Percutaneous sciatic nerve block with tramadol induces analgesia and motor blockade in two animal pain models

A.M. Souza, H.A. Ashmawi, L.S. Costa, I.P. Posso and A. Slullitel

Percutaneous sciatic nerve block with tramadol induces analgesia and motor blockade in two animal pain models

A.M. Sousa[1], H.A. Ashmawi[1], L.S. Costa[1], I.P. Posso[1] and A. Slullitel[2]

[1]LIM-08 - Anestesiologia Experimental, Faculdade de Medicina,
Universidade de São Paulo, São Paulo, SP, Brasil
[2]Departamento de Anestesiologia, Hospital Santa Paula, São Paulo, SP, Brasil

Abstract

Local anesthetic efficacy of tramadol has been reported following intradermal application. Our aim was to investigate the effect of perineural tramadol as the sole analgesic in two pain models. Male Wistar rats (280-380 g; N = 5/group) were used in these experiments. A neurostimulation-guided sciatic nerve block was performed and 2% lidocaine or tramadol (1.25 and 5 mg) was perineurally injected in two different animal pain models. In the flinching behavior test, the number of flinches was evaluated and in the plantar incision model, mechanical and heat thresholds were measured. Motor effects of lidocaine and tramadol were quantified and a motor block score elaborated. Tramadol, 1.25 mg, completely blocked the first and reduced the second phase of the flinching behavior test. In the plantar incision model, tramadol (1.25 mg) increased both paw withdrawal latency in response to radiant heat (8.3 ± 1.1, 12.7 ± 1.8, 8.4 ± 0.8, and 11.1 ± 3.3 s) and mechanical threshold in response to von Frey filaments (459 ± 82.8, 447.5 ± 91.7, 320.1 ± 120, 126.43 ± 92.8 mN) at 5, 15, 30, and 60 min, respectively. Sham block or contralateral sciatic nerve block did not differ from perineural saline injection throughout the study in either model. The effect of tramadol was not antagonized by intraperitoneal naloxone. High dose tramadol (5 mg) blocked motor function as well as 2% lidocaine. In conclusion, tramadol blocks nociception and motor function *in vivo* similar to local anesthetics.

Key words: Tramadol; Tramadol-N-oxide; Rats; Peripheral nerves; Local anesthesia; Analgesia

Introduction

Tramadol, (1-RS,2RS)-2-[(dimethyl-amino)-methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is a centrally acting analgesic commercialized as a racemic mixture of two enantiomers [(+) and (-) tramadol]. The analgesic activity of this mixture has been attributed to different mechanisms (1,2). Tramadol is metabolized by cytochrome P450 (CYP) 2D6 (3) and its major metabolite is mono-o-desmethyl-tramadol (M1), which has a 200 times higher affinity for μ opioid receptors than tramadol (2). In addition to its central analgesic effects, tramadol exhibits a local anesthetic effect following intradermal injection and acts as a sole anesthetic in tendon repair surgery (4,5). Consistent with these findings, tramadol, but not its metabolite M1, blocks voltage-dependent sodium channels and suppresses somatosensory potentials following intrathecal administration through a mechanism not involving opioid receptors (6-8). In a similar way, tramadol decreases sensory and

motor responses evoked by electroneurography of the ulnar nerve in volunteers (9) and acts as an adjuvant after intra-articular injection (10) and peripheral nerve blocks, prolonging sensory and motor effects (11).

The aim of the present study was to evaluate the effect of perineural tramadol on sensory and motor functions following a modified sciatic nerve block technique in two different pain models. In addition, we propose a model for motor block assessment in rats.

Material and Methods

Experimental design

The two doses of tramadol used in these experiments (1.25 and 5 mg) were selected on the basis of previous reports (12,13). Animals were assigned to groups receiving sciatic nerve block with 2% lidocaine (50 µL) or tramadol

Correspondence: A.M. Sousa, Av. Dr. Eneas de Carvalho Aguiar, 255, Prédio dos Ambulatórios, 8º andar, Cerqueira Cesar, 05403-000, São Paulo, SP, Brasil. Fax: +55-11-3885-0997. E-mail: angela.sousa@icesp.org.br

(5 mg) in order to test motor function after sciatic nerve blockade. Another group of animals was submitted to sciatic nerve block with tramadol (1.25 mg) after hyperalgesia induction by two pain models: paw formalin injection or plantar incision model. In the plantar incision model, rats were allocated to one of two groups respectively subjected to a mechanical or thermal test.

Animals were tested for only one pain modality (chemical, heat, or mechanical stimuli) one at a time and only once. Sham sciatic block with tramadol, perineural injection of saline and contralateral sciatic nerve block with tramadol (1.25 mg) were used as controls.

In order to investigate the role of opioid receptors in the effect of tramadol, intraperitoneal naloxone (1 mg/kg) was injected 10 min before sciatic nerve block with tramadol (1.25 mg) in both models.

Animals

The experiments were performed after approval by the Ethics Committee for Analysis of Research Projects of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (protocols #051/02 and #188/10) and according to IASP Ethical guidelines for investigations of experimental pain in conscious animals (14). All experiments were performed on male Wistar rats (280-380 g), 5 animals per group, supplied by the breeding facilities of the Faculdade de Medicina da Universidade de São Paulo. Rats were housed 2 per cage with bedding and free access to food and water under a 12-h light/dark cycle and with room temperature maintained at 22° to 24°C.

Drugs

Tramadol hydrochloride and naloxone hydrochloride were kindly provided by Cristália Prod. Quím. Farm. (Brazil). Two doses of tramadol (1.25 and 5 mg) and 1 mg/kg naloxone were used in these experiments. Lidocaine hydrochloride and isoflurane were purchased from Cristália Prod. Quím. Farm.

Isoflurane was administered as inhalatory anesthesia. The other drugs were dissolved in 0.9% saline and administered in volumes of 50 μL (perineural) or 200 μL (intraperitoneal). Saline was also used as control.

Anesthesia

Animals were anesthetized with isoflurane in a 4-5% isoflurane/oxygen mixture in a sealed chamber and maintained with 1% isoflurane via a nose mask throughout the procedure. Animals were allowed to recover from anesthesia before testing.

Sciatic nerve block

A 2.5-cm insulated non-beveled needle (B. Braun, Germany) attached to a nerve stimulation apparatus (B. Braun) was used for the sciatic nerve block. Anesthesia was induced in an isoflurane/oxygen mixture as described above. The greater trochanter was located by palpation in the right hind limb and a neurostimulation needle was introduced 1 mm from the right femoral shaft into the sciatic notch between the greater trochanter and the ischial tuberosity pointing toward the ischium. A ground electrode was fixed to the left ear of the animal and an initial current of 0.6 mA was used for searching the thigh muscle contraction. Contraction was progressively stronger as the needle approached the nerve. The current was then reduced to 0.2 mA and if the muscle response did not change, it was considered that the needle was close to the sciatic nerve. Fifty microliters of lidocaine or tramadol was injected through the needle, according to the study group. In the sham group, sciatic nerve block was performed as described above except that, after nerve localization, the needle was pulled back and lidocaine or tramadol was injected far from the nerve. Animals were allowed to recover from anesthesia before testing.

Assessment of motor block

Assessment of motor block after tramadol or lidocaine injection was performed using a scale ranging from 0 (no block) to 3 (complete motor block; Table 1). This scale was based on walking behavior observation and developed using a pattern score-based motor block, similar to the Bromage scale used in humans. All animals were observed from the time they received the injection until complete regression of motor blockade. The motor block occurring after perineural tramadol was assessed and compared to that induced by lidocaine.

Flinching behavior test

Prior to testing, animals were allowed to acclimate to the environment. The test consisted of observation through a clear glass box on a raised platform to allow an unobstructed view of the hind paw. After 15 min acclimation, rats were anesthetized as described earlier, received a sciatic nerve block according to the study group and 50 μL 1% formalin injected into the dorsal aspect of the right hind paw at the time when they recovered from anesthesia. Following injection, each rat was placed back in the observation box and the number of flinches was counted every 5 min over a 60-min period. Flinch varied from a simple lifting of the paw (not associated with locomotion) to a vigorous shaking of the limb, or a rippling of the back muscles associated with movement. The flinches were discrete and easily quantifiable. The test was divided into two phases, i.e., first phase (0-15 min) and second phase (16-60 min) (adapted from Ref. 15).

Plantar incision model

Animals were anesthetized as described earlier. After preparation in a sterile manner, a 1-cm longitudinal incision was made with a number 11 blade through the skin and fascia of the plantar aspect of the hind paw, as described by Brennan et al. (16). After hemostasis with slight pressure, the skin was closed with 4-0 nylon sutures. Sciatic

nerve block was performed according to the study group; rats were allowed to recover in a plastic cage and tested when awake.

Nociceptive tests in the plantar incision pain model

Mechanical threshold. The mechanical threshold was measured using von Frey filaments (Stoelting Inc., USA). Rats were placed in a clear elevated acrylic cage (21 x 27 x 15 cm) with a 12 x 12-mm nylon mesh floor that allowed clear access to the plantar surface of the right hind paw. A series of 15 von Frey filaments of logarithmically incremental stiffness (0.39 to 588 mN) was used. Before the experiments, animals were acclimated for 15 min and the mechanical threshold was tested before plantar incision. Each filament was applied from underneath the floor through the mesh, perpendicularly to the plantar surface, with sufficient force to bend the filament. Response was defined as the withdrawal of the stimulated paw. Each filament was applied five times and, in the absence of a response, the next stronger filament was used. When there was a response, the previous weaker filament was retested. The strongest filament inducing a response was considered to be the mechanical threshold. The cutoff value used was 588 mN. Rats were then anesthetized for plantar incision, sciatic nerve block was performed and mechanical hyperalgesia was tested around the incision as described above, at 5, 15, 30, and 60 min after the animals awoke.

Heat threshold. Paw withdrawal latency in response to radiant heat was measured using the Hargreaves test equipment (Ugo Basile, Italy) (17). Rats were placed in a clear plastic chamber (18 x 29 x 12.5 cm) with a glass floor and allowed to acclimate to their environment for 30 min before testing. A heat source was then positioned under the glass floor directly beneath the hind paw and activated with a light beam intensity set to elicit baseline latency withdrawal values of 9-11 s in control rats. A cutoff time of 20 s was used to prevent tissue damage in the absence of a response. Mean paw withdrawal latency was obtained from the average values of three consecutive measurements, taken at 1-min interval. After surgery, animals were tested for heat hyperalgesia in the incision area at different times during the first hour.

Statistical analysis

Data are reported as means ± SEM for 5 rats/group. The results from 1% formalin subcutaneously injected and the heat and mechanical thresholds were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney test (GraphPad Prism 5, GraphPad Prism Software Inc., USA). Duration of motor block was analyzed by the Wilcoxon test. $P < 0.05$ was considered to be significant.

Results

Anesthesia

All rats recovered fully from anesthesia after 5 min without isoflurane.

Sciatic nerve block

Perineural injection of 2% lidocaine completely blocked motor function in the hind paw of all rats, which was recovered over time in a gradual way that allowed the construction of a motor block score (Table 1). The score was also further used to measure the intensity of motor block induced by tramadol. Time to complete return to normal motor function after lidocaine injection was 44.94 ± 3.74 min.

Perineural tramadol (5 mg) was as effective as 2% lidocaine in blocking motor function (score 3, soon after injection of the drug) and recovery from the block was complete after 36.25 ± 3.19 min in all animals. Nevertheless, 1.25 mg tramadol induced a slight difference during gait (score 1), but the capacity of withdrawing the paw was not affected. Sham block elicited no change in motor function with either tramadol or lidocaine injection (data not shown).
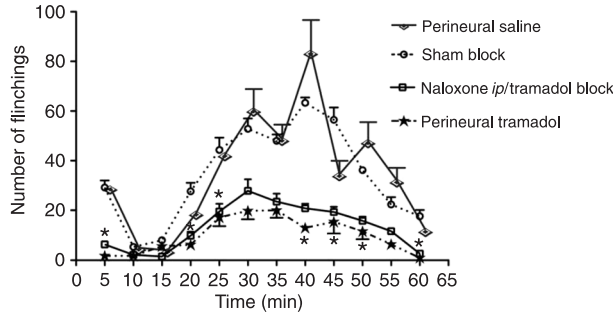
Flinching behavior test

Formalin induced the typical two-phase behavior of flinching in all animals. The number of flinching responses was 29.2 ± 6.06, 5 ± 1.48, 4 ± 1.09, 19 ± 6.14, 42.6 ± 6.75, 60.6 ± 18.49, 48.8 ± 12.76, 83.8 ± 28.78, 34.6 ± 11.89,

Table 1. Motor block score. Effect of the injection of 2% lidocaine via neurostimulation-guided sciatic nerve block on the walking behavior of 10 rats.

| Score	Reaction
0	Able to walk normally.
1	Weak or minimal motor blockade. Able to walk and to support the paw, but keeping the foot slightly sideways. Inversion or eversion of the foot.
2	Moderate motor blockade. Walking bending the forepart of the foot. The paw is flaccid. Thigh muscles are used to pull the leg. The animals do not support the plantar aspect of the foot on the surface.
3	Complete motor blockade. Paw is completely flaccid. Dragging the foot. Not bending the knee. Not using the thigh muscles. Failing to elevate the paw.

Score 3 indicates complete motor block, while score 0 indicates no motor block at all.

Figure 1. Effect of perineural sciatic tramadol (1.25 mg) in the presence or absence of intraperitoneal naloxone (1 mg/kg) on the flinching behavior induced by 1% formalin in the hindpaw. No difference was found between these groups, but they differed from the perineural saline injection and Sham block groups (*P < 0.05, Kruskal-Wallis test followed by the Mann-Whitney test). Data are reported as means ± SEM for 5 animals per group.
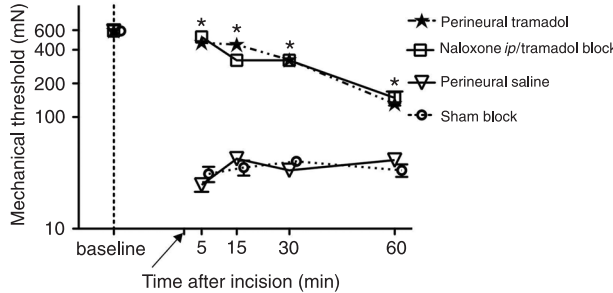
Figure 2. Effect of perineural sciatic tramadol (1.25 mg) in the presence or absence of intraperitoneal naloxone (1 mg/kg) on mechanical withdrawal threshold. No difference was found between these groups, but they differed from the perineural saline injection and Sham block groups at all times (*P < 0.05, Kruskal-Wallis test followed by the Mann-Whitney test). The arrow shows the time of surgery. Data are reported as means ± SEM for 5 animals per group.
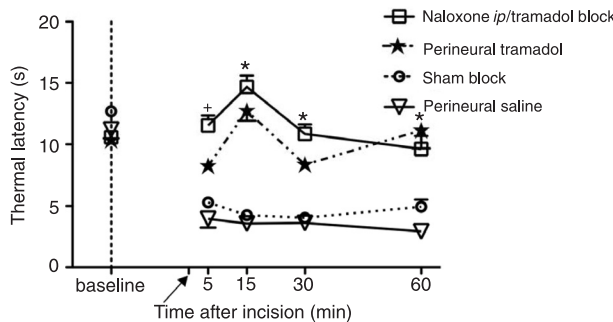
Figure 3. Effect of perineural sciatic tramadol (1.25 mg) in the presence or absence of intraperitoneal naloxone (1 mg/kg) on heat withdrawal threshold. The two groups were similar at 15, 30, and 60 min but differed significantly from the perineural saline and Sham block groups (*P < 0.05, Kruskal-Wallis test followed by the Mann-Whitney test). +P < 0.05 for perineural tramadol compared to naloxone *ip*/tramadol (Kruskal-Wallis test followed by the Mann-Whitney test). The arrow indicates the time of surgery. Data are reported as means ± SEM for 5 animals per group.

47.8 ± 17.35, 32 ± 11.54, 12.2 ± 6.7, measured every 5 min for 60 min. Perineural administration of 1.25 mg tramadol inhibited the first phase (1.8 ± 1.2, 1.8 ± 0.9, 5.6 ± 1.9) of formalin-induced hyperalgesia, while the second phase (6.2 ± 3.4, 17.2 ± 7.95, 19.8 ± 7.49, 20 ± 6.69, 13 ± 3.78, 15.4 ± 10.53, 11.6 ± 7.11, 6.4 ± 4.58, 1 ± 0.31) was markedly reduced. The effect of the same dose of tramadol injected as Sham block or in the contralateral paw (53.2 ± 10.81, 22 ± 11.02, 8.4 ± 2.63, 19.2 ± 7.57, 29.2 ± 8.58, 43.4 ± 11.96, 56 ± 18.67, 51.6 ± 9.62, 54.4 ± 10.54, 48 ± 17.41, 35.2 ± 8.32, 28.8 ± 8.29) was not different from perineural saline injection throughout the entire period of observation. Naloxone, 1 mg/kg, 10 min before sciatic nerve block with tramadol was not able to reduce the effect of tramadol throughout the period of observation (Figure 1).

Plantar incision model

Mechanical withdrawal threshold. Before surgery, rats did not exhibit paw withdrawal behavior after the thickest filament tested (588 mN). Soon after surgery, mechanical withdrawal threshold was reduced to 22.7 ± 7.4 mN at 5 min, and to 40.4 ± 9.7, 35.3 ± 3.9, and 39.2 ± 8.8 mN at 15, 30, and 60 min of observation in the saline group, respectively. Perineural sciatic tramadol (1.25 mg) prevented mechanical allodynia during the first 30 min of observation. Thresholds were 459 ± 82.8, 447.5 ± 91.7, 320.1 ± 120, 126.43 ± 92.8 mN at 5, 15, 30, and 60 min, respectively. Sham block (29.02 ± 10.855, 33.33 ± 12.15, 38.04 ± 7.16, and 31.37 ± 9.58 mN) was not different from perineural saline (described above) injection or contralateral perineural tramadol block (30.59 ± 9.97, 22.35 ± 7.32, 31.37 ± 4.80, and 29.41 ± 8.77 mN) at 5, 15, 30, and 60 min of observation, respectively. Intraperitoneal naloxone (1 mg/kg) 10 min before perineural sciatic tramadol did not antagonize the effect of tramadol (433 ± 96.41, 387.6 ± 81.81, 189.8 ± 26.21, 146.68 ± 48.02 mN at 5, 15, 30, and 60 min, respectively; Figure 2).

Thermal withdrawal latency. Thermal withdrawal thresholds were reduced from baseline values soon after surgery (11.2 ± 1.6 to 4 ± 0.7 s, 3.6 ± 0.3, 3.6 ± 0.4, and 3.9 ± 0.6 s after 5, 15, 30, and 60 min, respectively) after perineural saline injection. Perineural sciatic tramadol (1.25 mg) significantly increased thermal withdrawal latency towards preincisional values: 12.7 ± 1.8, 8.4 ± 0.8, and 11.1 ± 3.3 s after 15, 30, and 60 min, respectively (P < 0.05 compared to the perineural saline group). Sham perineural block (5.29 ± 0.62, 4.26 ± 1.06, 4.05 ± 0.7, and 4.95 ± 1.28 s) or contralateral perineural sciatic block (4.13 ± 0.66, 3.75 ± 0.52, 4.32 ± 0.35, and 4.21 ± 0.41 s) did not differ from perineural saline injection at 5, 15, 30, and 60 min. Intraperitoneal naloxone (1 mg/kg) 10 min before sciatic nerve block did not antagonize the effect of tramadol. In the first 5 min after sciatic nerve block, the combination of tramadol and naloxone was more effective than tramadol alone. Thermal latency was 11.54 ± 1.82, 14.68 ± 2.05, 10.84 ± 1.85, 9.64 ± 2.33 s at 5, 15, 30, and 60 min of observation, respectively (Figure 3).

Discussion

In the present study, we demonstrated that tramadol blocked nerve conduction in rats similar to lidocaine. Perineural tramadol blocked flinching behavior and increased withdrawal latencies after heat and mechanical stimuli. Such effects are reported using a method that mimics the clinical scenario of sciatic nerve blockade in humans. Previous studies have described sciatic nerve block in rats using anatomical topography for nerve location (18) or neurostimulation guidance (19), but the anatomical nerve location and equipment used for the sciatic nerve block were different from ours. In order to validate the nerve block technique and develop the scale for the magnitude of motor block, we used lidocaine as the standard local anesthetic before testing the effect of tramadol.

Sciatic nerve is a mixed nerve (containing sensory and motor fibers), and it was important to exclude that the changes observed in pain behavior were due to motor blockade. Quantifying such block was, therefore, mandatory. Objective quantification of motor block has been described before, consisting of series of experiments that are both time-consuming and difficult to reproduce (18). In the present study, motor effects of small dose of tramadol were seldom observed, making it difficult to use the previously described scale, a three-point motor scale (20). In the cited report, a score of 1 meant partial block, but the intensity of motor block described was stronger than the slight difference seen during gait after 1.25 mg perineural tramadol. In order to elucidate if this slight change in gait was, in fact, a motor blockade, sciatic nerve block with lidocaine was used as a gold standard. Lidocaine induced a profound motor block that progressively regressed, allowing the identification of minimal changes in gait, similar to the effect described with the lower dose of tramadol (1.25 mg), and the construction of an alternative score for motor block, that provides an additional grade of motor block compared to the previously described score (20). With the lowest score obtained on the present scale, rats were able to walk and grasp normally and had the strength to pull and raise their legs, reacting after noxious stimuli. Sciatic nerve block with a high tramadol dose (5 mg), however, induced complete motor blockade similar to 2% lidocaine.

If tramadol had a mechanism similar to that of local anesthetics, a differential rate of block would be expected. As a general rule, small fibers are more susceptible to the action of local anesthetics than large fibers, and clinical analgesia can be obtained without motor blockade. It is well known that the sciatic nerve consists of axons that have different diameters with different conduction velocities, blocked at different anesthetics concentrations. In the present study, tramadol blocked these fibers in a reversible manner that was not mediated by opioid receptors, in agreement with a previous report (21). While the highest dose completely blocked motor function, the lowest dose reduced pain behavior, but no effect was observed with Sham block (subcutaneous injection) or contralateral sciatic nerve block, in either the formalin or plantar incision models. In a previous publication, the same dose of tramadol administered at the site of the injury before formalin injection abolished the first phase of flinching behavior (13), leaving no doubts about the neural effect of tramadol observed in the present report.

The exact pharmacological mechanism of action of tramadol cannot be explained by these experiments. It has been reported, however, that local injection of tramadol inhibited pain behaviors not mediated by opioids (21), results replicated by our study, blocked the action potentials in isolated nerves (22) and presented a frequency-dependent block pattern like lidocaine (23,24). Moreover, intrathecal tramadol has been demonstrated to cause a dose-related suppressive effect on both sensory and motor neural conduction in the spinal cord (8). In our laboratory, intrathecal injection of tramadol induced complete motor block in the hind limbs of rats (data not shown), as did sciatic nerve block induced by the higher dose of tramadol. The current results also agree with Mert et al. (24), who reported dose-dependent inhibition of the action potentials of isolated nerves by tramadol and lidocaine, although tramadol was less efficient than lidocaine in inducing motor block. We showed that perineural administration of two different doses of tramadol resulted in blockade of nociception and motor function, not antagonized by naloxone, similar to local anesthetics. Such effect has been described by inhibition of action potentials in somatosensory fibers when directly applied over the sciatic nerve (12), with doses similar to those used in the present study. To our knowledge, complete motor block with tramadol has not been described *in vivo*. On the other hand, preclinical and clinical studies endorse the importance of this drug as an alternative adjuvant to local anesthetics (11,13,25).

Acknowledgments

References

1. Dayer P, Desmeules J, Collart L. [Pharmacology of trama-dol]. *Drugs* 1997; 53 (Suppl 2): 18-24.

2. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypi-cal' opioid analgesic. *J Pharmacol Exp Ther* 1992; 260: 275-285.

3. Stamer UM, Stuber F. Genetic factors in pain and its treat-ment. *Curr Opin Anaesthesiol* 2007; 20: 478-484.

4. Pang WW, Mok MS, Chang DP, Huang MH. Local anesthetic effect of tramadol, metoclopramide, and lidocaine following intradermal injection. *Reg Anesth Pain Med* 1998; 23: 580-583.

5. Kargi E, Babuccu O, Altunkaya H, Hosnuter M, Ozer Y, Babuccu B, et al. Tramadol as a local anaesthetic in tendon repair surgery of the hand. *J Int Med Res* 2008; 36: 971-978.

6. Katsuki R, Fujita T, Koga A, Liu T, Nakatsuka T, Nakashima M, et al. Tramadol, but not its major metabolite (mono-O-demethyl tramadol) depresses compound action potentials in frog sciatic nerves. *Br J Pharmacol* 2006; 149: 319-327.

7. Haeseler G, Foadi N, Ahrens J, Dengler R, Hecker H, Leu-wer M. Tramadol, fentanyl and sufentanil but not morphine block voltage-operated sodium channels. *Pain* 2006; 126: 234-244.

8. Jou IM, Chu KS, Chen HH, Chang PJ, Tsai YC. The effects of intrathecal tramadol on spinal somatosensory-evoked po-tentials and motor-evoked responses in rats. *Anesth Analg* 2003; 96: 783-788, table.

9. Ozturk E, Zinnuroglu M, Sezer OA, Gokyar I, Beyazova M, Kaya K. Effects of perineural tramadol on sensory and motor conduction of ulnar nerve. *J Opioid Manag* 2008; 4: 345-349.

10. Zeidan A, Kassem R, Nahleh N, Maaliki H, El-Khatib M, Struys MM, et al. Intraarticular tramadol-bupivacaine combi-nation prolongs the duration of postoperative analgesia after outpatient arthroscopic knee surgery. *Anesth Analg* 2008; 107: 292-299.

11. Gunes Y, Secen M, Ozcengiz D, Gunduz M, Balcioglu O, Isik G. Comparison of caudal ropivacaine, ropivacaine plus ketamine and ropivacaine plus tramadol administration for postoperative analgesia in children. *Paediatr Anaesth* 2004; 14: 557-563.

12. Tsai YC, Chang PJ, Jou IM. Direct tramadol application on sciatic nerve inhibits spinal somatosensory evoked poten-tials in rats. *Anesth Analg* 2001; 92: 1547-1551.

13. Sousa AM, Franco PA, Ashmawi HA, Posso IP. Local effect of tramadol on formalin evoked flinching behavior in rats. *Rev Bras Anestesiol* 2008; 58: 371-379.

14. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 1983; 16: 109-110.

15. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* 1977; 4: 161-174.

16. Brennan TJ, Vandermeulen EP, Gebhart GF. Characteriza-tion of a rat model of incisional pain. *Pain* 1996; 64: 493-501.

17. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988; 32: 77-88.

18. Thalhammer JG, Vladimirova M, Bershadsky B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. *Anesthesiology* 1995; 82: 1013-1025.

19. Kroin JS, Buvanendran A, Beck DR, Topic JE, Watts DE, Tu-man KJ. Clonidine prolongation of lidocaine analgesia after sciatic nerve block in rats is mediated via the hyperpolariza-tion-activated cation current, not by alpha-adrenoreceptors. *Anesthesiology* 2004; 101: 488-494.

20. Gianolio DA, Philbrook M, Avila LZ, MacGregor H, Duan SX, Bernasconi R, et al. Synthesis and evaluation of hydrolyz-able hyaluronan-tethered bupivacaine delivery systems. *Bioconjug Chem* 2005; 16: 1512-1518.

21. Mert T, Gunes Y, Gunay I. Local analgesic efficacy of trama-dol following intraplantar injection. *Eur J Pharmacol* 2007; 558: 68-72.

22. Dalkilic N, Tuncer S, Bariskaner H, Kiziltan E. Effect of tram-adol on the rat sciatic nerve conduction: a numerical analysis and conduction velocity distribution study. *Yakugaku Zasshi* 2009; 129: 485-493.

23. Guven M, Mert T, Gunay I. Effects of tramadol on nerve action potentials in rat: comparisons with benzocaine and lidocaine. *Int J Neurosci* 2005; 115: 339-349.

24. Mert T, Gunes Y, Ozcengiz D, Gunay I, Polat S. Compara-tive effects of lidocaine and tramadol on injured peripheral nerves. *Eur J Pharmacol* 2006; 543: 54-62.

25. Kaabachi O, Ouezini R, Koubaa W, Ghrab B, Zargouni A, Ben AA. Tramadol as an adjuvant to lidocaine for axillary brachial plexus block. *Anesth Analg* 2009; 108: 367-370.