Cardiovascular effects of low and a high dose of fentanyl in the isoflurane anesthetized dog: the influence of the anesthetic-sparing effect and the correction of bradycardia.

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

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Abstract

Fentanyl has historically been used to reduce inhalant anesthetic requirements in the dog, with the end goal of reducing detrimental cardiovascular effects seen with their use. While fentanyl has been investigated in this context with the older agent enflurane, this agent is no longer in common use. In the current literature, no studies exist that compare the effects of low and high doses of fentanyl on cardiovascular function in dogs anesthetized with isoflurane. In previous literature, a high dose of fentanyl improved cardiovascular function in enflurane anesthetized dogs only following correction of bradycardia associated with its use.

The objective of this study was to evaluate the effect of two doses of fentanyl on isoflurane requirement in the dog, followed by an evaluation of cardiovascular function in the isoflurane-anesthetized dog at equivalent depth of anesthesia. The hypothesis was that fentanyl would reduce inhalant requirements in a dose dependent fashion, and that cardiovascular function would increase with fentanyl administration only following correction of bradycardia.

A total of 8 healthy adult male beagle dogs were enrolled in this study, which was performed in a randomized cross-over design. Minimum Alveolar Concentration (MAC) was determined in these dogs via a 30 mA electric stimulation both before and after administration of a low (loading dose 30 µg/kg, continuous rate infusion (CRI) of 0.2 µg/kg/minute) or high (loading dose 90 µg/kg, CRI 0.8 µg/kg/min) dose of fentanyl. A 7-day washout was observed between experimental days. Following MAC determination, in a subsequent anesthetic episode animals

were placed at a MAC multiple of 1.3 and cardiovascular and blood gas parameters were evaluated before and after each fentanyl dose in the presence and absence of bradycardia.

Fentanyl decreased MAC in a dose-dependent fashion ($p < 0.001$), with the low dose reducing MAC by about 42% and the high dose by about 77%. MAC reduction, however, did not translate into improvement in cardiovascular function, with a significant reduction in cardiac index and oxygen delivery noted with both doses ($p < 0.01$) that was not different between treatments. Normal mean arterial pressures were maintained with both treatments despite these effects. Only with the high dose, however, correction of bradycardia caused an increase in both cardiac index and oxygen delivery ($p < 0.02$) when compared to isoflurane alone.

In clinically healthy dogs, administration of a high dose of fentanyl increased cardiac function following correction of bradycardia, but a decrease was observed when bradycardia went uncorrected. Further studies are needed in order to evaluate these effects in clinical patients.

Cardiovascular effects of low and a high dose of fentanyl in the isoflurane anesthetized dog: the influence of the anesthetic-sparing effect and the correction of bradycardia.

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General Audience Abstract

Fentanyl, a potent opioid painkiller, has historically been used to reduce inhalant anesthetic requirements in the dog, with the end goal of reducing detrimental effects on the heart and blood vessels seen with their use. While fentanyl has been investigated in this context with the older agent enflurane, this agent is no longer in common use. In the current literature, no studies exist that compare the effects of low and high doses of fentanyl on heart function in dogs anesthetized with isoflurane. In previous literature, a high dose of fentanyl improved blood flow in enflurane anesthetized dogs only following correction of the low heart rate associated with its use.

The objective of this study was to evaluate the effect of two doses of fentanyl on isoflurane requirement in the dog, followed by an evaluation of heart function and blood flow in the isoflurane-anesthetized dog at equivalent depth of anesthesia. The hypothesis was that fentanyl would reduce isoflurane requirements in a dose dependent fashion, and that heart function would increase with fentanyl administration only following correction of low heart rate.

A total of 8 healthy adult male beagle dogs were enrolled in this study, which was performed in a randomized cross-over design. Inhalant anesthetic requirement was assessed with an electric stimulation both before and after administration of a low or high dose of fentanyl. A 7-day washout was observed between experimental days. In a subsequent anesthetic episode animals

were placed at a surgical anesthetic depth and cardiac and blood gas parameters were evaluated before and after each fentanyl dose in the presence and absence of low heart rate.

Fentanyl decreased inhalant requirements in a dose-dependent fashion. This did not translate into improvement in cardiovascular function, with a significant reduction in blood flow and oxygen delivery noted with both doses that was not different between treatments. Normal blood pressure was maintained with both treatments despite these effects. Only with the high dose, however, correction of low heart rate caused an increase in both blood flow and oxygen delivery when compared to isoflurane alone.

In clinically healthy dogs, administration of a high dose of fentanyl increased heart function following correction of low heart rate, but a decrease was observed when the low rate went uncorrected. Further studies are needed in order to evaluate these effects in clinical patients.

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Attribution

The studies described herein were performed under the Department of Small Animal Clinical Sciences of Virginia-Maryland College of Veterinary Medicine. Dr. Williamson performed the experiments, collected data and ultimately produced this thesis under the supervision and guidance of Dr. Soares. Drs Pavlisko and Guerrero provided technical and logistical support and assisted in the revision of the manuscript. R.M. Council-Troche was responsible for the analysis of fentanyl plasma concentration.

Chapter I – Introduction

A variety of agents have been historically used to provide anesthesia in veterinary patients. These agents provoke unconsciousness, amnesia and muscle relaxation, allowing for provision of a variety of procedures from routine diagnostics to invasive surgical procedures. With few exceptions, however, these agents are also associated with a number of detrimental effects which are often dose dependent in nature (Steffey & Howland 1977). Most agents do not provide any form of anti-nociception or act efficiently to control response to sympathetic stimulation. This may contribute to hemodynamic instability during a procedure and necessitates analgesia following a procedure (Steffey et al. 2015). Further, volatile anesthetics have been noted to cause significant hemodynamic depression in patients. Cardiac output and mean arterial pressure have been noted to decrease significantly with the use of volatile agents (Pagel et al. 1991). This may prove detrimental to the patient under anesthesia as this can reduce the flow of blood and thereby oxygen to the tissues (Opie 1998; Walsh et al. 2013).

From these facts has come the philosophy of balanced anesthesia, which considers that the addition of several agents to an anesthetic protocol can provide a reduction of their individual doses while reducing undesired effects, with the ultimate goal to improve patient outcome (Lundy 1926; Tonner 2005). This philosophy is in common use today in both human and veterinary anesthesia, and is evident in the common use of analgesic techniques in association with inhalant anesthetics. A variety of different drugs are used in the provision of balanced anesthesia, depending on the clinical goals of the practitioner. Opioids are a commonly used drug, due to their relatively mild evident cardiovascular effects and, in the dog among other animal species, a noted ability to decrease inhalant requirements significantly (Murphy & Hug

1982a; Steffey et al. 1994). Additionally, these drugs provide analgesia in the awake animal postoperatively, reducing stress on the patient (Gutstein & Akil 2006). Other drug classes investigated in this context include the phenothiazines, benzodiazepines, alpha-2 agonists and dissociative anesthetics, among others (Webb & O'Brien 1988; Gutierrez-Blanco et al. 2013). Fentanyl is a particular opioid that is commonly used in the provision of balanced anesthesia (Ilkiw 1999). It has a relatively short elimination half-life compared to other opioids such as morphine (Barnhart et al. 2000), with a rapid equilibration time between body compartments (Sano et al. 2006), allowing for its use as a continuous rate infusion. Use of fentanyl during anesthesia has been suggested to improve cardiovascular stability due to its minimal effects on myocardial contractility, and high doses of fentanyl or its derivatives sufentanil or remifentanil are typical in anesthetic protocols for severely compromised humans in emergency procedures (Gutstein & Akil 2006). Fentanyl cannot by itself provoke surgical planes of anesthesia in the dog, unlike in other species such as humans (Limn et al. 1979; Bailey et al. 1987), however it is nonetheless used commonly as an adjuvant to anesthesia primarily due to its ability to reduce inhalant requirements (Murphy & Hug 1982b).

Fentanyl's dose-dependent ability to reduce inhalant anesthetic requirement in the dog has previously been explored in the context of enflurane-based anesthesia (Murphy & Hug 1982b). Enflurane, however, is no longer in common use due to significant side effects including decreased myocardial contractility and epileptogenesis (Steffey et al. 2015). Prior to our recent publication (Williamson et al. 2017), it was unknown as to whether or not the inhalant-sparing effects would be equivalent between inhalants or if differences would exist, with the literature suggesting both possibilities could occur (Webb & O'Brien 1988; Steffey et al. 1993).

The use of fentanyl has been shown to cause bradycardia in the anesthetized dog, which under enflurane anesthesia was shown to reduce cardiac output (Ilkiw et al. 1994). This reduction in cardiac output was reversible with the administration of an anticholinergic agent, atropine, following which cardiac output became superior to the use of enflurane alone (Ilkiw et al. 1994). While some effort has been made to address the use of fentanyl in the context of the more commonly used inhaled agents such as isoflurane (Simões et al. 2016) in dogs, no current literature exists to justify a particular dosing regimen or to suggest that a high dose would be superior or inferior to a low dose in terms of cardiovascular sparing effects.

Chapter II – Literature Review

Balanced Anesthesia

The concept of balanced anesthesia was pioneered by J. S. Lundy in 1926, and is fundamental to the practice of anesthesia, especially in critically ill patients (Lundy 1926; Ilkiw 1999; Tonner 2005). This principle involves the administration of a variety of anesthetic modalities such as drugs targeting multiple pathways or loco-regional anesthetic techniques in order to minimize side effects and maximize their benefits. The classical example of balanced anesthesia is the use of a longer-lasting premedication prior to induction of anesthesia, which has been shown to decrease inhalant requirements and induction doses, potentially reducing the negative side effects associated with inhalant use (Ilkiw et al. 1994; Pottie et al. 2008). In modern practice this also extends to the use of alternative modalities of analgesia such as local, regional or epidural blockade (Ilkiw 1999). The use of balanced anesthetic techniques is also likely to result in a reduction in patient morbidity and mortality (Ilkiw 1999; Bille 2012), however the full relationship has yet to be fully elucidated in dogs, and some studies suggest that at least in terms of premedication there may not be a benefit in terms of mortality (Brodbelt et al. 2007). Other benefits of balanced anesthesia include more efficient and potentially longer lasting control of sympathetic stimulation during surgery, as well as analgesia postoperatively (Ilkiw 1999). Balanced anesthesia techniques, in general, tend to reduce inhalant anesthetic requirements as represented by minimum alveolar concentration (MAC). This is important because of the significant cardiac, respiratory and vascular effects of these agents, which may be of greater detriment to patients with reduced cardiovascular stability (Ilkiw 1999; Ebert & Lindenbaum 2013). In particular, inhalants tend to reduce cardiac contractility and cause vasodilation, which can reduce venous return and thereby cardiac output (Steffey et al. 2015). The use of drugs

which may have less detrimental effects on the myocardium than the inhalants that they effectively replace may ultimately result in improved stability under anesthesia and thereby a better outcome (Ilkiw 1999).

Various drug classes have been used in the provision of balanced anesthesia. The phenothiazines have been shown to produce sedation and reduce MAC in the dog, however this beneficial effect may be somewhat limited by the propensity of these drugs to vasodilate (Webb & O'Brien 1988). Benzodiazepines have minimal cardiovascular effects and have been shown to moderately reduce MAC in the dog which may act co-operatively with other agents (Hellyer et al. 2001), however these drugs are unreliable sedatives in awake and healthy patients (Herron et al. 2008). Alpha-2 agonists such as dexmedetomidine have also been described, however this drug class has significant depression of cardiac output associated with its use, which may not be beneficial to unstable patients (Pascoe et al. 2006). Because of these factors, opioids are commonly used systemically to provide balanced anesthesia, as these drugs have been shown to reduce inhalant requirements with potentially little effect on cardiac function (Ilkiw 1999). Local anesthetic techniques, in addition to systemic pharmaceuticals, have been described to both provide analgesia and reduce anesthetic requirement, as reduction in nociception from a site necessarily reduces stimulation from surgery (Berg & Orton 1986; Ilkiw 1999).

Cardiovascular function during anesthesia

Cardiac Output

Clinical significance

Cardiac output (CO) is the volume of blood pumped by the heart on a per minute basis and together with arterial oxygen content constitutes the major determinants of oxygen delivery to the tissues (Haskins et al. 2005). It can be described as the product of heart rate and stroke volume, and as such any change in either factor will have significant effects on it (Haskins et al. 2005). The nature of the circulatory system, however, is that as heart rate increases, right atrial pressure and thus the cardiac filling pressure decreases (Hall 2011a). Cardiac output typically will increase despite this but in healthy adult humans this may occasionally result in little overall change in cardiac output within 10 minutes of administration of atropine, an anticholinergic drug that acts to increase heart rate with little other vascular effects (McMichael & Sharpey-Schafer 1944).

In general, the body strives to maintain a consistent cardiac output in order to maintain oxygen delivery to the tissues. Oxygen at the tissue level is utilized for basic cellular functions and a failure of oxygen delivery can constitute a serious event (Mellema 2001). Prolonged or pervasive failure of oxygen delivery on a whole-organism scale constitutes the clinical phenomenon of shock and can lead to severe consequences including acidosis, organ dysfunction or failure and death (Brady & Otto 2001). Maintenance of cardiac output in the anesthetized patient is therefore of paramount importance, especially in patients who are already compromised by their underlying clinical condition. In healthy animals, anesthetic maintenance with isoflurane at 1.2 MAC is characterized by little overall effect on cardiac output despite a reduction in systemic vascular resistance of approximately 20% (Bernard et al. 1990). In patients who are more

critically ill, however, the vasodilation induced by isoflurane may cause more significant impairment in circulation and worsening of perfusion, though the effects of this drug appear to be less significant than its predecessor, halothane (Teixeira Neto et al. 2007). In septic patients, for instance, myocardial contractility is significantly decreased and may not respond well to catecholamine stimulation. In human septic patients, left ventricular contractility is significantly impaired, resulting in a reduction of cardiac output which may be severe and non-responsive to therapy with beta-adrenergic agonists (Hunter & Doddi 2010). Blood pressure is typically decreased as well and may not respond to supportive therapy with vasopressors or inotropes as effectively as in the healthy patient (Brady & Otto 2001). In a canine hemorrhagic shock model, cardiac output following rapid hemorrhage (43 ml/kg) was reduced to approximately 26% of baseline (Cooper 2007).

Oxygen delivery

Cardiac output is utilized to deliver oxygen to the tissues. Tissue oxygen delivery (DO_2) is the product of arterial oxygen content (C_aO_2) and CO and is typically reported on a whole-organism basis, either in mL/min, mL/kg/min or mL/m^2/min. Delivery of oxygen is typically discussed in the context of whole-organism tissue oxygen consumption (VO_2), which represents the metabolic activity of the tissues. VO_2 is typically considered to be supply independent until DO_2 decreases below a specific point termed the critical DO_2. Below this point VO_2 decreases linearly with DO_2, and it is considered that DO_2 is likely inadequate to meet the needs of the tissue. In the anesthetized dog, this critical point is typically thought to lie around 9-10 mL/kg/min (Mellema 2001). When oxygen delivery decreases below this point, oxygen extraction ratio, the ratio of

VO_2 to DO_2 increases sharply and lactate becomes evident in the circulation, indicating tissue hypoperfusion and an increase in anaerobic metabolism (Mellema 2001).

Measurement

Several methodologies exist to measure cardiac output, each with its own benefits and drawbacks. The thermodilution technique for measurement of cardiac output is widely considered at this time to be the gold standard, though it is not definitive and may be subject to significant variation (Berthelsen et al. 2002). This methodology utilizes the injection of a bolus of cold or room temperature saline through the proximal port of a Swan-Ganz or similar pulmonary thermodilution catheter with the tip placed in the main pulmonary artery. A thermistor located at the distal tip of the catheter continuously measures the temperature of the blood surrounding it. After rapid injection of the bolus, cardiac output is determined by a modification of the Stewart-Hamilton equation (Berthelsen et al. 2002).

$$\dot{Q} = \frac{V \times (T_b - T_i) \times K}{\int_0^{t1} \Delta T(t)dt + 2\int_{t1}^{t2} \Delta T(t)dt}$$

Figure 1: The modified Stewart-Hamilton equation. V: Volume of injectate T_b: Temperature of blood T_i: Temperature of injectate K: Correction factor for specific heat and dead space, t1, t2: time when temperature increases to 20% and 60% above the nadir, respectively (Berthelsen et al. 2002).

This methodology requires the use of a pulmonary arterial catheter, the placement of which generally requires anesthesia or heavy sedation and can be challenging even with an experienced practitioner (Mellema 2001). Additionally, pathology can result from the placement of this device, including laceration/rupture of the main pulmonary artery, damage to cardiac valves and structures and arrhythmias. Because of this, transpulmonary thermodilution has been used

instead, which requires placement of a peripheral arterial and central venous catheter rather than a pulmonary arterial catheter and appears to have good agreement with traditional thermodilution (Friedman et al. 2006). Similar to arterial thermodilution, the lithium dilution method has been investigated in dogs and appears to have good agreement with thermodilution. This methodology instead of using changes in temperature following cold saline boluses involves the measurement of serum lithium concentrations following a bolus of lithium chloride. Similar to older dye dilution techniques, this technique is good for individual bolus use, however with multiple lithium infusions, accuracy may decrease due to accumulation of the lithium indicator. In addition the amount of blood drawn over the lithium sensor may preclude its use in very small patients. This method of measurement otherwise has a high degree of agreement with the standard method of thermodilution in dogs and has been used in several studies as the standard to which other methodologies are compared (Mason et al. 2001; Shih et al. 2011; Valverde et al. 2011).

Other less invasive methodologies for cardiac output include echocardiography and pulse-contour evaluation. Echocardiographic determination of cardiac output can be performed by transthoracic or esophageal echocardiography and involves estimation of stroke volume directly from two dimensional images of the heart chambers or via measurement of descending aortic flow and approximation to account for the quantity of flow diverted to the ascending portions of the arterial circulation (Ihlen et al. 1984). This methodology may be important for trending data, however it appears to have a variable error with a tendency to overestimate cardiac output.

Blood Pressure

Clinical Significance

Blood pressure is a derived value resulting from the product of CO with systemic vascular resistance (SVR). Three blood pressures are commonly measured, systolic arterial pressure, diastolic arterial pressure and mean arterial pressure. Most clinical significance is attributed to variations in the mean arterial pressure. Mean arterial pressure is commonly used clinically as a surrogate for CO in the peri-anesthetic setting largely due to the challenges associated with cardiac output described above. Blood pressure is thought to be representative of blood flow to the major organs in the body under anesthesia, with blood pressure within the limits of organ autoregulation (70-175 mmHg in most tissues (Hall 2011b)) suggesting adequate perfusion of tissues and thereby oxygen delivery. This is true to an extent, however, changes in vascular compliance and muscle tone are capable of increasing pressure with no change in flow simply through increases in resistance (Hall 2011c), which necessarily indicates that should this occur there will be no benefit to perfusion. A study evaluating septic patients showed little correlation between blood pressure and cardiac output outside of extreme hypotensive values, suggesting that in these patients maintenance of arterial tone is the mechanism by which blood pressure is maintained (Wo et al. 1993). Other studies have further attempted to characterize local tissue blood flow following administration of vasopressor agents and found that increases in blood pressure are not necessarily correlated with increases in local tissue perfusion (LeDoux et al. 2000; Dubin et al. 2009). This is important because at this time there is no clinically used methodology by which tissue perfusion can be directly observed. The net result of these studies is that in patients with hemodynamic alterations such as sepsis, blood pressure may not be necessarily indicative of either adequacy of cardiac function or tissue perfusion, and in fact

increases in blood pressure may instill a false sense of confidence in the clinician (Wo et al. 1993).

In healthy patients however, or those experiencing no increases in vasomotor tone, blood pressure may yet still represent the driving pressure by which the circulation is maintained, especially once it falls below the limits of autoregulation, at which point blood flow becomes linearly correlated with perfusion pressure (Hall 2011b). This is especially important in the peri-anesthetic setting as the inhaled volatile anesthetic agents have profound effects on blood pressure, largely through their interactions with vasomotor tone (Bernard et al. 1990). In the instrumented dog, isoflurane at a MAC multiple of 1.2 has been shown to decrease mean arterial pressure by 22%, entirely associated with a decrease in SVR of 22% and no significant change in cardiac output (Bernard et al. 1990). Further reduction in SVR may serve to increase venous capacitance and therefore reduce return and cardiac output (Hall 2011c).

Measurement

The gold standard for measurement of blood pressure is direct cannulation of a major artery such as the descending aorta, femoral or carotid artery (Barter & Epstein 2014). This is, however, impractical in most clinical settings as it requires invasive dissection into the neck, thigh or abdomen, with accompanying risk of significant bleeding (Schroeder et al. 2009). Other sites in the dog, such as the dorsal pedal or coccygeal arteries, have been investigated and found to closely correlate with aortic pressures (Soares et al. 2017) and require less invasive dissection. An important phenomenon to consider with more distal sites is that of pulse amplification, whereby the distensibility of the arteries diminishes as more distal sites with smaller diameters are cannulated. This typically does not impact the mean arterial pressure, instead increasing the

systolic pressure while shortening the time spent at peak pressures. At very distal sites, however, the mean blood pressure may also be impacted, with auricular pressures varying unpredictably and largely when compared to those in the distal aorta as blood pressure changes (Schroeder et al. 2009; Barter & Epstein 2014; Soares et al. 2017).

Direct measurement of blood pressure can be accomplished by several means. The most commonly used methodology is an electronic transducer, which allows for moment-to-moment measurement of blood pressure and often produces some form of visible tracing. This tracing represents the blood pressure data obtained by the transducer, and can provide the anesthetist further information on the hemodynamic status of the patient such as fluid responsiveness (Kramer et al. 2004; Schroeder et al. 2009). The transducer itself operates by means of a strain gauge coupled to a Wheatstone bridge which converts pressure signals to variations in resistance, which are determined by the monitor on a momentary basis (Szocik et al. 2009). This transducer is connected to the cannulated artery by a short length of noncompliant tubing with a natural frequency greater than the next 6-10 harmonics of the fundamental frequency (in this case, the heart rate). A patient with a heart rate of 120 beats/minute for instance, with a fundamental frequency of 2 Hz, requires a system with a natural frequency of 12-20 Hz or greater to faithfully replicate the arterial pressure waveform to avoid resonance. Generally, systems with a natural frequency less than 7.5 Hz are considered unacceptable in humans (Schroeder et al. 2009). As frequency increases, damping factors such as the presence of air within the tubing will additionally have less effect on the waveform.

Inhalant Anesthetics

Clinical Use

Inhalant anesthetics are chemical compounds that provoke general anesthesia following inhalation. In veterinary practice, these are typically volatile halogenated hydrocarbon molecules which are administered as vapor, typically either in 100% oxygen or some other mixture of gases (Steffey et al. 2015). Other inhalants can include nitrous oxide and xenon, which may see less common use due to lack of potency and high cost, respectively (Ebert & Lindenbaum 2013; Steffey et al. 2015) They are commonly used anesthetic agents due to their short onset and duration of action as well as limited metabolism. Isoflurane, sevoflurane and desflurane are among the most commonly used anesthetic agents and represent the most recent generation of inhalants (Ebert & Lindenbaum 2013; Steffey et al. 2015). Halothane and enflurane have also been historically used, however see little current use due to their greater cardiac effects than more modern agents (Klide 1976; Pagel et al. 1991; Steffey et al. 2015).

Inhalants are effective at inducing muscle relaxation, unconsciousness and amnesia. The mechanism by which they exert these effects has been incompletely characterized, however it is known that they interact with a number of cellular receptors including sodium, potassium and chloride channels, and may exert direct effects on the lipid and protein components of the neurons themselves (Campagna et al. 2003). The effect of these interactions is to depress central nervous system function in a dose dependent fashion, manifesting as a reduction in global supraspinal blood flow and metabolism, increased latency in somatosensory evoked potentials and an overall slowing of electroencephalographic (EEG) activity. Certain characteristic patterns are observed in the EEG waveform as well, including increased amplitude spikes interspersed with periods of little or no activity, a phenomenon noted at deeper planes of anesthesia termed

'burst suppression'. At lighter planes of anesthesia, waveforms observed are similar to EEG patterns seen during sleep (Campagna et al. 2003). Effects on the spinal column are primarily responsible for the development of muscle relaxation, and selective perfusion of the brain in the goat more than doubles required isoflurane concentrations to achieve immobility (Antognini 1993).

The action of inhalant anesthetics is dependent on the development of a certain partial pressure of gas within the tissue compartment of the central nervous system, rather than development of a concentration within the blood. As solubility in blood decreases, the partial pressure achieved in blood is greater for a given volume of agent that diffuses into the alveolar capillary beds. This allows for more rapid equilibration between compartments as less uptake is needed to achieve equivalent partial pressures (Ebert & Lindenbaum 2013). Newer agents are significantly less soluble than older agents in blood, though the magnitude to which this is true varies somewhat by species and agent (Soares et al. 2012).

Measurement of Inhalant Anesthetic Potency

Inhalant anesthetics are variably potent and typically in a manner proportional to their solubility in oil. This correlation is termed the Meyer-Overton hypothesis after its discoverers (Ebert & Lindenbaum 2013). The methodology to assess anesthetic potency was developed by Eger and successively refined by later generations of anesthesiologists (Eger et al. 1965; de Jong & Eger 1975; Quasha et al. 1980). Minimum alveolar concentration (MAC) is defined as the minimum alveolar concentration (in volumes percent at sea level) of any inhaled agent that is sufficient to prevent gross movement of the subject in response to a supramaximal noxious stimulation. It can also be generalized to a population level, where it is defined as the minimum alveolar

concentration of an agent that is needed to prevent such movement in 50% of the population (Quasha et al. 1980). Measurement of MAC has three key components: alveolar anesthetic concentration, the stimulus applied and the evaluation of the response to it.

Firstly, alveolar concentration of the agent must be determined by some methodology. This is typically done by sampling of gas at the end of expiration from an endotracheal tube as distally as is practicable. This gas is then ideally passed into a refractometry-based analyzer, however studies have shown that an infrared absorption spectrophotometer (among other methods) is also appropriate, providing it is calibrated with known standards (Rudolff et al. 2014). It is generally accepted that after approximately 15 minutes of equilibration partial pressures in the tissues of the vessel rich group such as the CNS and heart are in equilibrium with those in the alveoli, though with less-soluble modern anesthetics this may actually be shorter. This assumption may not hold true, however, in patients with compromised cardiac output, poor ventilation-perfusion matching or with highly soluble anesthetic agents such as methoxyflurane (Quasha et al. 1980).

Following determination of alveolar inhalant concentration and its maintenance, the stimulus is applied to the patient. This stimulus must be sufficient to cause a maximal level of stimulation but not provoke lasting harm to the patient. Studies done by Eger and others have shown that there is a level of stimulus beyond which no further likelihood of movement is observed; stimuli that reach and exceed this point are thereby termed supramaximal (Quasha et al. 1980). In human studies, a single incision is typically made for aesthetic and practical reasons, however in dogs this has not been found to be a supramaximal stimulus, and instead a tail clamping method is often used (Eger et al. 1965; Valverde et al. 2003). This is not without drawbacks, however, as improper or repeated application of this technique can cause injury to the underlying tissues including damage to the vertebrae of the tail itself. For this reason, other types of stimuli have

subsequently been evaluated for the determination of MAC, including electrical stimulation of the oral mucosa and appendages and clamping of the digits (Eger et al. 1988; Laster et al. 1993; Valverde et al. 2003; Figueiró et al. 2016). Electrical stimulation methodologies have been shown in the dog to be equivalent to tail clamping, providing a threshold current of 30 mA is surpassed when using constant-current electrical stimulus (Figueiró et al. 2016).

Following application of a stimulus, the patient's response must be observed. Eger chose to use gross movement as the endpoint for MAC, which is defined as a co-ordinated (though not necessarily conscious) movement of the appendages, head or neck. Stiffening of the patient, hyperventilation, coughing and chewing are not considered to be positive responses (Quasha et al. 1980). Other endpoints of MAC have been used in clinical studies, with the thought that they may be more relevant to patient care, as patients at MAC are, by definition, 50% likely to move which makes them poor surgical candidates. Alternatives, including the MAC to blunt autonomic responses to surgery (MAC_{BAR}) and MAC for responsiveness to verbal stimuli (MAC-awake) have been described, and may have more direct clinical relevance (Roizen et al. 1981; Gaumann et al. 1992; Aranake et al. 2013). Interestingly, limb isolation studies suggest that patients who are unconscious may still be capable of producing a motor response to verbal stimuli, which suggests a disconnect between the unconsciousness and muscle relaxation provoked by inhalants (Aranake et al. 2013).

MAC provides a standardized clinical reference frame from which we are able to directly compare the relative potency of inhalants under a variety of conditions. Many factors have been shown to directly influence it, including age, sex, oxygenation, carbon dioxide tension, body temperature and the administration of certain drugs (Quasha et al. 1980). Sedative drugs in particular, including the benzodiazepines, alpha-2 agonists, opioids and phenothiazines have

been shown to reduce MAC (Webb & O'Brien 1988; Pascoe et al. 2006; Ueyama et al. 2009; Monteiro et al. 2010b); central nervous system stimulants such as ephedrine will increase it, providing they can cross the blood-brain barrier (Steffey & Eger 1975). Certain disease states such as sepsis have also been shown to reduce MAC (Gill et al. 1995). The relative effects on MAC and thereby presumably anesthetic depth caused by any of these factors can give anesthetists an approximation of how these effects may influence the course of anesthesia for a given patient (Aranake et al. 2013).

Cardiovascular Effects

The development of a given partial pressure of anesthetic gas in the blood necessarily accompanies its distribution to all tissues of the body concordant with the blood flow to these tissues. As many of the putative sites of action of inhalants are not unique to the central nervous system this manifests in a number of less-desirable effects on several body systems, most notably the cardiovascular system. Administration of inhalants has been shown to decrease myocardial contractility in a dose dependent fashion in both in vitro and in vivo studies, with the degree of effect varying significantly by agent, with isoflurane and sevoflurane showing less effect than halothane and enflurane in the healthy dog (Bernard et al. 1990; Pagel et al. 1991; Hysing et al. 1992). The mechanism of action by which this occurs is posited to be through interaction with ionized calcium channels in the cell membrane and sarcoplasmic reticulum, reducing the overall influx of calcium and in the case of halothane actually serving to deplete the SR of calcium (Pagel et al. 2009). This results in a net decrease in left ventricular contractility and stroke volume which is partially compensated for by an increase in heart rate. This ultimately results in a decrease in cardiac output, with isoflurane showing roughly equivalent effect to sevoflurane,

and both showing less effect than halothane and enflurane (Bernard et al. 1990; Pagel et al. 1991). Mean arterial pressure is decreased as well through both the interactions of agents with the heart as described above, as well as direct effects on the calcium channels of the vascular smooth muscle causing a net reduction in SVR (Pagel et al. 2009). This decrease in vessel tone causes a reduction in preload due to venodilation, as well as a reduction in vascular resistance, one of the key components of blood pressure (Campagna et al. 2003; Hall 2011c).

Fentanyl

Clinical Use

Opioids are alkaloid derivatives of the compounds found in the opium poppy (Papaver somniferum) and are widely-used analgesic drugs in clinical practice. These compounds act by mimicking the biological activity of endogenous opioid compounds, including the dynorphins, enkephalins and endorphins. Morphine, one of the first compounds extracted from opium, is considered by many to be the prototypic opioid (Gutstein & Akil 2006).

Fentanyl (1-(2-phenethyl)-4-(*N*-propionylanilino)-piperidine) is a synthetic opioid derived from the molecule phenylpiperidine with principal action on the μ receptor family. It is estimated to be about 100 times as potent as morphine. Its clinical effects are similar to other μ-opioid agonist drugs and include analgesia and sedation (Gutstein & Akil 2006). Fentanyl and its derivatives are additionally noted for causing a dose-dependent bradycardia through activation of the parasympathetic nervous system in the brain (Griffioen et al. 2004).

Fentanyl has a rapid distribution time (~4.5 minutes), and also a short elimination half-life (~45 minutes) compared to other opioids such as morphine (80-95 minutes) (Barnhart et al. 2000; Sano et al. 2006) and hydromorphone (~60-80 minutes) (Guedes et al. 2008) and is often

administered as a continuous rate infusion to provide analgesic support. Its rapid onset of action is likely in part due to its highly lipid-soluble nature, which allows it to readily cross the blood-brain barrier (Gutstein & Akil 2006).

Fentanyl exerts its effects by interaction with opioid receptors, of which there are three classical receptor types MOP, KOP and DOP which are commonly denoted by the Greek letters μ, κ, and δ respectively. These receptors are G-protein coupled inhibitory receptors which interact to suppress the activity of adenylate cyclase, increase potassium conductance and decrease calcium conductance. The net effect of this process is hyperpolarization of the post-synaptic neuron and decreased neurotransmitter release; this acts to suppress neurotransmission, especially in the pain pathways (Griffioen et al. 2004; Gutstein & Akil 2006). The related orphanin FQ receptor has also been identified and is related to the classical opioid receptors. This receptor, however is notable for its excitatory effects on neurotransmission and has been incompletely characterized (Reinscheid et al. 1995).

Opioid receptors are located throughout the central nervous system, including the spinal cord and brain as well as within the peripheral tissues of the body. This structural localization is thought to explain the differences seen clinically between drugs which agonize only specific types of opioid receptor (Gutstein & Akil 2006).

Central nervous system effects

Opioids exert analgesic effect primarily through interactions within the brain and spinal cord, though peripheral mechanisms are thought to be of increased importance in recent scientific discussion especially in relation to their effects on control of inflammation (Gutstein & Akil 2006; Corder et al. 2017). Principally, opioids, especially agonists of the μ receptor, act by

inhibiting nociceptive transmission by activation of descending inhibitory pathways through the rostral anterior cingulate cortex, the rostral ventromedial medulla and the periacqueductal grey matter in the brain, as well by directly inhibiting neurotransmission at the level of the substantia gelatinosa and dorsal root ganglia of the spinal cord (Petrovic et al. 2002; Gutstein & Akil 2006). Opioids have been noted to produce several other central nervous system effects, including sedation, euphoria, dysphoria, respiratory depression, miosis and sedation, with effects varying based on dosage and species (KuKanich & Wiese 2015). These effects have not been completely characterized, but are thought to be in part due to the interactions of opioids with the dopaminergic system which is involved in reward behaviour (Gutstein & Akil 2006).

Metabolism

Fentanyl, like other opioids, is extensively metabolized in the liver, with as little as 1-5% excreted unchanged into the urine (Murphy et al. 1979; Murphy et al. 1983). Metabolism of fentanyl in the dog remains incompletely characterized. In humans, metabolism occurs principally through the action of the CYP3A4 enzyme, resulting in N-dealkylation of the amide group of fentanyl to produce norfentanyl (Feierman & Lasker 1996). Norfentanyl may have some weak agonist activity, but is largely considered to be an inactive metabolite (Feierman & Lasker 1996). The CYP3A12 enzyme appears to perform a similar functionality to CYP3A4 in the dog (Lu et al. 2005), however its inhibition with ketoconazole does not appear to affect metabolism, suggesting it is likely not involved in fentanyl metabolism in the dog (KuKanich & Hubin 2010). A single study found plasma concentrations of norfentanyl paralleling those of fentanyl in dogs under isoflurane anesthesia, suggesting that despite this fact, norfentanyl production may still be the primary mechanism through a different, unknown route, or simply

because the inhibition of CYP3A12 in the aforementioned study was not sufficient to reduce metabolism (Keating 2013).

One of the most relevant aspects of fentanyl's metabolism, however, is its relatively insaturable nature. Fentanyl's clearance has been shown in several studies to be relatively independent of dose and to roughly coincide with the quantity of hepatic blood flow (Björkman & Redke 2000; Keating 2013). The extraction ratio of fentanyl in the dog has been shown to approach 1.0 experimentally in awake animals, which means that all blood delivered to the liver is nearly completely cleared of fentanyl. (Björkman & Redke 2000). This therefore suggests that anything that reduces cardiac output without sparing hepatic blood flow preferentially would therefore necessarily reduce fentanyl clearance. This has been demonstrated in a study in human neonates, where a reduction in cardiac output secondary to increased intra-abdominal pressure was suspected to be the causative agent for a significant decrease in fentanyl clearance (Koehntop et al. 1986) and in human burn victims with increased cardiac output, where fentanyl clearance is significantly increased (Han et al. 2007). At this time, however, it is unknown the effect of changes in cardiac output on fentanyl disposition in the dog.

Anesthetic-Sparing Effects

The efficacy of fentanyl at reducing inhalant requirements has previously been studied in enflurane anesthetized dogs, where fentanyl with a loading dose of 15-270 µg/kg and an infusion rate of 0.05-3.2 µg/kg/min produced a maximal reduction in enflurane minimum alveolar concentration of 65% (Murphy & Hug 1982b). Reduction of MAC was notably dependent on plasma concentration and appeared to follow a logarithmic distribution, with little difference in MAC reduction being noted between doses of 0.8 µg/kg/min with a reduction of 64 ± 4% and 3.2

μg/kg/min with a MAC reduction of 66 ± 2% despite a reduction in fentanyl plasma concentration from 99 ± 33 ng/mL to 29 ± 7 ng/mL. This is likely due to saturation of the μ opioid receptors and may indicate the limit of MAC reduction in these individuals. In isoflurane anesthetized New Zealand white rabbits, a similar MAC reduction of approximately 60% was noted, though unlike in dogs, excessive movement was noted at the highest infusion rates (Barter et al. 2015).

Single doses of fentanyl have also been investigated in the context of the isoflurane anesthetized dog. A dose of 5 μg/kg followed by CRI of 0.15 μg/kg/min found a decrease in isoflurane MAC of 50% which did not depend on the duration of drug administration or administration of atropine CRI (0.04 mg/kg/hr) (Simões et al. 2016). A higher dose of fentanyl, 10 μg/kg followed by CRI of 0.3 μg/kg/min produced similar MAC reduction of 53% (Hellyer et al. 2001), which was further enhanced by diazepam to 71%. A 5 μg/kg bolus followed by an infusion of 0.5 μg/kg/min reduced isoflurane MAC by up to 66% (Steagall et al. 2006). Only the study by Hellyer et al. (2001) measured plasma fentanyl concentrations, which with fentanyl alone were up to 9.2 ng/mL. This is consistent with the values achieved by Murphy and Hug (1982) to achieve a MAC reduction of approximately 57%.

Cardiovascular effects

A study evaluating cardiac output in dogs anesthetized with enflurane showed a significant but mild decrease in cardiac output at equivalent anesthetic depth (1.3 MAC) with the addition of fentanyl sufficient to achieve steady state plasma concentrations of 71.7 ± 14.4 ng/mL, a level consistent with near-maximal MAC reduction. Cardiac index in these patients declined from 2.71 ± 0.48 to 2.24 ± 0.64 mL/m^2/min, a reduction of approximately 17%. These animals were

initially hypotensive (mean arterial pressure of 59 ± 6 mmHg) and became normotensive (82 ± 20 mmHg) despite this decrease in cardiac output (Ilkiw et al. 1994).

The decrease in cardiac output caused by fentanyl is characterized by minimal decreases in contractility in the healthy heart (Motomura et al. 1984) but rather a decrease in heart rate, provoked by activation of the parasympathetic nucleus ambiguus in the brain through inhibition of the inhibitory interneurons (Griffioen et al. 2004). Ilkiw et al. (1994) saw a 51% decrease in heart rate following administration of fentanyl, with a partially-compensatory increase in stroke volume of 83%. Following treatment of this bradycardia to achieve a rate of 93 ± 5 beats/minute, cardiac output was significantly increased by 74% compared to baseline values with enflurane alone. This value was actually 98% increased compared to the use of fentanyl alone.

Fentanyl and its derivatives have been shown to be associated with the release of vasopressin in a dose-dependent fashion, and may be associated with the release of other catecholamines (Weiskopf et al. 1987; Francis et al. 2008; Monteiro et al. 2010a). Vasopressin is a nonapeptide hormone produced by the hypothalamus that acts on the V_1 receptor; this is a G-protein coupled receptor activates inositol triphosphate production through phospholipase C, resulting in increased intracellular calcium concentrations (den Ouden & Meinders 2005). This increase in intracellular calcium causes an increase in vascular tone as it stimulates contraction of the vascular smooth muscle. Catecholamines act similarly through the alpha-1 and alpha-2 receptors, which is a G-protein coupled receptor with similar downstream pathways to the V_1 receptor (Opie 1998). Both of these substances serve to increase mean arterial pressure through increases in systemic vascular resistance (Francis et al. 2008).

Ilkiw et al. (1994) noted a significant increase in mean arterial pressure associated with the administration of fentanyl in the enflurane anesthetized dog at 1.3 times MAC despite no change

in CO. Systemic vascular resistance in these patients increased by 42% in these patients, resulting in an increase in mean arterial pressure of 28%. Similarly, administration of the related drug remifentanil at 1.3 times MAC in the isoflurane anesthetized dog increased systolic arterial pressure by up to 33% while decreasing cardiac output by up to 47%,

Chapter III – Isoflurane MAC-sparing effects in the Dog

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Abstract

Objective

To characterize the isoflurane-sparing effects of a high and a low dose of fentanyl in dogs, and its effects on mean arterial pressure (MAP) and heart rate.

Study design

Prospective, randomized crossover trial.

Animals

A total of eight healthy male Beagle dogs weighing 12.1 ± 1.6 kg (mean ± standard deviation) and approximate age 1 year.

Methods

Dogs were anesthetized using isoflurane and minimum alveolar concentration (MAC) was determined in duplicate by the bracketing method using an electrical stimulus on the tarsus. Animals were administered fentanyl: low dose (33 μg kg−1 loading dose, 0.2 μg kg−1 minute−1) or high dose (102 μg kg−1 loading dose, 0.8 μg kg−1 minute−1) and MAC was re-determined (MACISO-F). Blood was collected for analysis of plasma fentanyl concentrations prior to

administration and after MACISO-F determination. All values are presented as mean ± standard deviation.

Results

Isoflurane MAC (MACISO) was 1.30 ± 0.23% in the low dose treatment, which significantly decreased to 0.75 ± 0.22% (average MAC reduction 42.3 ± 9.4%). MACISO was 1.30 ± 0.18% in the high dose treatment, which significantly decreased to 0.30 ± 0.11% (average MAC reduction 76.9 ± 7.4%). Mean fentanyl plasma concentrations were 6.2 and 29.5 ng mL−1 for low and high dose treatments, respectively. MAP increased significantly only in the high dose treatment (from 81 ± 8 to 92 ± 9 mmHg). Heart rate decreased significantly in both treatments from 108 ± 25 to 61 ± 14 beats minute−1 with the low dose and from 95 ± 14 to 42 ± 4 beats minute−1 with the high dose.

Conclusions and clinical relevance

Fentanyl administration resulted in a dose-dependent isoflurane MAC-sparing effect with bradycardia at both doses and an increase in MAP only at high dose. Further evaluation is needed to determine the effects of fentanyl on the overall cardiovascular function.

Introduction

The minimum alveolar concentration (MAC)-reducing effects of different doses of fentanyl in enflurane-anesthetized dogs have been previously characterized (Murphy & Hug 1982a) but the authors have found no similar study with isoflurane published in the literature. Enflurane is no longer in routine clinical use (Steffey et al. 2015) and because differences exist in the mechanism of action of inhalants, there may be differences in the MAC-reducing properties of different inhalant anesthetics, as demonstrated for acepromazine when halothane was compared with isoflurane (Webb & O'Brien 1988).

While doses of fentanyl have been evaluated for their isoflurane MAC-sparing effect in dogs (Hellyer et al., 2001; Ueyama et al., 2009; Gutierrez-Blanco et al., 2013 ; Simões et al., 2016), the published studies did not compare multiple doses or plasma fentanyl concentrations.

The primary objective of this study was to measure the isoflurane-sparing effects of a high and a low dose of fentanyl in dogs, with a secondary objective of evaluating the effect of these doses on arterial pressure and heart rate (HR) at equipotent levels of anesthesia. The hypothesis was that fentanyl administration would result in a dose-dependent isoflurane-sparing effect in dogs. The secondary hypothesis was that the addition of fentanyl to isoflurane would increase blood pressure and decrease HR when compared with equipotent concentrations of isoflurane.

Materials and methods

Animals

A total of eight purpose-bred male adult Beagle dogs aged approximately 1 year and weighing 12.1 ± 1.6 kg (mean ± standard deviation) were enrolled in this study. Animals were determined to be healthy based on the results of a physical examination and routine basic blood work

(packed cell volume, serum biochemistry). Any animal displaying abnormalities that were suspected to affect MAC was removed from the study (Quasha et al. 1980). Animals were housed and maintained in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. Approval for this study was obtained from the Virginia Tech Institutional Animal Care and Use Committee prior to commencement (no.15-145). Animals were fasted for 12 hours prior to the experiment and water was freely available. This was a randomized crossover design study in which each animal underwent two anesthetic events separated by at least 1 week. Randomization was achieved using the RAND function of Excel (Version 14.6.7; Microsoft Corporation, CA, USA).

Instrumentation

Following preoxygenation using 100% oxygen delivered by a fitted facemask, the dog was anaesthetized using 5% isoflurane (FlurIso; VetOne, ID, USA) delivered in oxygen by a precision isoflurane-specific out of circuit vaporizer (Tec 7; Datex-Ohmeda Inc., WI, USA) until anesthesia was sufficient for endotracheal intubation. The dog was placed in lateral recumbency and the endotracheal tube was connected to a circle system (BleaseFocus; Spacelabs Healthcare Ltd, WA, USA). Spontaneous breathing continued with an end-tidal isoflurane concentration (Fe′Iso) ≥1.50% during the instrumentation period. Intravenous (IV) access was obtained through the cephalic vein using a 20-gauge 4.8-cm over-the-needle catheter (Jelco; Smiths Medical, UK). A 22-gauge 2.5-cm catheter (BD Insyte; Becton Dickinson Infusion Therapy Systems, Inc., UT, USA) was inserted aseptically into a dorsal pedal artery to facilitate invasive pressure measurement and collection of arterial blood.

The following measurements were obtained: pulse rate from the arterial pressure waveform, arterial hemoglobin oxygen saturation, rectal temperature, end-tidal carbon dioxide pressure, Fe′Iso, inspired oxygen concentration and respiratory rate, and arterial systolic, mean and diastolic pressures (SAP, MAP and DAP, respectively) using a standard anesthesia monitor (S/5 Anesthesia Monitor; GE Healthcare, WI, USA). Pulse rate was recorded as HR as no pulseless arrhythmia was detected during the experiments. The pressure transducer (APT300; Harvard Apparatus, MA, USA) was connected to noncompliant tubing continuously flushed with heparinized saline (2 units mL^{-1}). The transducer was calibrated using a mercury column and zeroed at the level of manubrium. The infrared gas analyzer was calibrated using three known concentrations of isoflurane (0.490, 1.504 and 2.530%, primary gas standards; Airgas Inc., PA, USA) at approximately 2-hour intervals. Fe′Iso was corrected for ambient pressure measured by a digital barometer (Fisher Scientific, NH, USA). A polypropylene catheter placed with its tip positioned at the midpoint of the endotracheal tube was used to obtain the gas samples for continuous analysis. Rectal temperature was maintained at 37.0–38.5 °C using a forced air-warming blanket (Bair Hugger; Arizant Inc., MN, USA). Lactated Ringer's solution (Hospira Inc., IL, USA) at 3 mL kg^{-1} $hour^{-1}$ was administered IV for the duration of anesthesia. Immediately prior to MAC determination, the dogs were mechanically ventilated with a tidal volume of 15 mL kg^{-1} and the respiratory rate was adjusted to maintain end-tidal carbon dioxide pressure at 35–45 mmHg (4.7–6.0 kPa).

Following instrumentation, a 1-hour period of equilibration was allowed prior to MAC determination.

MAC determination

MAC_{ISO} was evaluated by the bracketing technique using a nerve stimulator (NS242; Fisher & Paykel Healthcare Ltd, New Zealand) with the electrodes placed subcutaneously in the cranial aspect of the nondependent tarsus. A 50-Hz 30-mA square wave stimulus was applied for 1 minute or until movement, such as moving the head and/or limbs in response to the stimulus, was observed (positive response). Movement associated directly with the electrical stimulus, or responses such as chewing, swallowing or increased respiratory effort were not considered a positive response.

Once a positive or negative response was recorded, Fe′Iso was increased or decreased by 10%, respectively, and the dog was allowed to equilibrate at this concentration for 15 minutes prior to when the stimulus was repeated. This process was repeated until consecutive positive and negative responses were observed. MAC was calculated as the average Fe′Iso between these two responses. Two MAC values were determined and averaged to constitute an animal's MAC. If the response to the first Félso measurement after the first MAC determination resulted in an opposite response to the last Fe′Iso, the former was averaged with the latter to provide the second MAC value. If there was a difference >10% between the two MAC values, a third value was obtained and the average of all three values was used as the animal's MAC. This value was recorded as MAC_{ISO}. HR, SAP, MAP, and DAP were recorded prior to each stimulation event, with the values reported being the average of the data associated with all positive and negative responses used in calculating MAC.

On each study day, after the initial determination of MAC_{ISO}, animals were then randomly assigned to one of two IV fentanyl (Sublimaze; Hospira Inc.) treatments: administration of a low dose [30 μg kg^{-1} administered over 15 minutes and a continuous rate infusion (CRI) of 0.2 μg kg^{-1} $minute^{-1}$] or high dose (90 μg kg^{-1} administered over 15 minutes and 0.8 μg kg^{-1} $minute^{-1}$ CRI).

In both treatments, both infusions were started at the same time. Following infusion for 1 hour, MAC values were again determined and recorded as $MAC_{ISO\text{-}FENT\text{-}L}$ or $MAC_{ISO\text{-}FENT\text{-}H}$ for the low or the high dose of fentanyl, respectively. The noxious stimulation used for MAC determination was applied in the same limb during both experiment days.

Arterial blood (0.5 mL) was collected in nonheparinized plastic syringes after instrumentation and at the end of the experiment for immediate analysis of arterial pH, arterial partial pressure of carbon dioxide, arterial partial pressure of oxygen and base excess (i-STAT1 and CG4+ cartridges; Abaxis, CA, USA). An additional 5 mL arterial blood was collected immediately prior to MAC_{ISO} and $MAC_{ISO\text{-}FENT}$ determinations, and at the culmination of $MAC_{ISO\text{-}FENT}$ determination, for analysis of plasma fentanyl concentration. The blood was placed in a lithium heparin tube (BD Vacutainer; Becton Dickinson, UT, USA), centrifuged at 2500 g for 10 minutes and the plasma was separated and immediately stored at −80 °C for approximately 3 months prior to analysis.

Recovery from anesthesia

Fentanyl administration was stopped and isoflurane discontinued after determination of $MAC_{ISO\text{-}FENT}$. Carprofen (2.2 mg kg^{-1}; Rimadyl; Zoetis Inc., MI, USA) was administered subcutaneously and naloxone (0.02 mg kg^{-1}; Mylan Institutional, IL, USA) IV. The dog was monitored during recovery from anesthesia until coordinated ambulation was observed prior to being returned to its kennel. After ≥ 1 week, animals were re-anesthetized for determination of MAC using the alternate fentanyl dose.

Fentanyl analysis

Fentanyl was extracted from plasma samples using a modification of the method described by Coopman et al. (2007). Fentanyl-d5 (5 ng; internal standard) was added to 250 mg replicates of plasma and mixed briefly prior to adding 1 mL of water and 1 mL of 10.5% sodium carbonate. The fentanyl was extracted by adding 7 mL of a mixture of n-hexane:ethyl acetate (70:30) and mixed for 2 minutes. The samples were centrifuged for 5 minutes at 5,000 g and the top organic layer was transferred into 8 mL amber vials and dried down under a stream of nitrogen. The residues were then reconstituted in 0.5 mL of water:acetonitrile (60:40) and centrifuged at 13,000 g for 15 minutes. The supernatants were analyzed by high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS).

The LC-MS/MS analysis was performed using a liquid chromatograph system (1200 series; Agilent Technologies Inc., CA, USA) and a triple quadrupole mass spectrometer (G6470A; Agilent Technologies Inc.) with electrospray ionization operating in positive mode. Chromatographic separation was achieved using a column (4.6 × 50 mm × 2.7 μm) at 35 °C. The mobile phase was composed of 0.1% formic acid in water and 0.1% formic acid in acetonitrile (60:40) in isocratic mode, at 0.40 mL $minute^{-1}$. A rapid gradient was then performed to wash the column between runs. The injection volume was 10 μL. In these conditions, the retention time of fentanyl and internal standard were 2.1 minutes. Multiple reaction monitoring was performed to quantify fentanyl.

Standards for a four-point calibration curve were prepared in the same manner as the samples. Negative control dog plasma was spiked with fentanyl at concentrations ranging from 0.2 to 200 ng g^{-1}. The calibration curve was linear between these concentrations with $r2 > 0.999$. Quality control samples were also used to check for any potential variations between LC-MS/MS analyses. Three negative control plasma samples were spiked at approximately 1, 10 and 90 ng g^{-}

[1] in plasma and analyzed in duplicate with each batch of the fentanyl sample extracts. The lower limit of detection and the lower limit of quantification were 50 and 200 parts/per trillion, respectively. Multiplication of results by the specific gravity of canine plasma (1.025) converted the plasma fentanyl concentrations from ng g^{-1} to ng mL^{-1} (Remington & Baker 1958).

Statistical analysis

Data were evaluated using SAS JMP statistical software (SAS Institute, NC, USA). All data were evaluated for normal distribution by inspection of normal quantile plots and Shapiro–Wilk test.

Data for MAC were evaluated by ANOVA for repeated measures, with Tukey's test for intertreatment comparisons. Percentage changes in MAC value, total anesthesia time, fentanyl concentration, and fentanyl infusion time were compared by t test. Blood pressure and HR data were analyzed by mixed-model ANOVA using dose treatment as a fixed variable and dog identification as a random variable, with Bonferroni correction for intertreatment comparisons. Pearson correlation coefficient was calculated between each MAC value obtained in the low and high fentanyl doses with their respective time after the beginning of fentanyl CRI. Significance was set at $p < 0.05$.

Results

All data were normally distributed and are presented as mean ± standard deviation. There was no significant difference between treatments in MACISO prior to fentanyl administration (Table 1). MACISO-FENT was significantly lower in the high dose than the low dose treatment ($p < 0.0001$), with MAC reductions of 42.3 ± 9.4 and 76.9 ± 7.4% for the low and high doses, respectively (Table 1; $p < 0.0001$).

Fentanyl treatment	MAC_{ISO} (%)	$MAC_{ISO\text{-}FENT}$ (%)	MAC_{ISO} reduction (%)	Plasma fentanyl concentration (ng mL^{-1})
Low dose	1.30 ± 0.23	0.75 ± 0.22*†	42.3 ± 9.4†	6.2 ± 1.4†
High dose	1.30 ± 0.18	0.30 ± 0.11*	76.9 ± 7.4	29.5 ± 6.9

Table 1: Minimum alveolar concentration values with isoflurane alone (MAC_{ISO}) or with isoflurane-fentanyl ($MAC_{ISO\text{-}FENT}$) during the infusion of fentanyl at a low dose (33 μg kg^{-1} loading dose and 0.2 μg kg^{-1} $minute^{-1}$ constant rate infusion) or high dose (102 μg kg^{-1} loading dose and 0.8 μg kg^{-1} $minute^{-1}$ constant rate infusion) in eight Beagle dogs. The MAC_{ISO} reductions and plasma fentanyl concentrations at $MAC_{ISO\text{-}FENT}$ determinations are included. Data are mean ± standard deviation. *Significantly different within the treatment ($p < 0.05$); †significantly different between treatments ($p < 0.05$).

No fentanyl was detected in the blood samples collected prior to MAC_{ISO} determination. Plasma fentanyl concentrations were 7.7 ± 1.6 and 4.6 ± 1.2 ng mL^{-1} for the low-dose treatment prior to and at the end of MAC determination, respectively, and 33.9 ± 6.8 and 25.1 ± 6.9 ng mL^{-1} for the high-dose treatment at the same time points, respectively; mean values are presented in Table 1. Fentanyl concentrations were significantly different between the treatments ($p < 0.0001$) and between the time points within each treatment (low dose $p = 0.0007$, high dose $p = 0.0215$). The Pearson correlation coefficient between time of $MAC_{ISO\text{-}FENT}$ determination and its value was −0.29 and −0.33 for the low and high fentanyl doses, respectively.

SAP and MAP were significantly increased with the administration of the high dose of fentanyl (SAP $p < 0.0001$, MAP $p = 0.0076$), as compared with the low dose treatment, ($p = 0.09$; Table 2). DAP decreased with the administration of low dose of fentanyl ($p = 0.003$), but no significant change was noted with the high dose ($p = 1$). In both treatments, fentanyl administration resulted in significant decreases in HR ($p < 0.0001$).

Value	Dose	MAC_{ISO}	$MAC_{ISO + Fenta}$
Systolic Arterial Pressure (SAP) (mmHg)	Low	137 ± 26	151 ± 22^
	High	129 ± 21	187 ± 19*^
Mean Arterial Pressure (MAP) (mmHg)	Low	83 ± 12	79 ± 10^
	High	81 ± 8	92 ± 9*^
Diastolic Arterial Pressure (DAP) (mmHg)	Low	63 ± 9	55 ± 4*^
	High	63 ± 10	61 ±6^
Heart Rate (HR) (beats minute $^{-1}$)	Low	108 ± 25	61 ± 14*
	High	95 ± 14	42 ± 4*

Table 2: Cardiovascular variables measured at minimum alveolar concentration with isoflurane alone (MAC_{ISO}) or with isoflurane-fentanyl ($MAC_{ISO-FENT}$) during the infusion of fentanyl at a low dose (33 μg kg^{-1} loading dose and 0.2 μg kg^{-1} minute^{-1} constant rate infusion) or high dose (102 μg kg^{-1} loading dose and 0.8 μg kg^{-1} minute^{-1} constant rate infusion) in eight Beagle dogs. Data are mean ± standard deviation. *Significantly different within the treatment ($p < 0.05$); †significantly different between treatments ($p < 0.05$).

The time spent to finalize MAC_{ISO} determination was 160 ± 45 and 126 ± 104 minutes, and for $MAC_{ISO-FENT}$ was 154 ± 82 and 141 ± 61 minutes for the low-dose and the high-dose treatments, respectively. Total anesthesia duration was 446 ± 71 minutes for the low-dose treatment and 423 ± 97 minutes for the high-dose treatment. Neither anesthesia time nor time for MAC determination was significantly different between treatments.

Adequate oxygenation [arterial partial pressure of oxygen ≥ 450 mmHg (60.0 kPa)] and arterial partial pressure of carbon dioxide 35–50 mmHg (4.0–6.7 kPa) were measured in all dogs during the experiments.

Discussion

The principal findings of this study were that IV CRI of fentanyl in dogs: 1) decreased MAC in a dose-dependent fashion; and 2) caused a dose-dependent increase in blood pressure with bradycardia.

An average of 56 and 64% MAC reduction has been reported for enflurane (Murphy & Hug 1982a), as compared with 42% and 77% isoflurane MAC reduction noted for the low and high doses of fentanyl used in this study, respectively. The minor differences in MAC reduction noted between the two studies may be attributable to individual variation as MAC has been shown to vary by as much as 20% on an interindividual basis (Quasha et al. 1980). A second possibility is that MAC reduction of the same drug can be different when different inhalant anesthetics are used. Acepromazine (0.2 mg kg^{-1}) showed a different MAC reduction with halothane (28.2%) was compared with isoflurane (48.1%) (Webb & O'Brien, 1988), while no difference in the MAC-sparing effect of morphine was observed between the same inhalant anesthetics (Steffey et al. 1993). Should the difference in inhalant have made a significant difference in MAC, it would require a specific experimental design to exclude the possible differences in methodologies between different laboratories. Lastly, differences in plasma fentanyl concentration may have also had some influence in the differences of MAC observed by Murphy & Hug (1982a) and in the present study, especially in the low dose treatment. A 56% MAC reduction was achieved for enflurane with a plasma fentanyl concentration of 7.8 ng mL^{-1} (Murphy & Hug 1982a), while a 46% reduction in isoflurane MAC was achieved in the present study with an average fentanyl concentration of 6.2 ng mL^{-1}. An isoflurane MAC reduction of 53% was achieved in dogs with plasma fentanyl concentration between 6.4 and 9.2 ng mL^{-1} during an infusion rate of 0.3 μg kg^{-1} $minute^{-1}$ after a loading dose (LD) of 10 μg kg^{-1} (Hellyer et al. 2001), which is proportionally

similar to the results found in the present study. Regardless of the reasons, the magnitude of difference between the current study and the previous one with enflurane (Murphy & Hug 1982a) is low and unlikely to be of clinical significance.

Other opioids such as remifentanil (Allweiler et al., 2007 ; Monteiro et al., 2010a), morphine (Murphy and Hug, 1982b ; Steffey et al., 1993) and methadone (Credie et al. 2010) have been evaluated for their dose dependent MAC-reducing effects. Even though it is difficult to ascertain whether or not the higher dose of fentanyl used in this study achieved maximal MAC reduction (77%), other dose-response studies achieved similar maximal MAC reduction. Morphine has shown a maximal effect of up to 63% MAC reduction at 20 mg kg^{-1} in enflurane-anesthetized dogs (Murphy & Hug 1982b) and remifentanil achieved 71% isoflurane MAC reduction at 0.9 μg kg^{-1} $minute^{-1}$ (Monteiro et al. 2010a).

The selected LDs and infusion rates of fentanyl, as well as the duration of fentanyl infusion prior to starting MAC determination were chosen based on a previous study (Murphy & Hug 1982a). The aim of the LD is to decrease the time necessary to reach a steady state plasma concentration since without it the steady state is only achieved after 4–5 elimination half-lives (Stanski 1987). The LDs of fentanyl used in the original study of Murphy & Hug (1982a) and in the present study were calculated using a volume of distribution between the central compartment and the steady-state volumes of distribution (Murphy et al. 1979). The rationale of using a higher LD (33 μg kg^{-1}) than the ones reported in previous studies using similar fentanyl infusion rates (5–10 μg kg^{-1}) (Hellyer et al., 2001; Ueyama et al., 2009 ; Simões et al., 2016) was to provide a quicker steady-state plasma concentration of fentanyl (Stanski 1987) and to minimize variations during MAC determination. Higher LDs may not necessarily be needed clinically because small

variations in fentanyl plasma concentration were observed when a lower LD was used (Hellyer et al. 2001).

Surprisingly, fentanyl plasma concentration measured at the end of MAC determination was 40% and 26% lower than prior to MAC determination for the low and high fentanyl dose treatments, respectively. By contrast, Murphy & Hug (1982a) observed a maximal variation of 19% in fentanyl plasma concentration during MAC determination, which can be explained by the difference in the timing of blood sampling between the two studies. In the previous study (Murphy & Hug 1982a), blood was taken only after commencement of MAC determination as compared to immediately prior, as performed in the present study. In this regard, two possibilities can explain the lower fentanyl plasma concentration observed at the end of MAC determination: 1) the sympathetic stimulation caused during MAC determination could have caused a significant increase in cardiac output, which led to an increase in hepatic blood flow and consequently an increase in the clearance of fentanyl; and/or 2) the fentanyl plasma concentration observed in the initial sample was higher because a steady-state of fentanyl had not been reached at the end of 60 minutes of infusion. The fact that Simões et al. (2016) found no difference in the isoflurane-sparing effects of fentanyl with the use of atropine, which has been found to increase significantly cardiac output in enflurane anesthetized dogs (Ilkiw et al. 1994), may not support the possible increase in cardiac output occurring during MAC determination as a causative factor for the decrease in fentanyl plasma concentration observed in this study. Because Simões et al. (2016) did not measure plasma fentanyl concentration and significant variations on it were not associated with different enflurane MAC in dogs (Murphy & Hug 1982a), a decrease in plasma fentanyl concentration after the administration of atropine and its consequent increase in cardiac output is still possible, even in the absence of changes in MAC.

Conversely, Hellyer et al. (2001) found an increase of approximately 43% in plasma fentanyl concentration within 180 minutes of fentanyl infusion. Even though the authors suggested that this increase occurred because of saturation of biotransformation or tissue-uptake mechanisms (Hellyer et al. 2001), the different patterns of variation in plasma fentanyl concentration between the two studies may be simply related to the different LDs used and the timing of blood sampling. The higher LD used in the present study could have generated plasma concentrations of fentanyl higher than the steady-state at the end of 60 minutes of infusion, and because of that, a decrease in plasma fentanyl concentration was observed. By contrast, the lower LD (10 μg kg^{-1}) used in the previous study (Hellyer et al. 2001) was probably not enough to provide steady-state plasma concentrations of fentanyl at 30 minutes of infusion and a significant increase of plasma concentration over time was observed. The authors believe that the decrease in plasma fentanyl concentration noted did not affect the results of the study for two reasons: 1) the magnitudes of variations in plasma fentanyl concentration during the experiments were associated with minimal changes in MAC in enflurane anesthetized dogs (Murphy & Hug 1982a); and 2) the weak negative correlation between MAC and its time of determination after the fentanyl CRI was started. A stronger positive correlation could be expected if concentrations were still significantly decreasing during MAC determination. Future studies are necessary to clarify the causes for the decrease in plasma fentanyl concentration observed in this study. Independent of the reasons for fentanyl concentrations decreasing, the authors believe that the plasma fentanyl concentration measured at the end of MAC determination is a better representation of the observed MAC reduction because all MAC values were obtained closer to this time point.

Bradycardia has been reported with the use of high-dose opioid anesthetic techniques (Ilkiw et al., 1994 ; Michelsen et al., 1996) and was observed after both fentanyl treatments. This is thought to be secondary to the inhibition of GABAergic neurons in the nucleus ambiguous and a resultant increase in parasympathetic nervous system activity (Griffioen et al. 2004). In this study, no significant difference between treatments was noted for the fentanyl-mediated bradycardia, however, it is possible that the study was underpowered to detect this change due in part to the greater degree of variance in HR observed in the low-dose treatment. The increased blood pressure associated with the high dose of fentanyl was similar to the findings in the fentanyl–enflurane anesthetized dog and was caused by an increase in systemic vascular resistance with no effect on cardiac index (Ilkiw et al. 1994). In studies using high doses of remifentanil in dogs (Francis et al., 2008 ; Monteiro et al., 2010b), serum vasopressin was significantly elevated compared with the use of isoflurane alone, suggesting that increased vascular tone secondary to this effect might have played a role in the increased blood pressure seen, as has been demonstrated with fentanyl in humans (Weiskopf et al. 1987). DAP was lower in the dogs that were administered the low dose fentanyl treatment when compared with the high dose treatment and with the baseline isoflurane MAC probably because of the lower HR when compared to baseline and less vasoconstriction when compared to the high dose of fentanyl. Further investigation is therefore warranted to determine whether the effect on arterial pressure is solely due to increases in systemic vascular resistance or there is some component of increased cardiac output associated with lower levels of isoflurane. Nevertheless, the enflurane-sparing effect of fentanyl has not been associated with improvements in cardiac index if the bradycardia is not treated (Ilkiw et al. 1994). The more significant increase in blood pressure accompanying the fentanyl MAC reduction noted with the high dose seems to suggest that the use of higher

doses of fentanyl to maintain cardiovascular function in more critically ill anesthetized patients may be justified. However, this should be interpreted with caution, as cardiac output was not evaluated in this study. Further studies are warranted to explore the cardiovascular effects of high-dose fentanyl–isoflurane anesthesia, especially in the absence or presence of bradycardia. The noxious stimulus used for this study was electrical rather than mechanical and was chosen in part because of its quantifiability and repeatability. While many studies have been performed using the traditional tail clamping methodology, this technique is difficult to standardize and its use may contribute to the high degree of interexperimental variability seen with this technique (Valverde et al., 2003 ; Figueiró et al., 2016). Conversely, constant-current electrical stimulus is an easily standardized and repeatable technique which has been demonstrated to produce similar results to the tail clamp technique (Figueiró et al. 2016). Superficial electrical burns have previously occurred at high voltages but were not noted in either of the studies evaluating the technique or the present study. The MAC_{ISO} values obtained in the present study are similar to those obtained in prior studies (Monteiro et al., 2010a ; Figueiró et al., 2016) and as such it is unlikely that use of the electrical stimulus technique altered the outcome appreciably.

In conclusion, MAC of isoflurane was significantly reduced by the use of fentanyl CRI in a dose-dependent fashion. Bradycardia was observed with the low and high doses of fentanyl while MAP was increased only with the high dose. Further studies are needed to objectively determine its impact on the overall cardiovascular stability of the patient.

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Chapter IV – Cardiovascular and Respiratory Effects of Fentanyl in the Presence and Absence of Bradycardia

Abstract

Objectives

The aim of this study was to compare the cardiorespiratory effects of a low and high dose of fentanyl at equipotent doses of isoflurane in dogs both prior to and after the correction of bradycardia.

Study design

Prospective, randomized crossover trial

Animals

Eight healthy male Beagle dogs weighing 12.1 ± 1.6 kg [mean ± standard deviation (SD)] and approximate age 1 year.

Methods

The dogs were anesthetized with equipotent doses of isoflurane on two occasions when a low dose (33 μg kg^{-1} loading dose, 0.2 μg kg^{-1} $minute^{-1}$) or a high dose (102 μg kg^{-1} loading dose, 0.8 μg kg^{-1} $minute^{-1}$) of fentanyl was investigated. Cardiovascular and respiratory function was evaluated in three moments: before fentanyl administration (ISO), during fentanyl administration (ISO-F) and during fentanyl administration after normalization of heart rate (ISO-F_{NHR}). All values are expressed as mean ± SD.

Results

Fentanyl administration caused a dose-dependent bradycardia. When compared to ISO, cardiac index decreased during fentanyl infusion in both treatments, but was only greater at ISO-F_{NHR} in the high dose treatment. Mean arterial pressure was maintained within normal limits in both treatments at all time points, with the highest value (100 ± 16 mmHg) in the high dose treatment at ISO-F_{NHR}. Systemic vascular resistance increased in a dose dependent fashion after both doses of fentanyl, returning to baseline values at ISO-F_{NHR} in both treatments. Oxygen delivery index decreased and increased when compared to ISO at ISO-F and ISO-F_{NHR}, respectively, but only in the high dose-fentanyl treatment.

Conclusion and clinical relevance

An overall improvement in the cardiovascular function of dogs anesthetized with 1.3 isoflurane MAC was observed only after the treatment of bradycardia and only with the high dose of fentanyl.

Introduction

The MAC reducing effects of fentanyl have been previously documented in the isoflurane-anesthetized dog (Williamson et al. 2017), showing a significant reduction in minimum alveolar concentration (MAC) consistent with studies previously performed in enflurane-anesthetized dogs (Murphy & Hug 1982). This work, however, did not characterize the impact of this MAC reduction on the cardiovascular system. Previous studies with a single high dose of fentanyl in the anesthetized dog (Ilkiw et al. 1994), showed no improvement in cardiac output (CO) from the use of fentanyl-enflurane at 1.3 MAC as compared to enflurane alone unless heart rate (HR) was normalized with the use of atropine at which point a significant benefit was seen. Enflurane, however, is not in common use, likely due to its more pronounced cardiovascular depression when compared to isoflurane (Klide, 1976; Steffey et al. 2015). As such, cardiovascular effects of low and high doses of fentanyl in the isoflurane anesthetized dog merit further investigation, as the relatively milder effects of isoflurane on the myocardium may alter the ultimate hemodynamic effects.

The objective of this study was to compare the cardiorespiratory effects of two doses of fentanyl at equivalent depth of anesthesia in the isoflurane-anesthetized dog both prior to and after the correction of bradycardia. The hypothesis was that the use of fentanyl would improve CO and thereby cardiovascular function and oxygen delivery after correction of bradycardia in a dose dependent fashion.

Materials and methods

Animals

A total of eight healthy purpose-bred male adult Beagle dogs aged approximately 1 year and weighing 11.1 ± 1.3 kg (mean ± standard deviation) were enrolled in this study. Animals were determined to be healthy based on the results of a physical examination and routine basic blood work (packed cell volume, serum biochemistry). Approval for this study was obtained from the Virginia Tech Institutional Animal Care and Use Committee (no.15-145). Animals were fasted for 12 hours prior to the experiment; water was freely available.

This was a randomized crossover design study in which each animal underwent two anesthetic events separated by at least 1 week. Randomization was achieved using the RAND function of Excel (Version 14.6.7; Microsoft Corporation, CA, USA).

Instrumentation

Following preoxygenation delivered by a fitted facemask for 5 minutes, the dog was anesthetized using 5% isoflurane (FlurIso; VetOne, ID, USA) delivered in oxygen by a precision isoflurane-specific out of circuit vaporizer (Tec 7; Datex-Ohmeda Inc., WI, USA) until anesthesia was sufficient for endotracheal intubation. The dog was placed in lateral recumbency and the endotracheal tube was connected to a circle system (BleaseFocus; Spacelabs Healthcare Ltd, WA, USA). Spontaneous breathing continued with an end-tidal isoflurane concentration (FE′Iso) ≥1.50% during the instrumentation period. Intravenous (IV) access was obtained through the cephalic vein using a 20-gauge catheter (Jelco; Smiths Medical, UK). A 22-gauge catheter (BD Insyte; Becton Dickinson Infusion Therapy Systems, Inc., UT, USA) was inserted aseptically into a dorsal pedal artery to facilitate invasive pressure measurement and collection of arterial blood.

The following measurements were obtained using a standard anesthesia monitor (S/5 Anesthesia Monitor; GE Healthcare, WI, USA): pulse rate from the arterial pressure waveform, pulse oximetry, rectal temperature, end-tidal partial pressure of carbon dioxide ($P_E'CO_2$), F_E'Iso, inspired oxygen fraction (FIO_2), respiratory rate (f_R), and mean arterial pressure (MAP). Pulse rate was recorded as HR as no pulseless arrhythmia was detected during the experiments. The pressure transducer (Deltran II; Utah Medical, UT, USA) was connected to noncompliant tubing continuously flushed with heparinized saline (2 units mL^{-1}). The transducer was calibrated using a mercury column and zeroed at the level of manubrium. The infrared gas analyzer was calibrated using three known concentrations of isoflurane (0.490, 1.504 and 2.530%, primary gas standards; Airgas Inc., PA, USA) at approximately 2-hour intervals. F_E'Iso was corrected for ambient pressure measured by a digital barometer (Fisher Scientific, NH, USA). A polypropylene catheter placed with its tip positioned at the midpoint of the endotracheal tube was used to obtain the gas samples for continuous analysis. Rectal temperature was maintained between 37.0 and 38.5 °C using a forced air-warming blanket (Bair Hugger; Arizant Inc., MN, USA). Lactated Ringer's solution (Hospira Inc., IL, USA) at 3 mL kg^{-1} $hour^{-1}$ was administered IV for the duration of anesthesia. Dogs were mechanically ventilated with a tidal volume of 15 mL kg−1 and the respiratory rate was adjusted to maintain $P_E'CO_2$ at 35–45 mmHg (4.7–6.0 kPa).

Cardiac Output Measurement

Following instrumentation, the skin over the right external jugular was clipped and aseptically prepared. A cut down was performed and a 20 gauge catheter (BD Insyte; Becton Dickinson Infusion Therapy Systems, Inc., UT, USA) was introduced into the vessel. Following this, a 6Fr

7.5 cm introducer sheath (Arrow Percutaneous Sheath Introducer Set, Arrow International, PA, USA) was placed using the modified Seldinger technique into the vein and secured with suture (Ethilon® Nylon Suture; Ethicon US, USA). Through this, a 5 Fr 75 cm pulmonary arterial catheter (PAC) (Swan Ganz 132F5; Edwards Biosciences, CA, USA) was inserted. Each lumen of the catheter was connected to a pressure transducer (Deltran II; Utah Medical, UT, USA) via noncompliant tubing continuously flushed with heparinized saline (2 units mL^{-1}). Each transducer was calibrated with a water column and zeroed at the level of the manubrium. The tip of the PAC was positioned in the pulmonary artery, by observation of characteristic pressure waveforms.

Following placement of the PAC, F_E'Iso was adjusted to a level of 1.3 times a previously determined MAC for that animal (Williamson et al. 2017). F_E'Iso was allowed to equilibrate for 15 minutes at this anesthetic depth, following which arterial and mixed venous blood samples were withdrawn simultaneously for assessment of pH, oxygen and carbon dioxide tensions (I-Stat CG4+; Abaxis Medical, CA, USA), and hemoglobin concentration (Advia 2120; Tarrytown, NY, USA). Blood samples for gas analysis were drawn into dry lithium heparinized syringes (Line Draw; Smiths Medical, NH, USA). Hemoglobin samples were collected into a potassium EDTA tube (BD MAP; Becton Dickinson, UT, USA) and chilled until measurement. Ten mL of arterial blood was collected into a dry lithium heparin tube (BD Vacutainer; Becton Dickinson, UT, USA) for the measurement of plasma concentration of fentanyl. Plasma samples were separated in a refrigerated centrifuge at 2500 x ***g*** for 10 minutes, decanted into a separate container and stored at -80 ^{o}C for approximately 3 months prior to analysis.

CO was then determined via the thermodilution method by the injection of 3 mL of chilled saline through the proximal lumen of the PAC (S/5 Anesthesia Monitor; GE Healthcare, WI, USA).

The mean value of three measurements with a difference lower than 10% was recorded; measurements were repeated until 3 such values were obtained. Right atrial pressure (RAP), mean pulmonary arterial pressure (MPAP), MAP, pulmonary capillary wedge pressure (PCWP), HR and f_R were then recorded. All values were recorded at the end of expiration. Data from this time point is denoted by ISO.

Animals were then randomized to receive a low dose (30 μg kg^{-1} administered over 15 minutes and a continuous rate infusion (CRI) of 0.2 μg kg^{-1} $minute^{-1}$) or high dose (90 μg kg^{-1} administered over 15 minutes and 0.8 μg kg^{-1} $minute^{-1}$ CRI) of fentanyl, which was administered for 1 hour prior to further evaluation. In both treatments, the loading dose and CRI were started at the same time. After an infusion time of 1 hour, a 10 mL arterial blood was collected for the measurement of fentanyl plasma concentration, and all measurements including CO, hemoglobin and arterial blood gases were repeated. Data from this time point is denoted by ISO-F.

Following fentanyl administration, animals received glycopyrrolate 0.01 mg kg^{-1} intramuscularly (West Ward, NJ, USA) and additional intravenous doses as needed to maintain HR between 80-100 beats $minute^{-1}$ for 30 minutes continuously. Fentanyl infusion was continued during this period. Following this 30-minute period, a final set of measurements was taken. Data from this time point is denoted by ISO-F_{NHR}.

At the culmination of the experiment, the introducer was removed and manual pressure applied for 15 minutes to allow for clotting. Animals were administered carprofen 2.2 mg kg^{-1} subcutaneously (Rimadyl; Zoetis, MI, USA) and naloxone 0.02 mg kg^{-1} IV (Mylan Institutional, IL, USA) and allowed to recover.

Fentanyl concentration determination

Fentanyl concentration data was obtained as described in and simultaneously with data obtained from a previous study (Williamson et al. 2017) using a modification of the method described by Coopman et al. (2007). Briefly, fentanyl was extracted from plasma by use of a 70:30 *n*-hexane: ethyl acetate mixture and reconstituted in 0.5 mL of 60:40 water:acetonitrile, which was separated and the supernatant analyzed by high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS).

The LC-MS/MS analysis was performed using a liquid chromatograph (1200 series; Agilent Technologies Inc, CA, USA) with triple quadrupole mass spectrometer (G6470A; Agilent Technologies Inc., CA, USA). A calibration curve was prepared in the same manner as the sample and was found to be linear between 0.2-200 ng g^{-1}. The lower limits of detection and quantification were 0.05 and 0.2 ng g^{-1}, respectively. Multiplication of the resultant values by the specific gravity of canine plasma (1.025) elicited the final fentanyl concentrations determined in ng mL^{-1} (Remington & Baker 1958).

Data Analysis

Derived parameters including body surface area (BSA), cardiac index (CI), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), oxygen delivery index (DO_2I), oxygen consumption index (VO_2I), stroke volume indexed to body weight (SI) and oxygen extraction ratio (OER) were calculated using established formulae (Haskins et al. 2005).

Data were evaluated using SAS JMP statistical software (SAS Institute, NC, USA). All data were evaluated for normal distribution by inspection of normal quantile plots and Shapiro–Wilk test. Cardiovascular and respiratory data, as well as plasma fentanyl and vasopressin

concentrations were analyzed by mixed-model ANOVA using dose and time point as fixed variables and dog identification as a random variable, with Bonferroni post-hoc correction for comparisons between and within treatments. Fentanyl infusion times were compared between treatments by Student's t-test. Significance was set at $p < 0.05$.

Results

All results are presented as mean ± standard deviation, as all data was assessed as normally distributed. Fentanyl infusion time was 66 ± 7 minutes and 75 ± 12 minutes at ISO, and 143 ± 25 minutes and 143 ± 25 minutes at ISO-F_{NHR} for the low dose and the high dose fentanyl treatments, respectively, with no significant difference between treatments.

No fentanyl was detected in any dog at ISO. At ISO-F, fentanyl plasma concentrations were 8.82 ± 1.26 and 35.49 ± 6.46 ng mL^{-1} in the low and high dose treatments, respectively. This decreased to 4.74 ± 1.12 and 16.47 ± 1.62 ng mL^{-1} at ISO-$F_{NHR.}$ Values were significantly different between treatments in both time points ($p < 0.0004$) and within treatments in both treatments ($p = 0.04$ for low dose and $p < 0.0004$ for the high dose).

Cardiovascular parameters

One dog experienced a light plane of anesthesia during CO determination at ISO with markedly elevated CO, as such all cardiovascular values from this dog were discarded at this time point. Cardiovascular data is presented in Table 1.

CI decreased significantly in both treatments following administration of fentanyl ($p = 0.0036$ for low dose, $p < 0.0009$ for high dose) but was not different between treatments. Following restoration of HR, CI increased versus ISO-F in both treatments ($p < 0.0009$) but was only

increased versus ISO with the high dose ($p = 0.0099$), with no difference noted between treatments.

HR was not different between groups at baseline and decreased significantly ($p < 0.0009$) with the administration of fentanyl in both groups, with the high dose causing a greater ($p < 0.0009$) decrease than the low dose. At ISO-F_{NHR}, HR was increased versus ISO in both treatments ($p = 0.0027$ for low dose and $p < 0.0009$ for high dose) but was not different between treatments. SI increased significantly for all treatments from baseline at ISO-F and ISO-F_{NHR} ($p < 0.002$ for all groups) but was not different between treatments at any time point.

MAP was increased at ISO-F and ISO-F_{NHR} versus baseline ($p = 0.014$ and $p < 0.0009$, respectively) and increased versus ISO-F and ISO-F_{NHR} ($p = 0.0027$) in the high dose treatment. Conversely, MAP was not different from baseline at any time point in the low dose treatment, and waslower than with the high dose at ISO-F_{NHR} ($p < 0.0009$). Despite this, SVRI was increased in both treatments at ISO-F ($p = 0.0108$, $p < 0.0009$, respectively) and ISO-F_{NHR} ($p < 0.0009$ for both treatments) compared to baseline, with the high dose treatment showing a greater SVRI than the low dose at ISO-F ($p = 0.0018$). PVRI was higher in the high dose than the low dose treatment at ISO-F ($p = 0.0027$) and was lower in ISO-F_{NHR} than in ISO-F only in the high dose ($p = 0.0009$).

Respiratory and derived parameters

Data from one dog in the low dose treatment was excluded due to sampling errors. A further 3 data points were excluded from analysis due to failure of the blood gas cartridges. Data are presented in table 2.

Arterial and mixed venous pH decreased significantly in the high dose treatment at ISO-F alone ($p < 0.01$) but was not different from the low dose treatment. No significant differences were observed in either $P_{E'}CO_2$, arterial carbon dioxide tension (P_aCO_2) or arterial tension (P_aO_2). At ISO-F, both arterial oxygen content (C_aO_2) ($p = 0.0027$) and arterial hemoglobin ($p = 0.0012$) increased significantly in the high dose treatment versus baseline, but not in the low dose treatment. Mixed venous oxygen content ($C_{MV}O_2$), in contrast, increased significantly in the high dose treatment at ISO-F_{NHR} when compared to ISO-F ($p = 0.019$), but no significant difference was noted between treatments or at any other time point. Mixed venous hemoglobin increased significantly at ISO-F when compared to ISO ($p = 0.0009$) in the high dose and low dose treatments ($p = 0.02$) but returned to values not different from baseline afterwards.

Despite no significant change in VO_2I at any time in either treatment, DO_2I decreased in both treatments at ISO-F ($p = 0.0018$ and $p = 0.03$, respectively), which increased ($p < 0.0009$) at ISO-F_{NHR} and was higher than baseline only in the high fentanyl dose ($p = 0.02$). OER increased with the high dose treatment alone at ISO-F compared to ISO ($p < 0.0009$) but was not different between treatments. With both treatments at ISO-F_{NHR}, however, OER was reduced versus ISO-F ($p < 0.0009$ and $p = 0.01$, respectively). No change in lactate was observed within or between groups.

Discussion

The principal findings of this study were that an intravenous fentanyl infusion in isoflurane anesthetized dogs at equivalent anesthetic depth: 1) caused a non dose-dependent decrease in CI due to significant bradycardia; 2) decreased DO_2I and increased OER only at the high dose; 3) improved CI and DO_2I at the high dose only after the treatment of bradycardia; 4) caused an

increase in SVRI followed by a return to baseline values after the treatment of bradycardia, which maintained MAP within normal limits for the species despite changes in CI.

Of particular concern is the degree of bradycardia associated with fentanyl administration. Fentanyl is noted to increase vagal tone in the dog through centrally mediated factors, which causes a significant decrease in HR (Griffioen et al. 2004). HR decreased after fentanyl administration in a dose dependent fashion, with the high dose experiencing a 60% decline in HR as opposed to a 45% decrease after the low dose. This was to some degree compensated for by an increase in SVRI in both groups, however in neither group was it sufficient to maintain CI. CI decreased significantly after the administration of fentanyl at equipotent doses of isoflurane regardless of the dose, as demonstrated in a similar study using remifentanil (Monteiro et al. 2010b). However, the same results were not observed with a similar dose and anesthetic sparing effect of fentanyl in enflurane- anesthetized dogs (Ilkiw et al. 1994). In the former study, CI decreased by 12% with the administration of fentanyl and was not different when compared to an equipotent dose of enflurane alone, while in the present study CI significantly decreased by 42%. This difference between the studies can likely be attributed to the greater myocardial depressant effect of enflurane when compared to isoflurane (Klide 1976; Hysing et al. 1992). In spite of similar degrees of bradycardia observed in the previous study with enflurane (Ilkiw et al. 1994) and the present study at the high dose of fentanyl, CI seemed to decrease less with enflurane because a similar MAC sparing effect of fentanyl caused a higher increase in SI with enflurane (83%) than with isoflurane (36%).

An increase in arterial hemoglobin and CaO_2 similar to one achieved with remifentanil (Monteiro et al. 2010b) was observed after the high dose of fentanyl. However, it was not enough to prevent the decrease in DO_2I because of the magnitude of decrease in CI. The increase

in arterial hemoglobin and CaO_2 found in this study might be a result of splenic contraction caused by higher levels of vasopressin associated with the administration of fentanyl, as reported in humans (Weiskopf et al. 1987) and with remifentanil in dogs (Monteiro et al. 2010b). Decreases in DO_2I related to decreases in CI have been observed when doses of remifentanil equipotent to the ones used for fentanyl in this study were administered (Monteiro et al. 2010a; Monteiro et al. 2010b; Williamson et al 2017). Even though DO_2I seemed to decrease after both doses of fentanyl in the present study, it only reached statistical significance in the high-dose treatment. The exclusion of two blood gas analyses in the low-dose treatment, due to technical errors, decreased the statistical power of our study and could explain the lack of difference in DO_2I found before and after the administration of the low dose of fentanyl. As expected, any decrease in DO_2I was observed when a similar anesthetic-sparing effect using a similar dose of fentanyl was used in enflurane-anesthetized dogs because no change in CI was noted (Ilkiw et al. 1994). The changes observed in DO_2I were responsible for an increase in OER because no change in VO_2I was observed. The maintenance of normal mixed venous oxygen content and blood lactate levels in these dogs, however, suggests that the reduction in DO_2I is unlikely to be clinically relevant in these healthy dogs (Haskins et al. 2005).

Fentanyl is commonly used as part of balanced anesthetic protocols aiming to minimize the cardiovascular depression of inhalant anesthetics, due to its MAC-sparing effects (Ilkiw 1999). Nevertheless this effect, demonstrated by the improvement in CI and DO_2I at equipotent doses of isoflurane, was only achieved with the high dose of fentanyl, and only after the drug-induced bradycardia was treated. The same effect has been demonstrated with enflurane using a similar dose of fentanyl (Ilkiw et al. 1994). Consequently, the lack of improvement in cardiovascular function with a lower dose of fentanyl suggests that when the cardiovascular sparing effect of

isoflurane-fentanyl anesthesia is desirable, high doses of fentanyl need to be used and the bradycardia needs to be treated.

Despite the noted decline in CI, MAP actually increased with the high dose and remained unchanged with the low dose treatment, due to the significant increase in SVRI observed in both doses of fentanyl. These findings corroborate with the results of our previous study (Williamson et al. 2017) and also with Ilkiw et al.'s (1994) previous study using enflurane. The likely cause of increased SVRI with both doses of fentanyl is vasoconstriction, the mechanism of which is not fully elucidated at this time. This vasoconstriction seems to be partially due to increases in vasopressin secretion following fentanyl administration, as reported in humans (Weiskopf et al. 1987) and in dogs by other opioids like remifentanil (Francis et al. 2008; Monteiro et al. 2010) and methadone (Garofalo et al. 2012). MAP is often used clinically in the anesthetized patient as a surrogate for CO as it is effectively the product of CO and SVR (Hall 2011). In addition, changes in MAP are also generally considered to be indicative of changes in perfusion once blood pressure is above or below the limits of autoregulation for a given tissue (Patel & Drummond 2010). In these dogs, however, normal MAP in the presence of bradycardia was not indicative of maintained global tissue perfusion because CI decreased and MAP was only preserved by vasoconstriction. Consequently, normal MAP in isoflurane-anesthetized dogs may be misleading of maintenance of adequate cardiovascular function when bradycardia is present. Overall, these results suggest that in patients receiving fentanyl, an increase in MAP is not suggestive of improvement in cardiovascular function or tissue oxygen delivery. Whether or not the bradycardia caused by fentanyl is partially due to its vasoconstriction and increase in MAP still needs to be elucidated.

Interestingly, a reduction in SVRI was noted following the treatment of bradycardia both in the present study and by Ilkiw et al. (1994). This phenomenon may be the result of a reduction in fentanyl plasma concentrations after the treatment of bradycardia causing a reduction in vasopressin secretion (Weiskopf et al. 1987). However, this may not explain completely the decrease in SVRI associated with normalization of HR, as PVRI would not be expected to decrease with plasma vasopressin concentration because vasopressin is a pulmonary vasodilator (Walker et al. 1989). An alternative explanation, however, may be the mechanical properties of the vessels themselves, which exhibit passive stress relaxation following stretching (Mikami & Attinger 1968). Similar to what was found in this study, the correction of bradycardia caused by dexmedetomidine in dogs anesthetized with isoflurane decreased SVR (Bloor et al. 1992). This suggests that this effect observed with fentanyl was at least partially caused by a passive phenomenon, due to an increase in flow.

A 46% and 54% decrease in plasma fentanyl concentration was observed with the low and high dose treatments, respectively, following correction of bradycardia. A slightly smaller decrease in fentanyl plasma concentration was observed prior to and after determination of MAC in a previous study (Williamson et al. 2017). It is possible that the increase in CO associated with correction of bradycardia resulted in an increase in hepatic blood flow with concurrent increase in fentanyl clearance, as it is efficiently biotransformed by the canine liver, with plasma clearance approaching hepatic blood flow (Murphy et al. 1983; Björkman & Redke 2000). Dexmedetomidine, another drug which notably decreases CO and has a high extraction ratio, has been shown to affect its own metabolism in this manner, with decreases in CO of 3-19% in humans decreasing clearance by up to 12%, and a study in dogs showing an increase in clearance by 50% following reversal with atipamezole (Salonen 1995; Dutta et al. 2000). Alternatively, a

temporal decrease in fentanyl plasma concentration could be explained by a residual effect of the loading doses used, which did not seem to happen when the same doses were used in the enflurane study (Murphy & Hug 1982). However, these authors reported a 19% variation in fentanyl plasma concentration and others with lower loading doses reported no change (Ilkiw et al. 1994) or an increase (Hellyer et al. 2001) in fentanyl plasma concentration during the experiments. Despite this decrease, the authors do not believe that the effects on cardiovascular function seen in this study are attributable to a decrease in fentanyl concentration and a possible decrease in its anesthetic sparing effect as the increase in CO appears to be solely due to a change in HR. Additionally, these variations would not be likely to significantly influence MAC, based on the results seen in enflurane anesthetized dogs (Murphy & Hug 1982). The effects of the treatment of the fentanyl-mediated bradycardia in its pharmacokinetics and anesthetic sparing effects of fentanyl deserve future investigation.

In conclusion, in dogs anesthetized with isoflurane receiving a fentanyl infusion, cardiac index is decreased in the presence of bradycardia despite normal or increased mean arterial pressure. Cardiac index and oxygen delivery increases only with administration of a high dose and only after correction of bradycardia. Further investigation is needed to explore the clinical relevance of these findings.

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Variable	Dose	ISO	ISO-F	ISO-F_{NHR}
CI (L $minute^{-1}$ m^{-2})	Low	3.31 ± 0.80†	2.34 ± 0.67 a	3.79 ± 0.45 b
	High	3.12 ± 0.97	1.80 ± 0.49 a	4.03 ± 0.46 a,b
HR (Beats $minute^{-1}$)	Low	104 ± 10.5†	57 ± 14.7 a,*	89 ± 7.3 a,b
	High	105 ± 9	42 ± 7 a	85 ± 6 a,b
SI (mL $beat^{-1}$ kg^{-1})	Low	1.4 ± 0.4†	1.9 ± 0.4 a	1.9 ± 0.2 a
	High	1.3 ± 0.4	2.0 ± 0.4 a	2.1 ± 0.2 a
MAP (mmHg)	Low	75 ± 15†	79 ± 9	76 ± 13*
	High	72 ± 16	85 ± 17 a	100 ± 16 a,b
MPAP (mmHg)	Low	15 ± 2†	16 ± 3	16 ± 3
	High	16 ± 3	18 ± 3	18 ± 2
RAP (mmHg)	Low	7 ± 1†	10 ± 2 a	7 ± 2 b
	High	6 ± 2	11 ± 2 a	6 ± 1 b
PCWP (mmHg)	Low	8 ± 2†	11 ± 3	7 ± 2 b
	High	8 ± 2	12 ± 3 a	9 ± 2
SVRI (dyn sec cm^{-5} m^{-2})	Low	1689 ± 320†	2528 ± 968 a,*	1476 ± 299 b
	High	1762 ± 367	3457 ± 1108 a	1878 ± 406 b
PVRI (dyn sec cm^{-5} m^{-2})	Low	177 ± 52†	176 ± 50 *	192 ± 46
	High	214 ± 59	270 ± 73	169 ± 42 b

Table 1: Cardiovascular variables collected from dogs at 1.3 MAC of isoflurane (ISO), 1.3 MAC isoflurane with low or high dose of fentanyl (ISO-F), and 1.3 MAC isoflurane plus fentanyl with HR maintained 80-100 beats $minute^{-1}$ (ISO-F_{NHR}). Data are presented as mean ± standard deviation. a: different from T1, b: different from T2, *: different between dose groups, † n=7 for this measurement.

Value	Dose	ISO	ISO-F	ISO-F_{NHR}
P_aCO_2 (mmHg)	Low†	44.5 ± 3.3 n=6	48.2 ± 4.0	45.1 ± 2.4
	High†	42.8 ± 3.2	48.6 ± 7.0	44.0 ± 3.5
P_aCO_2 (kPa)	Low†	5.93 ± 0.44 n=6	6.43 ± 0.53	6.01 ± 0.32
	High†	5.70 ± 0.43	6.48 ± 0.93	5.87 ± 0.47
P_aO_2 (mmHg)	Low†	474 ± 13 n=6	487 ± 24	495 ± 25
	High†	472 ± 45	499 ± 24	479 ± 32
P_aO_2 (kPa)	Low†	63.18 ± 1.73 n=6	64.92 ± 3.20	65.99 ± 3.33
	High†	62.90 ± 6.00	66.52 ± 3.20	63.85 ± 4.27
C_aO_2 (mL dL^{-1})	Low†	17.95 ± 1.44, n=6	19.49 ± 2.86, n=6	17.89 ± 2.09
	High†	17.86 ± 1.32	20.27 ± 1.80 [a]	18.32 ± 1.42 [b]
Arterial Hemoglobin (mg dL^{-1})	Low	12.3 ± 0.9†	13.1 ± 2.1	12.1 ± 1.5
	High	12.4 ± 1.0	14.0 ± 1.4 [a†]	12.6 ± 1.1†
$C_{\bar{v}}O_2$ (mL dL^{-1})	Low†	14.68 ± 1.72, n=6	14.62 ± 1.93	15.43 ± 1.93
	High	14.97 ± 2.20	14.75 ± 2.20	16.30 ± 1.92 [b]
$\dot{V}O_2I$ (mL m^{-2} min^{-1})	Low†	102 ± 25, n=6	94 ± 24, n=6	95 ± 23
	High†	80 ± 13	101 ± 18	97 ± 21
DO_2I (mL m^{-2} min^{-1})	Low†	592 ± 165, n=6	415 ± 124, n=6 [a]	689 ± 132 [b]
	High†	552 ± 215	378 ± 109 [a]	728 ± 121 [a,b]
Oxygen Extraction (%)	Low†	18.3 ± 6.1, n=6	23.9 ± 8.1, n=6	13.8 ± 2.4 [b]
	High†	16.7 ± 7.5	28.2 ± 7.6 [a]	13.6 ± 3.3 [b]
Lactate (mmol L^{-1})	Low†	1.28 ± 0.49 n=6	1.19 ± 0.43	1.20 ± 0.30
	High†	1.47 ± 0.28	1.53 ± 0.70	1.19 ± 0.59

Table 2: Cardiorespiratory variables collected from dogs at 1.3 MAC of isoflurane (ISO), 1.3 MAC isoflurane with low or high dose of fentanyl (ISO-F), and 1.3 MAC isoflurane plus fentanyl with HR maintained 80-100 beats $minute^{-1}$ (ISO-F_{NHR}). Data are presented as mean ± standard deviation. a: different from T1, b: different from T2, *: different between dose groups, †: n=7 for this dose group unless otherwise specified.

Chapter V – Conclusions

Similar to Murphy and Hug (1982b)'s studies with enflurane, fentanyl decreased isoflurane MAC in a dose-dependent fashion in healthy dogs. This decrease in MAC, however, was not accompanied by a concurrent cardiovascular sparing effect, and in fact CO decreased as a function of the bradycardia associated with fentanyl administration. In the high dose group, CO was improved to greater than baseline following correction of bradycardia with glycopyrrolate. This suggests that in this group of patients, a cardiac sparing effect is only noted in the absence of bradycardia. Further studies are needed to evaluate how this effect may differ with different patient populations.

The decrease in fentanyl concentration associated with increases in cardiac output is highly suggestive of an increase in metabolism associated with increased hepatic blood flow. Further studies are needed to evaluate the degree to which this is true and how this affects the pharmacokinetic profile of fentanyl.

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