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Modification of local anesthetic-induced antinociception by fentanyl in rats

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Abstract:
In clinical practice, using the lowest doses of drugs for anesthesia or analgesia is the main goal. Opioid combinations with local anesthetics can be preferable for achieving adequate anesthesia or analgesia. The primary purpose of this study was to examine possible thermal antinociceptive effects of the opioid –fentanyl and the amide local anesthetics levobupivacaine and lidocaine when locally administered alone or in combination.
The paw withdrawal latencies to noxious thermal stimuli in rats were measured to assess the antinociceptive actions of drugs after subcutaneous intraplantar injection into the hind paw.
All drugs examined in this study produced dose- and time-dependent increases in the paw withdrawal latencies. Fentanyl is approximately 125 and 500 times more potent than levobupivacaine and lidocaine, respectively. At the same dose, the antinociceptive potency of levobupivacaine was 3.6-fold higher than that of lidocaine. Co-injection of the lowest doses of levobupivacaine and lidocaine dramatically increased the paw withdrawal latency. However, in the presence of fentanyl, the effects of levobupivacaine and lidocaine were different. Although co-injection of levobupivacaine with fentanyl both enhanced and prolonged antinociceptive action, the lidocaine-fentanyl combination did not significantly change the paw withdrawal latency.
These results suggest that intraplantar co-administration of fentanyl with levobupivacaine, but not lidocaine, may provide more effective antinociception without increasing the dose requirements.

Key words:
fentanyl, levobupivacaine, lidocaine, intraplantar, antinociception, rat

Introduction

Fentanyl, N-(1-phenethyl-4-piperidyl) propionanilide and its derivatives are very effective in the treatment of pain [25, 34]. Opioids are usually administered systemically to treat moderate to severe pain [21]. However, a large number of clinical and animal studies have demonstrated that opioids, such as fentanyl, can produce local anesthetic actions by interacting with peripheral opioid receptors localized at the peripheral terminals of thinly myelinated and unmyelinated cutaneous sensory fibers [12, 31, 36].

Local anesthetics are used for many clinical procedures, such as acute and chronic pain management. Lidocaine, an amide local anesthetic, has well-documented effects on nerve signaling [9, 26, 34]. The nerve signal conduction block of lidocaine is most likely mediated by its binding to specific receptors on voltage-gated sodium channels; thus, it has long been used to temporarily abolish pain in clinical practice [10, 23].

Levobupivacaine, an amino amide type of local anesthetic similar to lidocaine, is the S-(–) enantiomer of racemic bupivacaine [4]. Previous clinical and experimental studies have shown that although the pharmacological activities of levobupivacaine are similar to those of bupivacaine, levobupivacaine is less neurotoxic and cardiotoxic [4, 11, 15, 20].

From a clinical perspective, an ideal anesthetic must have low systemic toxicity, be harmless and provide enough time to perform the clinical procedures. In clinical practice, using the lowest doses of local anesthetics for anesthesia or analgesia is the main goal. For this purpose, local anesthetics-opioid combinations can be preferable for achieving adequate anesthesia or analgesia. Therefore, in the present study, we hypothesized that a combination of the minimum dose of local anesthetic with the minimum dose of fentanyl would potentiate or prolong the local antinociceptive effectiveness. To test this hypothesis, we examined the thermal antinociceptive actions of fentanyl and both levobupivacaine and lidocaine when intraplantarly injected into a rat paw alone or in combination.

Materials and Methods

Animals

The animals used in this study were adult female Wistar rats (retired breeders, 7–9 months old, weight 230–250 g). Rats were maintained in a climate-controlled room under a 12-h light/dark cycle (6:00 a.m. to 6:00 p.m.), and food and water were available *ad libitum*.

Female rats were used because they are less aggressive than adult male rats, and female rats are easier to handle during behavioral testing. Females are more sensitive than males to many pain conditions, and the majority of pain sufferers are women. However, only 8–10% of animal pain studies are performed with female animals [7, 17]. Therefore, studying female pain sensitivity could be important for improving or creating new approaches for pain management.

This study complied with the Ethical Guidelines of the International Association for the Study of Pain, and the experimental protocols were approved by the Institutional Animal Care and Use Committee of Cukurova University.

Assessment of thermal nociception

The plantar test (Hargreaves method, [18]) is used to assess the hind paw nociceptive withdrawal latency to thermal stimuli in a free-moving rat. The nociceptive response to heat was tested with a commercially available paw thermal stimulation system as described elsewhere [14, 18, 22, 26, 27].

Rats were brought into the colony room two weeks before the test day and were acclimated to their environment for 1–5 days prior to the test. Habituation to the experimental setup was accomplished by placing the rats on the thermal nociception test apparatus for at least 30 min, three times before the test days. On the testing day, the rats were brought into the test room 1 h prior to the test session to habituate them to the environment. Before the drug injections, rats were acclimated to the testing environment again for 15 min, and pre-treatment values were measured (2 rats were tested simultaneously). After the habitation- acclimation process, thermal nociception was determined by measuring paw withdrawal latency using a thermal stimulation system consisting of a clear plastic chamber (10 × 20 × 24 cm) that sits on a clear smooth glass floor, and temperature was maintained at 25°C. A noxious thermal stimulus was focused on the plantar aspect of the left or right hind paw until the animal lifted the paw away from the heat source. Basal withdrawal latency was determined to reduce the variability and to select animals that showed a basal latency between 5 and 7 s. In order to avoid excessive suffering, a cut-off latency of 25 s was used. The measurement was taken at 10-min intervals after drug injections for a total of 60 min.

Drugs and experimental procedures

To test the effects of drugs on the peripheral receptive fields of sensory neurons, paw withdrawal latencies were measured after intraplantar injection of drugs for both the injected ipsilateral (right paw) and non-injected contralateral paw (left paw) for all experimental groups. The paw withdrawal latencies were measured in the right hind paw to determine the local effects of injected drugs, and the paw withdrawal latencies of the contralateral paw were used as an indicator of the systemic effects of drugs.

All chemicals used in experiments were dissolved in saline (0.9% NaCl), and all doses are expressed in this study in mg or μg per 100 μl. The control group

comprised intact, non-injected animals. All drugs were administered subcutaneously into the plantar hind paw in a volume of 100 µl using a 30-gauge needle. The needle was inserted at the midline near the heel and advanced anteriorly to the base of the second or third toe, where the drug was injected, forming a swelling (which disappeared approximately 3–5 min after injection) that usually extended back to the initial point of entry. In the saline group, an equal volume of saline was injected into the animals. All experiments were performed by the same experimenter during the same time of day (9:00 a.m. to 1:00 p.m., at 22–24°C) to exclude diurnal variations in pharmacological effects. The animals were randomly assigned to treatment groups, and the observer was blind to the drug administrations.

The doses of the administered drugs were chosen in accordance with our previous studies [26, 27] and several pilot studies. Levobupivacaine (0.1, 0.25 and 0.5 mg), lidocaine (0.5 and 1 mg) and fentanyl (1 and 2 µg) were intraplantarly injected into the paws of the rats. Furthermore, to test the synergistic effects of levobupivacaine, the lowest dose of levobupivacaine (0.1 mg) was combined with lidocaine (0.5 mg). To examine the efficiencies of opioid-local anesthetic combinations, fentanyl (1 µg) was co-administrated with levobupivacaine (0.25 mg) or lidocaine (0.5 mg) in a total volume of 100 µl. For these experiments, each group comprised eight rats. All efforts were taken to minimize animal suffering. No signs of skin inflammation, discoloration or irritation were noted at the sites of injection with any of the test compounds.

Statistical analysis

In the presented data, each point is an average of eight animals, and values represent the means ± standard error of means (SEM). Data were tested for normal distribution using the Kolmogorov-Smirnov test. Data were analyzed statistically by one-way or two-way analysis of variance (ANOVA) using SPSS statistical software. To evaluate the effects of the drugs, the thermal withdrawal latencies (dependent variables) measured during pre-treatment and all of the post-treatment values were analyzed using one-way, repeated measures ANOVA. To evaluate the effectiveness of the drugs, difference between groups was tested using a two-way ANOVA (repeated time measurements and treatments as independent variables). Differences were considered significant at the 95% confidence level. When significant F values were noted using an ANOVA, Tukey *post-hoc* analysis was performed. Differences were considered statistically significant when $p < 0.05$.

Results

The mean paw withdrawal latency obtained from control group including intact rats was 6.3 ± 0.1 s, and latencies did not significantly change during the experiments. No statistically significant differences were found among the basal thermal latencies of the experimental groups ($p > 0.05$). Injection of saline did not cause any significant changes in latencies compared with the pre-treatment paw withdrawal latencies ($p > 0.05$). In addition, paw withdrawal latencies of the contralateral non-injected paw (6.1 ± 0.1 s) to thermal stimulation did not significantly change ($p > 0.05$), and intraplantar administration of all tested levobupivacaine, lidocaine or their combinations with fentanyl did not produce antinociception in the contralateral paw (data not shown).

Antinociceptive effects of fentanyl

Fentanyl caused a significant, dose-dependent increase in paw withdrawal latency to noxious thermal stimuli. The changes in thermal latencies induced by saline or

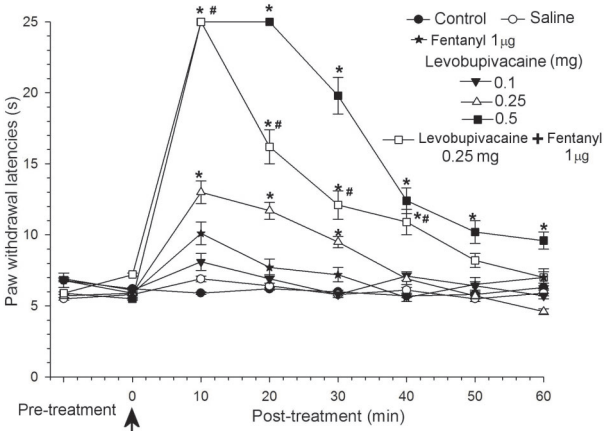

Fig. 1. Antinociception produced by fentanyl. Fentanyl increased the paw withdrawal latencies in a dose- and time-dependent manner. Each point represents the mean value of 8 rats, and the vertical bars indicate the SEM. On the x-axis, 0 indicates the time of drug injection (arrow). * $p < 0.05$ as compared to saline at each post-treatment time point

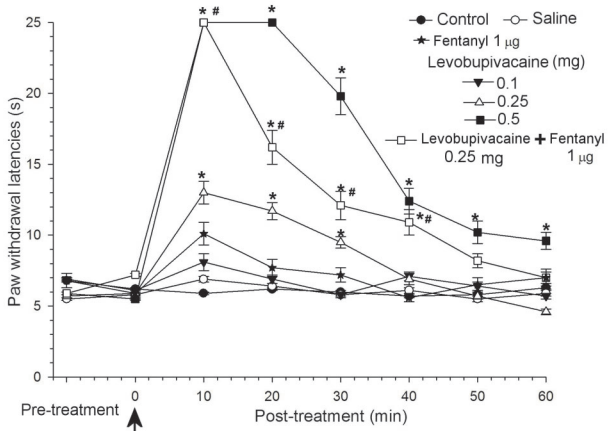

Fig. 2. The antinociceptive effects of levobupivacaine. Thermal paw withdrawal latency was significantly enhanced following the intraplantar administration of levobupivacaine. The effects of levobupivacaine are dose- and time-dependent. Co-injection of the lowest levobupivacaine and fentanyl doses significantly potentiated and prolonged the antinociception. Each point represents the mean value of 8 rats, and the vertical bars indicate the SEM. On the x-axis, 0 indicates the time of drug injection (arrow). * $p < 0.05$ as compared to saline at each post-treatment time point. # $p < 0.05$ as compared to 0.1 mg levobupivacaine at each time point

various doses of fentanyl are shown in Figure 1. One microgram of fentanyl caused a significant increase in paw withdrawal latency (10.1 ± 0.8 s) for a short time (10 min) (Fig. 1). However, 2 μg of fentanyl caused

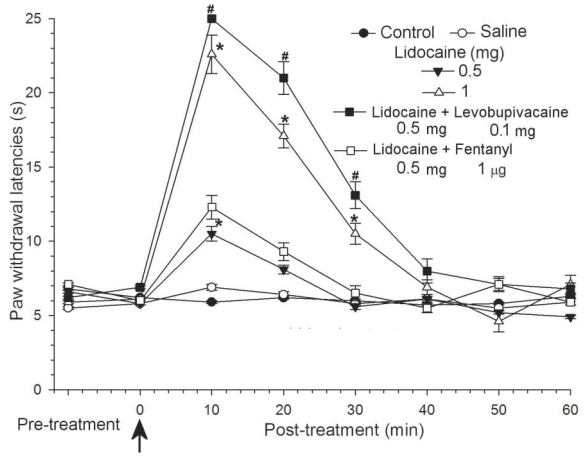

Fig. 3. The effects of lidocaine on thermal nociception. Lidocaine increased the latencies in a dose- and time-dependent manner. Co-injection of a low dose of lidocaine and levobupivacaine potentiated and prolonged the antinociception produced by lidocaine. In addition, antinociception produced by a combination of lidocaine and fentanyl did not differ from that of lidocaine alone. Each point represents the mean value of 8 rats, and the vertical bars indicate the SEM. On the x-axis, 0 indicates the time of drug injection (arrow). * $p < 0.05$ as compared to saline at each post-treatment time point. # $p < 0.05$ as compared to 0.5 mg lidocaine at each time point

a significant and longer lasting (30 min) increase in paw withdrawal latencies when compared to saline or pre-treatment paw withdrawal latencies ($p < 0.05$).

Antinociceptive effects of local anesthetics

The changes in paw withdrawal latencies induced by various doses of levobupivacaine are shown in Figure 2. Levobupivacaine produced a dose-dependent increase on the paw withdrawal latency. While 0.1 mg of levobupivacaine did not cause a statistically significant change in paw withdrawal latency, 0.25 mg and 0.5 mg of levobupivacaine significantly increased the latencies for 30 min and 60 min, respectively, when compared to saline and/or pre-treatment values ($p < 0.05$).

The dose-dependent effects of lidocaine on the paw withdrawal latencies to thermal stimuli are shown in Figure 3. The administration of 0.5 mg lidocaine caused a significant but short-term (10 min) antinociceptive effect ($p < 0.05$). However, a significant and longer lasting (10.5 ± 0.7 s at 30 min) increase in paw withdrawal latencies was produced by 1 mg of lidocaine ($p < 0.05$).

When the lowest ineffective dose levobupivacaine (0.1 mg) was added to 0.5 mg lidocaine, paw withdrawal latencies significantly increased, and antinociceptive action lasted for 30 min (Fig. 3). The antinociceptive effects of this combination were not significantly different from that of 1 mg lidocaine ($p > 0.05$).

Comparison of the antinociceptive actions of fentanyl and local anesthetics

In Figure 4, the antinociceptive effects of fentanyl, levobupivacaine and lidocaine 30 min after administration are compared. Fentanyl was approximately 125 and 500 times more potent than levobupivacaine and lidocaine, respectively, with 1 μg of fentanyl inducing approximately the same antinociceptive action as either 0.25 mg of levobupivacaine or 1 mg of lidocaine. A two-fold increase in both levobupivacaine and lidocaine dose resulted in an approximate two-fold increase in paw withdrawal latency. In addition, a four-fold higher dose of lidocaine (1 mg) was required to induce the same antinociceptive effect as 0.25 mg of levobupivacaine. When administered at the same dose (0.5 mg), the antinociceptive potency of levobupivacaine was 3.6-fold higher than that of lidocaine.

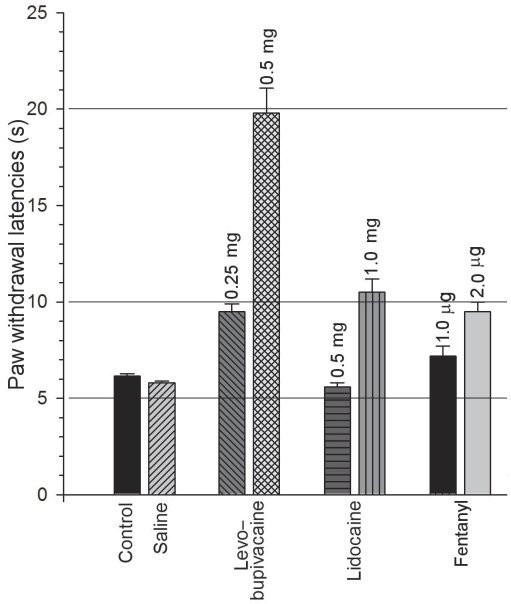

Fig. 4. A comparison of fentanyl-, levobupivacaine- and lidocaine-induced antinociception. The paw withdrawal latencies were measured 30 min after intraplantar injection. Fentanyl is approximately 125 and 500 times more potent than levobupivacaine and lidocaine, respectively. There was a 4-fold difference between the levobupivacaine and lidocaine dose at the same paw withdrawal latency. Although the levobupivacaine dose was 2-fold lower than that of lidocaine, its antinociceptive effect was 1.8-fold higher

Effects of fentanyl on the antinociceptive effects of local anesthetics

A combination of 1 µg of fentanyl and 0.5 mg of lidocaine did not significantly change the paw withdrawal latency when compared to lidocaine alone ($p > 0.05$) (Fig. 3). However, co-injection of 0.25 mg of levobupivacaine and 1 µg of fentanyl caused a dramatic increase in paw withdrawal latencies ($p < 0.05$) (Fig. 2). In addition, the latencies did not return to the baseline level in the presence of levobupivacaine-fentanyl combination for 60 min. Thus, the addition of fentanyl potentiated the antinociceptive action of levobupivacaine by 30–40% at each time point over the 60-min period.

Discussion

Previous studies have reported that the peripheral endings of sensory nerves have a repertoire of voltage-sensitive sodium channels similar to those channels found on the sensory nerves somata in dorsal root ganglia [1, 33]. The findings presented in this study also suggest that local administration of fentanyl, levobupivacaine, lidocaine or their combinations to the peripheral endings of sensory nerves can produce antinociceptive actions without eliciting potentially serious side effects.

Local injection (intraplantar) of agents into the peripheral receptive field is an administration method for determining the direct pharmacological actions of these agents on peripheral nerve endings. Although the pharmacological actions of agents are rather transient, their suppressive effects on paw withdrawals from noxious thermal stimuli are proven by evaluating their peripheral antinociceptive potencies. In the present study, plantar tests were used to assess drug-induced changes in rat hind paw nociceptive withdrawal latencies. Antinociceptive actions of locally applied agents on thermal withdrawal latencies suggested that local administration can be useful in pain states in which abnormal thermal nociception is associated with acute tissue injury, such as sun burns, surgical thermocoagulation and nerve plexus injury.

Nociceptive signals are produced by intense stimulation of primary afferent sensory Aδ and C nerve fiber terminals by noxious thermal stimuli [28, 31]. Voltage-sensitive sodium channels are present in these small-size peripheral sensory neurons (known as nociceptors) and play key roles in membrane excitability. A large number of clinical and experimental studies have reported that local anesthetics can reversibly prevent the generation and propagation of electrical signals in sensory nerve fibers by blocking voltage-sensitive sodium channels [24, 34, 35]. Therefore, levobupivacaine and lidocaine, two amide local anesthetics, are now widely preferred in clinical practice. These local anesthetics are used by clinicians to achieve adequate anesthesia or analgesia at several sites (e.g., epidural, subarachnoid, brachial plexus, peripheral nerve) and several situations (e.g., local administration, obstetrics and pain treatment) [10, 15, 16, 23]. Previous studies have suggested that levobupivacaine is more effective than lidocaine in the treatment of pain, and levobupivacaine has several advantages over lidocaine, such as lower arrhythmogenic potential, lesser inotropic effect on cardiac muscle and less depressing action on the central nervous system.

Intraplantar administration of levobupivacaine or lidocaine achieved dose- and time-dependent antino-

ciceptive effects in the present study. Levobupivacaine-induced antinociception was more effective and longer than that of lidocaine. When comparing the doses, a 3.6-fold lower levobupivacaine dose can produce the same level of antinociception as lidocaine. Local anesthetics that belong to the amide chemical class bind directly to intracellular voltage-dependent sodium channels. Therefore, their lipid solubility is the most important factor for their anesthetic potencies [11]. Because levobupivacaine has higher liposolubility than lidocaine [15, 23], fewer molecules of levobupivacaine can easily penetrate the membrane and produce ion channel blockade, resulting in enhanced anesthetic potency.

In addition to these individual effects, adding the lowest, ineffective, dose of levobupivacaine (0.1 mg) to lidocaine (0.5 mg) greatly increased the paw withdrawal latencies. This result suggests a potential synergism between lidocaine and levobupivacaine. When administering drug combinations, the dose requirements for effective local antinociceptive are relatively small; thus, some of the unwanted side effects associated with high dose administration can be prevented. The vascular properties of local anesthetics help to determine the effectiveness of their therapeutic activity [4, 8]. The high vasoconstrictor effect of levobupivacaine can enhance the antinociceptive efficacy of lidocaine.

Opioids are also used as frequently as local anesthetics as treatment to manage pain. Opioid receptors also are present on the peripheral terminals of thinly myelinated and unmyelinated cutaneous sensory fibers and play an important role in opioid-induced antinociception [12, 21, 36]. Fentanyl, a clinically used selective μ-opioid receptor agonist, is used to treat both acute and chronic pain [25, 34]. A large number of preclinical and clinical studies have examined the antinociceptive effects of fentanyl. Consistent with previous studies, our recent paper suggested that local administration of fentanyl can produce dose-dependent antinociception (early effect) and hyperalgesia (late effect) [26, 30]. Thus, 1 μg fentanyl was chosen as the proper dose in this study. This fentanyl dose caused only temporary antinociception.

In clinics, to increase the safety of local anesthetics, they can be combined with opioids. A number of studies have suggested that the intrathecal, intraarticular or perineural administration of opioids alone or in combination with low-dose local anesthetics can provide effective analgesia and reduce some of the unwanted side effects [13, 32].

Fentanyl is one of the most commonly used additives with local anesthetics [3]. Previous clinical and experimental studies reported that intrathecal administration of a combination of fentanyl and local anesthetics, such as levobupivacaine and bupivacaine, produces enhanced spinal anesthesia and post-operative analgesia [5, 6, 20, 37]. These studies suggested that the combination of fentanyl with a local anesthetic could greatly increase the safety of local anesthetics because adequate anesthesia could be achieved using much lower concentrations of local anesthetic. In contrast, some clinical studies have demonstrated that in combination with lidocaine, sufentanil does not affect lidocaine-induced analgesia [2, 19].

In the present study, although fentanyl did not potentiate and prolong the lidocaine-induced antinociception, adding fentanyl to levobupivacaine both potentiated and prolonged its antinociceptive action. Fentanyl exhibited an additive effect with only levobupivacaine. In the presence of fentanyl, a reduced levobupivacaine dose can provide more effective antinociception than lidocaine. Local anesthetics exert their effect by binding directly to the intracellular voltage-dependent sodium channels in peripheral sensory neurons. Lipid solubility appears to the primary determinant of intrinsic anesthetic potency [11]. Levobupivacaine has high liposolubility, similar to fentanyl, but higher than lidocaine [15, 23]. High lipophilic compounds can easily pass through the membrane, so fewer drug molecules are required for blockage, resulting in enhanced potency. By adding a lipophilic opioid fentanyl, a lower dose of levobupivacaine improved effective antinociception. This action may also be associated with conformational changes of opioid receptors that promote the binding of fentanyl. In addition to local anesthetic effects, levobupivacaine may expose opioid receptors to fentanyl.

The different effects of fentanyl in combination with levobupivacaine or lidocaine can be related to the local anesthetics' vasoactive properties. Local anesthetics are generally known as vasodilators [38]. However, levobupivacaine intrinsically produces a mild degree of vasoconstriction [29]. The vasoconstrictive effect of levobupivacaine may decrease the rate of local clearance of the intraplantarly injected drugs and thus may potentiate its effects.

In conclusion, as compared to lidocaine, levobupivacaine is a more potent local anesthetic and has long lasting antinociceptive action. Fentanyl potentiated and prolonged only levobupivacaine-induced antino-

ciception. In clinics, low concentrations of a local anesthetic with an acceptable safety profile are preferable. Therefore, a combination of levobupivacaine with fentanyl may be used for adequate topical anesthesia or analgesia with the lowest possible dose requirements.

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References:

1. Amaya F, Decosterd I, Samad TA, Plumpton C, Tate S, Mannion RJ, Costigan M, Woolf CJ: Diversity of expression of the sensory neuron specific TTX-resistant voltage-gated sodium ion channels SNS and SNS2. Mol Cell Neurosci, 2000, 15, 331–342.
2. Atanassoff PG, Brull SJ, Printsev Y, Silverman DG: The effect of intradermal administration of lidocaine and morphine on the response to thermal stimulation. Anesth Analg, 1997, 84, 1340–1343.
3. Axelsson K, Gupta A: Local anaesthetic adjuvants: neuraxial versus peripheral nerve block. Curr Opin Anaesthesiol, 2009, 22, 649–654.
4. Bardsley H, Gristwood R, Baker H, Watson N, Nimmo W: A comparison of the cardiovascular effects of levobupivacaine and rac-bupivacaine following intravenous administration to healthy volunteers. Br J Clin Pharmacol, 1998, 46, 245–249.
5. Beale N, Evans B, Plaat F, Columb MO, Lyons G, Stocks GM: Effect of epidural volume extension on dose requirement of intrathecal hyperbaric bupivacaine at caesarean section. Br J Anaesth, 2005, 95, 500–503.
6. Ben-David B, Solomon E, Levin H, Admoni H, Goldik Z: Intrathecal fentanyl with small dose dilute bupivacaine: better anesthesia without prolonging recovery. Anesth Analg, 1997, 85, 560–565.
7. Berkley KJ: Sex differences in pain. Behav Brain Sci, 1997, 20, 371–380.
8. Bouaziz H, Iohom G, Estebe JP, Campana WM, Myers RR: Effects of levobupivacaine and ropivacaine on rat sciatic nerve blood flow. Br J Anaesth, 2005, 95, 696–700.
9. Brau ME, Branitzki P, Olschewski A, Vogel W, Helpelmann G: Block of neuronal tetrodoxin-resistant Na^+ channels by stereoisomers of piperidine local anesthetics. Anesth Analg, 2000, 91, 1499–1505.
10. Butterworth JF, Strichartz GR: Molecular mechanisms of local anesthesia: a review. Anesthesiology, 1990, 72, 711–734.
11. Chaplan SR, Bach FW, Shafer SL, Yaksh TL: Prolonged alleviation of tactile allodynia by intravenous lidocaine in neuropathic rats. Anesthesiology, 1995, 83, 775–785.
12. Coggeshall RE, Zhou S, Carlton SM: Opioid receptors on peripheral sensory axons. Brain Res, 1997, 764, 126–132.
13. David BB, Maryanovsky M, Gurevitch A, Lucyk C, Solosko D, Frankel R, Volpin G, DeMeo PJ: Comparison of minidose lidocaine-fentanyl and conventional-dose lidocaine spinal anesthesia. Anesth Analg, 2000, 91, 865–870.
14. Dirig DM, Salami A, Rathbun ML, Ozaki GT, Yaksh TL: Characterization of variables defining hind paw withdrawal latency evoked by radiant thermal stimuli. J Neurosci Meth, 1997, 76, 183–191.
15. Foster RH, Markham A: Levobupivacaine: a review of its pharmacology and use as a local anaesthetic. Drugs, 2000, 59, 551–579.
16. Gautier P, De Kock M, Huberty L, Demir T, Izydorczic M, Vanderick B, Izydorczic M: Comparison of the effects of intrathecal ropivacaine, levobupivacaine, and bupivacaine for caesarean section. Br J Anaesth, 2003, 91, 684–689.
17. Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB, Gold MS et al.: Consensus Working Group of the Sex, Gender, and Pain SIG of the IASP. Studying sex and gender differences in pain and analgesia: a consensus report. Pain, 2007, 132, 26–45.
18. Hargreaves K, Dubner R, Broun F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain, 1988, 32, 77–78.
19. Hartmannsgruber MWB, Atanassoff PG, Budde A, Brull SJ, Kain ZN, Silverman DG: Intradermal sufentanil does not improve lidocaine-induced local anesthesia. Can J Anesth, 2003, 50, 153–158.
20. Jain K, Grover V, Mahajan R, Batra Y: Effect of varying doses of fentanyl with low dose spinal bupivacaine for caesarean delivery in patients with pregnancy-induced hypertension. Int J Obstet Anaesth, 2004, 13, 215–220.
21. Janson W, Stein C: Peripheral opioid analgesia. Curr Pharmaceut Biotech, 2003, 4, 270–274.
22. Jevtovic-Todorovic V, Meyenburg AP, Olney JW, Wozniak DF: Anti-parkinsonian agents procyclidine and ethopropazine alleviate thermal hyperalgesia in neuropathic rats. Neuropharmacology, 2003, 44, 739–748.
23. Leone S, Cianni SD, Casati A, Fanelli G: Pharmacology, toxicology, and clinical use of new long acting local anesthetics, ropivacaine and levobupivacaine. Acta Biomed, 2008, 79, 92–105.
24. Mao J, Chen LL: Systemic lidocaine for neuropathic pain relief. Pain, 2000, 87, 7–17.
25. Martin TJ, Eisenach JC: Pharmacology of opioid and nonopioid analgesics in chronic pain states. J Pharmacol Exp Ther, 2001, 299, 811–817.
26. Mert T, Gunes Y, Gunay I: Local analgesic efficacy of tramadol following intraplantar injection. Eur J Pharmacol, 2007, 558, 68–72.
27. Mert T, Gunes Y, Ozcengiz D, Gunay I: Magnesium modifies fentanyl-induced local antinociception and hyperalgesia. Naunyn Schmiedebergs Arch Pharmacol, 2009, 380, 415–420.
28. Millan MJ: The induction of pain: An integrative review. Prog Neurobiol, 1999, 57, 1–164.

29. Newton DJ, Burke D, Khan F, McLeod GA, Belch JJ, McKenzie M, Bannister J: Skin blood flow changes in response to intradermal injection of bupivacaine and levobupivacaine, assessed by laser Doppler imaging. Reg Anesth Pain Med, 2000, 25, 626–631.
30. Obara I, Przewlocki R, Przewlocka B: Local peripheral effects of μ-opioid receptor agonists in neuropathic pain in rats. Neurosci Lett, 2004, 360, 85–89.
31. Ossipov MH, Lai J, King T, Vanderah TW, Malan TP, Hruby VJ, Porreca F: Antinociceptive and nociceptive actions of opioids. J Neurobiol, 2004, 61, 126–148.
32. Parpaglioni R, Baldassini B, Barbati G, Celleno D: Adding sufentanil to levobupivacaine or ropivacaine intrathecal anaesthesia affects the minimum local anaesthetic dose required. Acta Anaesth Scand, 2009, 53, 1214–1220.
33. Pinto V, Derkach VA, Safronov BV: Role of TTX-sensitive and TTX-resistant sodium channels in Aδ- and C-fiber conduction and synaptic transmission. J Neurophysiol, 2008, 99, 617–628.
34. Sawynok J: Topical and peripherally acting analgesics. Pharmacol Rev, 2003, 55, 1–20.
35. Scholz A, Vogel W: Tetrodotoxin-resistant action potentials in dorsal root ganglion neurons are blocked by local anesthetics. Pain, 2000, 89, 47–52.
36. Stein C, Machelska H, Binder W, Schafer M: Peripheral opioid analgesia. Curr Opin Pharmacol, 2001, 1, 62–65.
37. Wang C, Chakrabarti MK, Whitwam JG: Specific enhancement by fentanyl of the effects of intrathecal bupivacaine on nociceptive afferent but not on sympathetic efferent pathways in dogs. Anesthesiology, 1993, 79, 766–773.
38. Willatts DG, Reynolds F: Comparison of the vasoactivity of amide and ester local anaesthetics: An intradermal study. Br J Anaesth, 1985, 57, 1006–1011.

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