

Effect of medetomidine, midazolam, ketamine, propofol and isoflurane on spinal reflexes in healthy dogs

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Abstract

Background: Sometimes it is necessary to use sedatives or even general anaesthetics to examine animals with spinal cord injuries. These drugs may affect spinal reflexes, alter the outcome of neurological examinations, and make it difficult to diagnose location of the lesion.

Objectives: The aim of this study was to evaluate the effects of five pre-anaesthetic and anaesthetic agents commonly used in clinics on spinal reflexes in dogs.

Methods: Ten native adult dogs were participated in three groups. In all groups, the dogs were premedicated with medetomidine and midazolam; then, in the first group, ketamine, in the second group, propofol and in the third group, isoflurane were used for induction of anaesthesia. The spinal reflexes were evaluated before injection, 15 min after medetomidine, 20 min after midazolam, and at 15, 30, 45 and 60 min after induction of anaesthesia.

Results: Medetomidine did not reduce monosynaptic reflexes (patellar and cranial tibial reflexes) but increased them while it had no effect on the polysynaptic limb withdrawal reflexes. Midazolam had no effect on the spinal reflexes; Ketamine did not affect the patellar, cranial tibial and extensor carpi radialis reflexes, but reduced polysynaptic pain-related reflexes; and propofol and isoflurane abolished the all spinal reflexes.

Conclusions: Medetomidine, midazolam and ketamine have no effect on reducing monosynaptic reflexes (patellar and cranial tibial reflexes) and may be used for neurological examination of restless animals in the clinic. Propofol and isoflurane eliminated all spinal reflex responses and are not suitable for neurological examinations.

KEYWORDS

isoflurane, ketamine, medetomidine, midazolam, propofol, spinal reflexes

1 | INTRODUCTION

The nervous system is a very complex system that controls the rapid functions of the body (Guyton & Hall, 2006). The Motor neurons that stimulate muscles or organs are divided into two general categories. The first group is the upper motor neurons (UMN), which are

the central control system and originate in the brain and brainstem and terminate within the brainstem or spinal cord. These neurons do not communicate directly with the muscles and exert their effects through the lower motor neurons. The second category is the lower motor neurons (LMN), which are in direct contact with the muscles and originate in the spinal cord or brainstem and are mainly

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responsible for performing reflexes (De Lahunta et al., 2015). Spinal reflexes are used to differentiate LMN and UMN lesions. LMN lesions cause a lack or decrease in reflexes, while in UMN lesions the spinal reflexes increase, which may sometimes be undetectable. The most reliable spinal reflexes that respond well are the patellar and the limb withdrawal reflexes (Nelson and Couto, 2020; Schatzberg, 2010).

Spinal reflexes are affected after spinal cord injuries and may increase or decrease depending on the location of injury. In a significant number of animals with spinal cord injury, neurological examination may be difficult due to the lack of cooperation of animals, and various analgesics or even general anaesthetics may be required to restrain the animal and perform various diagnostic and therapeutic measures. These drugs may affect spinal reflexes, altering the results of neurological examinations and making it difficult to identify the location of the lesion. Some of the most important drugs that may be used for this purpose are medetomidine, midazolam, ketamine, propofol and isoflurane.

Medetomidine is an alpha 2 agonist, which stimulates alpha 2 adrenergic receptors in the central nervous system (CNS), resulting in decreased release of norepinephrine in the CNS, leading to sedation, drowsiness, muscle relaxation and analgesia (Mckelvey and Hollingshead, 2003; Tranquilli et al., 2013). Medetomidine is usually used as a pre-anaesthetic medication before ketamine, sodium thiopental, propofol, or inhalation anaesthesia (Mckelvey and Hollingshead, 2003; Tranquilli et al., 2013).

Midazolam is a short-acting, water-soluble benzodiazepine with a pH of about 3.5, which, after injection and exposure to the body's normal pH, becomes fat soluble by chemical deformation and closure of its diazepine ring. Then, it can cross the blood-brain barrier and depresses the central nervous system (Clarke et al., 2014; Tranquilli et al., 2013).

Ketamine, a phencyclidine derivative, is a selective and non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, which exerts its anaesthetic effect by blocking the binding amino acid glutamate to these receptors. In addition to N-methyl-D-aspartate receptors, ketamine interacts with other receptors such as opioid, monoaminergic, muscarinic and voltage-sensitive calcium channels. Ketamine is a cataleptic analgesic that theoretically blocks pain receptors in the spinal cord and causes analgesia without causing respiratory depression (Posner, 2018).

Propofol (2,6-diisopropylphenol) is an alkylphenol derivative that is insoluble in water but highly soluble in fat. Propofol reduces the metabolic activity of the brain by acting on the GABA receptors (Ying and Goldstein, 2005). Clinical use of propofol includes: Short-term and long-term sedations, induction of general anaesthesia, maintenance of anaesthesia and treatment of epilepsy (Krasowski et al., 2002; Lagerkranser et al., 1997).

Isoflurane is a general inhalation anaesthetic commonly used clinically to induce and maintain the general anaesthesia, characterised by end points including immobility, amnesia, analgesia and loss of consciousness (Stachnik, 2006). Isoflurane activates the calcium-dependent enzyme ATPase in the sarcoplasmic reticulum. On the other hand, by acting on glutamate, glycine and GABA_A receptors and inhibiting the exocytosis of synaptic vesicles and releasing neurotransmitters

from nerve terminals, it induces anaesthesia and perpetuates it. This drug induces muscle relaxation and reduces pain sensitivity (Speigel and Hemmings, 2020).

In a number of studies, the effects of premedication and anaesthetic drugs on some spinal reflexes in small animals have been studied (Horsley et al., 2021; Truchetti et al., 2020; Tudury et al., 2017). In some of these studies, the effect of some sedatives on the angles of reflexes has been investigated which is very valuable, but in the neurological examination of animals, the angle of the reflexes is not routinely measured, rather, reflexes are examined visually. In these studies, only the effects of dexmedetomidine and butorphanol on the patellar and limb withdrawal reflexes (Horsley et al., 2021), and the effect of nerve block on the extensor carpi radialis and cranial tibial reflexes (Tudury et al., 2017), have been investigated. On the other hand, there is still no specific protocol according to which a specific drug can be used for chemical restraint of fearful or aggressive dogs. Therefore, the aim of this study was to investigate the effect of some sedatives and anaesthetics that are routinely used in the clinic (medetomidine, midazolam, propofol, ketamine, isoflurane) on common spinal reflexes in a clinical setting. We hypothesised that these drugs would reduce all spinal reflexes, but our hypothesis was rejected and our results surprisingly proved the opposite for some drugs. The results of this study can be used by clinicians in fearful or aggressive animals who need sedation or even anaesthesia for neurological examination.

2 | MATERIALS AND METHODS

2.1 | Study conditions

The study protocol was assessed by the Research Committee of the Faculty of Veterinary Medicine and approved by the Research Ethics Committee of Ferdowsi University of Mashhad, Mashhad, Iran (Approval ID: IR.UM.REC.1399.125). The study was performed on 10 healthy intact adult mixed breed dogs (6 females and 4 males) in the age range of 1–3 years with a mean age of 24.4 ± 6.6 months and a mean weight of 22.2 ± 4.6 kg. All dogs received the antiparasitic medication 2 weeks before the start of the study [Praziquantel forte (Endopet, Santavet, Albarfarma, Istanbul, Turkey), which contains a combination of the praziquantel, pyrantel pamoate and febantel; one tablet per 10 kg body weight]. All 10 dogs in the study underwent each of the 3 treatments with a 1-week washout period between treatments, but their participation was randomly divided into three drug groups. In other words, it was randomly determined that each dog would first fall into one of the ketamine, propofol or isoflurane groups and then, with a 1-week washout period from the previous experiment, be randomly assigned to one of the other two groups.

In each dog, an IV line was placed into the cephalic vein and Ringer's solution was administered at a rate of 10 ml/kg/h. Vital signs of dogs including heart rate (via ECG), respiratory rate (via auscultation) and rectal temperature (with a medical thermometer) were measured at all timepoints. Also, non-invasive blood pressure was measured at all timepoints with a suitable cuff placed on the radius area with the dog in

lateral recumbency using a cardiopulmonary monitoring device (CardioSet ARAD P10, Sairan Electro Optics Industries Co., Esfahan, Iran). The dog's ECG was recorded with an ECG device (BCM-600, BIONICS, gangwon-do, South Korea). The dogs were breathing normally. All the dogs recovered uneventfully from each episode of anaesthesia.

In order to conduct the study blindly and to avoid errors, all neurological examinations and evaluation of reflexes were performed by the same investigator (AAS); however, since the use of sedatives was consistent in all three groups and also the method of using anaesthetics and their effects were different and this could make the examiner aware of the type of medication, all cases were recorded with videos to be re-examined blindly. In all three groups, after the restraining of dogs in lateral recumbency, spinal reflexes were evaluated and recorded before injection of pre-anaesthetics. Then, for sedation, medetomidine (Dorbene 1 mg/ml, Syva Laboratories S.A., Leon, Spain) was used intramuscularly at a dose of 15 µg/kg (Tranquilli et al., 2007); and spinal reflexes were assessed again 15 min later. Midazolam (Midazolam, Exir Pharmaceutical Company, Boroujerd, Iran) was then injected intramuscularly at a dose of 0.25 mg/kg (Jones et al., 1979) to complete the pre-anaesthesia and spinal reflexes were re-evaluated after 20 min. In the first group, ketamine hydrochloride (Ketamin 10%, Bremer Pharma GmbH, Warburg, Germany) with a dose of 6 mg/kg was intravenously used for induction and 5 mg/kg/h to continue anaesthesia (Iida et al., 1997). In the second group, propofol (Lipuro 10 mg/ml, B. Braun Melsungen AG, Melsungen, Germany) was intravenously injected at a dose of 6.5 mg/kg for induction and 2.5 mg/kg/h for continuing of anaesthesia (Muir 3rd and Gadawski, 1998) and in the third group, anaesthesia was induced with 5% isoflurane (Piramal Critical Care LTD, West Drayton, UK) in oxygen and maintained with 2% isoflurane in oxygen (Mutoh et al., 1997). In all three groups, spinal reflexes were evaluated at 15, 30, 45 and 60 min after induction of anaesthesia. For groups 2 and 3, the dogs were given a 1-week rest period and then the next method was tested.

Dogs were considered sedated when they remained in lateral recumbency and did not react to environmental stimuli such as touching and handclap. The dog's sedation levels were assessed and recorded using the reference scales (Grint et al., 2009; Wagner et al., 2017).

After administration of anaesthetics, the level of anaesthesia was assessed using the palpebral reflex and maintained in a constant level for all three groups by increasing or decreasing the drugs.

2.2 | Classification of reflexes

To examine spinal reflexes, the animals were placed on lateral recumbency. For better and more accurate comparison, the testing order of reflexes was consistent throughout the procedure: patellar, cranial tibial, extensor carpi radialis, pelvic limb withdrawal, thoracic limb withdrawal, cutaneous trunci (panniculus), palpebral and gag reflexes.

To evaluate the patellar reflex, the pelvic limb was held in a partial flexion position and the patellar ligament was struck with a patellar hammer. The response is a brisk extension of the stifle.

The cranial tibial reflex was tested by striking the belly of the cranial tibial muscle immediately distal to the proximal end of tibia with the relaxed limb and slightly extension of hock. The normal response is flexion of the hock.

The extensor carpi radialis reflex was evaluated by striking the belly of extensor carpi radialis muscle immediately distal to the elbow joint with the relaxed limb and flexion of carpus. The normal response is extension of the carpus.

Thoracic and pelvic limb withdrawal reflexes were elicited by compressing the skin of forelimb and rear limb digits respectively (fourth digit) by a tissue forceps. A normal response is the flexion of the entire limb after noxious stimuli.

To perform the cutaneous trunci (panniculus) reflex, the skin on both sides of the spine was stimulated in the lumbar region. The normal response is cutaneous trunci muscle contraction on both sides as evidenced by the skin moving over the thorax.

To perform the palpebral reflex, the corners of the eyelids were gently touched. The normal response to this reflex is immediate and complete closure of the eyelids.

The gag reflex was assessed by applying external pressure to the hyoid region. A healthy animal will swallow.

To ensure consistency of stimulus intensity, all reflexes were performed by the same investigator (AAS).

The response of each reflex, scored based on (Taylor, 2020) with some variation as follow: zero (none), +1 (reduced), +2 (normal), +3 (exaggerated), +4 (clonic = several consecutive contractile movements), +5 (throwing and clonic = consecutive contractile movements with limb throwing).

2.3 | Statistical analysis

The scores of reflexes at trends of times in each group were compared by non-parametric Friedman statistical method and if they were significant, the Wilcoxon signed rank test was used to compare them. All analyses were performed using spss24 software (IBM, Armonk, New York) and the significance level was considered $p < 0.05$.

3 | RESULTS

The results showed that the median scores of patellar reflex in all three groups increased significantly after injection of medetomidine compared to the pre-injection scores. This reflex increased after midazolam injection in all three groups but was not significant. Also, at all timepoints after ketamine administration, it did not change compared to before drug injection. This reflex significantly decreased in 15 min after propofol injection compared to before drug injection and at all timepoints after isoflurane administration compared to before baseline values and compared to after injection of medetomidine and midazolam values ($p < 0.001$; Figure 1).

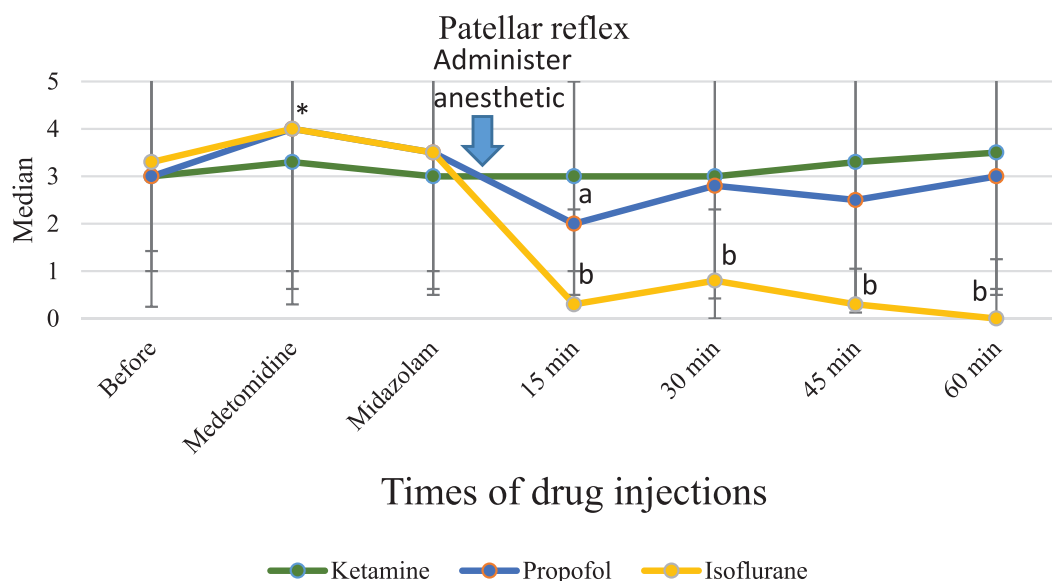


FIGURE 1 Patellar reflex response after administration of pre-anaesthetic and anaesthetic agents in 10 healthy dogs. Midazolam and ketamine did not change the patellar reflex ($p > 0.05$). *Significant increase ($p < 0.05$) in patellar reflex between baseline and post-drug administration of medetomidine. *a* indicates significant decrease ($p < 0.05$) in patellar reflex between baseline and 15 min after propofol injection. *b* indicates significant decrease ($p < 0.05$) in patellar reflex between baseline and post-drug administration of isoflurane at all timepoints.

The cranial tibial reflex was significantly increased after injection of medetomidine compared to before injection. This reflex did not change much after midazolam injection compared to before injection, and increased at all timepoints after injection of ketamine which was not significant ($p > 0.05$) while was significantly reduced at 15, 30 and 60 min after propofol and at all timepoints after isoflurane administrations ($p < 0.001$).

The extensor carpi radialis reflex, in the ketamine group, did not change after injection of medetomidine and midazolam and at all timepoints after ketamine administration. In the propofol and isoflurane groups, it decreased after injection of medetomidine, which was not significant; however, it decreased significantly after injection of midazolam and at all timepoints after administrations of propofol and isoflurane compared to before drug values ($p < 0.05$).

The Pelvic limb withdrawal reflex, in all three groups, increased after medetomidine and decreased after midazolam injections which both of them were not significant ($p > 0.05$) but significantly decreased at all timepoints after ketamine, propofol and isoflurane administrations compared to before drug values ($p < 0.05$) (Figure 2).

The thoracic limb withdrawal reflex did not change after injection of medetomidine and increased after midazolam injection, which was not significant ($p > 0.05$) while significantly decreased at all timepoints after ketamine, propofol and isoflurane administrations compared to before drug values ($p < 0.05$) (Figure 3).

The cutaneous trunci (panniculus), palpebral and gag reflexes were significantly decreased after administrations of medetomidine, midazolam, ketamine, propofol and isoflurane compared to before drug values. The palpebral and gag reflexes also decreased significantly after administrations of propofol and isoflurane compared to after injection of medetomidine and midazolam ($p < 0.05$).

4 | DISCUSSION

The present study was performed to determine the effect of pre-anaesthetic and anaesthetics of medetomidine, midazolam, ketamine, propofol and isoflurane on spinal reflexes to determine which drug can be used for tranquilisation or anaesthetising the dogs with spinal cord injury, so that it does not affect spinal reflexes and does not change the results of neurological examinations. In a number of previous studies, the effects of anaesthesia drugs on humans, cats, mice and dogs have been studied (Dahm et al., 1989; Lervik et al., 2012; Murrell & Hellebrekers, 2005; Paquette et al., 2019; Siegenthaler et al., 2020; Takatsuki & Ohtsuka, 2012; Tudury et al., 2017); however, based on our knowledge, there is no study that has clinically investigated the effect of pre-anaesthetic and anaesthetic drugs on spinal reflexes in dogs.

In a monosynaptic reflex such as the patellar, cranial tibial and extensor carpi radialis reflexes, a neural arch is established between the spinal cord and the corresponding muscle fibres. On the other hand, reflexes in which there are more interneurons between afferent and efferent neurons are called polysynaptic reflexes, in which the number of synapses in these arcs varies from two to one hundred synapses. A more complex reflex arc is required in polysynaptic reflexes, such as withdrawal and cutaneous trunci (panniculus) reflexes, which occur with a painful stimulus (Barrett et al., 2010; Guyton & Hall, 2006).

Among the spinal reflexes, the patellar reflex (which is a monosynaptic reflex) and the thoracic limb and pelvic limb withdrawal reflexes (which are pain-dependent and polysynaptic reflexes) are the most important reflexes commonly examined on neurological examination. Other spinal reflexes, even in normal animals, may sometimes not respond well (De Lahunta et al., 2015).

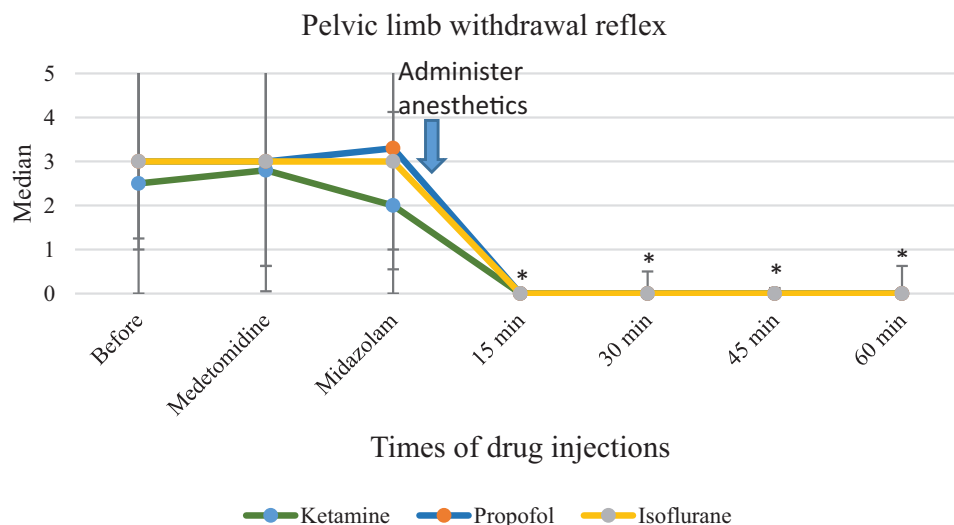


FIGURE 2 Pelvic limb withdrawal reflex response after administration of pre-anaesthetic and anaesthetic agents in 10 healthy dogs. Medetomidine and midazolam did not significantly change the reflex ($p > 0.05$). *Significant decrease ($p < 0.05$) in pelvic limb withdrawal reflex between baseline and post-drug administration of ketamine, propofol and isoflurane at all timepoints.

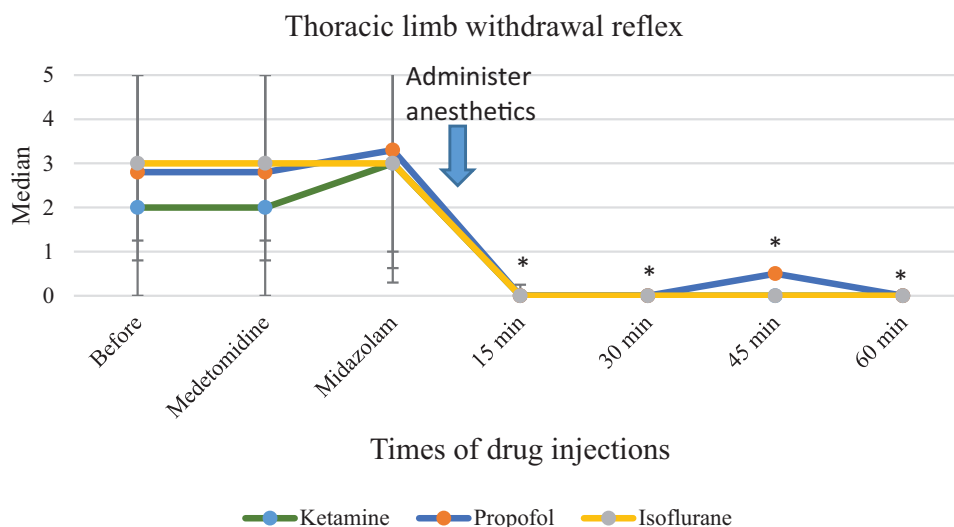


FIGURE 3 Thoracic limb withdrawal reflex response after administration of pre-anaesthetic and anaesthetic agents in 10 healthy dogs. Medetomidine and midazolam did not significantly change the reflex ($p > 0.05$). *Significant decrease ($p < 0.05$) in thoracic limb withdrawal reflex between baseline and post-drug administration of ketamine, propofol and isoflurane at all timepoints.

In the present study, intramuscular administration of medetomidine resulted in profound sedation in all dogs. However, medetomidine did not significantly change the thoracic and pelvic limb withdrawal reflexes ($p > 0.05$) and, contrary to our expectations, increased the monosynaptic tendon reflexes (patellar and cranial tibial reflexes). Medetomidine is an alpha-2 adrenergic agonist that blocks the release of noradrenaline in the synaptic cleft in the pons and the dorsal horn of the spinal cord, thereby relieving pain and causes analgesia and muscle relaxation (Siegenthaler et al., 2020). It has been reported that medetomidine severely reduces the pain and the response of withdrawal reflexes, but increases the non-pain-dependent reflexes (Kuusela et al., 2000; Lervik et al., 2012; Siegenthaler et al., 2020). This may be because

the drug prevents muscle contraction that may be caused by severe stress in the animal (Murrell & Hellebrekers, 2005). As a result, the dogs relax enough and the muscles involved in the reflex relax, while without relaxation, they tighten themselves so much that the stifle joint cannot be opened with a hammer blow (Horsley et al., 2021). This condition was clearly observed in the present study during the examination of animals; however, after anaesthesia with isoflurane and dexmedetomidine (the active isomer of medetomidine), it reduces pain-dependent withdrawal reflexes (Lervik et al., 2012). Therefore, the results of the present and other studies show that relaxation of a healthy animal with medetomidine causes better observation of these reflexes and may be confused with UMN symptoms. With this explanation, it seems

that the administration of medetomidine in nervous and restless animals will provide better examination results; however, for this decision, further studies are needed in animals with spinal cord injury, because the present study was performed in healthy animals and its results (although necessary) may differ from the results of animals with spinal cord injury.

In the present study, midazolam reduced the polysynaptic pain-dependent reflex of the panniculus and the monosynaptic non-pain-dependent reflex of the extensor carpi radialis. It had little effect on other reflexes, especially the patellar and thoracic and pelvic limb withdrawal reflexes. Although we expected that midazolam injection after medetomidine would have a synergistic effect and severely impair all reflexes, this attenuating effect did not occur in any of the major spinal reflexes. Midazolam, as a benzodiazepine agonist, increases the entry of chlorine ions into cells, causing the cell to become hyperpolarised, thereby enhancing the inhibitory effect of gamma aminobutyric acid. So, by reducing the excitability of neurons, this drug has a debilitating effect on brain activity and causes muscle relaxation, and in combination with ketamine, enhances the effect of ketamine (Hadley et al., 2012). In 1983, Leah et al. investigated the effect of midazolam on the spinal cord in cats and concluded that intravenous doses of midazolam increased the depolarisation of Ia afferents by GABA and piperidine-4-sulphonate, and presynaptic inhibition of spinal monosynaptic reflexes (Leah et al., 1983). Berti and Nistri (1983) also studied the effects of caffeine and midazolam on the frog spinal cord and concluded that midazolam-mediated enhancement of gamma-aminobutyric acid causes depolarisation of afferents in frog spinal cord (Berti and Nistri, 1983). Koch et al. (2008) examined the pain and sensitivity of skin reflexes in rats and reported that midazolam reduced the pain threshold by acting on GABA receptors in dorsal horn neurons and had no effect on C-fibre activity in healthy animals (Koch et al., 2008). Some studies have suggested that midazolam alone may increase animal agitation and aggressive behaviours in dogs (Clarke et al., 2014; Riviere and Papich, 2018). This stimulant effect is species-dependent, so that in humans, pigs, cattle and goats, midazolam has sedative-hypnotic effects (Mirakhur et al., 1984; Smith et al., 1991; Stegmann, 1998) but stimulant effects have been reported in dogs, cats and horses (Court & Greenblatt, 1992; Ilkiw et al., 1996; Muir et al., 1982; Siegenthaler et al., 2020). However, in the present study, midazolam did not cause any stimulating effect in any of the dogs, rather provided significant sedation, so that dogs could be easily manipulated and examined after injection. One of the reasons for these differences could be the method of drug injection, because in the previous studies, midazolam was injected intravenously but in the present study it was injected intramuscularly. Another reason could be the administration of medetomidine before the injection of midazolam in the present study; therefore, medetomidine has prevented the stimulatory effects of midazolam. Our goal in using of these drugs together was to mimic that used in a comparable clinical setting.

In the present study, ketamine reduced the pain-dependent polysynaptic reflexes but did not significantly change the non-pain-dependent monosynaptic reflexes. Lodge and Anis investigated the effects of ketamine and the three other anaesthetics, Alfax-

alone/alfadolone, methohexitone and diisopropylphenol on spinal reflexes in 14 cats (Lodge & Anis, 1984). They concluded that the reduction of polysynaptic (not monosynaptic) reflexes by ketamine indicates that ketamine acts by a different mechanism. In fact, ketamine blocks the postsynaptic stimulatory action of N-methyl-D-aspartate (NMDA), which are more present in the polysynaptic reflexes and dorsal roots of renshaw cells, thus reduces these reflexes. Guirimand et al. (2000) reported that a gradual increase in response to repetitive pain stimulation (wind-up phenomenon) occurs in pain-dependent reflexes; that is, if there is repeated stimulation of pain, the response gradually increases, but there is no response after only one stimulation. The mechanism of this gradual increase in response due to repeated stimulation is the stimulation of NMDA receptors located in the postsynaptic membrane (Guirimand et al., 2000). On the other hand, monosynaptic reflexes, including the patellar reflex, contain non-NMDA receptors (AMPA/Kainate receptors) and ketamine does not affect these glutamate receptors (Brockmeyer & Kendig, 1995).

In the present study, propofol reduced all reflexes (non-pain-independent monosynaptic reflexes and pain-dependent polysynaptic reflexes). Propofol is an alkyl-phenol derivative that induces anaesthesia by potentiating the effect of the GABA inhibitory neurotransmitter and opening the chlorine channels and reduces the metabolic activity of the brain (Fossum, 2012; Riviere & Papich, 2018). Grasshoff and Antkowiak evaluated the effects of propofol *in vitro* in the absence and presence of bicuculline (a GABA receptor antagonist) and concluded that propofol acts exclusively through gamma-aminobutyric acid receptors and increases synaptic transmission through these receptors; as a result, it causes anaesthesia and depression (Grasshoff & Antkowiak, 2004). Baars et al. (2009) examined the effect of propofol and sevoflurane anaesthetics on H and RIII reflexes. The H reflex arc is an analogue of tendon reflex (monosynaptic) and the RIII reflex arc is a polysynaptic pain-dependent withdrawal reflex performed by a complex network of interneurons in the fifth layer of the dorsal horn of the spinal cord. They concluded that propofol has a weaker effect on monosynaptic reflexes but a stronger effect on polysynaptic reflexes (Baars et al., 2009). Contrary to their study, Kim et al. (2007) in a study in mice showed that propofol mainly acts on the motor neurons of the ventral horn of the spinal cord and causes immobilisation of the limbs through these neurons, but stated that this effect occurs in higher doses, and their effect on dorsal spinal cord neurons is small (Kim et al., 2007). According to the above explanations, it seems that in addition to the animal species, the dose of the drug used is also effective in the mechanism of action of the drug (Kim et al., 2007). It has been reported that, due to the synergistic effect, the duration of anaesthesia and smooth muscle relaxation is significantly increased in the combination of medetomidine and midazolam with propofol (Koruk et al., 2020). In the present study, this synergistic effect was seen and spinal reflexes were reduced in the propofol group.

In the present study, isoflurane, like propofol, reduced pain-dependent and non-pain-dependent reflexes. By acting on glutamate, glycine and GABA_A receptors, isoflurane prevents exocytosis of synaptic vesicles and the release of neurotransmitters from nerve terminals, inducing anaesthesia and its continuation (Speigel & Hemmings, 2020).

These anaesthetics mainly affect the ventral root components of the spinal cord and reduce their effects drastically and reversibly (Rivera-Arconada et al., 2016). According to Baars et al. (2009), sevoflurane in contrast to propofol significantly reduced the H reflex (a monosynaptic reflex). This inhibitory effect on the monosynaptic reflex confirms that the ventral horn is an important target for volatile anaesthetics. As a result, volatile anaesthetics reduce monosynaptic reflexes (Baars et al., 2009). Sevoflurane and other volatile anaesthetics such as isoflurane act through several molecular targets in the spinal cord. Not only in the presence of bicuculline (GABA receptor antagonist), but also in the presence of strychnine (glycine receptor antagonist) and a combination of bicuculline and strychnine reduce nerve activity and thus reduce polysynaptic reflexes (Grasshoff & Antkowiak, 2004). In the present study, as in previous studies, a synergistic effect for the duration of anaesthesia and smooth muscle relaxation was observed in the concomitant use of isoflurane with medetomidine and midazolam (Koruk et al., 2020).

The results of the present study showed that the palpebral reflex, which is routinely used to determine the depth of anaesthesia, sometimes disappears earlier than deep pain of limbs; therefore, making decisions based on it may cause the animal suffering during surgery. Accordingly, we suggest that assessing of deep pain in the limbs for beginning of surgery is better than the assessing of palpebral reflex, although more studies are still needed to make this decision.

One of the limitations of the present study was the subjective evaluation of spinal reflexes that may affect the scoring of these reflexes; to avoid this effect, an attempt was made to have the same method of examining reflexes by the same researcher at all timepoints. Nevertheless, our aim in this study was to evaluate sedatives and anaesthetics in a normal clinical setting to compare them with what routinely occurs in the clinics.

An additional limitation of this study was the small number of dogs studied, which may affect statistical comparisons. To reduce statistical errors as much as possible, dogs of approximately similar age and size were selected to reduce data scatter.

In the present study, the order of reflexes had no effect on the study results. Another limitation of this study was that a special protocol of sedation and anaesthesia was performed in this study; therefore, with the exception of medetomidine, which was initially prescribed, the effects of other drugs may be overlapped to some extent and their individual effects may be different. So the results are related to this protocol. Results may differ from other methods of anaesthesia. This study was performed on neurologically healthy dogs; thus, these results may vary in dogs with neurological or orthopaedic problems.

5 | CONCLUSION

The results of the present study showed that the pre-anaesthetics, medetomidine and midazolam do not reduce the monosynaptic pain-dependent reflexes (patellar and cranial tibial reflexes) rather, by relaxing the muscles opposite the extension, can improve visibility of these reflexes; however, they reduce polysynaptic pain-related

reflexes. Ketamine does not affect the patellar, cranial tibial and extensor carpi radialis reflexes but reduces polysynaptic pain-related reflexes. Therefore, since medetomidine, midazolam and ketamine have little effect on monosynaptic reflexes (patellar and cranial tibial reflexes), these drugs may be used before neurological examination of aggressive animals. Propofol and isoflurane, on the other hand, eliminate all spinal reflexes and are not suitable for neurological examination. Although each of these drugs works by different mechanisms, the combined use of these drugs may mask some of their effects, so more physiological studies are needed to determine their exact effects individually.

AUTHOR CONTRIBUTIONS

Donya Saberfard: Methodology; writing – review & editing. Ali Asghar Sarchahi: conceptualisation; methodology; supervision; writing – original draft; writing – review & editing. Hossein Kazemi Mehrjerdi: Methodology; writing – review & editing.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article.

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PEER REVIEW

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ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

ETHICAL APPROVAL

The study protocol was assessed by the Research Committee of the Faculty of Veterinary Medicine and approved by the Research Ethics Committee of Ferdowsi University of Mashhad (Approval ID: IR.UM.REC.1399.125).

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