Comparative Somatic and Visceral Antinociception and Neurotoxicity of Intrathecal Bupivacaine, Levobupivacaine, and Dextrobupivacaine in Rats

Tomoko Muguruma, M.D.,* Shinichi Sakura, M.D.,† Yumiko Kirihara, D.V.M.,‡ Yoji Saito, M.D.§

Background: The current study investigated whether racemic bupivacaine and its S(−)- and R(+)−enantiomers, levobupivacaine and dextrobupivacaine, differ in somatic and visceral antinociception and neurotoxicity when administered intrathecally in rats.

Methods: In experiment 1, rats intrathecally received 15 µl saline or 0.125, 0.25, 0.5, or 1% bupivacaine, levobupivacaine, or dextrobupivacaine. The tail-flick and colorectal distension tests were performed to assess somatic and visceral antinociceptive effects, respectively, for 180 min after injection. In experiment 2, rats given 0.25% anesthetic solutions were evaluated with colorectal distension–induced response in blood pressure and heart rate. In experiment 3, four groups of rats received a 1-h infusion of saline or 2.5% bupivacaine, levobupivacaine, or dextrobupivacaine. Additional rats received either 1.25% bupivacaine or levobupivacaine for 1 h. Four days after infusion, animals were assessed for persistent sensory impairment using the tail-flick test. Spinal cords and nerve roots were obtained for histologic analysis.

Results: In experiment 1, the three drugs produced similar time course effects and dose–effect relations in tail-flick latency. Colorectal distension thresholds and motor paralysis were slightly lower and less apparent, respectively, at some concentrations in rats given levobupivacaine than in those given the other agents. In experiment 2, colorectal distension–induced response in heart rate was less depressed in rats given levobupivacaine than in those given the other anesthetics. In experiment 3, three groups of rats given 2.5% anesthetic solutions developed similar significant increases in tail-flick latency and incurred similar morphologic damage. Two groups of rats receiving 1.25% anesthetic solutions were similar in functional impairment and nerve injury scores.

Conclusions: The results suggest that, when administered intrathecally in rats, bupivacaine and its R(+)− and S(−)-enantiomers are similar for somatic antinociception and neurotoxicity but slightly different in visceral antinociception and motor paralysis, in which levobupivacaine is less potent than the others.

BUPIVACAINE, a racemic mixture of S(−)- and R(+)− enantiomers, has been one of the most widely used local anesthetics because of its long duration of action and beneficial ratio of sensory to motor blockade. Recently, the pure S(−)-enantiomer, levobupivacaine, has been developed for clinical use as an agent that has lower risk of cardiotoxicity.1–6 Because the both enantiomers have different binding affinities to sodium channels,7,8 anesthetic effects of levobupivacaine may be different from those of racemic bupivacaine. Although previous studies9 have investigated the effects of bupivacaine, levobupivacaine, and the R(+)−enantiomer dextrobupivacaine on somatic pain and motor function, the effect on visceral pain has never been compared, nor have the stereoselective effects been investigated with the three agents administered intrathecally.

Bupivacaine has enjoyed its popularity for spinal anesthesia also because of its infrequent incidence of permanent neurologic injury10 and transient neurologic symptoms.11–14 Our previous laboratory data confirm that its neurotoxicity is less than that of lidocaine.15 However, it is not known whether the enantiomers of bupivacaine are similar in neurotoxicity.

Accordingly, we performed three experiments using rats to answer the above questions: In the first, somatic and visceral antinociception of intrathecal bupivacaine, levobupivacaine, and dextrobupivacaine were compared. The second experiment was performed to confirm the visceral antinociceptive results obtained in the first experiment. Finally, we sought to examine whether these three agents are similar with respect to sensory impairment and histologic damage. Because neurotoxicity is dose dependent, the three drugs were administered in equipotent doses.

Materials and Methods

This study was approved by the Animal Research and Use Committee of Shimane University, Izumo City, Japan (permission No. 04-025), and was comprised of three experiments. All experiments were conducted in male Sprague-Dawley rats (250–350 g), which were maintained on a 12-h light–dark schedule and were housed individually with free access to food and water. To reduce the influences of handling on behavioral reactions, all rats were trained in the test situation at least two times before intrathecal catheterization.

Surgical Procedure

For intrathecal catheterization, anesthesia was induced with sodium pentobarbital (Dainippon Pharmaceutical, Osaka, Japan; 30 mg/kg intraperitoneally) and maintained with 1.5% halothane. Using an aseptic technique, a midline skin incision was made over the spinous pro-
cesses of the L2–L5 vertebrae with a foam block placed under the animal’s abdomen. The fascia was opened, and superficial muscles around the spinous process were dissected and retracted laterally. With the use of fine forceps, the ligament was pierced, and a heat-connected catheter of stretched polyethylene tubing PE-10 (1.1 cm), PE-10 (10 cm), and PE-20 (6 cm) was introduced into the subarachnoid space. Catheters were passed through the L3–L4 or L5–L6 intervertebral space in experiments 1 and 2, or 3, respectively, and advanced 1.1 cm in the caudal direction. Before starting experiments, rats were allowed at least 2 days to rest for recovery from the operation. Rats having any problem with tail movements or motor dysfunction in the hind limbs were not used in the ensuing experiments.

Behavioral Examination

The tail-flick (TF) test was performed on rats placed in an acrylic restraint to measure the response to noxious somatic stimulus by using TF equipment (model-DS20; Ugo Basile, Comerio-Varese, Italy) and monitoring latency to withdrawal from a heat source focused on a distal segment of the tail approximately 5 cm from the tip. A cutoff time of 10 s was used to avoid damage to the tail.

The colorectal distension (CRD) test was performed using a modified method of Ness and Gebhart to measure the response to noxious visceral stimulus. CRD involves air inflation of an 8-cm flexible latex balloon. The system consists of a large proximal stimulating balloon and a small distal sensing balloon, both of which were connected to a catheter. Pressure in each balloon was continuously monitored via in-line pressure transducers and recorded. The balloon was inserted intrareally into the descending colon rectum, 1.5 cm from the base of the balloon to the anus, during light halothane anesthesia and kept in place with the catheters fixed to the tail using tape during experiment on each test day. Animals were placed in an acrylic restraint and tested while awake after recovery from anesthesia. In experiment 1, to measure the visceromotor response, pressure within the stimulating balloon was steadily increased at a rate of 2.5 mmHg/s until a rapid increase of the pressure (spike-like waves) in the sensing balloon was detected. The minimal pressure in the stimulating balloon at which the increase of pressure in the sensing balloon was triggered was defined as the threshold response for CRD. A cutoff distension pressure was 60 mmHg to prevent tissue damage. In experiment 2, pressure within the stimulating balloon was steadily increased at a rate of 2.5 mmHg/s until it reached 70 mmHg, which was kept 20 s. CRD-induced hemodynamic change was noted with a tail cuff blood pressure monitor (model-MK2000; Muromachi Kikai, Tokyo, Japan).

The paw pressure (PP) test was performed to measure the response of the legs to noxious mechanical stimulus. Pressure was applied to the dorsal surface of hind paws and progressively increased at a rate of 15 g/s with a device (type 7200; Ugo Basile, Comerio-Varese, Italy). Rats were wrapped with a cloth for the test. The pressure at which rats withdrew the paw from the device was defined as the PP threshold. Both hind paws of each rat were tested, and the mean value was used for analysis. A cutoff pressure of 400 g was used to avoid damage to the paws.

Motor function (MF) in the lower limbs was assessed by bilaterally grading the motor block as follows: 0 = none, 1 = partially blocked, and 2 = completely blocked. The normal baseline score was 0, and the score with bilateral complete block was $2 + 2 = 4$.

Experimental Protocols

The investigator administering a test solution and performing behavioral examination was blinded to the solution administered to each animal. Local anesthetic solutions were prepared by dissolving crystalline bupivacaine hydrochloride, levobupivacaine hydrochloride, or dextrobupivacaine hydrochloride in saline. All of the drugs were donated by Maruishi Pharmaceutical Co., Ltd., Osaka, Japan.

Experiment 1. To compare the anesthetic effects of bupivacaine, levobupivacaine, and dextrobupivacaine, rats were randomly given 0.125, 0.25, 0.5, or 1% solutions of each local anesthetic, or saline. A group of seven animals received the same solution. After the baseline values of TF latency and CRD threshold were determined, all drug injections were given in a volume of 15 µl followed by 10 µl saline to flush the catheter. The TF, CRD, and MF tests were performed 5, 10, 15, 20, 30, 45, 60, 90, 120, and 180 min after injection. At the end of an experiment, each rat intrathecially received 15 µl lidocaine, 2%. Data obtained from rats that did not show motor block or an increase in TF latency after the intrathecal lidocaine were not included in the data analysis. Some rats were tested on multiple days (not more than 4) but never received the same solution twice, and only rats that recovered completely were used. Each rat received only one solution on any one day. Animals were killed by an overdose of intraperitoneal pentobarbital, and the location of the distal end of the intrathecal catheter verified by postmortem examination.

Experiment 2. To compare the visceral analgesic effect of bupivacaine, levobupivacaine, and dextrobupivacaine, seven rats received the 0.25% solutions in a double-blind, crossover fashion. The anesthetics were separated by at least 24 h in each animal. Awake rats were subjected to CRD at 70 mmHg lasting 20 s before, and 5, 10, 15, 20, and 30 min after the intrathecal injection of 15 µl of each anesthetic solution followed by 10 µl of saline to flush the catheter. Blood pressure and heart rate were measured while CRD was applied.

Experiment 3. After the measurement of baseline TF and PP latency, 90 rats were divided into four groups to...
intrathecally receive a 1-h infusion of saline (group C, n = 19) or 2.5% bupivacaine (group B, n = 23), levobupivacaine (group S, n = 25), or dextrobupivacaine (group R, n = 23) in saline at a rate of 2 μl/min. In addition, 30 rats intrathecally received 1.25% bupivacaine (n = 14) or levobupivacaine (n = 16). The osmolarities of 2.5% bupivacaine, levobupivacaine, and dextrobupivacaine and 1.25% bupivacaine and levobupivacaine solutions were 422, 429, 430, 356, and 361 mOsm/l, respectively, and the pH values were 4.92, 4.91, 4.95, 5.10, and 5.17, respectively. All infusions were administered by a mechanical infusion pump (model 975; Harvard Apparatus, South Natick, MA) into rats which were placed in a horizontal acrylic restraint. A segment of calibrated polyethylene tubing was inserted between the syringe and the intrathecal catheter, and the injection was monitored by observing the movement of a small air bubble within the tubing. The TF, PP, and MF tests were performed 5 min after infusion, and animals that did not show the cutoff value of the TF latency were excluded from the study. All of the tests were repeated 4 days later. Then the rats were killed by intraperitoneal pentobarbital and perfused intracardially with a phosphate-buffered 2.0% paraformaldehyde-2.5% glutaraldehyde fixative. Methyl green solution was injected to confirm the location of the catheter after the perfusion. The spinal cord and nerve roots were dissected out and immersed in the same fixative for 5 h. Two specimens (10 mm rostral and caudal to the conus medullaris) from each rat were postfixed with cacodylate-buffered 1% osmium tetroxide, dehydrated in a series of graded alcohol solutions, and embedded in epoxy resin. From the embedded tissue, 1-μm transverse sections were obtained using the microtome (MT6000; RMC, Tucson, AZ) and stained with toluidine blue dyes. Histopathologic evaluation was performed using light microscopy by a pathologist blinded to the intrathecal solution received and to the results of behavioral measurements. Sections obtained from 10 mm rostral to the conus (caudal spinal cord) were used for qualitative evaluation. Quantitative analysis of nerve injury was performed using the sections obtained from 10 mm caudal to the conus (cauda equina). Each fascicle present in the cross section was assigned an injury score of 0-3 (where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe) as described previously.17 The injury score for each rat was then calculated as the average score of all fascicles in the cross section. In addition, ultrathin sections were obtained using the same microtome described above and double-stained with uranyl acetate and lead citrate for electron microscopy (EM-002B; Topcon, Tokyo, Japan).

Statistical Analysis

Data are presented as mean ± SEM unless otherwise stated. In experiment 1, TF latencies and CRD thresholds were converted to the percent maximal possible effect (%MPE), calculated as (postdrug value − baseline value) / (cutoff value − baseline value) × 100% and were analyzed by analysis of variance (ANOVA) with repeated measures followed by the Scheffé and Dunnett tests. MF scores are presented as median (10th–90th percentiles) and were compared using the Kruskal-Wallis test followed by the Dunn test. Time to recovery from motor paralysis was compared with one-way ANOVA followed by the Scheffé test. To determine the relative concentration of local anesthetic solutions used for experiment 3, the area under the time–effect curve was calculated by accumulating the effect (%MPE in TF test) measured at the discrete time intervals using the trapezoidal integration method. The dose–effect relation for anesthesia was determined by using the area under the time–effect curve values, and the potency ratio was calculated and tested for significance with a computer-based program (GraphPad Prism; GraphPad Software, Inc., San Diego, CA). In experiment 2, baseline changes in mean blood pressure and heart rate in response to CRD before injection was assessed for significance using a paired t test and compared using ANOVA with repeated measures. Changes in those after drug injection were converted to percent change, calculated as (post drug change/base-line change) × 100% and analyzed by ANOVA with repeated measures followed by the Holm test. In experiment 3, the results of the TF and PP tests were converted to %MPE and analyzed using one-way ANOVA followed by the Scheffé test or an unpaired t test, as appropriate. Injury scores were compared using the Kruskal-Wallis test followed by the Dunn test or the Mann-Whitney U test, as appropriate. Sample size for experiment 3 was determined by a power analysis based on the variability observed in our previous study (SD 28%) and an expected difference of 33% in the TF latency (%MPE) with β set at 0.2 and α set at 0.05. A minimal sample of 68 and 26 rats (17 and 13 in each group) met these criteria for the first and second parts of the experiment comparing four and two groups, respectively. P < 0.05 was considered significant.

Results

Experiment 1

A total of 53 rats received test solutions. Baseline TF latencies and CRD thresholds did not differ among groups. Every intrathecal anesthetic solution produced a significant increase in TF latency and CRD threshold, whereas saline did not show any change in either test (figs. 1 and 2 only showing the results of 0.25% solutions). At all drug concentrations, the prolongation of TF latency occurred similarly for the three anesthetics. The dose–effect (%MPE in TF test) curves of the three anesthetics were almost identical (fig. 3). In contrast, levobupivacaine solutions produced a slightly smaller increase in CRD threshold than the others; significance
was found between 0.125% bupivacaine and levobupivacaine at 5 min after the injection and between 0.25% and 0.5% levobupivacaine and dextrobupivacaine at 15 and 10 min after the injection, respectively. The peak effect on motor function was observed 5 min after the injection of any anesthetic solutions and did not differ among the three local anesthetics of any concentration except for 1% (table 1). Rats given 1% levobupivacaine developed less motor paralysis than those given 1% bupivacaine. The duration of motor paralysis was similar for bupivacaine and dextrobupivacaine, whereas rats given 0.125% levobupivacaine recovered from paralysis significantly earlier than those given 0.125% bupivacaine.

Experiment 2
Colorectal distension significantly increased heart rate but not blood pressure before injection. Baseline changes in heart rate in response to CRD did not differ among groups. Five minutes after injection, the three anesthetics similarly depressed the response, which gradually returned to the baseline value by 20 min.

Percent change 10 min after 0.25% levobupivacaine was significantly larger than that after 0.25% bupivacaine and dextrobupivacaine (fig. 4).

Experiment 3
Sixteen rats were excluded from the study comparing 2.5% anesthetic solutions: 5, 4, and 3 rats in groups B, S, and R, respectively, did not develop anesthesia during infusion; 1 animal each in groups B and R died after infusion; postmortem examination revealed that the catheter was located in the epidural space in 2 rats in group C. Therefore, the remaining 74 rats were included in the data analysis: group C, n = 17; group B, n = 17; group S, n = 21; group R, n = 19.

Baseline TF latencies and PP thresholds did not differ among groups. TF latency and PP threshold did not change in rats in group C throughout the experiment. When assessed 4 days after infusion, TF latencies (%MPE) of rats in groups B, S, and R increased significantly from the baseline, and all differed significantly from latencies of rats in group C (fig. 5). No groups of rats developed an increase in PP threshold or MF scores.

Sections obtained from the cauda equina of rats in group S contained moderate to severe injury in the fascicles (fig. 6). Histologic changes in nerve roots were characterized by edema and axonal degeneration including appearance of swelling, atrophy, and loss of axons with macrophage infiltration. Similar findings were observed in specimens from rats in groups B and R. The nerve injury scores for three groups of anesthetic-treated rats were similar, and all differed significantly from a group of rats given saline (fig. 7).

Sections obtained from the spinal cord of animals in groups S were almost intact but with minimal axonal degeneration in the white matter (fig. 8). Findings observed in specimens from rats in groups B and R were similar.

Typical electron microscopic findings are shown in figure 9. In the specimen from a rat given saline, axons and myelin lamellae of myelinated fibers were almost
Table 1. Motor Function after Drug Injection

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Values are presented as median (10th, 90th percentiles). The grading of the motor block was as follows: 0 = none, 1 = partially blocked, and 2 = completely blocked. The normal baseline score was 0, and the score with bilateral complete block was 2 + 2 = 4.

* P < 0.05 vs. 1% bupivacaine.

Discussion

Although in vitro studies have found that dextrobupivacaine is more potent in inhibiting and slower in dissociating from the normal sodium channel than levobupivacaine, the stereoselectivity has been strikingly less apparent in previous in vivo studies. For example, Vladimirov et al. performed a neurobehavioral study to compare R(+), S(−), and racemic bupivacaine using rat sciatic nerves only to find a modest stereopotency for peak analgesia achieved at the lowest doses examined. Kanai et al. injected bupivacaine and levobupivacaine epidurally and intrathecally in rats and found that the two drugs were similar for somatic antinociceptive effects.

In the current study, the three drugs produced similar prolongation of TF latency in experiment 1. Because TF latency only reflects the function of the tail, it is possible that a difference in somatic antinociceptive effects ex-
histed in other parts of the body. However, because the time course effects of the three drugs on TF latency were almost identical at every concentration tested, it is unlikely that the difference would have been clinically relevant.

The results of MF test have confirmed previous findings on a difference in motor blocking effect between bupivacaine and levobupivacaine. Kanai et al. found that duration of motor block was shorter with levobupivacaine than with bupivacaine at lower concentrations tested for epidural and intrathecal anesthesia using rats. In contrast, the current study observed a significant difference in motor blocking effect between the two anesthetics at 1%, the highest concentration tested. However, the difference in the concentrations at which statistical significance was found between the two studies can be probably explained by the nature of techniques used, both of which were crude and are different from each other, and a type II error.

To our knowledge, this is the first study to compare the effects of intrathecal bupivacaine and its enantiomers on visceral antinociception as well as neurotoxicity. The CRD test was used to assess visceral antinociceptive effects in the present study. In experiment 1, the effects of the three drugs on CRD threshold were almost similar, but there were some time points when the CRD threshold of levobupivacaine was slightly lower than that of the others. Because CRD threshold is measured with abdominal musculature contractions, the influence of motor block on the results should be considered. In fact, the effect on motor function of hind limbs was less with levobupivacaine; 1% levobupivacaine produced less motor paralysis than 1% bupivacaine. In addition, it is possible that the three anesthetic solutions differed in anesthetic spread, so that abdominal musculature might have been affected differently. However, it is unlikely that difference in motor palsy of the abdominal muscles was responsible for the difference in CRD threshold for the following reasons. First, there was a considerable difference in time to show peak effects between CRD and motor function tests. Second, because the intrathecal catheter was inserted through L3–L4 with the tip directed caudal, the rats receiving the drugs seldom developed apparent motor blockade in the abdomen. In fact, lower CRD threshold with levobupivacaine was observed even at the lowest concentration of the solutions tested, with which no rat given any drugs developed complete motor block even in the hind limb. Therefore, the visceral antinociceptive effect of levobupivacaine seems to be a little weaker than that of the others.

Nevertheless, we conducted experiment 2, in which responses in blood pressure and heart rate to CRD was evaluated in rats given 0.25% solutions. Mechanical stimulation of the gut has been demonstrated to produce cardiovascular responses as well as visceromotor responses. The selection of the concentration was based on the results of experiment 1 that showed a moderate effect of 0.25% solutions on CRD threshold. Before injection, heart rate significantly increased after CRD. In contrast, as opposed to the results of a previous study by Ness and Gebhart, no apparent pressor response was observed in the current study. Therefore, only heart rate was analyzed to compare the effects of the three drugs. As expected, the response in heart rate was significantly decreased immediately after injection but then gradually returned to the baseline values in all

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Fig. 6. Transverse sections obtained from 10 mm caudal to the conus 4 days after a 1-h intrathecal infusion of saline (A) and 2.5% levobupivacaine (B). Arrows indicate damaged fascicles in cauda equina. Histologic changes in cauda equina were characterized by edema and axonal degeneration, including appearance of myelin ovoid, and swelling, atrophy, and loss of axons with macrophage infiltration.

Fig. 7. Nerve injury score for sections obtained 4 days after a 1-h intrathecal infusion of 2.5% levobupivacaine (n = 21), 2.5% bupivacaine (n = 17), 2.5% dextrobupivacaine (n = 19), or normal saline (n = 17). Nerve injury was evaluated for sections obtained 10 mm caudal to the conus 4 days after infusion. Each fascicle present in the cross section was assigned an injury score of 0–3 (where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe). The injury score for each rat was then calculated as the average score of all fascicles in the cross section. Data are presented as mean ± SEM. *P < 0.05 compared with saline.
of the animals tested. However, in the course of study, we found that change in heart rate after CRD was less decreased in rats given levobupivacaine than in the others. The results also suggest that levobupivacaine is weaker than bupivacaine and dextrobupivacaine in visceral antinociception.

The stereoselectivity has also been examined in clinical studies. Most of the researchers have observed similar effects of epidural bupivacaine and levobupivacaine on sensory and motor functions. However, Gautier et al. recently intrathecally administered low doses of anesthetics with sufentanil to detect differences. In the study, the success rate of intrathecal levobupivacaine was lower than that of bupivacaine, and the duration of analgesia and motor block with the former solution was shorter than with the other. In addition, because the concentration of commercially available bupivacaine and levobupivacaine solutions for clinical use is calculated differently (i.e., the concentration of levobupivacaine is expressed as mg/ml of base, whereas that of bupivacaine as mg/ml of hydrochloride), the levobupivacaine solutions used in those studies must have contained more active local anesthetic than bupivacaine. Therefore, the stereoselectivity might be more apparent on the molar base.

In the third experiment, rats received a 1-h infusion. In our previous study, we found that TF latencies and nerve injury scores of rats intrathecally given 20 μl bupivacaine, 2.13%, did not differ from those of rats given saline. Therefore, although a bolus injection was used in the first two experiments where anesthetic effects were compared, a different technique was needed to increase the occurrence of neurotoxic injury to compare the anesthetics that were expected to be similarly less neurotoxic. The infusion technique has the advantage of producing consistent and restricted anesthetic distribution, which has been considered one of the causative factors to produce clinical injury. As a result, rats incurred injuries that were similar to those observed with lidocaine in previous studies. The sensory and motor impairment was mostly limited to the tail, and histologic changes occurred selectively in the sacral nerve roots.

In the first part of experiment 3, the three drugs were administered at the same concentration because their dose–effect curves obtained in experiment 1 were almost identical. According to the results comparing the three anesthetics of 2.5%, the three induced similar functional impairment and histologic damage, which were different from those observed in saline-treated animals. However, the degree of injury obtained in the experiment may have raised concern regarding the concentration of the anesthetics used and suggested that there might have been a ceiling effect and differences in effects might be present at lower concentrations, especially between bupivacaine and levobupivacaine (fig. 5). Therefore, we conducted the additional experiment comparing the two in 1.25% solutions and confirmed similar neurotoxic effects. Because our previous study showed that bupivacaine is less neurotoxic than lidocaine, levobupivacaine and dextrobupivacaine seem to be similarly less neurotoxic than lidocaine.

In conclusion, the results suggest that, when administered intrathecally in rats, bupivacaine and its
Fig. 10. Sensory function (A) and nerve injury score (B) for sections obtained 4 days after a 1-h intrathecal infusion of 1.25% bupivacaine (n = 14) and levobupivacaine (n = 15). Tail-flick (TF) latencies were converted to the percent maximal possible effect (%MPE) and calculated as (postdrug value – baseline value)/(cutoff value – baseline value) × 100%. Nerve injury was evaluated for sections obtained 10 mm caudal to the conus 4 days after infusion. Each fascicle present in the cross section was assigned an injury score of 0–3 (where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe). The injury score for each rat was then calculated as the average score of all fascicles in the cross section. Data are presented as mean ± SEM. There were no differences between groups.

S(−)-enantiomers are similar for somatic antinociception and neurotoxicity. In terms of visceral antinociception and motor paralysis, levobupivacaine is slightly less potent than the others, but the difference may be of little clinical importance.

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References


21. Facenda KA, Simpson AM, Henderson DJ, Smith D, McGrady EM, Morris LMM: A comparison of levobupivacaine 0.5% and racemic bupivacaine 0.5% for extradural anesthesia for caesarean section. Reg Anesth Pain Med 2003; 28:394–400

