

Anesthesiology
2000; 93:744-55
© 2000 American Society of Anesthesiologists, Inc.
Lippincott Williams & Wilkins, Inc.

Potency of Bupivacaine Stereoisomers Tested In Vitro and In Vivo

Biochemical, Electrophysiological, and Neurobehavioral Studies

Marina Vladimirov, M.D., Ph.D.,* Carla Nau, M.D.,† Wai Man Mok, Ph.D.,* Gary Strichartz, Ph.D.‡

Background: Chiral local anesthetics, such as ropivacaine and levobupivacaine, have the potential advantage over racemic mixtures in showing reduced toxic side effects. However, these *S*- (levo, or “-”) isomers also have reportedly lower potency than their optical antipode, possibly resulting in no advantage in therapeutic index. Potency for local anesthetics inhibiting Na⁺ channels or action potentials depends on the pattern of membrane potential and so also does the stereopotency ratio. Here the authors have quantitated the stereopotencies of *R*-, *S*-, and racemic bupivacaine, comparing several *in vitro* assays of neuronal Na⁺ channels with those from *in vivo* functional nerve block, to establish relative potencies and to understand better the role of different modes of channel inhibition in overall functional anesthesia.

Methods: The binding of bupivacaine to Na⁺ channels was assessed indirectly by its antagonism of [³H]-batrachotoxin binding to rat brain synaptosomes. Inhibition of Na⁺ currents by bupivacaine was directly assayed in voltage-clamped GH-3 neuroendocrine cells. Neurobehavioral functions were disrupted by bupivacaine percutaneously injected (0.1 ml; 0.0625–1.0%) at the rat sciatic nerve and semiquantitatively assayed. Concentration-dependent actions of *R*-, *S*-, and racemic bupiv-

acaine were compared for their magnitude and duration of action.

Results: Competitive batrachotoxin displacement has a stereopotency ratio of *R*:*S* = 3:1. Inhibition of Na⁺ currents with different prepulse potentials shows that *S* > *R* potency when the membrane is hyperpolarized, and *R* > *S* potency when it is depolarized from normal resting values. Functional deficits assayed *in vivo* usually demonstrate no consistent enantioselectivity and only a modest stereopotency (*R*:*S* = 1.2–1.3) for peak analgesia achieved at the lowest doses. Other functions displayed no significant stereopotency in either the degree, the duration, or their product (area under the curve) at any dose.

Conclusion: Although the *in vitro* actions of bupivacaine showed stereoselectivity ratios of 1.3–3:1 (*R*:*S*), *in vivo* nerve block at clinically used concentrations showed much smaller ratios for peak effect and no significant enantioselectivity for duration. A primary role for the blockade of resting rather than open or inactivated Na⁺ channels may explain the modest stereoselectivity *in vivo*, although stereoselective factors controlling local disposition cannot be ruled out. Levo- (*S*-) bupivacaine is effectively equipotent to *R*- or racemic bupivacaine *in vivo* for rat sciatic nerve block. (Key words: Action potential; local anesthetic; sodium channel; state-dependence; stereopotency.)

* Instructor in Anesthesia, Harvard School of Public Health.

† Research Fellow of the Deutsches Forschungsgemeinschaft. Current position: Klinik für Anästhesiologie der Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany.

‡ Professor of Anaesthesia (Pharmacology).

Received from the Pain Research Center, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts. Submitted for publication September 1, 1999. Accepted for publication January 3, 2000. Supported by a grant from Chiroscience, Ltd., Cambridge, United Kingdom; and by grant No. GM35647 from the National Institutes of Health, Bethesda, Maryland (to Dr. Strichartz). Presented in part at the annual meeting of the American Society of Regional Anesthesia, Seattle, Washington, May 15, 1998.

Address reprint requests to Dr. Strichartz: Anesthesia Research Laboratories, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115. Address electronic mail to: gstrichz@zeus.bwh.harvard.edu

Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org

THE development of chiral local anesthetics, such as ropivacaine and levobupivacaine, was a response to reports of cardiovascular collapse after accidental intravenous injections of racemic bupivacaine.¹ Both of these chiral compounds, *S*-stereoisomers of closely related piperidine local anesthetics, are reported to have reduced cardiotoxicity compared with the racemic mixture of the *R*-isomer.^{2,3} However, these *S*-enantiomers are also reported to be less potent than their *R*- counterparts, and, if equal clinical actions require higher doses, the apparent advantage of reduced toxicity observed at a lower dose might be offset with no gain in therapeutic index.

The primary action of local anesthetics on the peripheral nervous system is a blockade of nerve impulses resulting from direct binding of these drugs to neuronal sodium channels. This action has some, albeit weak, stereoselectivity. For the stereoisomers of bupivacaine,

the dextro-(*R*-)enantiomer is slightly more potent than the levo-(*S*-)enantiomer for tonic block and increasingly more potent for the phasic block of nerve impulses *in vitro*.⁴ This observation implies that functional local anesthetic activity of dextro- and levobupivacaine *in vivo* might also be different.

Peripheral nerve fibers conduct trains of impulses at different frequencies to accomplish the functional transmission of information. Trains of efferent impulses in motor fibers lead to smoothly developing, sustainable muscle contractions and trains of afferent impulses in both myelinated and nonmyelinated fibers conduct information about intensity, rate of change, and duration of sensory input to the central nervous system. High-frequency impulses are both more sensitive to local anesthetic (LA) blockade and, important here, display greater stereoselectivity. If such phasic block accounts for functional losses during anesthesia *in vivo*, then a relatively larger stereopotency will be present, but if tonic block with little use-dependent contribution occurs *in vivo*, then the functional stereopotency will be small.

In this study we have extended earlier observations by using additional *in vitro* tests and also have conducted *in vivo* nerve block studies. The original assay of impulse blockade was complemented by experiments testing the competitive displacement of radiolabeled batrachotoxin (³H-BTX) from the Na⁺ channels in rat brain synaptosomes. Direct actions on Na⁺ channels were measured by inhibition of voltage-clamped Na⁺ current in clonal neuroendocrine (GH-3) cells.

In these electrophysiological experiments, several mechanistic questions are addressed, including whether there is stereoselectivity for the "tonic" inhibition of infrequently stimulated channels, as in nerves at rest, how such stereoselectivity depends on the resting ("holding") membrane potential, and if the use-dependent inhibition that results during frequent depolarization of the membrane, as would occur during a train of action potentials, demonstrates stereoselectivity. Variations of use-dependent test protocols also are applied to reveal the kinetic basis for increased drug affinity of inactivated channel states and, importantly, a mechanism for the previously reported stereoselectivity of cardiac actions. Altogether, the diverse assays provide a comprehensive approach to assess the Na⁺ channel-related pharmacodynamics of bupivacaine enantiomers and of the racemic mixture.

To compare their functional effectiveness, we examined the actions of bupivacaine stereoisomers and their

racemic mixture on neurobehavioral assays in rats. This involves semiquantitative assessment of changes in neurologic responses that are reversible and dose-dependent.⁵ By comparing the actions of drugs on isolated tissues and cells *in vitro* with the neurobehavioral results *in vivo*, one is able to separate local disposition from neuronal pharmacodynamic influences for the percutaneous block of sciatic nerve used here.

Methods

In Vitro Assays

Batrachotoxin Binding Experiments. The ability of bupivacaine enantiomers to displace ³H-BTX from Na channels was assayed with a technique adapted from Creveling *et al.*⁶ as described in McHugh *et al.*⁷ Incubations were conducted in a total volume of 250 μ l containing synaptosomes at a final concentration of 0.8 μ g total protein/ μ l, 50 nM ³H-BTX-B (50 Ci/mmol), 1 μ M tetrodotoxin, 0.03 mg *Leiurus quinquestriatus* venom dissolved in synaptosome storage buffer, composed of 130 mM choline \cdot Cl, 5.5 mM glucose, 5.4 mM KCl, 0.8 mM MgSO₄, 50 mM HEPES, pH adjusted to 7.4 with Tris base (\approx 22 mM). Incubations for 30 min at 37°C were terminated by dilution of the reaction mixture with 3 ml of wash buffer (163 mM choline Cl, 5 mM HEPES, 1.8 mM CaCl₂, and 0.8 mM MgSO₄, pH adjusted to 7.4 with Tris base) and filtered through Whatman GF/C filters (Whatman International Ltd., Maidstone, England). Filtration was accomplished by vacuum perfusion through a 10 sample filtration device (Unipore Model 25; BioRad Laboratories, Richmond, CA). Each filter was washed three times with wash buffer at 0°C (1 ml/wash, approximately several seconds per wash). Filters and filtrate were counted in 15 ml of Aquasol-2 (DuPont-NEN, Boston, MA) on a Beckman scintillation counter (model LS6800; Beckman, Inc., Palo Alto, CA). The counting efficiency was approximately 35%. Nonspecific binding was determined by parallel experiments in the presence of 300 μ M veratridine. Specific binding was approximately 75% of total binding at 50 nM ³H-BTX-B ($K_d \approx$ 82 nM in 1 μ M *Leiurus quinquestriatus* toxin; K_d of *Leiurus quinquestriatus* for enhancement of ³H-BTX-B binding = 35 nM⁸).

Scorpion venom was prepared as described by Wang and Strichartz.⁹ Briefly, 5 mg/ml dry weight of lyophilized venom was incubated overnight in synaptosome storage buffer (see above) at 4°C and spun at 20,000g for 15 min to pellet the undissolved mucopolysaccharide.

The pellet was discarded and the supernatant was then used as a 5-mg nominal protein/ml solution of scorpion venom.

Preparation of Synaptosomes. Synaptosomes were prepared using a method adapted from Dodd *et al.*¹⁰ A sheep (white-faced Dorset-Ewes, 40–60 kg) was killed using a barbiturate overdose according to a protocol approved by the Harvard University Standing Committee on Animals for Dr. Hal Feldman. The cerebral cortex was harvested (5 g wet weight) and placed in 200 ml of 0.32 M sucrose at 0°C. Three- to four-gram sections were placed in 30 ml of 0.32 M sucrose at 0°C and were homogenized in a Teflon/glass homogenizer (Fisher Scientific, Woburn, MA; clearance \approx 0.15–0.23 mm, 12 up-and-down strokes at 800 rpm) while the mortar of the homogenizer was kept in an ice-water bath. The crude homogenates were pooled and distributed as 20-ml aliquots into polycarbonate centrifuge bottles. These were centrifuged at 5,100 rpm (1,500g) on a 70.1 Ti rotor for 12 min at 4°C in a Beckman L-80 preparative ultracentrifuge (Palo Alto, CA). The pellet (P1) was discarded. The supernatant (nominally in 0.32 M sucrose) was removed, layered onto 8 ml of 1.2 M sucrose at 0°C, and spun at 50,000 rpm (170,000g) for 17 min on a 70.1 Ti rotor at 4°C. Four milliliters of supernatant was removed from the gradient interface and mixed with 10 ml of 0.32 M sucrose at 0°C. The pellet (P2) and other material was discarded. The diluted interface was layered onto 8 ml of 0.8 M sucrose and spun at 50,000 rpm for 17 min on a 70.1 Ti rotor. The supernatant was discarded and the pellets (P3) were resuspended in synaptosomal storage buffer. The synaptosomes were snap frozen in 250- μ l aliquots on ethanol-dry ice and stored at -80°C . Storage of synaptosomes in a similar fashion has been shown to have little effect on toxin binding or Na^+ flux.¹¹ Total protein concentration was measured to be 3.41 mg/ml after solubilizing membranes in 1.2% (wt/vol) sodium dodecyl sulfate by Peterson's modification of Lowry's method.¹²

Whole Cell Voltage Clamp of Na^+ Currents. The GH-3 cell line was purchased from American Type Culture Collection (Rockville, MD) and maintained as described by Dubinsky and Oxford.¹³ Cells were kept in a humidified incubator at 37°C under 5% CO_2 and 95% air. Cells were replated to a lower density every 7 days in 35-mm culture dishes. The culture medium (89% Dulbecco's Eagle Medium, Gibco BRL #11965, Life Technologies, Gaithersburg, MD; 10% fetal calf serum, HyClone, #SH-30070, Logan, UT; 1% penicillin-streptomycin,

Gibco BRL, #15140) was replaced every 3–4 days. Cells were used for experiments at days 1–5 after replating.

Na^+ currents in GH₃ cells were recorded at room temperature ($23 \pm 2^{\circ}\text{C}$) using the whole cell configuration of the patch clamp method.¹⁴ Patch pipettes were pulled from borosilicate glass tubes (TW150F-3, World Precision Instruments, Sarasota, FL) and heat polished at the tip before use to give a resistance of $1 \pm 0.5 \text{ M}\Omega$.

Currents were recorded using an Axopatch 200A patch clamp amplifier (Axon Instruments, Foster City, CA), filtered with a 4-pole low-pass Bessel filter at 2–5 kHz, and digitized at 20 kHz (TL-1, DMA Interface, Axon Instruments). All experiments were conducted under voltage clamp with series resistance compensation, leakage current was subtracted by the P/4 method⁷ with exception of the experiments for phasic inhibition. pCLAMP 6.0 software (Axon Instruments) was used for acquisition and analysis of currents. Microcal Origin software (Microcal Software, Northampton, MA) was used for creating figures and applying fitting procedures.

Experiments were performed with external solution containing 130 mM NaCl, 0.1 mM CaCl_2 , 5 mM KCl, 5 mM MgCl_2 , 10 mM glucose, 10 mM HEPES (adjusted to pH 7.4 with NaOH), and internal solution containing 110 mM CsF, 10 mM NaCl, 5 mM MgCl_2 , 10 mM EGTA, 10 mM HEPES (adjusted to pH 7.2 with CsOH). Control solutions as well as test solutions were applied to cells with a multiple-barrel perfusion system. *R*(+)- and *S*(-)-bupivacaine were obtained as crystalline HCl salts from Chiroscience, Ltd. (Cambridge, United Kingdom) and dissolved in water to give 50-mM stock solutions.

In Vivo Assay

Neurobehavioral Experiments. Neurobehavioral experiments were performed on male Sprague-Dawley rats, weighing 250–300 g (age, 10–12 weeks). All procedures were approved by the Harvard Committee on animals and are in accordance with published guidelines for animal experimentation of the International Association for the Study of Pain. Rats were delivered at an age of 40 days and were handled daily over the next 10–14 days. The goal of handling is to familiarize the animal with the experimenter, the environments in which the studies are conducted, the manipulations involved in the neurologic evaluation, and the injection procedure.⁵ This familiarization minimizes the contamination from stress response during the experiment and improves experimental performance. Criteria for sufficient handling were an absence of signs of behavioral stress (absence of locomotor freezing and decreased number of

fecal boli in the open field) and somatic stress (a regular gain in weight) and a lack of response to the tactile (non-noxious) stimulation of the area to be tested, followed by a robust, distinctive response to noxious stimulation. Animals that did not meet all criteria were not used in this study.

General behavioral reactions, indicating the mental status of the animals, were monitored throughout the observation period under free behavior conditions, observed in a 1 × 1-m uncovered box. Mental status was considered normal when the animal was responsive to its environment and exhibited exploratory activity. These behavioral aspects are known to be altered by systemic (toxic) local anesthetics.^{5,15} All solutions were prepared on the day of the experiment, not more than 30 min before injection.

Regional Block. An experiment consisted of monitoring the specific neural functions, mediated by the sciatic nerve of either hind limb: proprioception, motor function, and nociception before and after injection of drug close to the sciatic nerve of one limb.⁵ The noninjected limb served as a control for evaluation of neurologic changes. Time intervals for testing were 1, 5, 15 min, and every 15 min until 2 h after the injection, and thereafter every 30 min until functions were fully recovered.

A total of 142 experiments were performed on 71 rats. The following drugs were injected, all in 0.1-ml volumes:

- 0.0625% racemic bupivacaine (n = 8); *R*-bupivacaine (n = 8); *S*-bupivacaine (n = 8);
- 0.125% racemic bupivacaine (n = 7); *R*-bupivacaine (n = 6); *S*-bupivacaine (n = 5);
- 0.25% racemic bupivacaine (n = 12); *R*-bupivacaine (n = 12); *S*-bupivacaine (n = 12);
- 0.5% racemic bupivacaine (n = 10); *R*-bupivacaine (n = 8); *S*-bupivacaine (n = 7);
- 1% racemic bupivacaine (n = 12); *R*-bupivacaine (n = 12); *S*-bupivacaine (n = 12).

Each hind limb was injected once only; each rat received two injections, spaced no closer than 3 days. Separate control experiments showed that the first injection did not change the response to the second one on the contralateral leg.

Administration of Drugs. Injections were performed without any sedation of the rats. The rat was placed in lateral recumbency; the greater trochanter and ischial tuberosity were localized by palpation; the injection needle was advanced until encountering the ischium. Then 0.1 ml of the drug was injected within 5 s.

Assessment of Sciatic Nerve Functions. The order of testing was always as follows: proprioception, motor function, nocifension.⁵ The evaluation of proprioception was based on quantification of the "hopping" and "tactile placing" reactions. *Hopping* tests the ability to do passive lateral movement; *tactile placing* is testing of the ability to reposition the knuckled toes. The functional status was graded as follows: 3 (normal), 2 (slightly impaired), 1 (severely impaired), or 0 (absent).

Motor function was evaluated by the extensor postural thrust (measured in grams with a platform balance) as the force that resists contact of the platform by the heel. Normal motor function was established by measuring the applied force necessary to bring the heel to the platform before injection. The reduction in extensor thrust was considered block of motor function.

Nociception was evaluated by the nocifensive reaction to thermal and mechanical stimulation, *i.e.*, to heat and pinch. The latency of the withdrawal response to application of a hot (51.0 ± 0.5°C) metal probe (3-mm diameter) to the dorsal surface of the lateral and medial margin of the metatarsus was monitored. Prolongation of latency to 10 s (cutoff time) was taken as complete nociceptive block. The nocifensive response to mechanical noxious stimulation (pinch of little toe) was scored as follows: 3 (normal), 2 (slightly impaired), 1 (severely impaired), or 0 (absent).

Changes of each function were evaluated as percent of maximal possible effect (%MPE) in each experiment at specified time after injection. %MPE was used for general comparison of functions measured with different numeric scales.

The definition of %MPE is given by

$$\%MPE = [(control\ response - drug\ affected\ response)/(control\ response)] \times 100$$

When the drug abolishes the response, %MPE = 100; when the affected response of the drug is identical to the control response, %MPE = 0. %MPE was calculated for every functional change considering 100% of MPE as a complete block of function, and by comparing the magnitude of evoked response with the magnitude of the predrug control measurement on the same leg. The duration of the complete block of each function was also measured.

Treatment of Behavioral Data. Comparisons of the drugs were made by (1) comparing the dose-response for the magnitude (%MPE) of different functional defi-

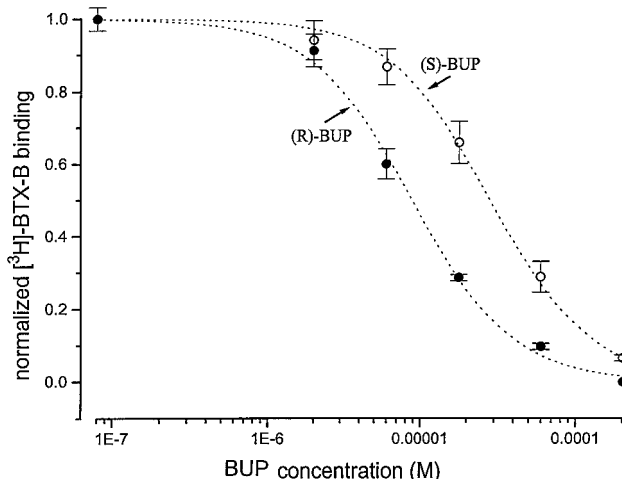


Fig. 1. Concentration-dependent inhibition of saturable batrachotoxin (^3H -BTX-B) binding by *R*- and *S*-bupivacaine. The dashed lines are fits to the data of the modified Hill equation: $Y/Y_{\text{max}} = ([\text{bupivacaine}]^n)/([\text{bupivacaine}]^n + \text{IC}_{50}^n)$. Data points show the mean of three experiments \pm SD.

cits induced by racemic *versus R*- *versus S*-bupivacaine; (2) comparing the fraction of animals that had complete functional block at a specified time after injection of racemic *versus R*- *versus S*-bupivacaine; (3) comparing the duration of functional changes after injection of racemic *versus R*- *versus S*-bupivacaine; and (4) comparing area under the curve for motor and nociceptive blockade.

Statistical Comparisons

Effects of *R*- and *S*-bupivacaine on batrachotoxin binding and Na^+ currents were compared by paired Student *t* test. *In vivo* actions producing functional deficits were compared for *R*-, *S*-, and racemic bupivacaine by analysis of variance with Bonferonni correction for graded actions and by the Fisher exact test for the proportion of the population that was fully blocked. *P* values are listed in most cases, and $P < 0.05$ was taken as the criterion for significant differences.

Results

Batrachotoxin Binding

Both *R*- and *S*-bupivacaine displaced ^3H -BTX from Na^+ channels of rat brain synaptosomes (fig. 1). The concentration dependence of this antagonism was well fit by a Hill equation (see fig. 1 legend) with IC_{50} values of $8.9 \pm 0.5 \times 10^{-6}$ M and $2.9 \pm 0.1 \times 10^{-5}$ M and Hill coefficients (n_H) of 1.30 ± 0.10 and 1.20 ± 0.06 for *R*- and *S*-bupivacaine, respectively. These data show that bind-

ing of only one bupivacaine molecule to the Na^+ channel can displace a molecule of batrachotoxin,^{16,17} and that the stereopotency ratio (*R*:*S*) equals 3:1.

Inhibition of Na^+ Currents

Sodium currents under voltage clamp were inhibited by bupivacaine enantiomers in tonic (fig. 2) and use-dependent (phasic, fig. 3) modes. Both prolonged, single depolarizations and brief, repetitive depolarizations increased the inhibition of bupivacaine, showing that this drug binds more to open (activated) and inactivated states than to resting, closed states of the Na^+ channel.¹⁸⁻²¹

The degree of block was altered by the membrane potential in ways that differ between the two enantiomers. In assays of tonic inhibition, when the membrane was held for 5 s at -80 mV, the *R*-enantiomer was slightly but significantly more potent than the *S*-enantiomer, with IC_{50} values of 101.7 ± 7.5 μM and 132.1 ± 8.8 μM , respectively, giving a stereopotency ratio (*R*:*S*) of 1.3 ($P = 0.02$). Depolarization of the membrane's holding potential increased the affinity for both enantiomers; at -70 mV, IC_{50} values equaled 34.8 ± 2.4 μM and 37.6 ± 2.6 μM , and at -60 mV, IC_{50} values equaled 18.6 ± 0.8 μM and 21.6 ± 1.2 μM for *R*- and *S*-bupivacaine, respectively. Although *R*-bupivacaine was the slightly more potent enantiomer at these potentials, the stereopotency ratios were small (1.08 and 1.16, respectively) and not statistically different from 1.0.

Hyperpolarization of the membrane's holding potential, however, decreased the affinity for both enantiomers; at -100 mV, the IC_{50} for *R*-bupivacaine increased to 317.4 ± 10.5 μM and that for *S*-bupivacaine to 264.0 ± 19.4 μM . At this potential, the *R*-enantiomer became significantly less potent than the *S*-enantiomer (*R*:*S* = 0.83; $P = 0.036$), as previously reported.²²

The kinetic basis for bupivacaine's stereoselective block of Na^+ channels can be gleaned from an analysis of use-dependent block (fig. 3). Repetitive depolarizations prompted further channel blockade, as reflected by decreasing Na^+ current amplitude during a train of short pulses applied at 5 Hz (fig. 3, current traces). At the same bupivacaine concentration, and stimulation frequencies of 1 and 5 Hz, *R*-bupivacaine produced a slightly slower developing block that reached a greater steady state than *S*-bupivacaine at 5 Hz (fig. 3B). For example, at 5 Hz and 100 μM , *R*-bupivacaine yields a reduction in peak amplitude of tonic block of $58.5 \pm 0.6\%$ with a decay constant τ of 4.6 ± 0.8 pulses, whereas *S*-bupivacaine yields a reduction in peak amplitude of tonic block of $48.5 \pm 1.1\%$ with a τ of 3.8 ± 0.2 pulses.

IN VITRO AND IN VIVO ACTIONS OF BUPIVACAINE ENANTIOMERS

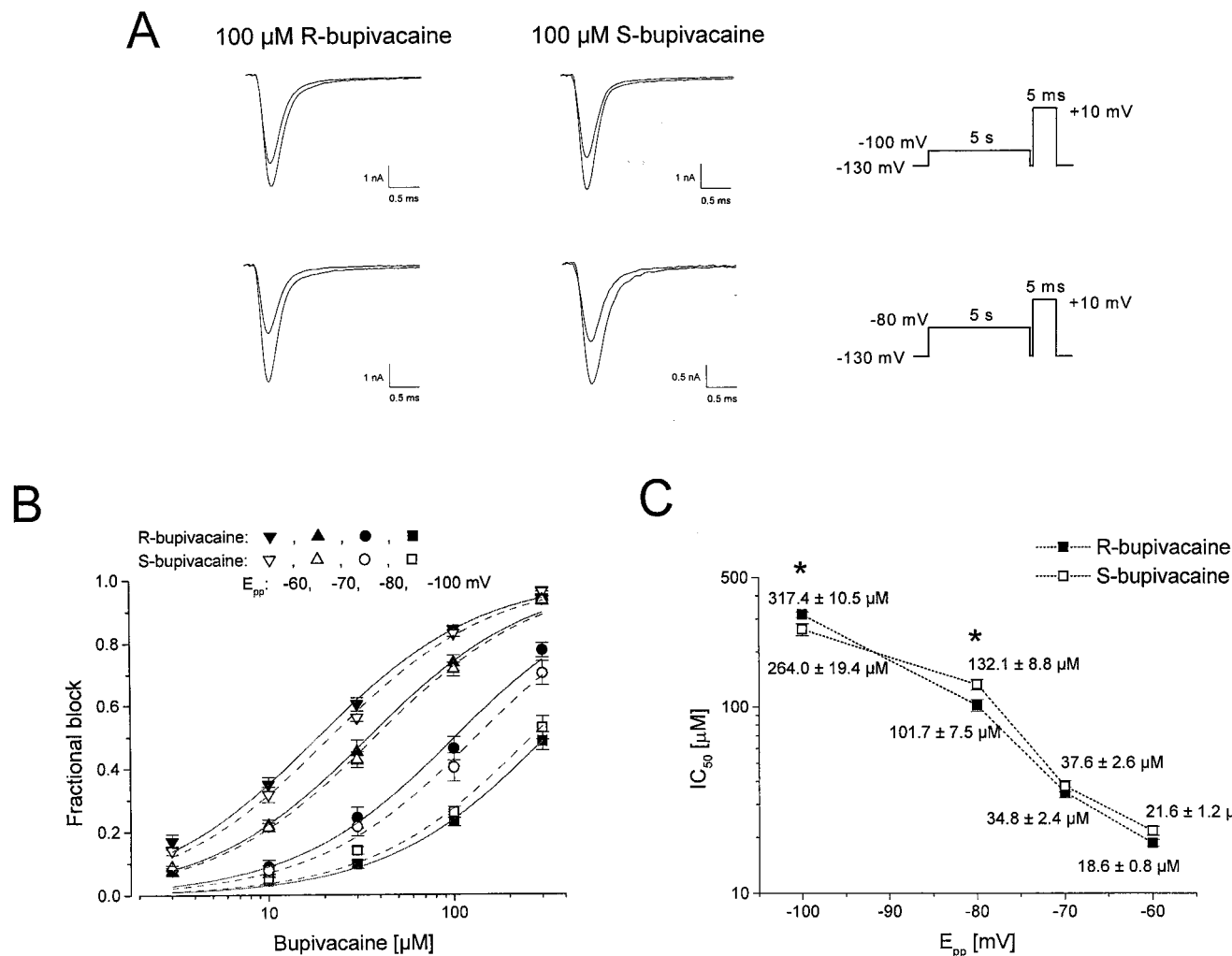


Fig. 2. Tonic inhibition of Na⁺ currents by bupivacaine enantiomers. (A) Na⁺ currents in control and in the presence of 100 μ M *R*- or *S*-bupivacaine were evoked by the test pulses shown to the right, delivered at 20-s intervals. (B) Concentration dependence of Na⁺ current block by *R*-bupivacaine (solid symbols) and *S*-bupivacaine (open symbols). Five-second prepulses to -60 mV through -100 mV were applied in the protocol shown in (A). Solid lines (*R*-bupivacaine) and dashed lines (*S*-bupivacaine) represent nonlinear least squares fitting of the function $f_b = 1/(1 + (IC_{50}/[bupivacaine]))$ to the data points. (C) IC₅₀ values, from the fitting to concentration-inhibition data (B), graphed as a function of prepulse potentials. Data are presented as fitted values \pm SE of the fit. An unpaired Student *t* test (SigmaStat, Jandel Scientific Software, San Rafael, CA) was used to evaluate the significance of differences in fitted values between *R*- and *S*-enantiomers. *P* values < 0.05 (*) were considered to be statistically significant.

Rates of dissociation of bupivacaine isomers from Na⁺ channels were assessed by increasing the recovery interval during which the membrane was unstimulated after a 10-s depolarizing conditioning pulse to +10 mV and measuring the recovery current as a function of recovery time. These recovery kinetics show two phases in the control situation without drug—a fast and a slow one—that are attributable to the recovery from fast (Hodgkin-Huxley type) and slow inactivation, respectively (table 1). Both bupivacaine enantiomers slightly but insignifi-

cantly increased the time constant for fast inactivation but their major effect was to increase the fraction of channels and the time constant of slow recovery, now reflecting, in part, the dissociation of bupivacaine from inactivated channels. The slowly recovering fraction is increased approximately fourfold over control by *R*-bupivacaine and threefold by *S*-bupivacaine, and the slow recovery rate is slowed by approximately the same factors by the respective drugs. In other words, *R*-bupivacaine dissociates less rapidly from Na⁺ channels than

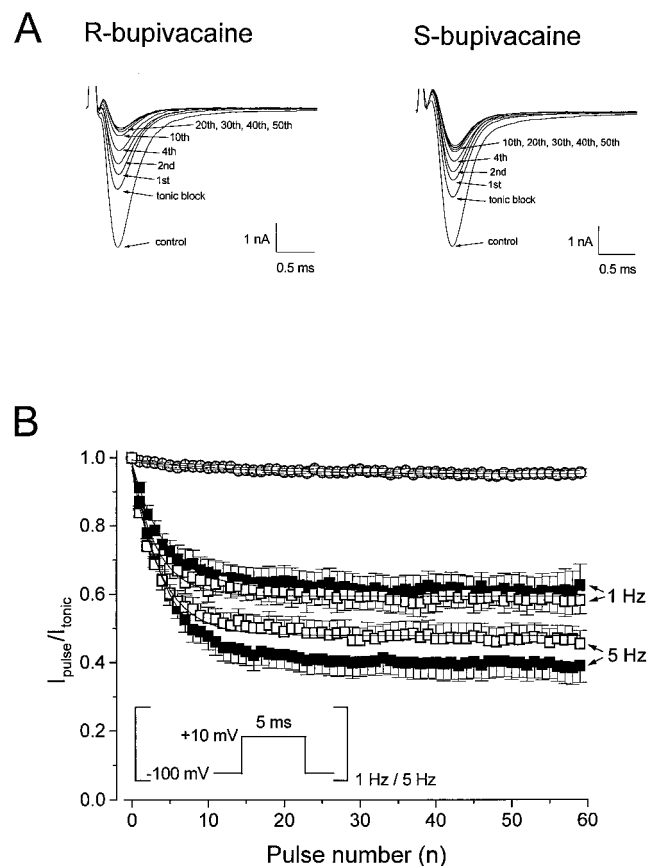


Fig. 3. Use-dependent inhibition of Na⁺ currents by bupivacaine enantiomers. (A) Na⁺ currents in the presence of 100 μM *R*- or *S*-bupivacaine obtained with repetitive 5-ms pulses to +10 mV at a frequency of 5 Hz. Holding potential was -100 mV. (B) Time course for the development of phasic inhibition by 100 μM *R*-bupivacaine (filled boxes) and *S*-bupivacaine (open boxes). Repetitive 5-ms pulses to +10 mV were applied at a frequency of 1 or 5 Hz after tonic block had reached steady state. Holding potential was -100 mV. The peak amplitudes were normalized to the amplitude obtained at tonic block and plotted against the pulse number. Data are from four experiments each.

S-bupivacaine. Thus, the basis of stereoselectivity for channel inhibition is found primarily in tighter binding to the receptor.

In Vivo Sciatic Nerve Block

All neural deficits occurred at the appropriate anatomic locus, the ipsilateral leg of the injected sciatic nerve. No effects on the contralateral side were observed, nor were there any generalized indications of systemic toxicity (abnormal gaits, twitching, or sedation). All neurologic deficits disappeared fully within 3–3.5 h at the longest. There were no residual changes in

Table 1. Kinetic Parameters for the Recovery of Na⁺ Current after Prolonged Depolarization*

	τ_1 (ms)	τ_2 (s)	A_2 †
Control	6.3 ± 0.7	1.4 ± 0.4	0.18
<i>R</i> -bupivacaine‡	7.7 ± 0.7	5.2 ± 0.1	0.71
<i>S</i> -bupivacaine‡	7.8 ± 0.9	4.1 ± 0.3	0.58

* Cells were conditioned with a 10-s depolarizing pulse to +10 mV from a holding potential of -100 mV and the recovery at -10 mV after the long depolarization was determined by giving a test pulse to +10 mV at various times after the conditioning pulse. The data were normalized to the amplitude of the test pulse obtained after a 50-s recovery time and fitted by the sum of two exponentials.

† Fraction of current recovering with the slower time constant.

‡ Both enantiomers at 0.3 mM; n = 4 for all values.

function or in gross anatomic appearance of the leg, nor any signs of local inflammation.

Changes in resting posture and in gait were observed before any other functional impairment, and they lasted longer. The magnitude and the duration of these changes appeared to increase with increasing dose, and their pattern is similar to that previously reported with lidocaine in the rat⁵: for postural changes, the toes first became fully extended and then ventroflexed and curled, and the stifle was extended and the hock dropped; for changes of gait, the injected limb touched the supporting surface with more weight bearing on the lateral margin or with weight bearing on the knuckle paw, then the leg dragged on the supporting surface.

The graded deficits in the four neurobehavioral functions mediated by the sciatic nerve are exemplified in figure 4 for blockade by the three drugs at a dose of 0.125%. Inhibition of nocifensive responses, to both noxious heat and pinch, is smaller in degree and shorter in duration than inhibition of proprioception and motor function, a differential functional block that occurs at all doses. Data such as those in figure 4 were used to compare the effects of the three drugs in terms of maximum graded effects (%MPE), the period after injection during which the maximum effect occurred, and the duration of significant partial deficits (*i.e.*, > 20% MPE for both motor activity and the nocifensive response to pinch). The first two of these parameters are shown in table 2, the last in table 3.

No stereoselective actions of drugs on maximum %MPE was observed, with the exception of inhibition of the response to pinch at 0.125%, where *R* = *S* > racemic. Durations of the maximum effect (table 2) as well of partial deficits (table 3) generally increased with increasing dose for all three drugs and, for the partial deficit of both motor and nociception, the duration of effect from

IN VITRO AND IN VIVO ACTIONS OF BUPIVACAINE ENANTIOMERS

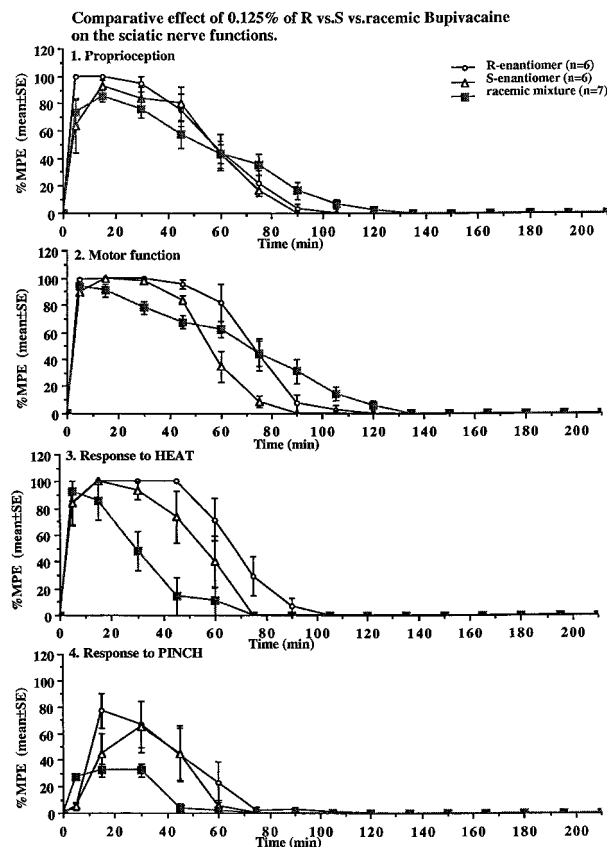


Fig. 4. The time course of graded deficits in the four neurobehavioral functions after injection of *R*-, *S*-, or racemic bupivacaine at 0.125%. The number of animals for each data set is shown in parentheses. Values are mean \pm SD.

S-bupivacaine is less than, or occasionally equal to, that from *R*-bupivacaine at all doses. In contrast, the duration of effects from racemic bupivacaine has no consistent relationship to those from the single enantiomers, being sometimes greater than both, sometimes less than either, and sometimes between the two.

These complex actions cannot be explained by a single mechanism, such as binding to Na^+ channels, but probably arise from a combination of pharmacodynamic and pharmacokinetic differences. Both factors contribute to the integrated actions of the drugs, which can be compared by calculating the area under the curve for the %MPE functions *versus* time, such as those in figure 4. Such a comparison is made in figure 5, where integrated inhibition of motor functions (fig. 5A) and heat nociception (fig. 5B) are presented graphically for three bupivacaine doses: 0.125, 0.25, and 1%. No significant differences exist between the two stereoisomers or the racemic mixture when this integrated activity is compared.

Table 2. Maximum Effect* and Its Time of Occurrence† for Inhibition of Motor and Nocifensive Pinch Responses after Sciatic Nerve Block

Dose (%)	<i>R</i> -Bupivacaine	<i>S</i> -Bupivacaine	rac-Bupivacaine
Motor			
0.0625	96 \pm 5/5-15	82 \pm 16/	91 \pm 4/5-15
0.125	98 \pm 3/5-45	99 \pm 2/15-30	94 \pm 4/5-15
0.25	100 \pm 0/5-60	100 \pm 0/5-60	100 \pm 0/5-90
0.50	100 \pm 0/5-90	100 \pm /16-60	100 \pm 0/5-90
1.0	100 \pm 0/5-60	100 \pm 0/5-90	98 \pm 2/5-105
Nocifensive pinch			
0.0625	12 \pm 13/5-15	15 \pm 26/2-15	18 \pm 31/15
0.125	78 \pm 12/15	68 \pm 15/30	32 \pm 8/15-30
0.25	100 \pm 0/30-45	99 \pm 2/30-45	100 \pm 0/15-60
0.50	100 \pm 0/30-90	96 \pm 5/45	100 \pm 0/5-75
1.0	100 \pm 0/15-45	98 \pm 3/15-60	100 \pm 0/5-75

* Value to the left of the diagonal line is the maximum % MPE (mean \pm SD)

† Values to the right of the diagonal line are the inclusive times (min) at which maximum % MPE were observed. n = 6 or 7 for all points.

‡ Significantly different from effects of *R*- and *S*-bupivacaine at the same dose

Another measure of local anesthetic effect having strong analogy to clinical anesthesia is the fraction of injected animals that become completely blocked. Scoring by presence or absence of response quantizes the population, and is shown in figure 6 for all four behavioral functions inhibited by bupivacaine doses of 0.125 and 0.5%. As for the graded response (%MPE), the fully blocked fraction increased with dose in both degree and duration. Interestingly, at the lower dose (0.0625) the fraction of thermal nocifensive responses that was fully blocked was greater than that of either proprioceptive or motor functions, the opposite relation from when the graded response (%MPE) was evaluated (fig. 4).

Table 3. Duration* of Deficits of Motor Activity and Pinch Nociception Induced by Sciatic Nerve Block *In Vivo*

Dose (%)	<i>R</i> -Bupivacaine	<i>S</i> -Bupivacaine	rac-Bupivacaine
Motor			
0.0625	60 \pm 12	45 \pm 10	60 \pm 16
0.125	75 \pm 18	60 \pm 5	90 \pm 23
0.25	115 \pm 25	115 \pm 19	130 \pm 31
0.50	160 \pm 22	145 \pm 17	160 \pm 40
1.0	175 \pm 22	145 \pm 20	130 \pm 15
Nocifensive pinch			
0.0625	0	0	0
0.125	45 \pm 10	30 \pm 12	25 \pm 18
0.25	85 \pm 14	70 \pm 18	100 \pm 9
0.50	130 \pm 27	75 \pm 25	100 \pm 16
1.0	145 \pm 26	100 \pm 28	130 \pm 19

* Longest measured time during which graded inhibition (% MPE) of function exceeded 20%; n = 6 or 7.

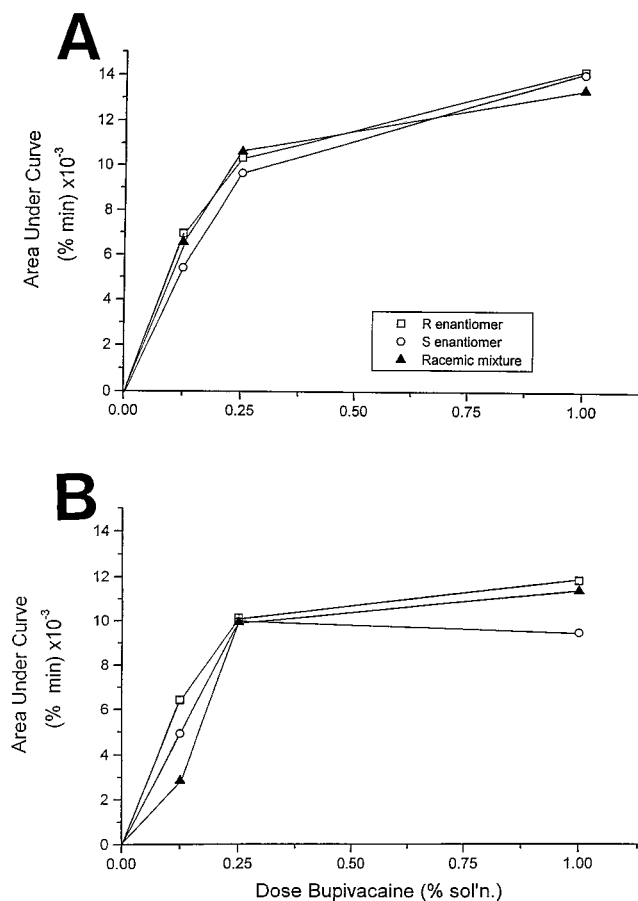


Fig. 5. Area under the curve for percent of maximal possible effect (%MPE) versus time for (A) motor block and (B) inhibition of the nocifensive response to thermal stimulation at three concentrations of the bupivacaine enantiomers and racemate. Areas were determined by measuring the profile from the averaged data, as graphed in figure 4, and the errors were estimated from the envelopes formed between the larger and smaller extremes of the SDs.

The fraction of animals fully blocked at the lower dose (0.125%, fig. 6A) also demonstrated a greater effect of *R*-over racemic bupivacaine, particularly for the later phases of motor block and heat nociception, and greater effects of the *S*-enantiomer over the racemate for heat nociception. However, at the higher dose (0.5%, fig. 6B), there was no difference among the three drugs in the extent (all near or at 100%) or the duration of full functional blockade.

Discussion

The experimental results show that both bupivacaine stereoisomers and the racemic mixture are safe and

effective for *in vivo* nerve block in the rat. Neither local nor systemic toxicity or irritation is apparent, judging by the calm, unседated behavior of the animals and their continuous ease in handling. The observed inhibitions were fully reversible in the dose range used here.

These doses can be compared with those used in humans. For example, for axillary (brachial plexus) blockade in adults, 20–30 ml (5 mg/ml) bupivacaine, 0.5%, might be delivered. This results in a dosing of 1.5–2 mg/kg total body mass for this block. For the sciatic nerve, injection of 0.1 ml bupivacaine, 0.5%, also produces a secure block, also corresponding to a dosing of 1.5–2 mg/kg. This is to some degree a fortuitous agreement, because local vascularity, surface:volume relationships and species-dependent pharmacokinetic factors may differ markedly between the two circumstances. Still, the agreement supports the extension of qualitative results from rats to humans. *R*-bupivacaine is generally more potent than *S*-bupivacaine when assayed *in vitro* for Na⁺ channel inhibitory activity, although the exact stereopotency depends on the the assay and the conditions. Antagonism of batrachotoxin binding shows a greater stereoselectivity (approximately 3:1) than the tonic blockade of resting or inactivated channels measured by voltage clamp (approximately 0.8–1.3:1). The same 3:1 stereopotency was found previously by Lee *et al.*⁴ for competitive antagonism by bupivacaine enantiomers of the depolarizing action of veratridine on frog sciatic nerves.²³

Inhibition of Na⁺ shows the resting channel conformation (favored at –100 mV) to have a slightly higher (1.2-fold) affinity for *S*- over *R*-bupivacaine, while the inactivated channel (favored at –60 mV) binds the *R*-enantiomer 17 times tighter and the *S*-enantiomer 11 times tighter than their resting-state affinities, resulting in only approximately a 1.2-fold stereopotency ratio (*R*:*S*) in the moderately depolarized membrane. These findings confirm and extend the earlier reports of weak but significant stereoselectivity (*R* > *S*) for the inhibition of neuronal action potentials^{4,24} and Na⁺ currents,^{22,23} both of which were enhanced by steady or repeated transient depolarization. In a nerve membrane at the “normal” resting potential of –70 to –80 mV, therefore, the *R*-bupivacaine enantiomer is approximately 1.3 times more potent for tonic blockade of Na⁺ channels.⁴ However, use-dependent actions, which result from increased local anesthetic binding to Na⁺ channels opened or inactivated by repeated stimulation, will lead to a stereoselective phasic block more favorable to *R*-bupivacaine. The underlying kinetic mechanism re-

IN VITRO AND IN VIVO ACTIONS OF BUPIVACAINE ENANTIOMERS

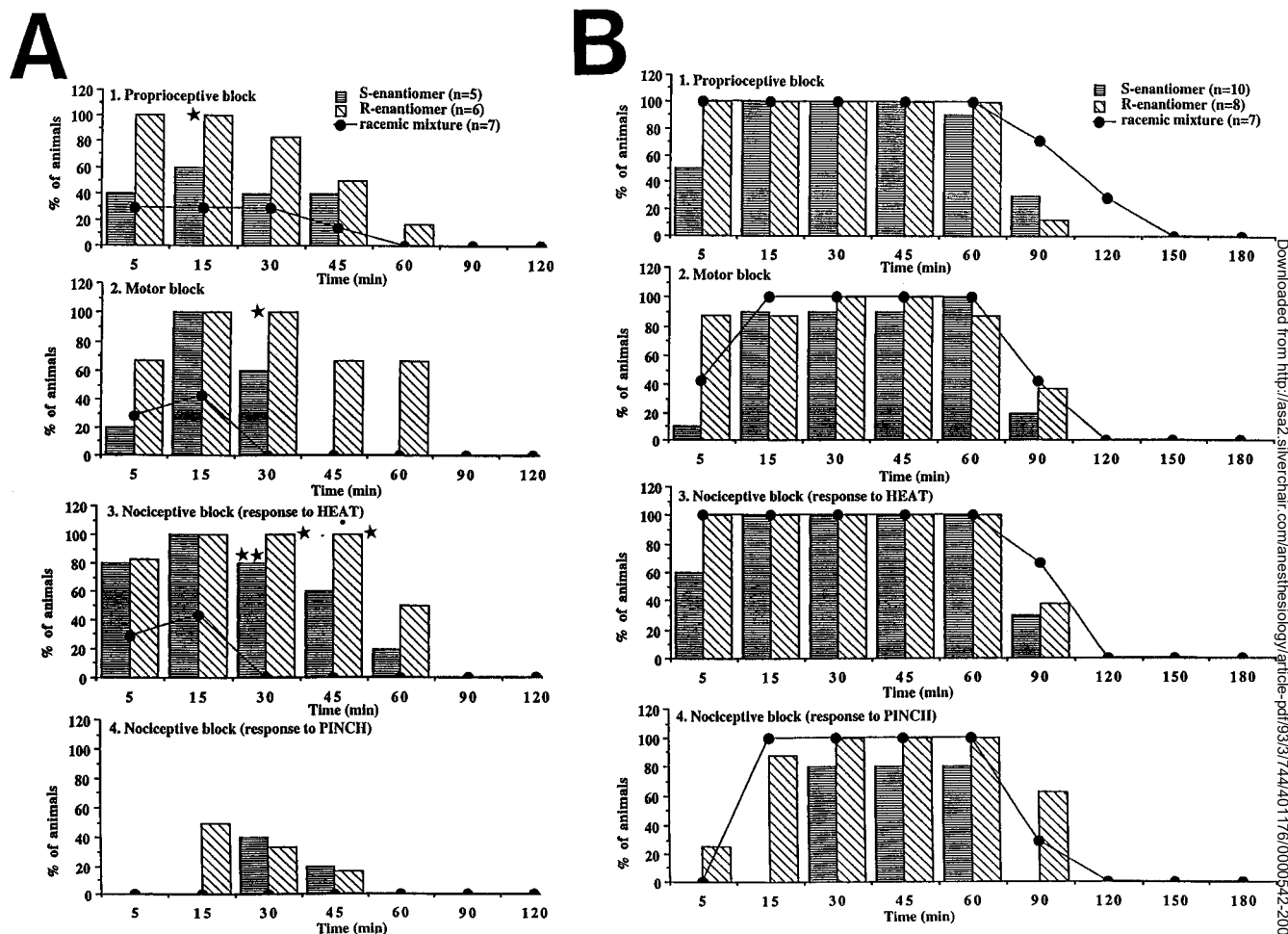


Fig. 6. Fraction of injected animals achieving full loss of the particular function after injection of bupivacaine enantiomers or racemic mixture, at concentrations of (A) 0.125% and (B) 0.5%. *R- significantly greater than racemate. **S- significantly greater than racemate by the Fisher exact test.

ported here describes a slower dissociation of *R*-bupivacaine from inactivated channels compared with *S*-bupivacaine. A similar, state-dependent affinity difference occurs with cardiac Na^+ channels²⁵ and would be particularly emphasized during the large and prolonged systolic depolarization of cardiac tissues. This may explain the pronounced stereoselectivity of cardiotoxicity (see below).

In contrast to the *in vitro* neuronal stereopotency, only small differences in enantiomer potency could be detected for the neurobehavioral deficits that characterize sciatic nerve block *in vivo*. Neither the maximum degree of blockade (%MPE) nor its duration was clearly favored by one enantiomer at all doses, for any of the four functions tested, although the fraction of animals completely blocked by 0.125% *R*-bupivacaine was

greater than when 0.125% of the racemate was used. Stereoselective ($R > S$) actions of bupivacaine isomers have been reported for infiltration anesthesia in animals and humans,²⁶ but other clinical trials have not detected a significant difference in degree (intensity), spread, or duration of block between bupivacaine enantiomers or between levobupivacaine and the racemic mixture.²⁷

There are several possible explanations for the difference between *in vitro* and *in vivo* stereoselectivity findings. First, the predominant mode of neural blockade that underlies functional deficits may be a result of tonic inhibition of resting nerve fibers. Stereoselectivity for this block *in vitro* is modest at best, as reported here (fig. 2C) and in other reports^{4,24} and might be undetected during typical clinical tests, where quantitation is not a primary objective and intraindividual variability is high.

The methods used here for assaying functional neural blockade can readily discriminate dose differences of threefold, near the ED₅₀ (e.g., see table 2, nociception); therefore, if potency differences of 3:1, as shown for batrachotoxin displacement or the competitive antagonism of veratridine's actions,⁴ were reflected in functional blockade they should be detectable.²⁷

Second, inhibition of K⁺ channels by bupivacaine, known in some cases to have a strong (*R* > *S*) stereoselectivity,^{28,29} may either potentiate³⁰ or antagonize³¹ impulse blockade that is primarily caused by inhibition of Na⁺ channels. Because different types of axons, classified by anatomy (nonmyelinated, myelinated) or by function (sensory, motor), appear to have different kinds of K⁺ channels,³²⁻³⁵ blockade of Na⁺ channels may not be the predominant factor determining the stereoselectivity of impulse blockade.

Third, the local disposition of drug that is a major determinant of degree and duration of neural blockade may favor the neuronal uptake of the intrinsically less potent enantiomer, thus nullifying any pharmacodynamic advantage. Studies of rat sciatic nerve block with radiolabeled lidocaine reveal that only a few percent of the total injected dose (in 0.1 ml) actually is present in the nerve during the peak of functional deficits.³⁶ Vasoconstriction by epinephrine (1:100,000) is known to potentiate and prolong this blockade and also elevates intraneural lidocaine approximately twofold (unpublished observations). The *S*-enantiomers of piperidine-deriving local anesthetics, e.g., *S*-mepivacaine, ropivacaine, and *S*-bupivacaine, are reported to be more vasoconstrictive than the *R*-enantiomers,^{26,37} and thus, through this vascular action, *S*-bupivacaine could enhance its own neuronal uptake and potentiate its own block, more so than the comparative actions of *R*-bupivacaine.

Finally, all of these factors could be contributing to the overall observed functional block *in vivo*, because the participation of any one factor does not preclude the others.

That neural blockade *in vivo* shows little to no significant stereoselectivity for sensory or motor deficits contrasts with the effects on cardiac activities,^{3,38,39} where *R*-bupivacaine is both more potent and more toxic than *S*-bupivacaine. The discrepancy between neuronal "therapeutic" and cardiac "toxic" actions may arise from the different states of the Na⁺ channel involved in the effects on these different tissues. Neural blockade requires relatively high concentrations of bupivacaine, likely binding to resting state Na⁺ channels in a nerve membrane most often at a potential of -70 to -80 mV

(where little stereoselectivity of block is present) and only briefly depolarized by neuronal impulses. In contrast, cardiac effects that can be produced by much lower drug concentrations arise from binding to inactivated Na⁺ channels (with higher *R* > *S* stereoselectivity) that are populated during the hundreds of millisecond-long depolarizations of cardiac action potentials.²⁵ One would predict from these state-dependent affinities that *R*-bupivacaine would be more cardiotoxic than *S*-bupivacaine, a prediction born out by *in vitro*^{3,38,39} and *in vivo* studies.^{40,41} These differences in cardiotoxicity between *R*- and *S*-enantiomers of bupivacaine (or between ropivacaine and its *R*-enantiomer), coupled with equipotency for nerve block *in vivo*, might provide an advantage in therapeutic index that justifies their clinical use.

References

1. Albright GA: Cardiac arrest following regional anesthesia with etidocaine or bupivacaine. *ANESTHESIOLOGY* 1979; 51:285-7
2. Åberg G: Toxicological and local anaesthetic effects of optically active isomers of two local anaesthetic compounds. *Acta Pharmacol Toxicol* 1972; 31:273-86
3. Mazoit J, Boïco O, Samii K: Myocardial uptake of bupivacaine. *Int J Pharmacokinetics and pharmacodynamics of bupivacaine enantiomers in the isolated perfused rabbit heart.* *Anesth Analg* 1993; 77:477-82
4. Lee Son S, Wang GK, Concus A, Crill E, Strichartz G: Stereoselective inhibition of neuronal sodium channels by local anesthetics: Evidence for two sites of action? *ANESTHESIOLOGY* 1992; 77:324-35
5. Thalhammer JG, Vladimirova M, Bershinsky B, Strichartz GR: Neurological evaluation of the rat sciatic nerve block with lidocaine. *ANESTHESIOLOGY* 1995; 82:1013-25
6. Creveling CR, McNeal ET, McCulloh DH, Daly JW: Membrane potentials in cell-free preparations from guinea pig cerebral cortex. Effect of depolarizing agents and cyclic nucleotide. *J Neurochem* 1980; 35:922-32
7. McHugh J, Mok WM, Wang GK, Strichartz G: Irreversible inhibition of sodium current and batrachotoxin binding by a photoaffinity derivatized local anesthetic. *J Gen Physiol* 1995; 105:267-87
8. Catterall WC, Morrow CS, Daly JW, Brown GB: Binding of batrachotoxin in A 20-a-benzoate to a receptor site associated with sodium channels in synaptic nerve ending particles. *J Biol Chem* 1981; 256:8922-7
9. Wang, GK Strichartz GR: Purification and characterization of neurotoxins from venoms of the scorpions *Centruroides sculptatus* and *Leiurus quinquestriatus*. *Mol Pharm* 1983; 23:519-33
10. Dodd PR, Hardy JA, Oakley AE, Edwardson JA, Perry EK, Delaunoy JP: A rapid method for preparing synaptosomes: Comparison with alternative methods. *Brain Res* 1981; 226:107-18
11. Tamkun MM, Catterall WA. Ion flux studies of voltage sensitive sodium channels in synaptic nerve ending particles. *Mol Pharmacol* 1981; 19:78-86
12. Peterson GL: A simplification of the protein assay method of Lowry et al: Which is generally more applicable. *Analytical Biochem* 1977; 83:346-56

13. Dubinsky JM, Oxford GS: Ionic currents in two strains of rat anterior pituitary tumor cells. *J Gen Physiol* 1984; 83:309-39
14. Hamill OP, Marty A, Neher E, Sackmann B, Sigworth FJ: Improved patch clamp techniques for high resolution current recording from cells and cell free membrane patches. *Pflugers Arch* 1981; 391: 85-100
15. Kohane DS, Yieh J, Lu NT, Langer R, Strichartz GR, Berde CB: A re-examination of tetrodotoxin for prolonged duration local anesthesia. *ANESTHESIOLOGY* 1998; 89:119-31
16. Postma SW, Caterall WA: Inhibition of binding of [³H]batrachotoxin A-20- α benzoate to sodium channels by local anesthetics. *Mol Pharmacol* 1984; 25:219-27
17. Sheldon RS, Cannon NJ, Duff HJ: A receptor for type I anti-arrhythmic drugs associated with rat cardiac sodium channels. *Circ Res* 1987; 61:492-7
18. Courtney KR: Mechanism of frequency dependent inhibition of sodium currents in frog myelinated by the lidocaine derivative GEA-968. *J Pharmacol Exp Ther* 1975; 195:225-36
19. Khodorov BL, Shishkova L, Peganov F, Revenko S: Inhibition of sodium currents in frog Ranvier node treated with local anesthetics: Role of slow sodium inactivation. *Biochim Biophys Acta* 1976; 433: 409-35
20. Hille B: Local anesthetics: Hydrophobic and hydrophilic pathways for the drug-receptor reaction. *J Gen Physiol* 1977; 69:497-515
21. Chernoff DM: Kinetic analysis of phasic inhibition of neuronal sodium currents by lidocaine and bupivacaine. *Biophys J* 1990; 58: 69-81
22. Wang GK, Wang SY: Altered stereoselectivity of cocaine and bupivacaine isomers in normal and batrachotoxin modified Na channels. *J Gen Physiol* 1992; 100:1003-20
23. Rando TA, Wang GK, Strichartz GR: The interaction between the activator agents batrachotoxin and veratridine and the gating processes of neuronal sodium channels. *Mol Pharmacol* 1985; 29:467-77
24. Akerman B, Camougis G, Sandberg RV: Stereoisomerism and differential activity in excitation block by local anesthetics. *Eur J Pharmacol* 1969; 8:337-47
25. Valenzuela C, Snyder DJ, Bennett PB, Tamargo J, Hondeghem LM: Stereoselective block of cardiac sodium channels by bupivacaine in guinea pig ventricular myocytes. *Circulation* 1995; 92:3014-24
26. Aps C, Reynolds F: An intradermal study of the local anaesthetic and vascular effects of the isomers of bupivacaine. *Br J Clin Pharmacol* 1978; 31:273-286
27. Cox CR, Faccenda KA, Gilhooly C, Bannister J, Scott NB, Morrison LMM: Extradural S(-)-bupivacaine: Comparison with racemic RS-bupivacaine. *Br J Anaesth* 1998; 80:289-93
28. Nau C, Vogel W, Hempelmann G, Bräu ME: Stereoselectivity of bupivacaine in local anesthetic sensitive ion channels of peripheral nerve. *ANESTHESIOLOGY* 1999; 91:785-95
29. Valenzuela C, Delpón E, Tarnkum MM, Tamargo J, Snyders DJ: Stereoselective block of a human cardiac potassium channel (Kv1.5) by bupivacaine enantiomer. *Biophys J* 1995, 69:4189-427
30. Drachman D, Strichartz GR: Potassium channel blockers potentiate impulse inhibition by local anesthetics. *ANESTHESIOLOGY* 1991; 75:1051-61
31. Strichartz GR, Cohen IS: V_{max} as a measure of G_{Na} in nerve and cardiac membranes. *Biophys J* 1978; 23:153-6
32. Kocsis JD: Functional characteristics of potassium channels. *Neural Membranes*. Edited by Ritchie JM, Keynes RD, Bolis L. New York, Alan R. Liss, 1986, pp 123-44
33. Bromm B, Schwarz JR, Ochs G: A quantitative analysis of combined potential and current clamp experiments on the single myelinated nerve fibres of *Rana esculenta*. *J Theor Neurobiol* 1981; 1:120-36
34. Bretag AH, Stämpfli R: Differences in action potentials and accommodations of sensory and motor myelinated nerve fibers as computed on the basis of voltage clamp data. *Pflügers Arch* 1975; 354:257-71
35. Gold MS, Shuster MJ, Levine JD: Characterization of six voltage-gated K^+ currents in adult rat sensory neurons. *J Neurophysiol* 1999; 75:2629-46
36. Popitz-Bergez FA, Leeson S, Strichartz GR, Thalhammer JG: Relation between functional deficit and intraneural local anesthetic duration of peripheral nerve block: A study in the rat sciatic nerve. *ANESTHESIOLOGY* 1995; 83:583-92
37. Aberg G, Wahlström B: Mechanical and electrophysiological effects of some local anaesthetic agents and their isomers on the rat portal vein. *Acta Pharmacol Toxicol* 1972; 31:255-66
38. Vanhoutte F, Vereecke J, Verbeke N, Carmeliet E: Stereoselective effects of the enantiomers of bupivacaine on the electrophysiological properties of the guinea-pig papillary muscle. *Br J Pharmacol* 1991; 103:1275-81
39. Harding DP, Collier PA, Huckle, RM, Gristwood R, Spridgen E: Comparison of the cardiotoxic effects of bupivacaine, levobupivacaine and ropivacaine: An in vitro study in guinea-pig and human cardiac muscle (abstract). *Br J Pharmacol* 1998; 125:127P.
40. Gristwood R, Bardsley H, Baker H, Dickens J: Reduced cardiotoxicity of levobupivacaine compared with racemic bupivacaine (Maccaine): New clinical evidence. *Exp Opin Invest Drugs* 1994; 3:1209-11
41. Mather LE, Huang YF, Veering B, Pryor ME: Systemic and regional pharmacokinetics of levobupivacaine and bupivacaine enantiomers in sheep. *Anesth Analg* 1998; 86:805-11