appropriate size of IV catheter was introduced into the cephalic vein. At 15 min after drug administration (before mask induction of anesthesia), the animal was assessed sedation score (Table 1), measured vital signs, and then, checked nociception by clamping the third or fourth digit of the hind limb (Figure 1) for 5 second using a 20-cm hemostat forceps with rubber protected jaws. The clamping was applied for a maximum of 1 minute, but was removed sooner if purposeful response occured. The purposeful response was jerking or twisting of the head or a running motion of the extremities. Shivering, stiffening, or changing of respiration was not considered the purposeful response. Number of dogs moving purposefully in response to clamping was recorded.

Table 1 Sedation scoring scale.

Score	Descriptive signs
0	Alert with normal startle reaction (head turn towards noise/cringe), standing
1	Less alert but still active, reduced startle reaction (reduced head turn/minimal cringe), if recumbent, animal can rise normally
2	Drowsy, minimal startle reaction, difficult rising if recumbent
3	Very drowsy or stuporous, absent startle reaction, unable to rise

(Modified from Grint et al. (2009) and Valverde et al. (2004))

Figure 1 Noxious stimulation by clamping the third or fourth digit of the hind limb.

After mask induction of anesthesia with isoflurane in oxygen, orotracheal intubation with a cuffed endotracheal tube of appropriate size was done. Non-rebreathing anesthetic circuit and a ventilator were used throughout the experiment. Initially, anesthesia was maintained with the isoflurane at 1.0 % for 15 min, then, vital signs were measured and noxious stimulation was performed. The isoflurane concentration was increased or decreased by 0.1-0.2 % when there was presence or absence of purposeful movement. Isoflurane at each new concentration was maintained for at least 15 min before repeating the noxious stimulation and recording the vital signs. Isoflurane MAC was the average concentration of isoflurane between the highest concentration that allows the purposeful movement and the lowest concentration that stops the movement. This was the isoflurane MAC of each animal. Then, the animals were castrated under anesthesia using isoflurane at 1-1.5 retrieved MAC. The isoflurane concentration was adjusted according to stage of anesthesia.

ECG and heart rate were recorded by Datascope Passport®V (Mindray DS USA, Inc., Mahwah, NJ, USA) (Figure 2). End-tidal isoflurane and end-tidal carbon dioxide concentrations were measured using a Multi-Gas Analyzer (Gas Module 3™, Mindray DS USA, Inc., Mahwah, NJ, USA) (Figure 3) and displayed on the Datascope Passport®V. An anesthesia ventilator (SAV 2500 Surgivet™, Smiths Medical PM, Inc., Waukesha, WI, USA) (Figure 4) was used to maintain respiratory rate at 12 breathes per minute and the end-tidal carbon dioxide concentration between 30 and 45 mmHg. Normal body temperature was maintained by a warming unit (3M Bair Hugger™ Temperature Management Unit Model 775, 3M Health care, St. Paul, MN, USA) (Figure 5). Systolic arterial pressure was measured using Doppler Flow Detector (Model 811-BL, Parks Medical Electronics, Inc., Aloha, OR, USA) (Figure 6). Oxygen saturation was measured by Pulse Oximeter (Model UT100V, Utech CO., LTD., Chongqing, China) (Figure 7).

Figure 2 Datascope Passport®V (Mindray DS USA, Inc., Mahwah, NJ, USA).

Figure 3 Multi-Gas Analyzer (Gas Module 3™, Mindray DS USA, Inc., Mahwah, NJ, USA).

Figure 4 An anesthesia ventilator (SAV 2500 Surgivet™, Smiths Medical PM, Inc., Waukesha, WI, USA).

Figure 5 A warming unit (3M Bair Hugger™ Temperature Management Unit Model 775, 3M Health care, St. Paul, MN, USA).

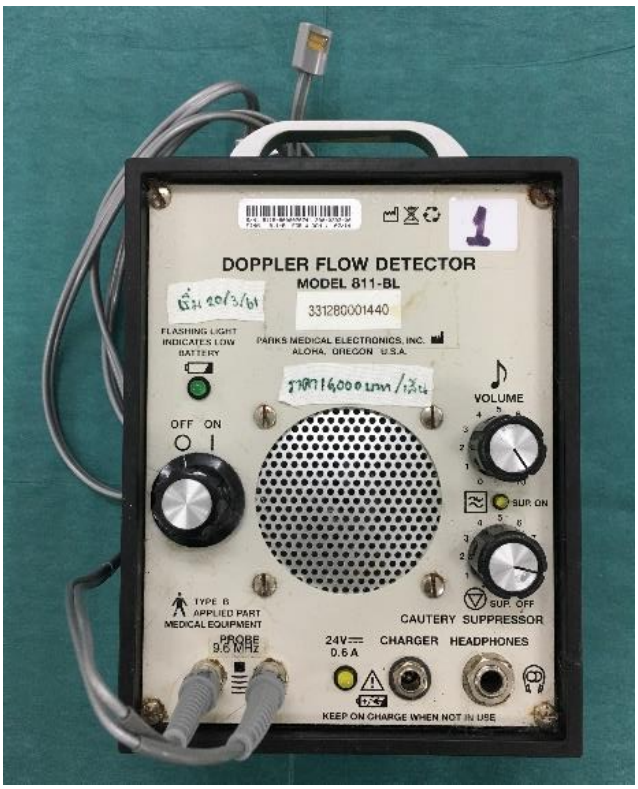

Figure 6 Doppler Flow Detector (Model 811-BL, Parks Medical Electronics, Inc., Aloha, OR, USA).

Figure 7 Pulse Oximeter (Model UT100V, Utech CO., LTD., Chongqing, China).

3.3 Statistical Analysis

Using Microsoft Office Excel, all data were analyzed by analysis of variance (ANOVA) and t-test. T-test paired two-sample for means (two-tail) was used for comparing data within groups, while ANOVA was used for comparing data between groups. Data to be analyzed were age, weight, sedation scores, respiratory rate, blood gases variables, heart rate, systolic arterial pressure, isoflurane MAC, end tidal isoflurane concentration for surgery, time to isoflurane MAC determination, and time to isoflurane concentration for surgery determination. Data are presented as mean ± SD, median and/or range. A p - value of less than 0.05 is considered significant.

CHAPTER IV
RESULTS

Breeds and number of dogs in each breed are shown in table 2. There was no significant difference (p >0.05) in age of dogs among the three groups (Table 2). Weight of dogs in Groups 2 and 3 were significant higher than the weight of dogs in Group 1 (Table 2). All dogs responded to the noxious stimulation before anesthesia induction. No dogs in Group 1 were sedated (Table 3). Mean sedation scores of dogs in Groups 2 and 3 were significantly higher than the score of dogs in Group 1, and the score of Group 3 was higher than the score of Group 2.

Table 2 Breeds, age and weight of dogs in Group 1 receiving placebo, Group 2 receiving dexmedetomidine, and Group 3 receiving dexmedetomidine combined with pethidine.

Variables	Group 1 (n = 6)	Group 2 (n = 12)	Group 3 (n = 12)
Breeds			
- Chi Hua Hua	2	4	2
- Cross breed	0	1	2
- Pomeranian	3	2	2
- Poodle	0	1	3
- Shih Tzu	1	4	3
Age (year)			
- Mean ± SD	2.9 ± 1.7	3.3 ± 2.2	3.9 ± 2.3
- Median (range)	3.3 (1.0 - 5.0)	2.5 (1.0 - 7.0)	4.0 (1.0 - 7.0)
Weight (kg)			
- Mean ± SD	3.3 ± 1.1[a]	4.9 ± 2.1[b]	5.1 ± 1.6[b]
- Median (range)	3.0 (2.1 - 5.4)	5.7 (2.3 - 8.7)	4.9 (2.4 - 8.0)

([a, b] - values with different superscript letters for each variable in the same row are significantly different (p <0.05)).

Mean isoflurane MAC were significantly different among the three groups (Table 3, Figure 8). The mean isoflurane MAC of Groups 2 (1.31 ± 0.33) and 3 (0.98 ± 0.24) were significantly less than Group 1 (1.63 ± 0.23), and the mean isoflurane MAC of Group 3 was significantly less than Group 2. When compared with the isoflurane MAC of Group 1, dexmedetomidine alone (Group 2) and dexmedetomidine combined with pethidine (Group 3) reduced the isoflurane MAC by 19.63% and by 39.88%, respectively. Mean ET isoflurane concentration for surgery of Group 3 was significantly less than Group 2 (Table 3). Ratios between the mean ET isoflurane concentration for surgery (of Groups 2

(2.01 ± 0.26) and 3 (1.73 ± 0.39)) and the mean isoflurane MAC of Group 1 (1.63 ± 0.23) were 1.23 for Group 2 and 1.06 for Group 3. Significant difference between the ratios was not observed. Mean time to isoflurane MAC determined were not significantly different between Groups 1 and 2, but significantly lower in Group 3 when compared with Groups 1 and 2 (Table 3, Figure 8). Mean time to isoflurane concentration for surgery determined of Group 3 was significantly less than Group 2 (Table 3). The mean time to isoflurane concentration for surgery determined in Groups 2 and 3 were significantly longer than the mean time to isoflurane MAC determined (Table 3). The ET isoflurane concentration for surgery, the ET isoflurane concentration for surgery/the isoflurane MAC ratio, and time to isoflurane concentration for surgery determined in Group 1 were not determined due to the use of different drug protocol during surgery.

Means ± SD (medians) of RR of dogs before and at 15 minutes after injection of the tested drug were shown in table 4 and figure 9. The RR before and at 15 minutes after drug injection were not significantly different among the three groups. The rates at 15 minutes after drug injection in Groups 2 and 3 were significantly less than the rate before drug injection. However, the RR and blood gases variables were within the clinically acceptable limit (Table 4). Significant difference in mean pH between before drug injection and after isoflurane MAC determined was not observed in Groups 1 and 2, but was found in Group 3. The mean pH before drug injection and after the isoflurane MAC determined in Groups 2 and 3 were not significantly different from Group 1, but those in Group 2 were significantly different from Group 3. Significant difference in PCO_2 between before drug injection and after the isoflurane MAC determined was observed in Group 3 but not in Groups 1 and 2. PCO_2 at all measurement points were not significantly different among the three groups. HCO_3^{-1} after the isoflurane MAC determined in Group 2 was significantly higher than that before drug injection, while no significant difference between the two intervals was observed in Groups 1 and 3. HCO_3^{-1} at all measurement points were not significantly different among the three groups.

Means ± SD (medians) of HR of dogs before and at 15 minutes after the tested drug injection, after the isoflurane MAC determined, and after the ET isoflurane concentration for surgery determined were shown in table 5 and figure 10. The HR at 15 minutes after drug injection, after the isoflurane MAC determined and after the ET isoflurane concentration for surgery determined in Groups 2 and 3 were significantly decreased from the rate before drug injection. There were no significant differences among the three groups for the HR before drug injection and after the ET isoflurane concentration for surgery determined. The HR at 15 minutes after drug injection and after the isoflurane MAC determined in Groups 2 and 3 were significantly lower than Group 1, but significant difference in the HR at the two

Table 3 Sedation score, isoflurane MAC (Iso MAC), ET isoflurane concentration for surgery (Iso Sx), time to Iso MAC determined, and time to Iso Sx determined of dogs in Group 1 receiving placebo, Group 2 receiving dexmedetomidine, and Group 3 receiving dexmedetomidine combined with pethidine.

Variables	Group 1 (n = 6)	Group 2 (n = 12)	Group 3 (n = 12)
Sedation score (1-3)			
- Mean ± SD	0.0 ± 0.0[a]	1.1 ± 0.7[b]	2.5 ± 0.8[c]
- Median (range)	0.0 (0.0 - 0.0)	1.0 (0.0 - 2.0)	3.0 (1.0 - 3.0)
Iso MAC (%)			
- Mean ± SD	1.63 ± 0.23[a]	1.31 ± 0.33[b]	0.98 ± 0.24[c]
- Median (range)	1.60 (1.35 - 1.95)	1.30 (0.70 - 1.90)	0.95 (0.55 - 1.45)
Iso Sx (%)			
- Mean ± SD	ND	2.01 ± 0.26[a]	1.73 ± 0.39[b]
- Median (range)	ND	1.95 (1.70 - 2.50)	1.70 (1.10 - 2.40)
Time to Iso MAC (min)			
- Mean ± SD	104 ± 39[a]	113 ± 43[a]	69 ± 40[b]
- Median (range)	92 (61 - 168)	108 (55 - 176)	57 (38 - 182)
Time to Iso Sx (min)			
- Mean ± SD	ND	150 ± 34*, [a]	122 ± 33*, [b]
- Median (range)	ND	150 (94 - 211)	113 (81 - 198)

(ND - not determined; *- significantly different (p <0.05) from Time to Iso MAC determined in the same column or group; [a, b, c] - values with different superscript letters for each variable in the same row are significantly different).

measurement points were not observed between Groups 2 and 3. The HR after the isoflurane MAC determined in Group 1 was significantly lower than the rates before and at 15 minutes after drug injection. The HR after the ET isoflurane concentration for surgery determined in Group 3 was significantly greater than the rate at 15 minutes after drug injection. The HR after the ET isoflurane concentration for surgery determined in Groups 2 and 3 were significantly higher than the rate after the isoflurane MAC determined. The HR after the ET isoflurane concentration for surgery determined in Group 1 was not determined due to the use of different drug protocol during surgery.

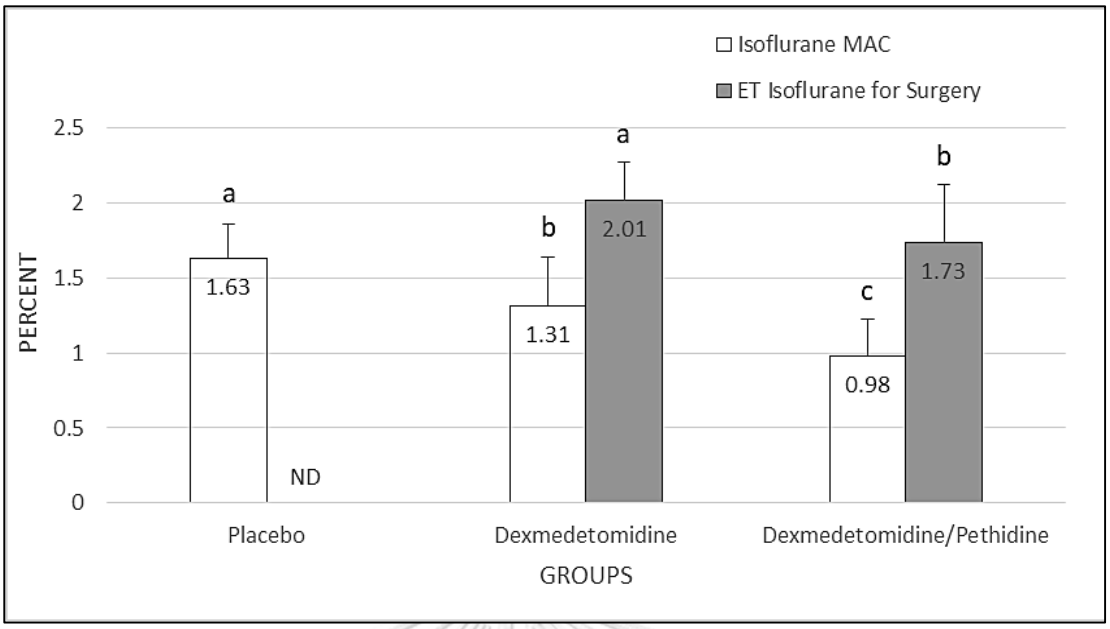

Figure 8 The minimum alveolar concentration of isoflurane (Isoflurane MAC) and the end tidal concentration of isoflurane for surgery (ET Isoflurane for Surgery) for dogs in groups receiving placebo, dexmedetomidine, and dexmedetomidine combined with pethidine (ND - not determined; a, b, c - values with different superscript letters for each variable are significantly different).

Means ± SD (medians) of SAP of dogs before and at 15 minutes after the tested drug injection, after the isoflurane MAC determined, and after the ET isoflurane concentration for surgery determined were shown in table 5 and figure 11. The SAP before drug injection, after the isoflurane MAC determined and after the ET isoflurane concentration for surgery determined were not significantly different among the three groups, while the pressure at 15 minutes after drug injection were significantly different. The pressure at 15 minutes after drug injection in Group 1 was significantly higher than those in Groups 2 and 3. The SAP at 15 minutes after drug injection in Group 3 was significantly lower than the pressure before drug injection. The SAP after the isoflurane MAC determined in all groups were significantly less than those before and at 15 minutes after drug injection. The SAP after the ET isoflurane concentration for surgery determined in Groups 2 and 3 were significantly lower than those before drug injection and after the isoflurane MAC determined. The SAP after the ET isoflurane concentration for surgery determined in Group 1 was not determined due to the use of different drug protocol during surgery.

Table 4 Respiratory rates (RR, breaths min $^{-1}$) before and at 15 minutes after the tested drug injection, venous blood gases variables before drug injection and after isoflurane MAC determined of dogs in Group 1 receiving placebo, Group 2 receiving dexmedetomidine, and Group 3 receiving dexmedetomidine combined with pethidine.

Variables	Group 1 (n = 6)	Group 2 (n = 12)	Group 3 (n = 12)
RR before drug injection			
- Mean ± SD	62 ± 24	72 ± 46	66 ± 50
- Median (range)	56 (40 - 100)	54 (35 - 160)	53 (24 - 200)
RR 15 min after drug injection			
- Mean ± SD	59 ± 25	30 ± 20*	23 ± 15*
- Median (range)	52 (36 - 96)	27 (12 - 88)	20 (8 - 60)
pH before drug injection$^{€}$			
- Mean ± SD	7.41 ± 0.02^{ab}	7.40 ± 0.03^{a}	7.43 ± 0.03^{b}
- Median (range)	7.41 (7.38 - 7.44)	7.4 (7.33 - 7.44)	7.43 (7.37 - 7.49)
pH after Iso MAC determined$^{€}$			
- Mean ± SD	7.41 ± 0.09^{ab}	7.43 ± 0.05^{a}	7.39 ± 0.04*$^{, b}$
- Median (range)	7.40 (7.28 - 7.55)	7.42 (7.34 - 7.51)	7.37 (7.34 - 7.46)
PCO_2 before drug injection$^{€}$			
- Mean ± SD	31.7 ± 3.6	31.9 ± 4.0	29.6 ± 7.53
- Median (range)	31 (27 - 36)	30.6 (24.8 - 39.3)	27.3 (21.1 - 45.9)
PCO_2 after Iso MAC determined$^{€}$			
- Mean ± SD	32.4 ± 7.8	31.5 ± 5.5	34.7 ± 5.2*
- Median (range)	31.3 (22 - 46)	31.4 (22.4 - 39.4)	35.8 (26.3 - 43.9)
HCO_3^{-1} before drug injection$^{€}$			
- Mean ± SD	20.9 ± 1.9	20.6 ± 1.4	21.1 ± 1.9
- Median (range)	20.8 (19 - 23)	21.2 (17.4 - 22.2)	20.6 (18.7 - 24.2)
HCO_3^{-1} after Iso MAC determined$^{€}$			
- Mean ± SD	21 ± 1.3	21.9 ± 1.6*	21.2 ± 1.7
- Median (range)	21 (19 - 23)	21.8 (19.6 - 24.7)	21 (17.6 - 23.6)

(* - significantly different (p <0.05) from the value before drug injection for each variable in the same column or group; $^{€}$ - n = 11 in Groups 2 and 3; $^{a, b}$ - values with different superscript letters for each variable in the same row are significantly different).

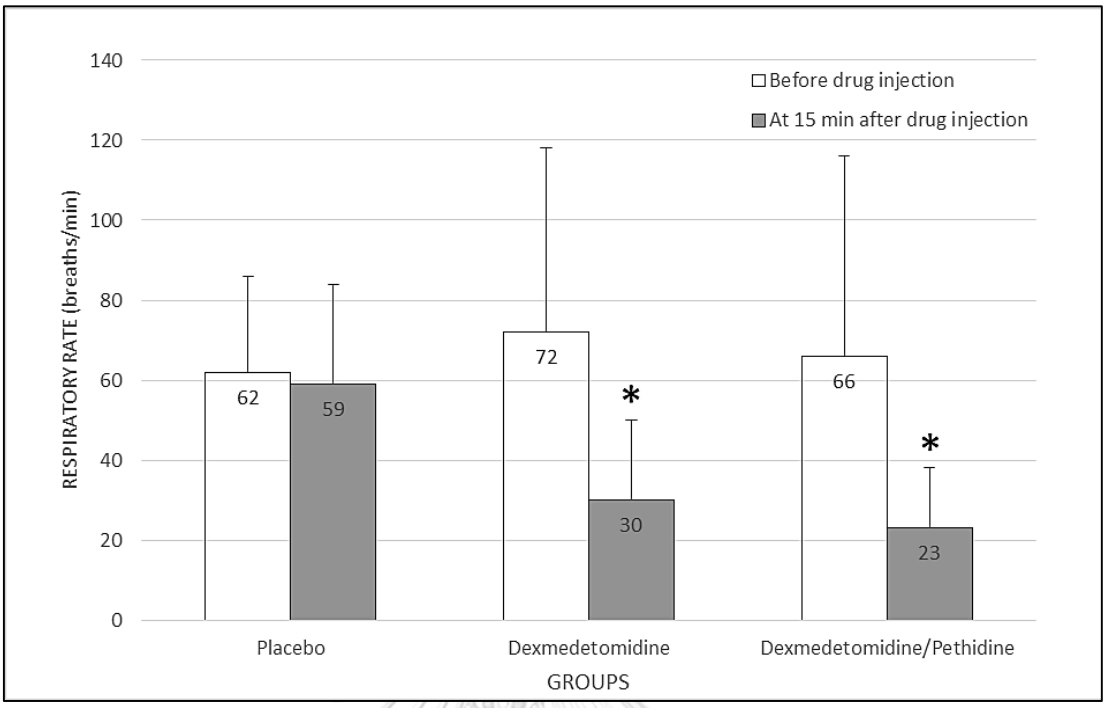

Figure 9 Respiratory rates before injection of the tested drug and 15 minutes after the injection of dogs in groups receiving placebo, dexmedetomidine, and dexmedetomidine combined with pethidine (* - significantly (p <0.05) less than the value before injection of the tested drug).

Table 5 Heart rates (HR, beats min^{-1}) and systolic arterial pressures (SAP, mmHg) before and at 15 minutes after the tested drug injection, after Iso MAC determined, and after ET Iso Surgery determined of dogs in Group 1 receiving placebo, Group 2 receiving dexmedetomidine, and Group 3 receiving dexmedetomidine combined with pethidine.

Variables	Group 1 (n = 6)	Group 2 (n = 12)	Group 3 (n = 12)
HR before drug injection			
- Mean ± SD	129 ± 22	108 ± 26	120 ± 36
- Median (range)	130 (100 - 160)	102 (81 - 164)	114 (60 - 200)
HR 15 min after drug injection			
- Mean ± SD	136 ± 28[a]	74 ± 25[*, b]	65 ± 28[*, b]
- Median (range)	136 (100 - 180)	74 (44 - 112)	56 (36 - 140)
HR after Iso MAC determined			
- Mean ± SD	108 ± 15[*, £, a]	67 ± 16[*, b]	58 ± 22[*, b]
- Median (range)	110 (86 - 127)	71 (43 - 95)	51 (38 - 112)
HR after ET Iso Surgery determined			
- Mean ± SD	ND	84 ± 17[*, #]	77 ± 20[*, £, #]
- Median (range)	ND	81 (60 - 120)	78 (39 - 111)
SAP before drug injection			
- Mean ± SD	166 ± 31	151 ± 38	156 ± 31
- Median (range)	168 (120 - 200)	135 (100 - 220)	160 (95 - 220)
SAP 15 min after drug injection			
- Mean ± SD	173 ± 21[a]	139 ± 41[b]	125 ± 18[*, b]
- Median (range)	180 (140 - 200)	123 (100 - 240)	125 (90 - 150)
SAP after Iso MAC determined			
- Mean ± SD	99 ± 12[*, £]	114 ± 22[*, £]	104 ± 16[*, £]
- Median (range)	103 (80 - 110)	114 (76 - 150)	101 (78 - 130)
SAP after ET Iso Surgery determined			
- Mean ± SD	ND	125 ± 22[*, #]	120 ± 20[*, #]
- Median (range)	ND	130 (80 - 150)	115 (95 - 160)

(ND - not determined; * - significantly different (p <0.05) from the value before drug injection for each variable in the same column or group; [£] - significantly different from the value at 15 minutes after drug injection for each variable in the same column or group; [#] - significantly different from the value after Iso MAC determined for each variable in the same column or group; [a, b, c] - values with different superscript letters for each variable in the same row are significantly different).

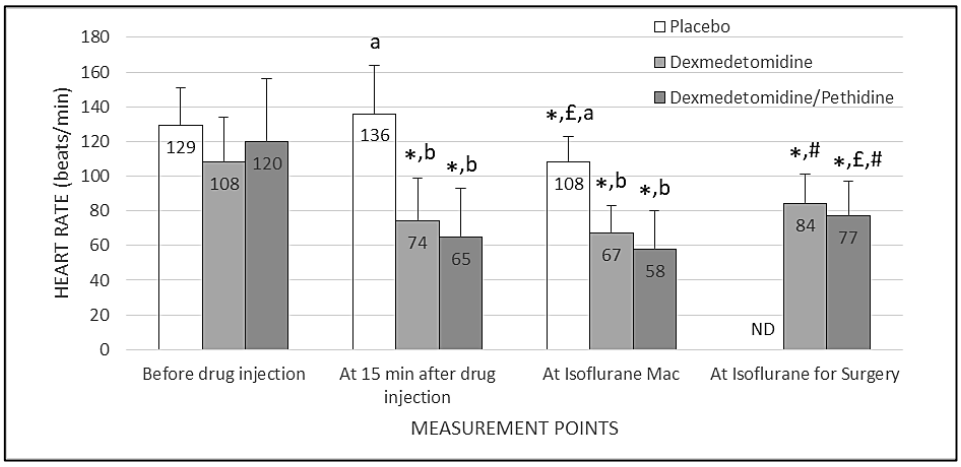

Figure 10 Heart rates before injection of the tested drug, 15 minutes after the injection, at the isoflurane MAC determination, and at the ET isoflurane concentration for surgery determination of dogs in groups receiving placebo, dexmedetomidine, and dexmedetomidine combined with pethidine (ND - not determined; * - significantly different (p <0.05) from the HR before drug injection in the same group; £ - significantly different from the HR at 15 minutes after drug injection in the same group; # - significantly different from the HR at the Iso MAC determination in the same group; a, b – HR with different superscript letters at the same measurement point are significantly different between groups).

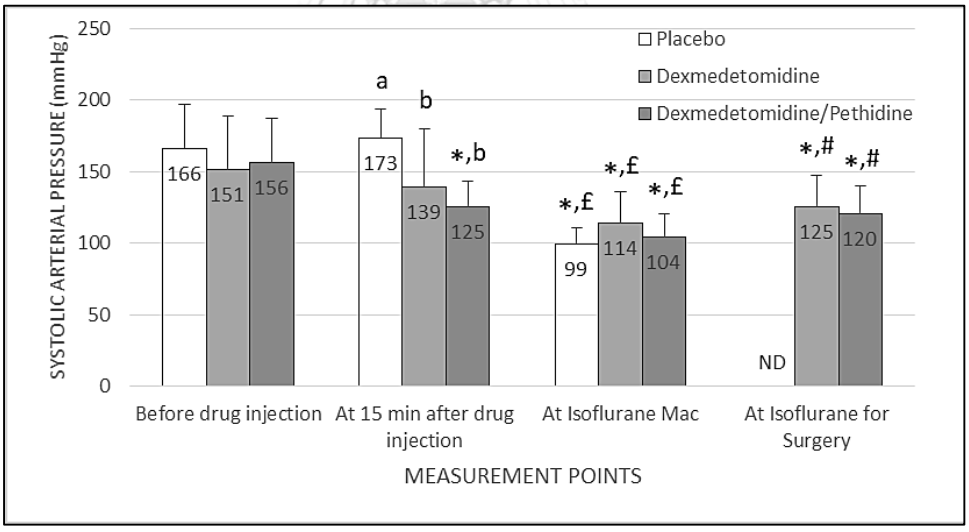

Figure 11 Systolic arterial pressures before injection of the tested drug, 15 minutes after the injection, at the isoflurane MAC determination, and at the ET isoflurane concentration for surgery determination of dogs in groups receiving placebo, dexmedetomidine, and dexmedetomidine combined with pethidine (ND - not determined; * - significantly different (p <0.05) from the SAP before drug injection in the same group; £ - significantly different from the SAP at 15 minutes after drug injection in the same group; # - significantly different from the SAP at the Iso MAC determination in the same group; a, b – SAP with different superscript letters of the same measurement point are significantly different between groups).

CHAPTER V
CONCLUSION AND DISCUSSION

5.1 Conclusion

This study evaluated the effects of dexmedetomidine combined with pethidine on the minimum alveolar concentration (MAC) of isoflurane and cardiorespiratory variables in thirty client-owned, healthy adult male dogs scheduled for castration at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The dogs were allocated in an alternate sequence into Group 1 (n=6) receiving sterilised water for injections as placebo IM (a negative control group), Group 2 (n=12) receiving 5 μg kg^{-1} of dexmedetomidine IM (a positive control group), and, Group 3 (n=12) receiving 5 μg kg^{-1} of dexmedetomidine and 5 mg kg^{-1} of pethidine IM. Sedation and antinociception were evaluated 15 minutes after injection of the tested drug. Then, anesthesia was induced via a face mask with 4% isoflurane in 4 L min^{-1} of oxygen. End tidal (ET) isoflurane concentration and ET carbon dioxide were monitored by a Multi-Gas Analyzer connected to the endotracheal tube. The ET isoflurane had been initially set at 1% for at least 15 minutes for anesthetic equilibration before the noxious stimulation was carried out by clamping the 3rd or 4th digit of the hind limb using a 20-cm hemostat for no longer than 1 minute. A positive response was considered when there was a gross purposeful movement of the head or extremities, including jerking or twisting of the head or movement of extremities. The ET isoflurane concentration was increased or decreased by 0.1 - 0.2%, depending on degree of the response, once the response was positive or negative, respectively. The new ET concentration was maintained for at least 15 minutes for anesthetic equilibration before the noxious stimulation was repeated. The isoflurane MAC is the average concentration of the ET isoflurane concentration between the highest ET concentration at which the purposeful movement is detected and the lowest ET concentration at which the movement is not detected. The isoflurane MAC and the time to the isoflurane MAC determination were recorded. After attaining the isoflurane MAC, the animal was castrated. The ET isoflurane concentration for surgery and the time to the ET isoflurane concentration for surgery determination were recorded. Cardiorespiratory variables included respiratory rates before and at 15 minutes after injection of the tested drug, heart rates and systolic arterial pressures before the tested drug injection, at 15 minutes after the injection, at the isoflurane MAC determination, and at the ET isoflurane concentration for surgery determination, and venous blood gases before the tested drug injection and at the isoflurane MAC determination. Data were analyzed by t-test and analysis of variance. A p – value of less than 0.05 was considered significant. Dexmedetomidine administered with pethidine provided significantly greater sedation and less isoflurane MAC than dexmedetomidine given alone. Sedation scores after

dexmedetomidine given alone and in combination with pethidine were significantly higher than after the placebo injection, and the score after the combination was significantly greater than that after dexmedetomidine alone. All thirty dogs responded to the noxious stimulation before anesthesia induction. Means ± SD (medians) of the isoflurane MAC of dogs were 1.63 ± 0.23% (1.60%), 1.31 ± 0.33% (1.30%), and 0.98 ± 0.24% (0.95%) for Groups 1, 2, and 3, respectively. The mean isoflurane MAC of Groups 2 and 3 were significantly less than that of Group 1, and the mean isoflurane MAC of Group 3 was significantly less than that of Group 2. Cardiorespiratory variables except the heart rate were within the clinically acceptable limits.

5.2 Discussion

Dexmedetomidine given with pethidine provided more sedation and significantly less isoflurane MAC than dexmedetomidine given alone, indicating synergism of the two drugs. Sedation was not found in any dogs of Group 1 while it was found in Groups 2 and 3. Sedation score of dogs receiving dexmedetomidine combined with pethidine was greater than the score of dogs receiving only dexmedetomidine supporting the finding of Grint et al. (2009). Mean isoflurane MAC of Group 1 receiving placebo in this study was 1.63 ± 0.23% which was more than those of Groups 2 and 3. This indicate sparing effect of dexmedetomidine and dexmedetomidine combined with pethidine on the isoflurane MAC. When compared with the isoflurane MAC of Group 1, dexmedetomidine alone (Group 2) and dexmedetomidine combined with pethidine (Group 3) reduced the isoflurane MAC by 19.63% and by 39.88%, respectively. Mean basal isoflurane MAC of 1.34 ± 0.11% was previously reported by a study using mask induction of anesthesia and noxious stimulation technique similar to the present study (Valverde et al., 2004). Moreover, another study reported mean ± SD of the basal isoflurane MAC of 1.58 ± 0.28% for inhibiting response to the noxious stimulation similar to ours in dogs receiving anesthesia induction with propofol and isoflurane for 90 minutes to minimize the effect of propofol (Acevedo-Arcique et al., 2014). Mean isoflurane MAC (0.98 ± 0.24%) for dogs receiving dexmedetomidine combined with pethidine in the present study is less than both of the reported mean basal isoflurane MAC. This additionally supports that dexmedetomidine combined with pethidine has sparing effect on the isoflurane MAC. In addition, the mean isoflurane MAC for dogs receiving dexmedetomidine and pethidine is less than means ± SD of the reported basal isoflurane MACs for inhibiting response to electrical stimulation at the buccal mucosa, including 1.80 ± 0.21% (Hellyer et al., 2001) and 1.20 ± 0.17% (Wilson et al., 2006) after mask anesthesia induction with isoflurane in oxygen, or 1.38 ± 0.08% (Muir et al., 2003) and 1.41 ± 0.10% (Ueyama et al., 2009) after anesthesia induction with propofol.

The present study started the trial with the end tidal isoflurane concentration of 1% which was less than the basal isoflurane MAC of 1.34 ± 0.11% for dogs receiving similar anesthesia induction and noxious stimulation (Valverde et al., 2004), and slightly higher than the isoflurane MAC of 0.90 ± 0.17% for dogs receiving dexmedetomodine after propofol anesthesia induction and the noxious stimulation similar to the present study (Acevedo-Arcique et al., 2014). Our study followed the technique of noxious stimulation used by (Valverde et al., 2004), but increased the end tidal isoflurane concentration by 0.1 - 0.2% once the purposeful response was present or absent, and allowed 15 minutes for anesthetic equilibration before repeating the noxious stimulation. The settings were comparable to those utilized in the previous studies on determination of isoflurane MACs for inhibiting response to the noxious stimulation in dogs. Once the purposeful response was present or absent, the end tidal concentration of isoflurane was increased or decreased, respectively, by 10-20% of the preceding concentration (Hellyer et al., 2001), increased by 10% or decreased by 20%, respectively (Muir et al., 2003; Wilson et al., 2006; Ueyama et al., 2009), and increased or decreased, respectively, by 0.1% (Valverde et al., 2004; Acevedo-Arcique et al., 2014). Then, the new end tidal concentration of isoflurane was maintained for at least 15 minutes for anesthetic equilibration in three studies (Muir et al., 2003; Wilson et al., 2006; Ueyama et al., 2009), but for at least 20 minutes in other three studies (Hellyer et al., 2001; Valverde et al., 2004; Acevedo-Arcique et al., 2014).

The ET isoflurane concentration for surgery was greater than the isoflurane MAC due to more severe pain during castration than clamping the digit, and over-time reduction in serum concentrations of dexmedetomidine and/or pethidine (Kuusela et al., 2001; Uilenreef et al., 2008). Waterman and Kalthum (1989) reported plasma concentrations of pethidine of 0.4 mcg/ml produced complete analgesia and of above 0.2 mcg/ml produced useful though not complete analgesia in dogs. These concentrations were maintained for 120 minutes after an IM dose of 3.5 mg/kg. Steffey et al. (1977) reported plasma concentrations of pethidine of above 0.4 mcg/ml remaining for 100 minutes and of above 0.2 mcg/ml remaining for 180 minutes following 5.5 mg/kg IM pethidine in anesthetized dogs. For dexmedetomidine or medetomidine, duration of the effect ranges from 30-180 minutes depending on the dose administered. The addition of an opioids may prolong analgesia depending on the opioid chosen, the dose, and the route of administration (Pypendop, 2015). Dexmedetomidine administration of 20 mcg/kg IV reduced isoflurane anesthetic requirements by 89% at 30 min and by 50% at 165 min (Bloor et al., 1992). From time to isoflurane MAC determination and time to ET isoflurane concentration for surgery determination of Groups 2 and 3 serum concentrations of pethidine and dexmedetomidine still remained for analgesia. Ratios btween the end tidal concentrations of isoflurane during castration of Groups 2 and 3 were 1.23 and 1.06 times of the isoflurane MAC of Group 1, respectively. Light to deep levels of anesthesia usually require 1 to 2 MAC of an inhalant anesthetic (Tranquilli et al., 2007).

Decreased respiratory rate, bradycardia and instant hypertension followed by hypotension are adverse clinical effects of α-2 adrenergic agonists (Dart, 1999; Pypendop, 2015). The combination of dexmedetomidine with other opioids such as butorphanol, buprenorphine (Leppanen et al., 2006), morphine, methadone, and tramadol (Cardoso et al., 2014) decreased the respiratory rates of dogs. The respiratory rate in this study was decreased after injection of dexmedetomidine alone or in combination with pethidine. However, the rates were within the clinically acceptable limit and did not differ significantly between groups. Therefore, pethidine did not alter the respiratory rate when compared with the bradypnea induced by dexmedetomidine alone, though opioids can depress respiration by reducing the response to hypercapnia (Steffey et al., 1993). In the present study, none of the dogs were hypercapnic because a ventilator was used to control ET carbon dioxide and uptake of oxygen and isoflurane. All blood gas variables before injection of the tested drug and after isoflurane MAC determination were within the normal limit although some significant changes were observed. There were no significant differences in blood gas variables among the three groups.

From Group 1, significant decrease in HR after determining the isoflurane MAC when compared with those before and at 15 minutes after placebo injection indicated the effect of isoflurane. HR at 15 minutes after injection of the tested drug in Groups 2 and 3, but not Group 1, were significantly lower than the rate before the injection, and were significantly less than HR at 15 minutes after placebo injection in Group 1. Therefore, bradycardia in Groups 2 and 3 was the reflex response to the vasoconstrictive effects on the α-2 adrenoreceptors and a reducing effect on the sympathetic tone of dexmedetomidine. In addition, the decrease in HR at 15 minutes after drug injection in Groups 2 and 3 was the result of the lower awareness of surroundings and lower stimulation due to the sedative effect of dexmedetomidine and pethidine. From previous studies, HR decreased in dogs receiving dexmedetomidine alone and dexmedetomidine in combination with morphine, methadone, tramadol (Cardoso et al., 2014), butorphanol or buprenorphine (Leppanen et al., 2006). In the present study, there were no significant differences in the HR between Group 2 receiving only dexmedetomidine 5 μg kg^{-1} IM and Group 3 receiving dexmedetomidine 5 μg kg^{-1}IM and pethidine 5 mg kg^{-1} IM. Similarly, Grint et al. (2009) reported that the HR of dogs receiving a combination of dexmedetomidine 5 μg kg^{-1} IM with pethidine 5 mg kg^{-1} IM did not differ from the HR of dogs receiving only dexmedetomidine 5 or 10 μg kg^{-1} IM. Therefore, pethidine does not seem to aggravate the bradycardia induced by the dexmedetomidine. In contrast with Stoelting (1999)'s suggestion on the vagoytic effect of pethidine, this study did not find evidence that pethidine reverses the bradycardia induced by α-2 adrenergic agonists, supporting the finding of Grint et al. (2009) using similar IM dose rates of pethidine and dexmedetomidine.

In Groups 2 and 3, the HR measured after determining the ET isoflurane concentration for surgery was significantly higher than the rate after determining the isoflurane MAC. The higher HR was due to the reduction in drug effect caused by the reduction in the plasma concentrations of dexmedetomidine and/or pethidine over time. Insufficient anesthesia and surgical stimulation might be the cause of the increased HR, but the HR measured after determining the ET isoflurane concentration for surgery in both groups were within the normal range of the HR during anesthesia in small breed dogs.

The SAP measured after determining the isoflurane MAC in Group 1 was lower than the SAP before and at 15 minutes after placebo injection, suggesting the effect of isoflurane. All SAP after injection of the tested drug in Group 3, but only the SAP after determining the isoflurane MAC and after determining the ET isoflurane concentration for surgery in Group 2, were significantly lower than the SAP before drug injection. However, all SAP were within the clinically acceptable limit. The decrease in SAP in groups 2 and 3 were the result of the bradycardia that occurred as the reflex response to the vasoconstrictive effects and a reducing effect on the sympathetic tone of the α-2 adrenoreceptor agonists, dexmedetomidine (Sinclair, 2003). On contrary, Congdon et al. (2011) reported that mean arterial pressure was increased in dogs receiving dexmedetomidine 10 μg kg^{-1} IM. Due to no significant differences in SAP between Groups 2 and 3 at any of the measurement points, pethidine appeared to have no effect on vasoconstriction. Similarly, there were no significant differences between the SAP measured after administering dexmedetomidine alone and in combination with morphine, methadone or tramadol (Cardoso et al., 2014). The SAP during isoflurane anesthesia after determining the ET isoflurane concentration for surgery in Groups 2 and 3 were significantly higher than the SAP at Iso MAC determination. The reduction in plasma concentrations of dexmedetomidine and/or pethidine over time reduces their effects. Surgical stimulation might be the cause of the increased SAP, but the SAP measured after determining the ET isoflurane concentration for surgery in Groups 2 and 3 were within the normal range of the SAP during anesthesia in dogs.

Normothermia was maintained using a warm air mat to prevent hypothermia, which would increase the potency and prolong the duration of the drugs. Alpha2-adrenergic agonists depress thermoregulation, and together with a reduction in muscle activity reduce body temperature (Virtanen, 1989; Pypendop and Verstegen, 1998). Various opioids such as methadone, morphine, and tramadol could decrease body temperature in dogs (Monteiro et al., 2009; Cardoso et al., 2014).

From no significant differences between Groups 2 and 3 for the RR, HR and SBP at all measurement points, the reduction of the three vital signs were the clinical adverse effects of the α_2-adrenergic agonist (Pypendop, 2015) rather than pethidine. Though the RR were decreased, the rate

and blood gases remained within the clinically accepted limit. The decreased SAP after drug injection remained within the clinically accepted limit, though the HR were significantly decreased.

In conclusion, dexmedetomidine administered with pethidine provided significantly better sedation and a better sparing effect on the isoflurane MAC than dexmedetomidine alone. Cardiorespiratory variables did not differ significantly between the dogs receiving only dexmedetomidine and those receiving dexmedetomidine combined with pethidine. Only heart rate decreased to below the clinically acceptable limit. Similar to other opioids such as morphine and methadone, pethidine enhanced the sedative and analgesic effects of dexmedetomidine, and did not exacerbate deleterious effects on the cardiorespiratory variables such as the bradycardia and bradypnea induced by dexmedetomidine alone.

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APPENDIX

Appendix 1 Age, weight and breed of dogs in Group 1 receiving placebo IM.

Dog	Age (years)	Weight (kg)	Breed
1	1	3.4	Chi Hua Hua
2	5	5.4	Shih-Tzu
3	1	2.1	Chi Hua Hua
4	4	2.9	Pomeranian
5	4	3.05	Pomeranian
6	2.5	2.7	Pomeranian

Appendix 2 Age, weight and breed of dogs in Group 2 receiving dexmedetomidine 5 μg kg^{-1} IM.

Dog	Age (years)	Weight (kg)	Breed
1	3	6.4	Shih-Tzu
2	1.5	2.3	Chi Hua Hua
3	7	6.3	Poodle
4	2	2.7	Cross breed
5	4	6.3	Shih-Tzu
6	5.5	6	Shih-Tzu
7	3	3.2	Chi Hua Hua
8	1	8.7	Shih-Tzu
9	2	6	Pomeranian
10	7	2.7	Chi Hua Hua
11	1	2.7	Pomeranian
12	2	5.3	Chi Hua Hua

Appendix 3 Age, weight and breed of dogs in Group 3 receiving dexmedetomidine 5 μg kg^{-1}IM combined with pethidine 5 mg kg^{-1}IM.

Dog	Age (years)	Weight (kg)	Breed
1	7	2.4	Cross breed
2	2	5.1	Cross breed
3	4	8	Poodle
4	7	5.5	Cross breed
5	1	5.8	Shih-Tzu
6	2	6	Shih-Tzu
7	2	3.7	Chi Hua Hua
8	1	7.8	Shih-Tzu
9	6	4.6	Poodle
10	5	4.5	Pomeranian
11	4	3.7	Pomeranian
12	6	4.2	Poodle

Appendix 4 Sedation scores, isoflurane minimum alveolar concentrations (Iso MAC), and time to the Iso MAC determination of dogs in Group 1 receiving placebo IM.

Dog	Sedation score (1 – 3)	Iso MAC (%)	Time to Iso MAC (min)
1	0	1.95	100
2	0	1.65	168
3	0	1.35	61
4	0	1.45	84
5	0	1.55	80
6	0	1.85	129

Appendix 5 Sedation scores, isoflurane minimum alveolar concentrations (Iso MAC), end tidal isoflurane concentrations for surgery (ET Iso Surgery), ratios between the ET Iso Surgery of Group 2 and the Iso MAC of Group 1, time to the Iso MAC determination, and time to the ET Iso Surgery determination of dogs in Group 2 receiving dexmedetomidine 5 μg kg^{-1}IM.

Dog	Sedation score (1 – 3)	Iso MAC (%)	ET Iso Surgery (%)	ET Iso Surgery/ Iso MAC of Gr.1	Time to Iso MAC (min)	Time to ET Iso Surgery(min)
1	1	0.7	1.8	1.10	56	100
2	0	1.35	1.8	1.10	138	159
3	1	1.15	1.8	1.10	106	151
4	1	1.25	2	1.23	176	211
5	1	1.45	1.9	1.17	166	177
6	1	1.05	2.1	1.29	69	94
7	2	1.9	2.5	1.53	164	176
8	1	1.25	2	1.23	102	135
9	0	0.95	1.7	1.04	55	141
10	2	1.75	2.5	1.53	131	183
11	2	1.35	1.9	1.17	82	126
12	1	1.55	2.1	1.29	110	149

Appendix 6 Sedation scores, isoflurane minimum alveolar concentrations (Iso MAC), end tidal isoflurane concentrations for surgery (ET Iso Surgery), ratios between the ET Iso Surgery of Group 3 and the Iso MAC of Group 1, time to the Iso MAC determination, and time to the ET Iso Surgery determination of dogs in Group 3 receiving dexmedetomidine 5 μg kg^{-1}IM combined with pethidine 5 mg kg^{-1}IM.

Dog	Sedation score (1 – 3)	Iso MAC (%)	ET Iso Surgery (%)	ET Iso Surgery/ Iso MAC of Gr.1	Time to Iso MAC (min)	Time to ET Iso Surgery (min)
1	3	0.55	1.6	0.98	69	152
2	3	1.05	1.3	0.80	66	100
3	3	1.35	1.9	1.17	182	198
4	2	1.45	1.8	1.10	109	161
5	3	0.95	1.1	0.67	54	112
6	3	0.95	1.9	1.17	45	113
7	3	0.95	1.4	0.86	45	119
8	3	0.75	1.4	0.86	59	102
9	1	0.95	1.6	0.98	49	114
10	3	0.95	2.1	1.29	42	110
11	2	0.95	2.4	1.47	38	99
12	1	0.95	2.3	1.41	66	81

Appendix 7 Respiratory rates and venous blood gas variables of dogs in Group 1 receiving placebo IM.

Dog	RR (breaths/min) before drug Inj	RR (breaths/min) 15 min after drug Inj	pH before drug Inj	pH at Iso MAC	PCO_2 (mmHg) before drug Inj	PCO_2 (mmHg) at Iso MAC	HCO_3^{-1} (mEq/L) before drug Inj	HCO_3^{-1} (mEq/L) at Iso MAC
1	100	96	7.443	7.452	32.3	29.9	23.0	22.2
2	52	36	7.416	7.553	36.1	21.7	22.9	22.5
3	40	36	7.402	7.393	35.5	34.6	22.0	21.4
4	60	60	7.381	7.283	29.2	45.7	19.0	20.1
5	80	80	7.423	7.410	27.3	30.7	19.2	20.1
6	40	44	7.392	7.370	29.6	32.0	19.5	19.4

(Inj – injection)

Appendix 8 Respiratory rates and venous blood gas variables of dogs in Group 2 receiving dexmedetomidine 5 μg kg^{-1}IM.

Dog	RR (breaths/min) before drug Inj	RR (breaths/min) 15 min after drug Inj	pH before drug Inj	pH at Iso MAC	PCO_2 (mmHg) before drug Inj	PCO_2 (mmHg) at Iso MAC	HCO_3^{-1} (mEq/L) before drug Inj	HCO_3^{-1} (mEq/L) at Iso MAC
1	160	30	ND	ND	ND	ND	ND	ND
2	35	16	7.395	7.492	29.9	26.3	19.4	22.6
3	40	20	7.442	7.453	24.8	26.3	19.7	20.7
4	48	16	7.333	7.404	30.6	28.4	17.4	19.6
5	36	28	7.370	7.343	39.3	39.4	21.8	20.8
6	44	28	7.392	7.511	29.8	22.4	20.1	21.8
7	100	12	7.439	7.407	29.1	35.1	21.3	22.3
8	60	40	7.408	7.391	34.9	37.7	22.0	22.7
9	160	88	7.400	7.427	34.3	37.8	21.2	24.7
10	36	20	7.386	7.497	36.0	29.4	21.3	24.4
11	80	26	7.440	7.418	30.1	31.4	22.2	21.3
12	68	40	7.405	7.387	32.0	32.4	20.5	20.3

(ND – not determined, Inj – injection)

Appendix 9 Respiratory rates and venous blood gas variables of dogs in Group 3 receiving dexmedetomidine 5 μg kg^{-1}IM combined with pethidine 5 mg kg^{-1}IM.

Dog	RR (breaths/min) before drug Inj	RR (breaths/min) 15 min after drug Inj	pH before drug Inj	pH at Iso MAC	PCO_2 (mmHg) before drug Inj	PCO_2 (mmHg) at Iso MAC	HCO_3^{-1} (mEq/L) before drug Inj	HCO_3^{-1} (mEq/L) at Iso MAC
1	36	9	ND	ND	ND	ND	ND	ND
2	200	8	7.408	7.415	39.8	36.5	23.8	23.6
3	28	20	7.447	7.399	24.5	30.5	20.1	20.2
4	68	48	7.371	7.400	45.9	38.0	24.2	23.2
5	40	20	7.390	7.362	27.3	38.7	18.7	21.6
6	45	20	7.421	7.348	32.5	43.9	21.9	22.5
7	60	20	7.485	7.361	21.1	35.8	20.6	20.5
8	24	12	7.427	7.354	27.4	27.9	19.7	17.6
9	25	24	7.462	7.461	26.9	26.3	21.1	21.0
10	60	60	7.474	7.343	22.7	38.1	20.6	20.4
11	100	16	7.428	7.368	25.2	33.4	19.0	19.9
12	100	24	7.449	7.434	32.1	32.2	22.8	22.4

(ND – not determined, Inj – injection)

Appendix 10 Heart rates of dogs in Group 1 receiving placebo IM.

Dog	HR (beats/min) before drug injection	HR (beats/min) at 15 min after drug injection	HR (beats/min) at Iso MAC
1	112	150	118
2	120	112	116
3	140	132	97
4	140	140	127
5	100	100	86
6	160	180	104

Appendix 11 Heart rates of dogs in Group 2 receiving dexmedetomidine 5 μg kg^{-1}IM.

Dog	HR (beats/min) before drug injection	HR (beats/min) at 15 min after drug injection	HR (beats/min) at Iso MAC	HR (beats/min) at ET Iso Surgery
1	112	80	76	111
2	148	45	60	69
3	81	80	51	83
4	92	44	45	71
5	104	68	73	82
6	84	44	59	120
7	100	80	81	87
8	84	108	69	79
9	120	108	43	60
10	108	64	95	92
11	100	56	74	69
12	164	112	82	79

Appendix 12 Heart rates of dogs in Group 3 receiving dexmedetomidine 5 μg kg^{-1}IM combined with pethidine 5 mg kg^{-1}IM.

Dog	HR (beats/min) before drug injection	HR (beats/min) at 15 min after drug injection	HR (beats/min) at Iso MAC	HR (beats/min) at ET Iso Surgery
1	108	36	39	62
2	100	48	48	65
3	140	60	86	94
4	160	140	112	111
5	100	52	40	39
6	93	72	49	80
7	140	80	72	91
8	120	80	54	58
9	120	44	38	65
10	200	72	42	93
11	60	48	57	95
12	100	52	53	75

Appendix 13 Systolic arterial pressures (SAP) of dogs in Group 1 receiving placebo IM.

Dog	SAP (mmHg) before drug injection	SAP (mmHg) at 15 min after drug injection	SAP (mmHg) at Iso MAC
1	190	200	110
2	200	180	110
3	185	180	90
4	150	180	100
5	120	160	105
6	150	140	80

Appendix 14 Systolic arterial pressures (SAP) of dogs in Group 2 receiving dexmedetomidine 5 μg kg^{-1}IM.

Dog	SAP (mmHg) before drug injection	SAP (mmHg) at 15 min after drug injection	SAP (mmHg) at Iso MAC	SAP (mmHg) at ET Iso Surgery
1	115	240	142	150
2	220	140	115	150
3	200	100	112	130
4	100	110	130	130
5	120	120	108	150
6	134	105	106	110
7	130	135	80	80
8	160	160	150	130
9	135	125	130	130
10	190	120	76	96
11	130	120	100	120
12	180	195	122	120

Appendix 15 Systolic arterial pressures (SAP) of dogs in Group 3 receiving dexmedetomidine 5 μg kg^{-1}IM combined with pethidine 5 mg kg^{-1}IM.

Dog	SAP (mmHg) before drug injection	SAP (mmHg) at 15 min after drug injection	SAP (mmHg) at Iso MAC	SAP (mmHg) at ET Iso Surgery
1	190	130	100	160
2	220	125	130	120
3	170	115	80	110
4	165	140	98	95
5	95	100	78	120
6	135	145	110	120
7	160	120	120	120
8	130	124	122	160
9	160	120	100	110
10	150	90	100	110
11	160	150	110	110
12	140	140	102	102

VITA

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