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ANIMAL SCIENCE

Analgesic, cardiorespiratory effects and motor block characteristics of epidural levobupivacaine alone or in combination with methadone or dexmedetomidine in bitches undergoing unilateral total mastectomy

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Abstract: The aim of this study was to evaluate the latency, extent of analgesia, and duration of motor block of levobupivacaine alone and combined with methadone or dexmedetomidine after epidural administration during and after mastectomy in dogs. Twenty-four mature, mixed-breed female dogs were randomly divided into three experimental groups with eight animals each, according to the agents used in lumbosacral epidural analgesia: levobupivacaine 0.75% alone (1.5mg/kg - control group), levobupivacaine 0.75% (1.5 mg/kg) + methadone 1% (0.3 mg/kg), or levobupivacaine 0.75% (1.5 mg/kg) + dexmedetomidine 0.05% (3 µg/kg). During surgery, cardiorespiratory parameters were evaluated. Rescue analgesia was given when there were signs of nociception and was necessary in all three treatment groups. Since all animals received rescue analgesia during the surgery and immediately post-surgery, the duration of the sensitive block were not evaluated. The extent of sensory block was between the 12º and 13º thoracic vertebrae for the control group, 7º thoracic vertebra to 5º lumbar vertebra (methadone group), and 8º thoracic vertebra to 4º lumbar vertebra for the dexmedetomidine group. Methadone or dexmedetomidine combined with levobupivacaine increased the extent of the sensory block and the duration of the motor block in bitches when administered via the epidural route.

Key words: α-2 agonists, analgesia, motor block, opioids, sensory block.

INTRODUCTION

Epidural analgesia, when performed using local anesthetics, enables inguinal, perianal, and pelvic limb procedures to be performed with excellent analgesic quality and minimal cardiorespiratory depression. However, the extent of its block limits its use in surgeries involving extensive areas encompassing the thoracic region, such as laparotomy or mastectomy (Cassu et al. 2008, Bernardi et al. 2012). Thoracolumbar epidural analgesia is an alternative for obtaining a greater degree of analgesia; however, the effects and intensity of sympathetic block are not yet well understood. Neurological changes, such as the Horner syndrome, tremors, and the Schiff-Sherington position; and respiratory changes, such as hypoventilation and bronchoconstriction, can occur more frequently after this technique (Groeben 2006). To increase analgesic area, reduce latency, and increase the intensity and duration of regional anesthesia, different

drug classes have been combined with local anesthetics, such as opioids and α-2 adrenergic agonists (Cassu et al. 2008, Valverde 2008, Pohl et al. 2012).

In dogs, methadone has a short elimination half-life, rapid clearance, and a low oral bioavailability (Kukanich et al. 2005). It is a complete μ-agonist receptor and an N-methyl-D-aspartate (NMDA) receptor antagonist that reduces norepinephrine reuptake in the nerve terminal (Murrell 2011), and is best known for its action on NMDA receptors, which increases its antinociceptive action and has been indicated for the treatment of severe and neuropathic pain states (Murrell 2011). Thus, it is the drug of choice when highly painful procedures are performed. Epidural methadone has been shown to promote a more intense and prolonged analgesic effect when compared to the same dose intravenously (Leibetseder et al. 2006, Campagnol 2011, Bernardi et al. 2012, da Silva et al. 2016). Furthermore, the cardiovascular effects were similar when these two pathways were compared (Vieira et al. 2017). The preference for using the epidural route can be attributed to the pharmacological antagonism of NMDA receptors located in the spinal cord dorsal horn, causing initiation and maintenance of central sensitization (Inturrisi 2014), which is an important pain control tool in hyperalgesic states (Mathews 2008, Mathews et al. 2015).

The combination of α-2 adrenergic agonists in epidural analgesia reduces the anesthetic dose because of its ability to cause analgesia and enhance the blocking area, thus promoting nerve tissue hyperpolarization and changing the transmembrane potential and conductance of the *locus coeruleus* ions in the brain stem (Scheinin & Pihlavisto 2000). In dogs, when administered via the epidural route, dexmedetomidine is effective in promoting analgesia in the pelvic limbs (Campagnol et al. 2007); however, it can cause a significant reduction in temperature and cardiorespiratory parameters (Imani Rastabi et al. 2019), sedation, and prolonged duration of action (Nour et al. 2013).

The hypothesis of the research is that the epidural association of dexmedetomidine or methadone with a local anesthetic may achieve a more extensive and longer-lasting block when compared to local anesthetic alone. Therefore, the aim of this study was to determine whether levobupivacaine alone or in combination with methadone or dexmedetomidine via epidural route could promote analgesia in sufficient extension for unilateral total mastectomy in bitches. If it can promote analgesia throughout surgery, the duration of the sensitive block can be evaluated in the post-surgical period. In addition, the latency and duration of motor blockade of the three protocols were evaluated.

MATERIALS AND METHODS

Animals

The study was approved by the local animal ethics committee (Protocol 690/2015). Sample size calculation was performed according to the maximum difference in the observed mean and standard deviation of the variable "respiratory rate" using the Student's t-test for independent samples with equal standard deviation and setting of the number of animals required per group to 6. For the safety margin, we decided to standardize the number of animals per group to 8. Twenty-four bitches of different ages and breeds were selected from the local community to undergo unilateral mastectomy due to the presence of mammary tumors. All animals included in the study were classified as ASA II according to the physical status classification system of the American Society of Anesthesiologists.

The inclusion criteria were the presence of malignant cells by fine needle aspiration cytology, as well as non-ulcerated tumors, which were limited to 10 cm in diameter, and not adhering to the muscles. Moreover, the dogs were deemed healthy based on normal physical examination, blood count, and biochemical serum tests (creatinine, albumin, and alanine aminotransferase). In addition, the animals have not undergone previous treatment before surgery.

The animals were fasted for 12 h for food and 2 h for water before the procedure. They were received in the early morning and immediately referred for pre-anesthetic procedures. During post-surgical monitoring, they were kept in individual 2.25 m^2 masonry cages next to the surgical center, where there was a blanket, water, and food *ad libitum*. The blankets and food were provided by the tutors.

Anesthetic and surgical procedure

On the day of the surgery, prior to the preanesthetic medication, a physical examination was performed and the parameters for baseline time (M0) were collected. Rectal temperature (RT) was measured with a digital thermometer. Respiratory rate (f_R) and heart rate (HR) were measured with auscultation using a stethoscope for one min, and recorded in movements per minute and beats per minute, respectively. The Systolic (SAP), mean (MAP), and non-invasive diastolic (DAP) blood pressure were recorded using the oscillometric method with a cuff positioned at the distal radioulnar region and measured by a multi-parameter monitor. The cuff used was 40% of the limb width.

Acepromazine (0.05 mg/kg) (Acepran® 0.2%, Vetnil Ind. e Com. de Produtos Veterinários Ltda, São Paulo, SP, Brazil) was administered with midazolam (0.2 mg/kg) (Dormire, Cristália Produtos Químicos e Farmacêuticos Ltda, São Paulo, SP, Brazil) intramuscularly as preanesthetic medication. After 20 min, all parameters were measured again, and this was recorded as the preanesthetic time (M1).

Immediately thereafter, the animals were induced with 4 mg/kg of intravenous propofol (Propovan, Cristália Produtos Químicos e Farmacêuticos Ltda, São Paulo, SP, Brazil) to lose eyelid and swallowing reflexes, thus allowing for tracheal intubation with an endotracheal tube appropriately sized for each patient. The endotracheal tube was then attached to a semi-closed inhalation anesthesia circuit (Dixtal Dx5020 anesthesia equipment, Manaus-AM, Brazil) with a continuous flow of 1 L/min 100% oxygen, and the patient was kept under general anesthesia with isoflurane (Isoforine, Cristália Produtos Químicos e Farmacêuticos Ltda, São Paulo, SP, Brazil). The anesthetic gas was adjusted to 1.5–2.5% with a calibrated vaporizer (HB, São Paulo, Brazil) according to the requirement of each patient based on clinical signs (the patients were in stage III plane 3 throughout the intraoperative period, as per Guedel's stages of anesthesia classification), and was monitored by a digital gas analyzer (Dixtal 2020 gas analysis module, Manaus-AM, Brazil) for anesthesia maintenance.

Experimental design

The dexmedetomidine (Dexdomitor; Zoetis Indústria de Produtos Veterinários Ltda, SP, Brazil) and methadone (Mytedom; Cristália Produtos Químicos e Farmacêuticos Ltda, SP, Brazil) dosages were determined from previous studies (Sabbe et al. 1994, Campagnol et al. 2007, Campagnol 2011, Odette & Smith 2013). The dogs were randomly allocated to one of the three experimental groups, with each group comprising eight animals, using a raffle system with a random number generator (Excel 2010; Microsoft Corp., WA, USA). All animals

received the same pre-medication but differed according to the agent(s) used in epidural analgesia as follows: Levobupivacaine group (L) (control): levobupivacaine 0.75% alone (1.5 mg/kg) - Novabupi, 0.75%; Cristália Produtos Químicos e Farmacêuticos Ltda, SP, Brazil); Methadone group (LM): levobupivacaine 0.75% (1.5 mg/kg) + methadone 1% (0.3 mg/kg); and Dexmedetomidine group (LD): levobupivacaine (1.5 mg/kg) + dexmedetomidine 0.05% (3 µg/kg);

Immediately after stabilization, the lumbosacral epidural area was shaved and sterilized. Soon after, a pediatric Tuohy needle for epidural analgesia (22G gauge) was inserted into the intervertebral space. The location of the epidural space was confirmed using the hanging drop technique associated with loss of resistance to the injection. In all three groups, the volume was made up with saline to obtain a total of 0.36 mL/kg for all treatments, and the entire volume was applied within one min. The veterinarian who performed the applications as well as intra- and postsurgical monitoring was unaware of the treatment, thereby making this a blind study.

Anesthetic monitoring

Soon after application of epidural analgesia, the animals were kept in sternal recumbency, and the isoflurane vaporization rate was reduced until the animal started to show eyelid reflexes, eyeball centralization, and mydriasis (stage III, plan 1 of Guedel's stages) to prevent anesthesia from causing loss of other reflexes.

The time for the establishment of the motor block was assessed through the time elapsed from the end of the application of the epidural to the relaxation of the anal sphincter (as observed by a lack of response to the touch of a hypodermic needle in the region), as well as loss of reflex removal of the pelvic limbs, which was carried out every 30 seconds. The extent of the motor block was assessed by the cutaneous reflex test of the trunk, so that, to the point where there was a response to the clamping, the extension of the block was considered. The motor blocking duration was also noted, which was measured from the lack of postural tone until complete recovery of the animal in the standing position without ataxia.

The time for the onset of sensory block was assessed using the superficial pain reflex test, which was performed using interdigital pinching with Kocher clamps coated with a latex tube and closed up to the second tooth on both pelvic limbs. The extent of the sensory block was assessed by monitoring hemodynamic and respiratory parameters during the surgical procedure according to the response to analgesic stimuli in the mammary region being operated on (inguinal, abdominal, and cranial thoracic areas).

After establishment of the sensory and motor blocks, the animals were placed in the dorsal decubitus position on the surgical table to start the surgical procedure, which was initiated 20 min after blocking.

The experimental time points were considered as showed in figure 1.

The following parameters were evaluated at each time point: heart rate (HR) in beats per minute (bpm) using a multiparameter monitor (Multiparametric monitor; Dixtal, Manaus-AM, Brazil), respiratory rate (f_R) in breaths per minute (bpm) using a multiparameter monitor and inspection of chest movement; end-tidal CO_2 ($ETCO_2$) in mmHg using a capnograph positioned between the anesthetic system and endotracheal tube; oxygen saturation (SpO_2 percentage), measured using an oximeter positioned on the patient's tongue; rectal temperature (RT) in °C measured using a digital clinical thermometer; and non-invasive blood pressure measured

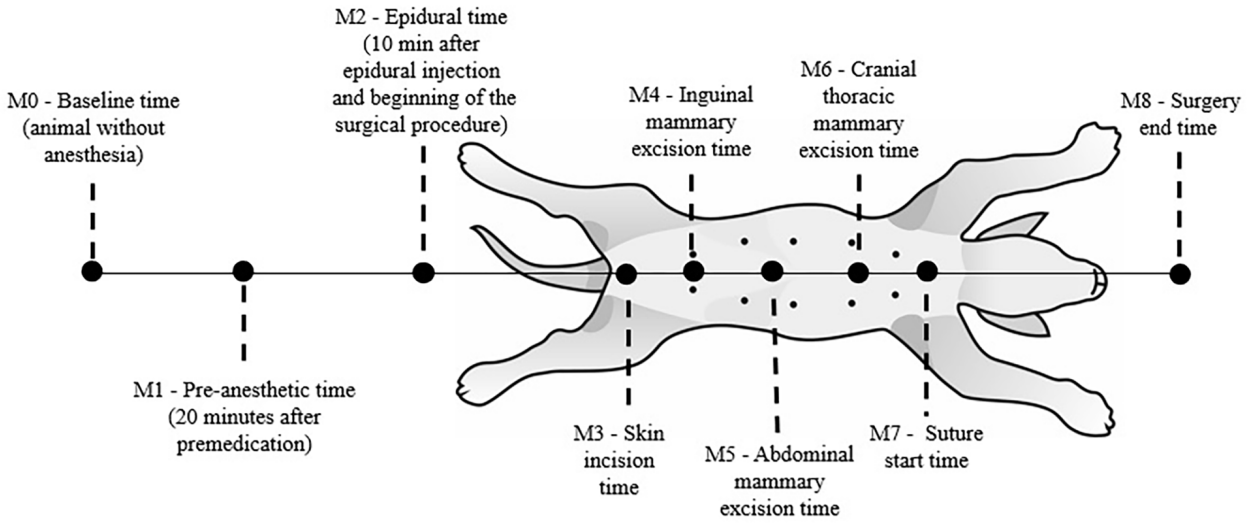

Figure 1. Schematic demonstration of the moments of recording cardiorespiratory parameters, according to the location of the surgery.

using a multi-parameter monitor, as was done in the pre-anesthetic moment.

All animals were cannulated with a 22G peripheral intravenous catheter and received fluid therapy at a maintenance rate of 10mL / kg/h of Ringer's lactate solution throughout the surgical procedure. In cases of hypotension (PAM<60 mmHg), volume loading (22.5mL/kg/h of Ringer's lactate solution), and vasopressor drug therapy (dopamine: 10µg/kg/min) was instituted (Davis et al. 2013); which were recorded in individual files and evaluated by two reviewers. In the case of bradycardia (HR<60bpm), atropine (0.044 mg/kg) was administered intravenously.

The surgical procedure was a unilateral total mastectomy. At any time during the procedure, when there were signs of nociception (20% elevation of HR, fR, or PA basal parameters), rescue analgesia was applied.

Evaluation of postoperative analgesia

At the end of the surgery, the surgical wound was dressed with chlorhexidine and protected with micropore tape. If the extension of the sensitive block was sufficient, that is, it guaranteed the analgesia of the entire operated region without performing analgesic rescue, the assessment of the duration of the sensitive block was maintained, which would consist of the time elapsed from the moment the block was established until the first anesthetic rescue. The degree of analgesia achieved by the epidural block was evaluated every 60 min using the Melbourne scale (Firth & Haldane 1999) and Glasgow composite pain scale (Reid et al. 2007). The Melbourne scale uses objective (SAP, HR, and f_R) and subjective variables (Firth & Haldane 1999), and analgesic rescue is performed when the sum of the values is above a score of 7. The Glasgow composite pain scale (Reid et al. 2007) analyzes the subjective parameters, and when the sum of this scale's scores was 5 (minimum), pain was considered.

Rescue analgesic medication administered intraoperatively or postoperatively

During the surgery, rescue analgesia was performed using an initial intravenous fentanyl bolus of 2 µg/kg, repeated as many times as needed, every 2 minutes intravenously until cardiorespiratory parameters returned to baseline values.

After the surgery, when the animal reached the rescue cutoff point on at least one of the scales, rescue analgesia was performed with 0.5 mg/kg morphine (Dimorf Cristália Produtos Químicos e Farmacêuticos Ltda,SP, Brazil) (intramuscularly) associated to 25 mg/kg metamizole (Analgex V, Agener União Saúde Animal, SP, Brazil)–(intravenous) as well as 0.1 mg/kg meloxicam (Maxicam 2%, Ourofino Saúde Animal, SP, Brazil) (intravenous). At the end of the experiment, the animals returned home to their owners. Analysis of the sensory block was interrupted when analgesic rescue (during or after surgery) was performed.

Statistical analysis

The study was conducted in a completely randomized design. Prior ro the analysis of variance, the Shapiro-Wilk normality test was used to confirm the normal distribution of the parametric data. The variables heart rate, respiratory rate, systolic, diastolic and mean blood pressure, CO2 end-tidal and rectal temperature were then analyzed by the mixed linear model as repeated measures in time as follows:

$$y_{ijk}=\mu+P_i+T_k+(\alpha\beta)_{ik}+\varepsilon_{ijk}$$

Where:

y_{ijk} = measurement taken at hour *k* on the j^{th} dog assigned to protocol *i;*

μ= overall mean

P_i= effect of the protocol *i*

T_k= effect of time *k* when the measurement was taken

$(PT)_{ik}$= effect of interaction between protocol i and time k

ε_{ijk} = random error with mean 0, assuming normal distribution.

Based on the Corrected Criterion of Information of Akaike (AICC) the first-order autoregressive covariance matrix was selected. The analysis was conducted using Proc Glimmix in SAS University Edition and differences were considered statistically significant when $p<0.05$.

The non-parametric variables (latency, duration of motor block and extension of sensory block and fentanyl doses) were evaluated using the Kruskal-Wallis test for inter-group comparisons, followed by Dunn's multiple comparison test by Biostat 5.0 software (Ayres et al. 2007). Differences were considered statistically significant when $p<0.05$.

RESULTS

The animals were aged 9.4±1.5 (7.9–10.9) years and weighed 14.7±3.4 kg (11.3–18.1). In all animals anesthetized for surgery, epidural analgesia was successful, as confirmed by the findings of sensory and motor blockade of the pelvic limbs. There were no complications such as the Horner syndrome, tremors, Schiff–Sherington position, respiratory changes, venipuncture, or inadvertent spinal injection. After the latency evaluation period, the animal was positioned and prepared for the surgery, and the position, local asepsis, and dressing of the surgeon and assistant were changed, which took an average of 21±4 minutes. There was no difference in the time to surgery between the animals.

The extent of sensory blocking was between T12 and T13 for the L group, T7 to L5 for the LM, and T8 to L4 for the LD; while the spine length was 60±12 cm in the L group, 59±14 cm in the LM group, and 55±13 cm in the LD group.

There was no significant difference in the latency time between the three groups (Table I). However, there was a significant difference in the duration of the motor block. In the LD group, the times for anal sphincter reflex recovery ($p=0.005$), tail tone recovery ($p=0.0057$), and time to normal walking ($p=0.04$) were longer than those in L groups, with no difference to LM. In

Table I. Mean ± standard deviation of the latency period and duration of motor blocking (absence/return of tail tone, relaxation/return of anal sphincter, absence/return of postural tone and normal walking) and sensory blocking (interdigital reflex) evaluated after the administration of levobupivacaine alone (L), combined with methadone (LM) or dexmedetomidine (LD) in bitches undergoing unilateral total mastectomy.

Protocol	Latency (seconds)			Recovery (minutes)			
	Anal sphincter relaxation	Loss of interdigital reflex	Loss of cutaneous reflex	Anal reflex	Tail tone	Interdigital reflex	Normal walking
L	90±60	80±29	101±35	243±15a	235±18a	305±102a	405±81a
LM	65±41	69±51	88±33	326±82ab	336±81ab	384±90ab	495±110ab
LD	52±43	52±43	40±35	551±153b	571±122b	518±198b	863±218b

*** Means followed by different lowercase letters differ significantly among the groups (p<0.05).**

the LD, 9.5±2.2 h, 8.8±3.35 h and 14.3±3.6 h were needed for the return of anal sphincter motor activity, tail tone and until the animal could walk without ataxia versus 3.9±0.2 h, 5±1.6 h and 6.8±1.46 h for L and 5.5±1.3 h, 5.5±1.3 h and 8.0±1.8 h for LM, respectively (Table I).

Heart rate analysis revealed a significant difference between the LD and both LM and L groups (p=0.049) at the following time points: M4 (p=0.006), M5 (p=0.012), M6 (p=0.012), M7 (p=0.019), and M8 (p=0.009). No significant differences were found between the groups for any of the other analyzed variables (p>0.05) (Table II).

Rescue analgesia was necessary during the intraoperative period in all animals. The LD was given a mean 2.1 µg/kg (± 2.1 µg /kg) intravenous fentanyl during the intraoperative period, which is a significantly lower value than that used in the other groups (5.7±2.4 µg/kg and 3.3±1.7 µg/kg for the L and LM, respectively).

All animals received rescue analgesia immediately after surgery. As none of the protocols offered sufficient analgesia in extension and all animals received other analgesic drugs, the evaluation of the duration of sensory block exclusively by drugs via the epidural route was impaired in all groups. Since it was not possible to determine the duration of the sensory block, the analog scales were not used, and the results were not demonstrated.

Due to bradycardia (FC<60bpm), in the LD, one bolus of atropine was administered shortly after the end of the epidural administration to four of the eight anesthetized animals compared with one animal in the LM and none of the animals in the L. No animals received vasopressors during or after surgery.

DISCUSSION

The results obtained in this study support the hypothesis that peridural dexmedetomidine or methadone combined with levobupivacaine increases extension of sensory blockade and duration of motor block, but the extension was not sufficient to promote analgesia in unilateral mastectomy. In all groups, analgesic rescue needed to be performed after cranial abdominal mammary gland excision, which were similar to the results obtained by Souza et al. (2010) and Campagnol (2011).

Several factors can alter the extent of the epidural block, such as the size and amount of fat in the epidural space (Reina et al. 2009), patient position (Gorgi et al. 2006), application speed (Son et al. 2014), concentration and volume of the anesthetic solution (Lee et al. 2004, Son et al. 2015), and the physicochemical

Table II. Mean ± standard deviation of the heart rate (HR), respiratory rate (f_R), rectal temperature (RT) systolic (SAP), mean (MAP) and diastolic (DAP) blood pressure, end tidal CO_2 ($EtCO_2$) and micrograms per pound of fentanyl bolus applied (FE) in bitches undergoing epidural anesthesia with levobupivacaine alone or combined with methadone or dexmedetomidine for unilateral total mastectomy.

Parameters	Protocol	Moments							
		M1	M2	M3	M4	M5	M6	M7	M8
HR	L	136±38	148±30*	104±17*	108±12a*	104±20a*	103±21a*	105±22a*	121±18a*
	LM	124±33*	120±20	101±19	97±27a*	107±18a	104±19a	106±19*a	118±14a
	LD	130±39	141±39*	95±16	85±18b	79±15b	82±14b	79±11*b	91±15b
f_R	L	32±12*	47±13*	13±12*	14±12*	12±9*	13±11*	13±10*	17±11*
	LM	45±20	54±20	14±7	11±5	10±3	10±6	9±6	17±5
	LD	36±15	39±10	13±8	19±13	17±8	13±11	12±9	17±6
RT	L	38.7±0.5*	38.4±0.3*	36.7±0.5	36.6±0.3	36±0.3*	36.1±0.2*	35.7±0.2*	35.9±0.3*
	LM	38.6±0.4*	38.4±0.3	37.3±0.3*	36.7±0.5*	36.4±,0.4*	36.2±0.3*	36±0.4*	36.0±0.4
	LD	38.9±0.4*	39.3±0.2	36±1.6*	35.8±0.2*	35.1±1.7*	35.7±1.2*	35.7±0.9*	35.2±1.2
SAP	L	122±8	110±17	81±19	87±20	86±17	83±22	89±17	88±16
	LM	118±21	108±19	93±27	86±14	89±12	90±20	94±13	102±18
	LD	111±15*	117±13*	94±17	93±22	89±20	85±10	77±12*	91±19
MAP	L	91±6	94±22	63±20	65±10	71±20	65±15	71±19	71±19
	LM	88±15*	86±15*	71±16	63±15	68±13	57±11*	67±14*	73±15
	LD	89±11*	85±18*	70±19	67±21	65±22	60±10*	53±11*	62±27*
DAP	L	75±19	73±20	52±25	46±14	54±18	44±19	52±19	52±18
	LM	68±17*	68±6*	49±11*	43±14*	47±11*	48±6*	51±13*	54±14
	LD	76±16*	68±19*	53±19	51±23	45±19	46±8*	37±6*	54±21*
$EtCO_2$	L			54±10	53±7	53±11	58±9	47±13*	57±16
	LM			50±13	49±18	50±5	52±5	63±14	57±5
	LD			55±6	57±8	58±8	61±8	58±5	49±11*
FE	L			1,1±0,4	0,6±0,3	0,9±0,4	0,7±0,2	1,5±1,3	1,2±0,9
	LM				2,1±1,3	0,5±0,3	0,6±0,4	1,5±0,8	0,7±0,4
	LD				0,3±0,1	0,3±0,2	0,2±0,2	0,6±0,5	0,4±0,3

***Significantly different from BT (p<0.05). M1: at baseline; M2: 20 minutes after preanesthesia medication administration; M3: surgery start time; M4: inguinal mammary gland excision time; M5: abdominal mammary gland excision time; M6: thoracic mammary gland excision time; M7: suturing start time; M8: suturing end time.**
****Means followed by different lowercase letters differ significantly among the groups (p <0.05).**

characteristics of the drugs (Valverde 2008). In this study, epidural methadone combined with a local anesthetic did not provide adequate analgesia for the surgical procedure without rescue analgesia. Upon awakening, the animals in this group also manifested pain, possibly due to the low diffusion of the drug through the epidural space, which is attributed to its high lipid solubility, thus demonstrating the segmentary effect of this opioid. Due to its physical nature (lipid soluble), methadone has low dispersion through the cerebrospinal fluid and tends to remain at the application site, with limited spread to the adjacent dermatomes (Payne et al. 1996).

Sabbe et al. (1994) reported the local antinociceptive effect of epidural dexmedetomidine after observing different responses to pelvic and thoracic limb pinching, which differ from the results obtained with intravenous dexmedetomidine administration. A sensory block achieved with a combination of local anesthetic and dexmedetomidine has been effective for performing orthopedic

procedures on the hind limbs of dogs (Odette & Smith 2013) and in human abdominal surgery (Kamal & Tallat 2014), but it was not efficient in total mastectomy as demonstrated in our study. Systemic administration of α-2-adrenergic agonists occurs due to modulation of G protein activity on the ion channels present in the cell membrane, which is associated with a change in norepinephrine release in the brain stem (Gaynor & Muir 2009). The analgesic effect after epidural application is mediated by central connections to the α-2 receptors, leading to pain suppression through C fiber hyperpolarization and reduced release of nociceptive substances, such as substance P and glutamate, present in the spinal cord dorsal horn (Eisenach et al. 1994, Angst et al. 2004, Weerink et al. 2017). This hyperpolarization could explain the prolonged motor blocking observed when dexmedetomidine is associated with local anesthetics (Salgado et al. 2008, Odette & Smith 2013).

Motor block duration differed significantly between groups, with dexmedetomidine associated with levobupivacaine leading to the longest immobilization period than the L group. Similar results were observed by Kanazi et al. (2006), Salgado et al. (2008), Nour et al. (2013), and Kamal & Tallat (2014), who observed a significant increase in the time and intensity of the motor block when different local anesthetics were combined with dexmedetomidine. This effect is believed to be due to synergistic activity between the drugs, because the use of epidural dexmedetomidine alone does not lead to a reduction in motor activity (Sabbe et al. 1994, Eisenach et al. 1996, Campagnol et al. 2007).

The latency period was similar in the three groups, as also observed by Campagnol et al. (2007) and Kamal & Talaat (2014), who evaluated levobupivacaine alone or combined with dexmedetomidine, and found no significant difference between groups regarding motor block establishment.

The duration and extent of the blocks promoted by local anesthetics are directly related to the degree of protein binding and liposolubility. Lidocaine has a lower degree of protein binding (65%) and is less lipophilic (2.9) than levobupivacaine (97% and 30%) or ropivacaine (90% and 6.1%) (Skarda & Tranquilli 2013). Thus, if lidocaine had been used in this study instead of levobupivacaine, a greater extension of the block would probably occur, but with a shorter duration. The baricity of the solution can also affect the dispersion of drugs administered via the epidural route (DeRossi et al. 2005, Valverde 2008). Hypobaric drugs, such as lidocaine 1% (1,005), bupivacaine 0.05% (1,003), and ropivacaine 1% (1,006) result in greater extensions of blockade when compared to hyperbaric drugs (Valverde 2008). However, their results in veterinary medicine are contradictory, with advantages observed in the case of the use of local anesthetics with different basicity in horses (DeRossi et al. 2005) but not in dogs (Vilela 2012).

Of all cardiorespiratory parameters analyzed, heart rate was the only variable with statistical difference, at the M2 moment, when the LM group showed significantly lower values when compared to the L group. However, the interference of sympathomimetic drugs (atropine in four animals in the LD group and in one animal in the LM group shortly after the epidural application) and analgesics (in all animals throughout the surgery) was crucial for maintaining all cardiorespiratory parameters close to baseline values. This resulted in masking of the real effects of anesthetics applied by the epidural route. As these animals attended to in the clinical routine, the administration of these drugs was essential both for maintaining the safety of the anesthetic procedure and for

welfare. Thus, the evaluation of the effects of the HR, fR, PAS, PAM, and PAD variables exclusively by epidural block was impaired.

There was significant bradycardia in the LD group shortly after the epidural block, in which atropine was applied. Similar results were observed by Souza et al. (2010) and Shaikh et al. (2017), and are explained by the direct effect of α-2 agonist drugs on the autonomic nervous system, in which presynaptic activation of the α-2 adrenoceptors reduces peripheral norepinephrine release (Talke et al. 2000, Fantoni et al. 2017), thus resulting in sympathetic block and consequent bradycardia. The significant heart rate reduction caused by dexmedetomidine can endanger the patient's life because of coronary vasoconstriction, which may lead to ischemia and subsequent myocardial dysfunction (Gerlach et al. 2009). The activation of postsynaptic α-2 and peripheral α-1 receptors present in vascular smooth muscle produces vasoconstriction (Ruffolo Junior 1985), which explains the momentary pressure increase that occurs after intravenous application of these drugs. In our study, however, this effect did not occur due to the route of application; rather, there was predominance of central effects (Klimscha et al. 1995).

The use of fentanyl explains why the increase in the cardiorespiratory parameters of the animals was not evident in the table. It was observed that in the LD group, the fentanyl dose required for rescue analgesia was significantly lower than that used in the other two groups. This finding may result from the analgesic and sedative actions of α-2-adrenergic agonists that occur through molecular mechanisms that lead to cellular response modulation, neuronal hyperpolarization, inhibition of neurotransmitter exocytosis, and suppression of neurotransmission. However, the direct action of the drug on the autonomic nervous system can lead to increased difficulty for the anesthesiologist in determining patient pain, which may result in the use of lower doses of analgesic drugs (Weerink et al. 2017).

All animals in the study presented with hypercapnia. The increase in EtCO2 may have a metabolic origin (fever, malignant hyperthermia, seizures, tourniquet release), or may be due to abnormal pulmonary perfusion, increased blood pressure or cardiac output, hypoventilation, rebreathing, or technical errors (such as expired CO2 absorption, fresh gas flow inadequate, circuit failures, or valve problems) (Marshall 2004). In our study, the animals did not present with metabolic or pulmonary changes, and oxygen flow was adequate. Thus, the hypercapnia present in all animals in the study can possibly be explained by the failure to absorb CO2 in the system, circuit, or valves.

The relevance of this study is that it demonstrates the extent of sensory blocks, as well as the latency and duration of motor blocks with the combined use of levobupivacaine with dexmedetomidine or methadone applied through the epidural route in dogs. The study's limitations include its inability to determine the duration of the sensory block, due to the great extent of the surgical procedure and the use of drugs that interfered with the cardiorespiratory parameters.

The combined use of methadone or dexmedetomidine with levobupivacaine increased the extent of the sensory block and the duration of the motor block in bitches when administered via the epidural route. However, the extension was insufficient to provide analgesia in unilateral total mastectomy. Severe bradycardia is a common occurrence following epidural administration of dexmedetomidine.

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