

## The effects of bupivacaine combined with different adjuvants on block onset and duration and on ion channel expressions (SCN9A, TRPM) in sciatic nerve block in rats

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**Background/aim:** The objective of this experimental study was to examine the effects of epinephrine, dexmedetomidine, and clonidine added as adjuvants to bupivacaine on block onset and effect times, as well as the effects on the Na<sup>+</sup> and Ca<sup>2+</sup> channel gene expressions, which may indicate cell damage in the sciatic nerve cell membrane.

**Materials and methods:** Rats were divided into five groups: Group S (sham), saline solution; Group B, bupivacaine; Group BD, bupivacaine + dexmedetomidine; Group BC, bupivacaine + clonidine; and Group BE, bupivacaine + epinephrine. For each group, 0.2 mL of local anesthetic was injected into the sciatic nerve bifurcation point of the right leg. Sensory (proprioceptive and nociceptive block) and motor block onset and ending times were recorded.

**Results:** The shortest onset time for the examined sciatic block was observed in the BC group, whereas the longest sensory and motor block times were observed in the BD group. The present data suggest suppressed *TRPM7* and increased *TRPM2* in the groups other than the BE group.

**Conclusion:** Clonidine is more suitable for fast onset of peripheral nerve blocks, whereas the addition of dexmedetomidine is better in terms of duration. Because the *SCN9A* and *TRPM2,4,7* expression ratios of the BE group showed the least amount of change, this group had the best cellular integrity.

**Key words:** Sciatic nerve block, bupivacaine, TRPM, rat

### 1. Introduction

The sciatic nerve block is a regional anesthetic method that provides muscle relaxation and pain control in knee and foot surgery and has a low risk of local anesthetic toxicity. The use of peripheral nerve blocking has increased in line with developments in algology. Therefore, the need for long effect agents with low neurotoxic effects has grown. Bupivacaine is a local anesthetic that has a more prolonged effect, is more successful compared with other local anesthetics, and is less expensive (1).

The frequent use of regional anesthetic has precipitated an increase in the use of adjuvant agents added to local anesthetics to decrease the effect onset time while increasing the duration and quality of the block. There are many studies in which agents such as opioids,  $\alpha_{1-2}$  agonists, and NaHCO<sub>3</sub> have been used. However, we found no study

that compares the sensory and motor block duration of bupivacaine with the addition of dexmedetomidine, epinephrine, and clonidine as adjuvants.

Voltage-gated sodium channels are expressed at different levels in the central nervous system, the peripheral nervous system, and muscle cells based on development and take part in important mechanisms such as the onset and transfer of action potential. It has also been suggested that *TRP* channels play an important role in the mechanism of calcium as a second messenger (2), that they are sensitive to cellular death in many cellular functions (3), that they are related to cation flow in unexcited cells and are effective in calcium permeability (4), that they are generally independent of voltage (5), and that they have many functions related to cellular proliferation, fertility, and the sense of touch (6). The

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members of the *TRPM* family are divided into four groups: *TRPM 1–3*, *TRPM 2–8*, *TRPM 4–5*, and *TRPM 6–7* (4,7–11). *TRPM* channels have high cation permeability, ranging from high  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  permeabilities (*TRPM 6–7*) to  $\text{Ca}^{+2}$  impermeability. *TRPM2* channels are located in the brain and are nonselective  $\text{Na}^+$ ,  $\text{Ca}^{+2}$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  permeable channels. *TRPM2* is activated by  $\text{H}_2\text{O}_2$  and acts as a cellular redox sensor. It also plays an important role in oxidative stress, TNF $\alpha$ -mediated  $\text{Ca}^{+2}$  intake, and cellular death (12–14). *TRPM4* is found in high amounts in the heart, pancreas, and placenta. *TRPM4* and *TRPM5* are permeable to monovalent cations but impermeable to  $\text{Ca}^{+2}$  (15). *TRPM7* is nonselectively  $\text{Na}^+$  and  $\text{Ca}^{+2}$  permeable and is also important in attaining intracellular  $\text{Mg}^{+2}$  homeostasis. The decrease in intracellular  $\text{Mg}^{+2}$  in the case of *TRPM7* insufficiency shows that *TRPM7* plays a role in  $\text{Mg}^{+2}$  intake and homeostasis (16,17).

The objective of the present study was to compare the block onset and effect times of bupivacaine, which is one of the most commonly used local anesthetics, with those of epinephrine, dexmedetomidine, and clonidine, which are added to bupivacaine as adjuvants. Another objective was to examine their effects on  $\text{Na}^+$  and  $\text{Ca}^{+2}$  channel gene expressions, which may indicate cell damage after anesthesia in the sciatic nerve cell membrane, in the groups in this study.

## 2. Materials and methods

This study was carried out at the Gaziantep University Physiology Department Experimental Animals Research Laboratory with approval number 2011/53-3 from the Gaziantep University Faculty of Medicine Ethics Council.

### 2.1. Animals

A total of 30 mature, healthy male Wistar albino rats with weights ranging between 200 and 250 g were used in this study. The rats were held in an environment with a moisture ratio of 50%–60% at an ambient temperature of 24–26 °C in circadian rhythm with 12 h of light and 12 h of darkness. Standard commercial pellet feed and city tap water were used to feed the rats. No change was made in their feeding habits. Daily close monitoring of the rats was performed for 15 days prior to the study. The reason for this was to ensure that the rats got used to the researcher who was going to carry out the neurological and behavioral examination, the experiment environment, and the experiment methods such as the neurological evaluation. The aim of this adaptation period was to minimize stress during the experiment and thus to increase experiment performance. Focus was on the rear extremity of the rat for a correct neurological evaluation.

### 2.2. Doses and groups

Bupivacaine (Marcaine, 0.5%, Astra Zeneca, Kırklareli, Turkey) was used as a local anesthetic, whereas

dexmedetomidine (Precedex 100 µg/mL, Abbott, Chicago, IL, USA), clonidine (Clonidin-Ratiopharm 0.15 mg/mL, Ratiopharm, Blaubeuren, Germany), and epinephrine (Adrenalin 0.5 mg/mL, Osel, İstanbul, Turkey) were used as adjuvants.

The subjects were divided into 5 groups with 6 rats in each group. A total of 0.2 mL solution 27 gauge needle point injectors were prepared for each rat in the five groups blinded to surgeons. The groups were as follows:

Group (S): sham, (physiological saline solution)

Group (B): bupivacaine (5 mg/mL)

Group (BD): bupivacaine (5 mg/mL) + dexmedetomidine (0.005% (5 µg/mL))

Group (BC): bupivacaine (5 mg/mL) + clonidine (0.00075% (7.5 µg/mL))

Group (BE): bupivacaine (5 mg/mL) + epinephrine (0.0005% (5 µg/mL))

### 2.3. Surgical procedure

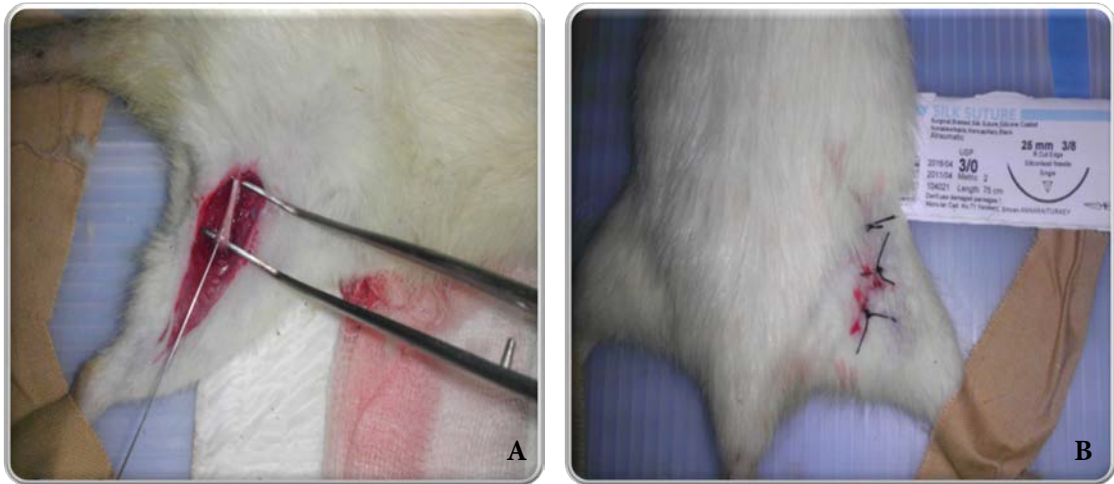
The rats were anesthetized with a 5 mg/kg intraperitoneal ketamine (Ketalar, 50 mg/mL, Pfizer, İstanbul, Turkey) injection. The trochanter major and ischial tuberosity were marked on the right legs with a posterior approach. The area between the marked points was shaved, after which a lateral subcutaneous incision was made and the surface fascia and muscles were excluded. All injections (local anesthetics) were given after full recovery from the ketamine anesthesia so that the block onset times could be evaluated. The 0.2 mL of local anesthetic that was prepared previously was injected under the epineurium without damaging the nerve integrity at the proximal of the sciatic nerve bifurcation point (Figure 1A). The surface muscle plans were brought closer and 4/0 silk was used for suturing. The skin was sutured with 3/0 silk (Figure 1B).

### 2.4. Experimental strategy

A double blind, randomized, controlled experimental model was used to evaluate motor and sensory blocks. The rats and the local anesthetic solutions were assigned unique identification numbers, to which the investigators were blinded. The same experiment row was used for the motor and sensory evaluations. Proprioceptive tests were carried out first, followed by motor evaluation and nociceptive tests. The opposite extremities of the rats were used as controls to evaluate channel expressions. The block evaluation was carried out as either available (+) or not available (–), without taking into account the block intensity.

### 2.5. Proprioceptive sensory function

Proprioceptive and nociceptive sensory evaluation and motor function were evaluated as described previously by Estebe and Thalhammer et al. (18,19). The proprioceptive sensory evaluation was carried out in a resting posture, and postural reactions (tactile placing response and hopping response) were observed. The tactile placing response was



**Figure 1.** The 0.2 mL local anesthetic injection under the epineurium at the proximal of the sciatic nerve bifurcation point (A). The skin suture with 3/0 silk (B).

evaluated based on the ability of the rats to bring their paws back to the original position after flexing the paw in a normal resting position until the dorsal surfaces touched the ground. The front half of the rat was lifted up from the surface in order to evaluate the hopping response. One hind leg at a time was lifted off the ground, and the animal's body moved laterally. As soon as this happens, an animal normally hops with the weight bearing limb in the direction of movement to avoid falling over. If the motor block is stronger, a rapid but weaker hopping response is observed right after the onset of the lateral movement. In contrast, if the proprioceptive block is stronger, then the hopping response is delayed and the passive lateral movement required to induce hopping should be wider.

## 2.6. Motor function

Motor function was evaluated based on the rats putting their weight on the rear foot, their hopping ability, their ability to grasp with their paw when hung upside down by their tails, and their ability to walk.

## 2.7. Nociceptive sensory function

The nociceptive sensory evaluation was carried out according to the flexor reflex of the rat. The flexor reflex occurs by the contraction of the flexor muscles in the hip, knee, and ankle. It is a polysynaptic reflex and is induced by giving a painful stimulant to the extremity. The intensity and duration of the flexor reflex depend on the intensity of the painful stimulant applied. The painful stimulant was applied mechanically in the study. Pressure was applied to the skin fold on the lateral metatarsus for surface sensation of pain, whereas pressure was applied to the distal phalanx of the fifth toe via tissue forceps for deep sensation of pain. A single painful stimulant was applied to the predefined regions by the same researcher at the same intensity and for the same duration of time.

## 2.8. RNA isolation and cDNA synthesis

Sciatic nerve samples were acquired from both legs of the rat for genetic evaluation following the process. Tissues were stored at  $-80^{\circ}\text{C}$  for RT-PCR studies. A Qiagen TissueLyser (Catalogue no 85600, Poison Information Center Mainz, Germany) device and Qiazol lysis reagent kit were used for tissue disruption and homogenization during the RNA isolation stage. A Roche high pure RNA tissue kit (Catalogue No. 12 033 674 001, Mannheim, Germany) was used for the isolation stage. RNA isolation was carried out in accordance with the directions provided by the manufacturing company.

A Biolabs Kit was used in accordance with the directions provided by the manufacturing company for cDNA synthesis. The reaction was prepared as shown below for cDNA synthesis with a total volume of  $20\ \mu\text{L}$  and cDNA synthesis was carried out under the following conditions:  $\text{d(T)}_{23}\text{VN}$  ( $50\ \mu\text{M}$ ) ( $2\ \mu\text{L}$ ), RNase free water ( $5\ \mu\text{L}$ ), RNA ( $2\ \mu\text{L}$ ), AMV reaction mix ( $10\ \mu\text{L}$ ), AMV enzyme mix ( $2\ \mu\text{L}$ ).

## 2.9. Reverse-transcriptase PCR (RT-PCR)

Optimum PCR conditions for each gene region were determined prior to the RT-PCR stage taking into consideration the GC % and  $T_m$  (melting temperature), obtained as a result of the design of the primers of the genes to be analyzed, as well as the base length values of the genes. The gradient PCR stage carried out to determine the optimum conditions was executed via Corbett Research (Model: RG-600, Australia). The PCR conditions determined for each gene were realized via Applied Biosystem Geneamp PCR system 9700 PCR device. The denaturation operation for the obtained cDNAs was carried out under the PCR conditions determined as follows: 2 min at  $94^{\circ}\text{C}$  (for  $\beta$ -actin housekeeping gene), for

4 min at 94 °C (*TRPM2*), and for 5 min at 94 °C (*TRPM4*, *TRPM7*, *SCN9A*); the 2nd denaturation stage was carried out for 30 s at 94 °C (for all genes); the binding stage was carried out for 45 s at 60 °C ( $\beta$ -*actin*, *TRPM2*), for 30 s at 60 °C (*TRPM4*), for 30 s at 60.4 °C (*TRPM7*), and for 30 s at 62.4 °C (*SCN9A*); the elongation stage was carried out for 30 s at 72 °C (for all genes); and the final elongation stage was carried out for 2 min at 72 °C ( $\beta$ -*actin*) and for 5 min at 72 °C (*TRPM2*, *TRPM4*, *TRPM7*, *SCN9A*).

The PCR products of the gene regions of all sample groups were run over 2% agarose gel at the end of RT-PCR (Figure 2). The gel images acquired as a result of electrophoresis were transferred to the digital environment. Image J software was used to measure the expressions levels of the studied genes.

The gene expression levels of *TRPM2*, *TRPM4*, *TRPM7*, and *SCN9A* were compared with the *beta-actin* gene as well as cDNA light brightness level.

### 2.10. Evaluation of results

The nerve tissues of the same rats (tissues with block and normal tissues) were compared with one another and studied simultaneously during the study. *Beta-actin*, which was used as a housekeeping gene, was studied as RT-PCR in the same sample. mRNA expression value was calculated by placing the measurement values obtained for each gene (*SCN9A*, *TRPM2*, *TRPM4*, *TRPM7*) in the following formula. Normal (0.75–1.25), low (0–0.75), and high (>1.25) parameters were used for the evaluation of the acquired results.

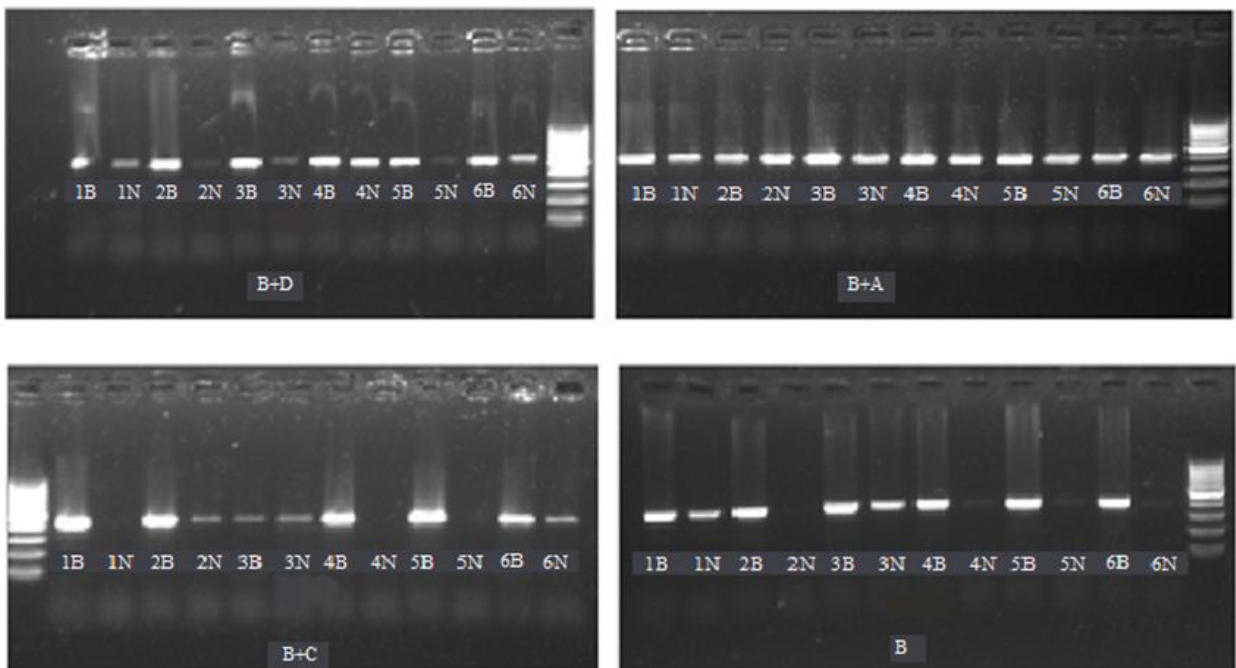
$$\text{mRNA expression value} = \frac{\text{Blocked gene expression} / \text{Normal gene expression}}{\text{Blocked } \beta\text{-actin} / \text{normal } \beta\text{-actin}}$$

### 2.11. Statistical method

SPSS 11.5 was used for the evaluation of the data about block times. One-way variance analysis (one way ANOVA) and Tukey HSD of the post hoc tests were used for the evaluation of the differences between groups since the groups displayed normal distribution. The values were given as average  $\pm$  standard deviation. Values of  $P < 0.05$  were accepted as statistically significant.

### 3. Results

No proprioceptive, motor, or nociceptive block developed in the counter extremities following the local anesthetic injections to the sciatic nerve in the rats of the 5 groups included in the study. In addition, no proprioceptive, motor, or nociceptive block developed in the Sham (S) group following injection, for which saline solution was used. Therefore, the Sham group indicated that our injection under the epineurium did not damage nerve integrity. Nociceptive block onset times were taken as the basis when recording the block onset times since the proprioceptive, motor, and nociceptive times were very close to each other. The block onset times and the proprioceptive, motor, and nociceptive block durations are given in the Table.



**Figure 2.** Gel electrophoresis image of the Na<sup>+</sup> channel gene *SCN9A* expression measurements. B (Group 2), B+D (Group 3), B+C (Group 4), B+A (Group 5), B: Sciatic nerve with block, N: Normal (sciatic nerve without block).

**Table.** The block onset and end times of groups (mean  $\pm$  standard deviation (SD)).

Groups	Group B (n = 6)	Group BD (n = 6)	Group BC (n = 6)	Group BE (n = 6)
Block onset time (s)	288 $\pm$ 35.87	168 $\pm$ 31.41*	115 $\pm$ 13.19*#	162 $\pm$ 30.78*
Proprioceptive block durations (min)	82 $\pm$ 21.77	131 $\pm$ 17.15*	105 $\pm$ 5.4#	117 $\pm$ 7.3*
Motor block durations (min)	84 $\pm$ 20.03	124 $\pm$ 16.57*	91# $\pm$ 13.62	113 $\pm$ 7.79*
Nociceptive block durations (min)	144 $\pm$ 18.20	266 $\pm$ 23.89*	176 $\pm$ 14.44*#	183 $\pm$ 14.37*#

(\*): Statistically significant compared to group B ( $P < 0.05$ ) (#): Statistically significant compared to group BD ( $P < 0.05$ )

The block onset times were respectively as follows  $BC < BE < BD < B$ , whereas the block end order was proprioceptive, motor, and nociceptive in the B group; it was motor, proprioceptive, and nociceptive block for the BD, BC, and BE groups. Accordingly, the fastest block onset time was that of the BC group and the longest block time was that of the BD group.

A statistically significant decrease was observed in the block onset times in all groups with adjuvant addition in comparison with the group to which pure bupivacaine was administered ( $P < 0.001$ ). A statistically significant decrease in duration was also observed in the group to which clonidine ( $P = 0.025$ ) was added as adjuvant in comparison with the group to which dexmedetomidine was added (Figure 3).

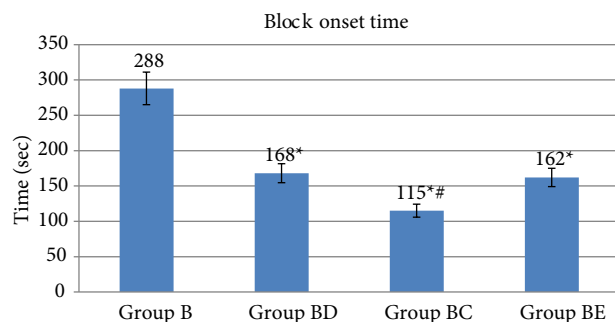
The proprioceptive block durations were determined to be longer at a statistically significant level for the group to which dexmedetomidine ( $P < 0.001$ ) and epinephrine ( $P = 0.002$ ) were added as adjuvants in comparison with the group to which pure bupivacaine was added. The increase in the proprioceptive block duration was not statistically significant in the group to which clonidine ( $P = 0.051$ ) was added in comparison with the group to which pure bupivacaine was administered. The proprioceptive block duration of the dexmedetomidine ( $P = 0.029$ ) added group

was longer at a statistically significant level in comparison with the group to which clonidine was added (Figure 4).

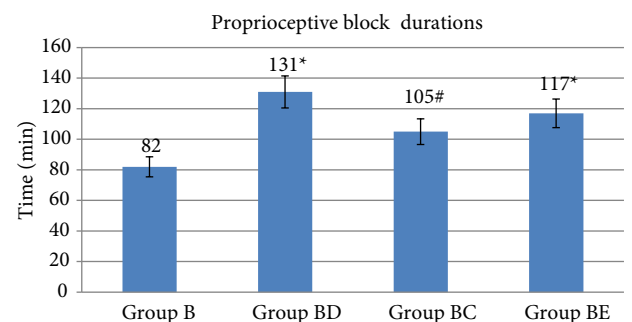
The motor block durations were determined to be longer at a statistically significant level in groups to which dexmedetomidine ( $P = 0.001$ ) and epinephrine ( $P = 0.019$ ) were added as adjuvants in comparison with the group to which pure bupivacaine was added. No statistically significant elongation in the motor onset duration was determined for the group to which clonidine ( $P = 0.854$ ) was added in comparison with the group to which pure bupivacaine was added. Dexmedetomidine ( $P = 0.006$ ) also increased the motor block duration at a statistically significant level in comparison with clonidine (Figure 5).

The nociceptive block duration for the groups to which adjuvants were added increased at a statistically significant level in comparison with the group to which pure bupivacaine was added (BD;  $P < 0.001$ , BC;  $P = 0.031$ , BE;  $P = 0.007$ ). Dexmedetomidine ( $P < 0.001$ ) addition increased the nociceptive block duration significantly in comparison with the clonidine and epinephrine added groups (Figure 6).

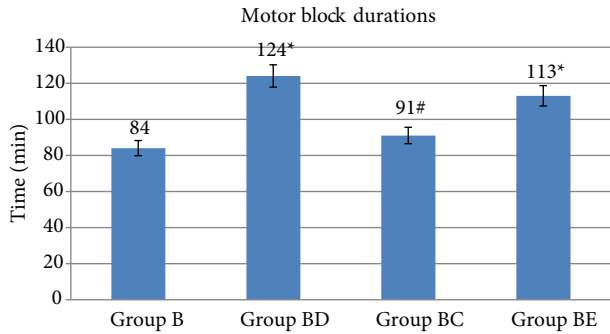
Complete recovery was determined in the rats that were included in the study without any neurologic deficit that could be clinically detected.



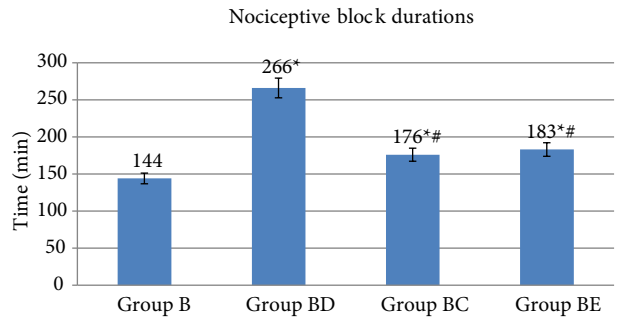
**Figure 3.** Block onset time of groups. (\*): Statistically significant compared to group B ( $P < 0.05$ ), (#): Statistically significant compared to group BD ( $P < 0.05$ ).



**Figure 4.** Proprioceptive block durations of groups. (\*): Statistically significant compared to group B ( $P < 0.05$ ), (#): Statistically significant compared to group BD ( $P < 0.05$ ).



**Figure 5.** Motor block durations of groups. (\*): Statistically significant compared to group B ( $P < 0.05$ ), (#): Statistically significant compared to group BD ( $P < 0.05$ ).



**Figure 6.** Nociceptive block durations of groups. (\*): Statistically significant compared to group B ( $P < 0.05$ ), (#): Statistically significant compared to group BD ( $P < 0.05$ ).

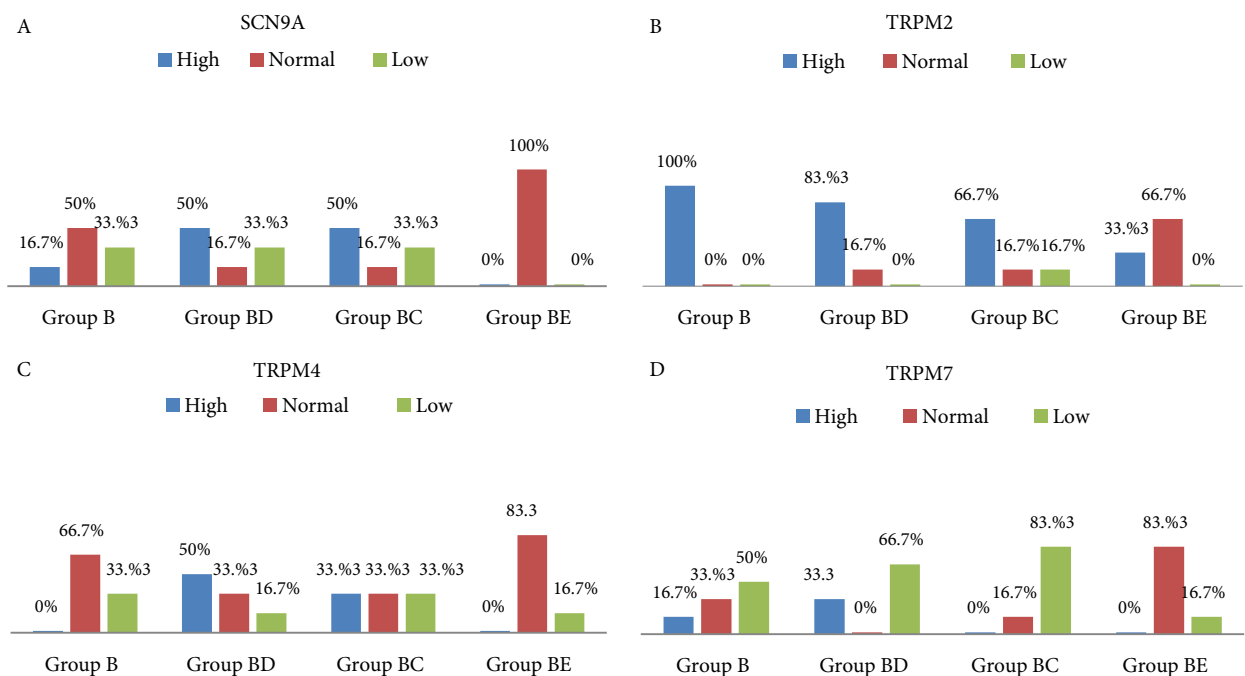
### 3.1. RT-PCR results

While the proportioning carried out showed that the *SCN9A* gene expression of the Na channel was 100% in the group to which epinephrine was added as adjuvant, it was determined to be 50% in groups to which dexmedetomidine and clonidine were added. It was determined to be 50% for the group to which pure bupivacaine was administered (Figure 7A). *TRPM2* gene expression of the  $Ca^{+2}$  channel was high in groups to which pure bupivacaine was administered and dexmedetomidine and clonidine were added as adjuvants; it was determined to be normal in the group to which epinephrine was added (Figure 7B). *TRPM4* gene expression of the  $Ca^{+2}$  channel was determined to be normal in the group to which pure bupivacaine was administered and epinephrine was added

as adjuvant, it was determined to be high in the group to which dexmedetomidine was added and it was found to be at the same ratio in the group to which clonidine was added (Figure 7C). *TRPM7* gene expression of the  $Ca^{+2}$  channel was determined to be 50% lower in the group to which pure bupivacaine was administered; it was also determined to be low in groups to which dexmedetomidine and clonidine were added as adjuvants, whereas it was found to be normal in the group to which epinephrine was added (Figure 7D).

### 4. Discussion

This randomized, blinded study examined the effects of epinephrine, dexmedetomidine, and clonidine added as adjuvants to bupivacaine in the sciatic nerve block of rats.



**Figure 7.** Expression levels of target genes in groups.

Before the under epineural injection of local anesthetics, intraperitoneal ketamine was used for the surgical procedure. The local anesthetics were injected after full recovery from the ketamine anesthesia and previous studies used the same procedure with isoflurane (20,21). Our results indicate that in all animal groups administered adjuvants the sensory and motor block durations increased and the block onset time decreased compared to the group that received only bupivacaine. Brummett et al. (21) have reported that 0.005% dexmedetomidine with the addition of 0.5% bupivacaine increases sensory and motor block times. Another study reported that ropivacaine added to dexmedetomidine is more likely to cause a sensory block than a motor block (22). Dexmedetomidine increased both the sensory and the motor block in our study, but the increase in the sensory block was greater. This is in accordance with normal nerve conduction physiology. Dexmedetomidine can be a useful adjuvant to add to local anesthetics during the postoperative period to provide analgesia without affecting mobilization. The perineural administration of dexmedetomidine in humans has not been approved by the Food and Drug Administration (22). On the other hand, there have been no reports of neurotoxicity. Clinical data have shown that no neurotoxicity is observed in the sciatic nerve block of rats when high doses of dexmedetomidine are administered alone or in combination with bupivacaine (21). According to our literature review, there has been no study in which the effects of clonidine and dexmedetomidine were compared in the perineural model and no comparison with epinephrine. Recent studies have shown that adding dexmedetomidine and clonidine as adjuvants to bupivacaine provides adequate anesthesia and postoperative analgesia compared to fentanyl in caesarean sections (23) and that the addition of dexmedetomidine to epidural bupivacaine can be advantageous with respect to the increased duration of motor and sensory blockade and arousable sedation (24).

Kanazi et al. (25) examined the effects of bupivacaine added to a low dose of dexmedetomidine or clonidine in spinal anesthesia and showed that both adjuvants decrease block onset time and increase the motor and sensory block times, which is in accordance with our study. Even though it has been shown in many studies that clonidine increases the anesthesia and analgesia times in peripheral nerve blocks, it has been reported that this effect is less distinct when combined with long-acting anesthetics (26–28). Another study has suggested a more effective blockade by clonidine in C-fibers, which was more effective than that in A $\alpha$ -fibers in rat sciatic nerve models (29). As is known through transmission physiology, C-fibers are responsible for the sensations of pain and heat, whereas A $\alpha$ -fibers are responsible for motor transmission. Knowing this, we can

explain the reason for the longer sensory block time in the group that received the addition of clonidine compared to the motor block time. Recent studies have shown that intrathecal dexmedetomidine is associated with prolonged motor and sensory block compared to clonidine, fentanyl, or bupivacaine alone (30) and that dexmedetomidine added to intrathecal bupivacaine produces longer postoperative analgesia than 50  $\mu$ g of clonidine among those undergoing lower limb surgery (31). In our study, we used perineural administration; however, the results match those of previous studies.

Kroin et al. (32) have shown that clonidine and epinephrine added to the sciatic nerve block with lidocaine increase the duration of analgesia and that the effect of clonidine on the nerve block is not only through  $\alpha$  receptors. Even though dexmedetomidine, which is also an  $\alpha_2$  agonist, has effects similar to those of clonidine, the fact that it causes longer sensory and motor blocks can be explained by the fact that its selectivity to  $\alpha_2$  receptors is eight times greater (33). Still, more studies that compare these two  $\alpha_2$  agonists are needed. In our study, compared to the group that received just bupivacaine, epinephrine (1/200,000) decreased the block onset time and increased the sensory and motor block durations of the group to which it was added. Another study showed that bupivacaine added to epinephrine and bicarbonate decreased the block onset time and similarly increased the sensory and motor block times in the sciatic nerve block of rats (34). Sinnott et al. (35) examined the mechanisms whereby epinephrine potentializes lidocaine in peripheral nerve blocks and reported that the addition of epinephrine increased the sciatic nerve block time while increasing the analgesic quality. There are two advantages to the addition of epinephrine in making a block. First, the plasma concentration of the local anesthetic is decreased, thereby decreasing the risk of systemic toxicity. Second, the block quality and time are increased. Many anesthetists accept that epinephrine increases the local anesthetic time via a vasoconstrictive effect. It excites the  $\alpha$ -adrenoreceptors in the neural veins, thus decreasing blood flow and the absorption of lidocaine from the nerves by causing vascular smooth muscle constriction (36). Apart from its vasoconstriction effect, epinephrine can also potentialize the effects of local anesthetics via its pharmacodynamic effects on nerve membranes. The activation of adrenoreceptors can be affected by various factors, such as Na<sup>+</sup>, Cl<sup>-</sup> channels, and Na-K pump that can regulate excitability (37). Partridge (38) administered anesthetics to the rat sciatic nerve topically and evaluated the blood flow using laser Doppler. The results showed that epinephrine causes more vasoconstriction when added to the local anesthetic than by itself. This effect decreases the block onset time and increases the block duration. It

should not be forgotten that the feeding of nerve fibers can be disrupted in the case of intrafascicular injection. Selander et al. (39) have shown that the nerve damage caused by the intrafascicular injection of local anesthetic can increase with the addition of epinephrine.

While the pain relieving effects of the analgesics that target sodium channels are preserved, they also have an increased potential for side effects. Mutation in the *SCN9A* gene that encodes the *Nav 1.7* sodium channel subtype creates two different phenotypes. People with this mutation either cannot feel the pain or have chronic familial pain syndrome (40). Reimann et al. (41) carried out studies on single nucleotide polymorphism (SNP) rs6746030 and determined that it changes pain sensitivity and pain experience in common diseases, such as osteoarthritis and spinal nerve root pain. They state that pharmacologic substances that can modify the functions of *SCN9A* will be beneficial in the treatment of diseases.

It has been shown that *TRPM 2* activity increases caspase 3, 8, 9, and, according to these data, *TRPM2* channels facilitate an increase in cellular  $Ca^{+2}$  in response to  $H_2O_2$  and thus contribute to apoptotic cell death induced by oxidants in endothelial cells. This means that vascular endothelial cells can be protected from apoptotic cell death by the downregulation of *TRPM2* (42). The increase in *TRPM2* in all groups except the one that received the addition of epinephrine can be interpreted as a preindication of cell apoptosis. What is interesting is that *TRPM2* increased in the group that received only bupivacaine, which shows that bupivacaine by itself disrupts cellular integrity.

*TRPM7* is activated by reactive oxygen and nitrogen species. There is an important physiological balance between *TRPM2* and *TRPM7* that is caused by oxidative stress. The decrease in or the blocking of *TRPM7* results in a decrease in anoxia-induced cell death.  $Ca^{+2}$  intake, the formation of reactive oxygen species, and anoxic cell death decrease with the suppression of *TRPM7* in cortical neurons (43). A decrease in *TRPM7* was observed in all groups in our study except the group that received the addition of epinephrine. *TRPM7* decreased in the groups

in which *TRPM2* increased, which is in accordance with the aforementioned literature. It seems that while the cell enters an apoptotic phase on the one hand, it tries to protect itself against anoxic cell death as a reflexive response on the other hand. Epinephrine is a natural catecholamine that is secreted in the human body, and thus the minimal change it causes in the  $Na^+$  and  $Ca^{+2}$  channel expression ratios in the cell membrane cannot be explained only by a vasoconstrictive effect. Results related to gene expression in this study have shown that epinephrine has a more protective effect at the cellular level than other adjuvants.

*TRPM4* activation in physiological conditions causes hyperpolarization and the flow of  $Na^+$  into the cell. In pathological cases, *TRPM4* activation causes  $Na^+$  loading. The cell volume increases, which results in the rupture of the cell membrane and thus irreversible cell necrosis (44). Gerzanich et al. (45) examined the role of *TRPM4* in progressive secondary hemorrhage related to capillary fragmentation, which is the most destructive mechanism in secondary injury of the central nervous system (spinal cord injuries). Capillary structural integrity has been retained in rats for which in vivo *TRPM4* gene suppression has been achieved. In addition, secondary hemorrhage has been eliminated, a decrease in lesion volume of one third to one fifth has been obtained, and significant advancements in neurological functions have been observed (45). In this study, significant results have not been obtained regarding *TRPM4*.

In conclusion, the shortest onset of effect was observed in the BC group in our study, whereas the longest block and analgesic times were observed in the BD group. Clonidine has a faster onset in peripheral nerve blocks, whereas dexmedetomidine has a longer effect time. The increase in the *TRPM2* ratio in all groups except the BE group, which is a preindication of cell apoptosis and oxidative stress, and the decrease in the amount of *TRPM7*, which protects cells against oxidative stress and anoxic cell death, are noteworthy. We also observed that the use of bupivacaine with epinephrine compared to bupivacaine alone results in early block onset and a longer duration while being safer at the cellular level. We think that our study will help inform future studies.

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