Anesthesiology
2000; 92:1350–60

Spinal Tonicaine

Potency and Differential Blockade of Sensory and Motor Functions

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Background: **Long-acting local anesthetics are beneficial for the management of postoperative pain and chronic pain. The authors recently reported that a single injection of N-β-phenylethyl-lidocaine (tonicaine), a quaternary lidocaine derivative, effectively blocks rat sciatic nerve function four to nine times longer than lidocaine, with a predominance of sensory *versus* motor blockade. The purposes of this study were to measure directly the potency of this charged drug by internal perfusion of cultured neuronal cells, and to evaluate the differential blockade of sensory *versus* motor function *via* spinal route in rats.**

Methods: **The tonic and additional use-dependent blockade of Na^+ currents by internal tonicaine was assayed in cultured GH_3 cells during whole cell voltage-clamp conditions. In addition, tonicaine was injected into the intrathecal space of rats at intervertebral space L4–L5, and the proprioceptive, motor, and sensory functions, and tissue integrity, subsequently were evaluated.**

Results: **Internal application of tonicaine in GH_3 cells revealed that it was ~80 times more potent in blocking Na^+ currents than was externally applied lidocaine. *In vivo* testing in a rat neuraxial anesthesia model showed that tonicaine at 0.5 mM produced blockade that lasted much longer than that produced by bupivacaine even at ~a 55 times higher concentration (28.8 mM). Tonicaine spinal block also produced a longer duration of sensory than motor blockade (112.5 ± 16.3 min *vs.* 45.8 ± 7.1 min). Evidence of neurotoxicity was seen at a concentration of 1.0 mM.**

Conclusion: In vitro **testing shows that tonicaine displays a higher affinity for the local anesthetic binding site than does lidocaine; *in vivo* testing indicates that tonicaine elicits sensory blockade of a duration significantly longer than that elicited by bupivacaine. Tonicaine, however, has a narrow therapeutic index, with substantial neurotoxicity at 1 mM in rats, and may have limited clinical value. (Key words: Analgesia; pain management; sodium channel blocker.)**

EINHORN[1] introduced the first synthetic local anesthetic, procaine, more than 10 yr ago. Since then, numerous attempts have been made to increase sensory blockade relative to motor blockade, decrease toxicity, and prolong the duration of blockade.[2] Long-acting local anesthetics would be especially beneficial for the management of postoperative and chronic pain; they also could play an important role in preemptive analgesia.[3] Innumerable compounds have been synthesized, but only a few drugs have become clinically useful, and even the most clinically potent agent, bupivacaine, has a rather limited effectiveness for pain management after a single injection. To date, most new compounds that produce ultralong nerve blockade with a single agent (such as tetraethylammonium derivatives, cyclizing compounds, and tetrodotoxin) have been found to be neurotoxic.[4–6] Therefore, our goal was to develop a sensory-selective long-lasting local anesthetic, test its physicochemical properties *in vitro*, and define effectiveness and toxicity *in vivo* in rats.

We reported that the local anesthetic binding site contains two large hydrophobic domains.[7,8] With this information, we have begun to synthesize new derivatives that introduce an additional hydrophobic moiety to the tertiary amine of local anesthetic drugs. Such a modification yields a quaternary ammonium derivative (fig. 1) that may exhibit a higher affinity for its receptor and also may penetrate readily the lipid bilayer because of its

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Received from the Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Boston, Massachusetts. Submitted for publication March 8, 1999. Accepted for publication December 1, 1999. Supported by the National Institutes of Health (research grant No. GM-48090), Bethesda, Maryland. Presented in part at the 42nd annual meeting of the Biophysical Society, Kansas City, Missouri, February 22–26, 1998.

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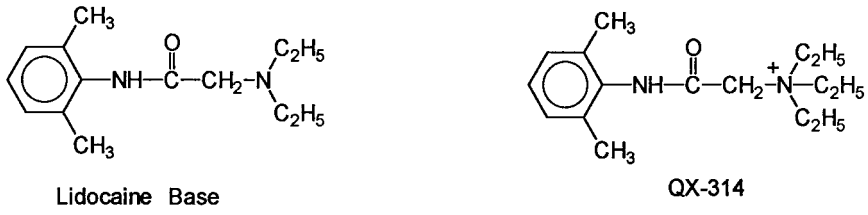


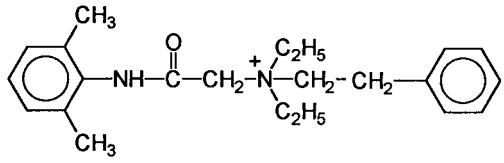


Fig. 1. Chemical structure of lidocaine and its derivatives.

amphipathic property (as opposed to external QX-314, which is also a permanently charged quaternary ammonium derivative but has not been found to be effective).[9] We synthesized tonicaine, a novel quaternary ammonium derivative of lidocaine, and reported that at 100 μM it inhibits peak Na^+ currents by 55.0 *versus* 27.1% for lidocaine after 30 min of external drug administration. Also, because of the slower rate of onset, a steady state was not reached with tonicaine.[10] Prolonged and complete sciatic nerve blockade in rats was achieved for 3–7 h, and sensory blockade was more prolonged than motor blockade. For example, tonicaine produced blockade of withdrawal response to pinching that lasted 9.3 times longer than the blockade produced by its parent drug lidocaine.[10]

Because tonicaine possesses a permanent charge, this molecule penetrates the membrane much more slowly than its parent drug lidocaine, with a half-time of more than 25 min to reach its steady state level of blockade of Na^+ currents. If tonicaine is applied externally, it is effective, but inhibition develops at a relatively slow rate, especially at lower concentrations. Often, in the whole cell configuration, the cell destabilizes before the steady state level of blockade has been reached. Therefore, to determine the potency of tonicaine *in vitro*, internal application is necessary. Conversely, lidocaine can exist as an uncharged drug; it has been applied externally because we noted in previous experiments that internal application of lidocaine actually reduces its potency compared with external application, probably because of its concentration gradient across the vast external sink. For example, internal lidocaine at 50 μM elicits no tonic blockade of Na^+ currents in GH_3 cells (n = 9; data not shown). Such an external sink is presumably negligible for tonicaine and QX-314 because of the lipid bilayer barrier in the diffusion of charged molecules. If lidocaine is applied internally, it penetrates the membrane so quickly (and gets rapidly diluted in the bathing solution of the cell, which, in addition, is replaced constantly) that the concentration near the membrane channels is only a small fraction of the concentration inside the pipette, the targeted intracellular concentration. Therefore, lidocaine by internal application produces blockade only at unphysiologically high drug concentrations.

The true binding affinity of tonicaine for the local anesthetic receptor remains unknown. The differential blockade of tonicaine, which shows a greater blockade of sensory function than of motor function, particularly intrigues us. Such a drug may have significant clinical advantages for management of acute and chronic pain. It is not known whether this differential-blockade phenomenon is also evident with spinal tonicaine injection. Therefore, we investigated the potency of tonicaine in blocking Na^+ currents *in vitro* by internal perfusion and evaluated the differential functional blockade of tonicaine *in vivo via* the spinal route in rats.

Material and Methods

Chemicals

Lidocaine and bupivacaine were purchased from Sigma (St. Louis, MO) and (2-bromo-ethyl)benzene was purchased from Aldrich (Milwaukee, WI); QX-314 chloride was donated by Astra Pharmaceutical Products (Worcester, MA). Tonicaine was synthesized from lidocaine (base) and (2-bromo-ethyl)benzene, as described previously.[10] Glycerol was added in previous formulations to increase the solubility of tonicaine, but because glycerol can be neurotoxic[11] in spinal injection, it was omitted for the preparation of the drug used in this study. Tonicaine at 10 mM stock solution was dissolved in 5% dextrose without glycerol, although the dissolving process was prolonged significantly.

Whole Cell Voltage Clamp Experiments and Cell Culture

The whole cell configuration of the patch clamp technique[12] was used to record macroscopic Na^+ currents at room temperature (20–24°C). The pipette electrodes had a resistance ranging from 0.8–1.2 MΩ. Command voltages were controlled by pCLAMP software (Axons Instruments, Foster City, CA) and delivered by a List-EPC7 patch clamp amplifier (List-Electronic, Darmstadt/Eberstadt, Germany). After the establishment of the whole cell configuration, cells were dialyzed for 30 min before data were acquired. Data were filtered at 5 kHz, sampled at 50 kHz, collected, and stored with pCLAMP software. Leak and capacitance currents were subtracted using the P/−4 protocol and further by a leak-and-capacitance subtractor, as described by Hille and Campbell.[13] The P/−4 protocol was not applied in the use-dependent blockade of Na^+ currents. Whole cell recordings were maintained for more than 1 h in this preparation, with little or no rundown of the Na^+ current. Pipette electrodes were filled with an internal solution containing 100 mM NaF, 30 mM NaCl, 10 mM ethyleneglycoltetraacetic acid (EGTA), and 10 mM HEPES titrated with CsOH to a pH 7.2. The external solution consisted of 150 mM choline chloride and 10 mM HEPES titrated with tetramethylammonium hydroxide to pH 7.4. These solutions create an outward Na^+ gradient and current, further reducing potential problems associated with series resistance errors.[14]

Rat clonal pituitary GH_3 cells were purchased from the American Type Culture Collection (Rockville, MD). Cells were split twice a week and maintained in Dulbecco modified Eagle medium (Hyclon Labs, Logan, UT) supplemented with taurine (1%), penicillin–streptomycin (1%), HEPES (20 mM), and heat-inactivated fetal bovine serum (10%), as described previously.[14] The 35-mm culture dishes in which the cells were grown also were used as recording chambers. Internal perfusion of tonicaine and QX-314 was accomplished using perfusing patch pipettes, as described by Tang *et al.*[15] Lidocaine was used externally for reasons stated previously, and bupivacaine was not used for our *in vitro* studies because the 50% inhibitory concentration (IC_{50}) had been determined in previous work.[16]

Rat Intrathecal Blockade and Neurobehavioral Evaluation

The protocol for animal experimentation was reviewed and approved by the Harvard Medical Area Standing Committee on Animals, Boston, Massachusetts. Male Sprague-Dawley rats were purchased from Taconic Farm (Germantown, NY) and kept in the animal-housing facilities of Brigham and Women's Hospital, with controlled humidity (20–30% relative humidity) and room temperature (24°C) and a 12-h (6:00 AM–6:00 PM) light-dark cycle. Rats were handled before the procedure to familiarize them with the experiment and to minimize stress-induced analgesia. At the time of injections, animals weighed 300–350 g and showed no signs of neurobehavioral impairment.

Normal neurobehavioral status was seen in all tested animals after no more than 8 days of handling by the same experimenter (once in the morning and once in the afternoon). This was defined as follows: The animal was observed for normal exploratory activity, responsiveness to its environment, rearing, grooming, and number of fecal boluses; normal gait and posture were first qualitatively described and observed for absence of asymmetry and then quantified by specific tests for proprioception (tactile placing and hopping response) and motor function (extensor postural thrust).

Spinal injections were performed using a method modified from one by Papir-Kricheli *et al.*[17] Briefly, rats were anesthetized lightly by inhalation of sevoflurane, the inhalation agent was discontinued, and, after optimal flexion of the lumbar spine was obtained, the L4–L5 intervertebral space was identified. A 30-gauge needle attached to a 100-μl precision syringe (Hamilton, Reno, Nevada) was inserted in the midline and advanced at a slightly caudal angle until a tail flick indicated entrance into the intrathecal space.[18] Ninety microliters of 37 mM lidocaine in 5% dextrose (n = 6), 28.8 mM bupivacaine in 5% dextrose (n = 6), or 0.5 mM tonicaine in 5% dextrose

(n = 6) was injected, and the rat was observed for development of spinal blockade, indicated by complete paralysis of the hind limbs. Rats that showed unilateral or no blockade (0–2 per group) were excluded from the study and killed using an overdose of sevoflurane.

Neurobehavioral examination[19] consisted of evaluation of motor function, proprioception, and nocifensive reaction at 3 min before spinal injection and at 2, 5, 10, 15, 20, 25, and 30 min afterward (lidocaine, bupivacaine, and tonicaine), then again at 15-min intervals until 2 h, at 30-min intervals until 4 h, at 1-h intervals until 6 h, and at 12-h intervals for up to 9 days (tonicaine and bupivacaine only).

Motor function of hind limbs, measured as the gram force of extensor postural thrust, was tested three times at each interval, and the highest value was considered to be the maximal possible effect. Motor blockade was considered to be resolved after the maximal possible effect was within 10% of or exceeded the preinjection maximal possible effect. Proprioception was based on resting posture and postural reactions ("hopping" and "tactile placing"). Nocifensive reaction was evaluated by the withdrawal reflex or vocalization to pinching at the back, hind limbs, and tail. Details of the neurobehavioral evaluation can be found elsewhere.[19]

Pathology

To evaluate morphologic changes in the spinal cords, especially at higher doses, 17 rats were killed using an overdose of sevoflurane 2 weeks after the injection. Spinal cords from each of the following groups were evaluated by a blinded neuropathologist experienced in peripheral neurotoxicity: 0.5 (n = 4), 0.625 (n = 5), 0.75 (n = 2), and 1 mM tonicaine (n = 2), 28.8 mM bupivacaine (n = 2), and dextrose 5% in water (n = 2) as a control group. The spinal cords were excised and placed in 4% glutaraldehyde in 0.1 M cacodylate buffer. After fixation, cross-sections of the spinal cord and of proximal and distal portions of the spinal roots were taken, embedded in paraffin, and stained with hematoxylin and eosin, as described previously.[20]

Statistical Analysis

An unpaired Student *t* test or one-way analysis of variance was used to calculate the significance of differences in the IC_{50} (among lidocaine, tonicaine, and QX-314) or the inhibition of Na^+ current at the sixtieth pulse (among control, tonicaine, and QX-314), respectively. An unpaired Student *t* test (conducted using Origin software; Microcal Software, Northhampton, MA) also was used to detect significant differences among the proprioceptive, motor, and sensory functions of the animals after tonicaine, bupivacaine, or lidocaine injection. Statistical significance was considered to occur at $P < 0.05$.

Results

Dose–Response Curve of Internally Perfused Tonicaine

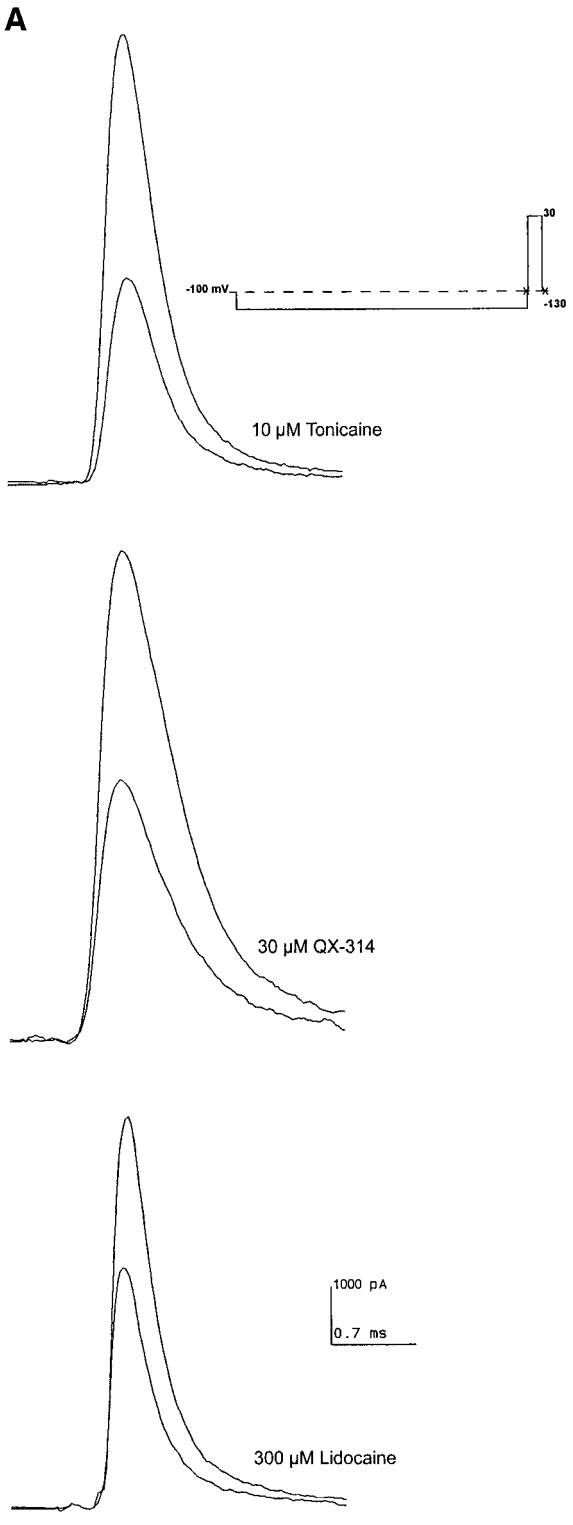
The dose–response curve of internally perfused tonicaine was constructed to determine the IC_{50}. The time to reach the steady state level of blockade is generally long with internal perfusion; because the drug flows through a narrow pipette tip, it usually takes up to 30 min at a low drug concentration. For this reason, each cell was used to determine the blockade of only one concentration of drug (fig. 2A). Internally applied tonicaine was found to be ~80 times more potent than externally applied lidocaine and ~2.5 times more potent than internally applied QX-314 (IC_{50} values 7, 569, and 18 μM respectively; fig. 2B). The Hill coefficient was approximately 1 for all three drugs, which suggests that one local anesthetic molecule blocks one Na^+ channel.

Use-dependent Blockade of Na^+ Channels by Tonicaine and QX-314

In addition to tonic inhibition of Na^+ currents by local anesthetic drugs during infrequent pulses, local anesthetic agents generally elicit use-dependent blockade of Na^+ currents during repetitive pulses at a frequency of 2 Hz. We found that additional use-dependent blockade of Na^+ currents by internal tonicaine and QX-314 also was present during repetitive pulses (fig. 3A). Currents were measured by 60 repetitive pulses at 2 Hz before and after the internal application of tonicaine or QX-314. The peak amplitudes of each data set were normalized with respect to the amplitude of the first pulse of the set. The percentage of the current obtained then was plotted against the pulse number. At pulse 60, approximately 50% of the remaining current was blocked further by tonicaine, whereas only approximately 30% was blocked by QX-314 (fig. 3B). Evidently, tonicaine elicits far greater additional use-dependent blockade than does its parent drug lidocaine.[10]

Spinal Application of Tonicaine and Lidocaine in Rats

To extend our previous study of tonicaine injection at the sciatic notch, we applied tonicaine, bupivacaine, and



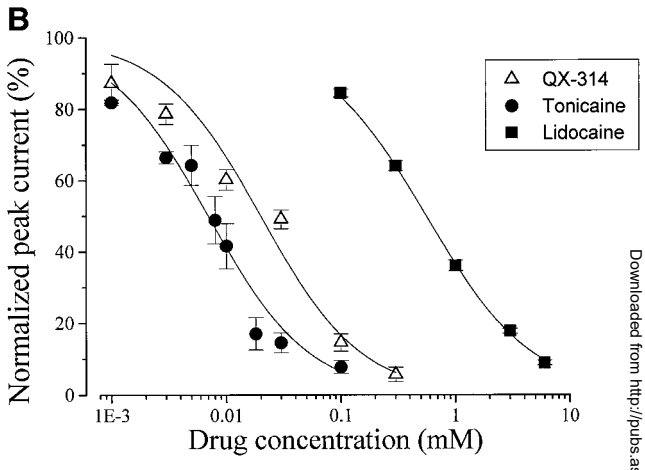


Fig. 2. (*A*) Representative tracings for phasic block of tonicaine, QX-314 (internal perfusion) and lidocaine (external perfusion). Tracings for tonicaine and QX-314 are for control and steady state concentrationss after 30 min; those for lidocaine are control and steady state concentrations after 1 min, if steady state concentration already was achieved. (*B*) Tonic block of Na^+ currents by tonicaine, QX-314 (internal perfusion), and lidocaine (external perfusion). Experiments were conducted under a reverse Na^+ gradient to minimize the series resistance artifact. The peak current amplitudes were measured, normalized with respect to the maximum inward Na^+ current, and plotted against the drug concentrations. Inhibition of Na^+ current by tonicaine was significantly greater than inhibition by QX-314 and lidocaine. The wash-in time course was slow for tonicaine. A prepulse was delivered at −130 mV for 100 ms, followed by a test pulse of +30 mV for 4 ms; the holding potential was set at −100 mV (the pulse protocol is illustrated in *A*). Data were fitted by Hill equation and n (Hill coefficient = 1.15 ± 0.25).

lidocaine through spinal injections of rats at the L4–L5 level and determined their effectiveness in blocking motor and sensory functions.

Onset. After spinal injection, the onsets of blockades for lidocaine (at 37 mM) and bupivacaine (at 28.8 mM) were found to occur sooner than that for tonicaine (at 0.5 mM). The onset time for tonicaine, however, was still rather short, measured at 2.5 ± 0.5 min. Considering the low concentration of spinal tonicaine injection, this fast onset for tonicaine probably results from its high potency in blocking Na^+ channels *in vivo* and the small spinal space. In fact, it took 5.7 min to block the withdrawal response to pinching if 40 mM tonicaine was injected through the sciatic-notch route. We could not measure the precise onset time for lidocaine and bupivacaine, because when animals awoke from sevoflurane anesthesia, the blockade already was established fully (generally less than 1 min after spinal injection). Because

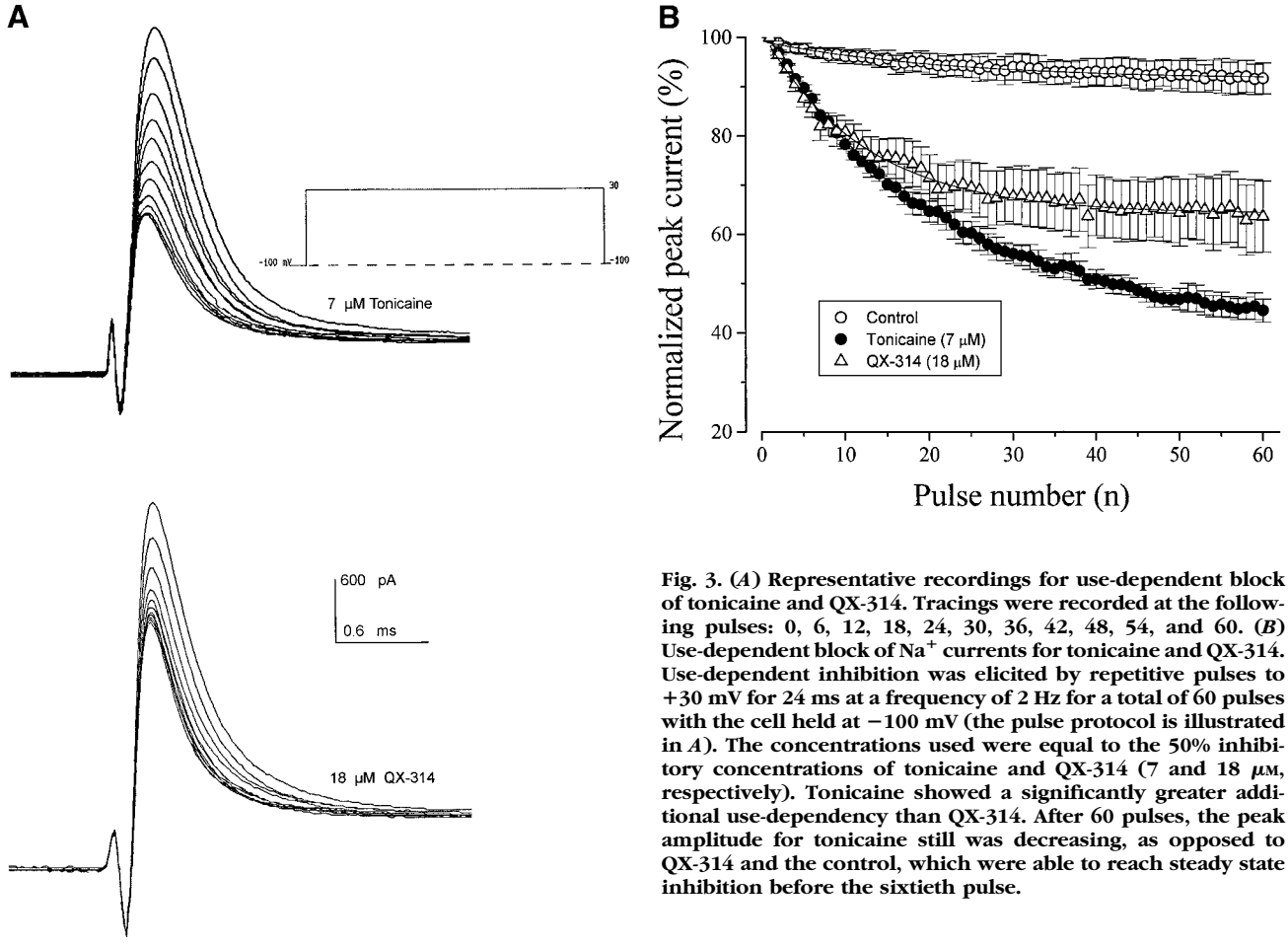


Fig. 3. (*A*) Representative recordings for use-dependent block of tonicaine and QX-314. Tracings were recorded at the following pulses: 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60. (*B*) Use-dependent block of Na^+ currents for tonicaine and QX-314. Use-dependent inhibition was elicited by repetitive pulses to +30 mV for 24 ms at a frequency of 2 Hz for a total of 60 pulses with the cell held at −100 mV (the pulse protocol is illustrated in *A*). The concentrations used were equal to the 50% inhibitory concentrations of tonicaine and QX-314 (7 and 18 μM, respectively). Tonicaine showed a significantly greater additional use-dependency than QX-314. After 60 pulses, the peak amplitude for tonicaine still was decreasing, as opposed to QX-314 and the control, which were able to reach steady state inhibition before the sixtieth pulse.

of the fast onset of blockade in the lidocaine and bupivacaine groups, and in the tonicaine group, and because of overlapping residual effects of sevoflurane inhalation anesthesia, we were unable to detect differences in onset of motor, proprioception, or sensory blockade.

Duration of Complete Blockade. The duration of complete blockade for motor function, proprioception, and nocifensive reaction to pinching were longer with tonicaine (9.2 ± 1.5, 11.7 ± 2.1, and 17.5 ± 2.8 min, respectively; n = 6 at 0.5 mM) than with bupivacaine (6.7 ± 2.1, 7.8 ± 1.4, and 10.0 ± 2.2 min, respectively; n = 6 at 28.8 mM) or lidocaine (7.0 ± 1.9, 7.8 ± 1.9, and 9.5 ± 2.1 min, respectively; n = 6 at 37 mM; fig. 4). Only the duration of complete blockade of nocifensive reactions, however, was statistically significantly longer for tonicaine than for bupivacaine or lidocaine; there was no statistically significant difference for motor function and proprioception. As noted with rat sciatic nerve injections, the sensory blockade by spinal tonicaine is longer than the motor blockade; lidocaine does not elicit such a differential blockade. Although bupivacaine shows a longer complete blockade of sensory than of motor functions, this is not statistically significant; tonicaine elicits a statistically significantly longer sensory blockade than motor blockade.

Regression of Blockade until Full Recovery of Functions. In contrast to the duration of complete blockade, the time needed for full recovery of all tested functions was statistically significantly longer for tonicaine than for lidocaine or bupivacaine (fig. 5). Notably, full recovery of sensory function took 6.4 times longer in the tonicaine group (112.5 ± 16.3 min) than in the lidocaine group (17.5 ± 2.8 min) and 3.2 times longer than in the bupivacaine group (35.0 ± 5.0 min). After the blockade resolved completely, no neurobehavioral abnormalities were detectable.

Half the animals in the tonicaine group showed a slight hyperreflexia to pinching in the area of blockade regression, which resolved completely by the time the blockade wore off. A similar effect, albeit shorter, was observed in 50% of animals in the bupivacaine group. No such hyperreflexia was observed after intrathecal lidocaine injection. A prolonged blockade of sensation to pinching of the distal part of the tail was seen in three animals in the tonicaine group, which fully recovered within 5 days, and in three animals of the bupivacaine group, with full recovery within 3 days.

Morphologic Changes after Spinal Tonicaine. At higher concentrations of tonicaine (1.0 mM) there were increasingly severe axonal degeneration in peripheral nerve roots (fig. 6) and degenerative changes in the spinal cord. In contrast, in all animals evaluated from the 0.5 mM (n = 4) and 0.625 mM (n = 5) injection groups, the spinal cord showed no abnormalities, and the number of degenerating fibers in peripheral nerve roots was minimal (single or several degenerating fibers in one or two roots of many roots evaluated; fig. 6). Similar minimal changes were found in the bupivacaine group: one animal showed two nerve roots of nearly 30 cross-sections with focal small clusters of degenerating axons, and another had two or three degenerating axons in the spinal cord and one degenerating axon in approximately 30 nerve cross-sections. Tonicaine at a concentration of 0.75 mM showed severe axonal degeneration in one or two nerve roots; however, tonicaine at 1 mM showed severe axonal degeneration in more than half of the many roots sampled. The histology of the animals in the dextrose in 5% water group was normal for spinal cord and roots.

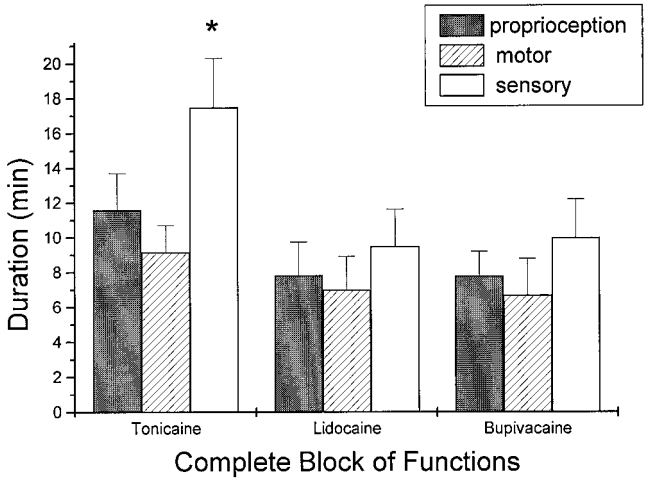


Fig. 4. Duration of complete blockade of proprioceptive, motor, and sensory block in minutes (mean ± SEM) after spinal injection of 0.5 mM tonicaine and 28.8 mM bupivacaine or 37 mM lidocaine. *$P < 0.05$ compared with lidocaine or bupivacaine.

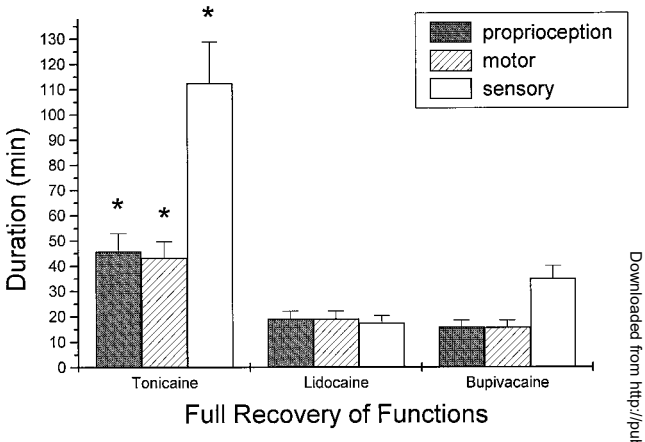


Fig. 5. Duration to full recovery of proprioceptive, motor, and sensory block in minutes (mean ± SEM) after spinal injection of 0.5 mM tonicaine and 28.8 mM bupivacaine or 37 mM lidocaine. *$P < 0.05$ compared with lidocaine or bupivacaine.

Discussion

In Vitro *Experiments*

External *N*-β-phenylethyl lidocaine (tonicaine), although it is a permanently charged quaternary ammonium compound, is able to cross the membrane barrier, probably because its additional hydrophobic arm shields the positive charge. Such a permanently charged amphipathic molecule is also a more potent Na^+ channel blocker than its parent drug lidocaine, as shown by its higher affinity to Na^+ channels in the dose–response study. The ease with which this drug can be trapped within the cytoplasm may contribute to its prolonged mode of local anesthetic action *in vivo*. Accordingly, it could be that the high affinity of tonicaine and its high intraneural concentration contribute significantly to the prolonged duration of blockade. A future ^{14}C radiolabeling of tonicaine and subsequent determination of intraneural uptake of tonicaine may help to clarify the importance of intraneural local anesthetic trapping.[21]

In addition to its higher tonic block of Na^+ current, tonicaine also elicits significantly higher use-dependent blockade than does QX-314 or lidocaine. After the sixtieth pulse, the use-dependent blockade of tonicaine was more than 50%, an indication that tonicaine may be especially valuable for pathologic pain states, which are characterized by high rates of spontaneous discharge.

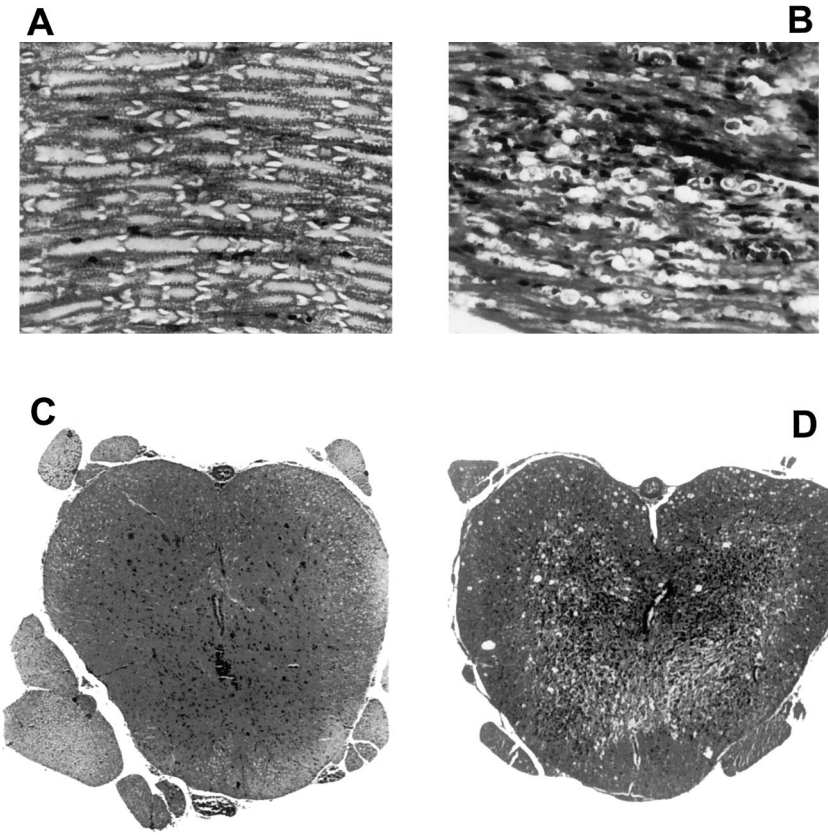


Fig. 6. Histologic sections of the spinal cords and peripheral nerve roots of rats after spinal injection of tonicaine. (*A*) Peripheral nerve root with normal histologic appearance, 2 weeks after injection of 90 μl tonicaine, 0.625 mM, (hematoxylin and eosin, 435×). (*B*) Peripheral nerve root showing severe axonal degeneration, 2 weeks after injection of 90 μl tonicaine, 1.0 mM (hematoxylin and eosin, 435×). (*C*) Spinal cord with normal histologic appearance, 2 weeks after injection of 90 μl tonicaine, 0.625 mM (hematoxylin and eosin, 60×). (*D*) Spinal cord with central necrosis and neuronophagia, 2 weeks after injection of 90 μl tonicaine, 1.0 mM (hematoxylin and eosin, 70×).

In Vivo *Experiments*

Tonicaine (37 mM, 0.1 ml) has been shown to achieve a complete and prolonged sciatic nerve block in rats for a duration of 3–7 h, with the sensory blockade lasting longer than the motor blockade. Our results indicate the same longer duration of sensory than of motor blockade with spinal application of tonicaine in rats. Therefore, this drug potentially offers an advantage over other local anesthetics, such as lidocaine or bupivacaine, in that it has a more sustained duration of action, thereby reducing the need for subsequent administrations, and also has a beneficial ratio of sensory to motor blockade. This ratio is important for the management of postoperative and chronic pain, but it is particularly appealing for obstetric anesthesia, because it affords appropriate sensory blockade and yet allows patient mobility and active participation to remain undiminished by motor blockade.

Limitation of Rat Spinal Injection Model

We chose our single-shot injection technique instead of the various catheter techniques[22–24] because our pilot studies at a concentration of 10 mM with a catheter technique resulted in permanent and more severe neurologic deficit than that observed after single-shot tonicaine injection. Therefore, we avoided the confounding variable of catheter-induced neurologic damage. Conversely, bupivacaine application with the single-shot technique also causes modest histologic changes in the spinal cord, as evident through the finding of minimal degenerative findings in the spinal cord. The major drawback of this model appears to be that the spinal cord in rats, as opposed to humans, does not end at vertebral level L2 but extends into the entire sacral part of the vertebral column.[25] In addition, there are technical difficulties in confirming the intrathecal space because of the inability to aspirate cerebrospinal fluid in our model. Therefore, it is possible that a variable dose of drug was injected not into the subarachnoid space, but into the spinal cord itself.

In the tonicaine group, a partial sensory blockade at the distal portion of the tail lasted up to 5 days. A similar finding, albeit of shorter duration, was made in the

bupivacaine group but not in the lidocaine group. Because this finding represented only minimally detectable sensory blockade, the overall shorter duration of lidocaine made it probably impossible to detect this subclinical changes with lidocaine. Nevertheless, this prolonged blockade seems to be similar to the various reports of transient radicular irritation and cauda equina syndrome after single-shot lidocaine injection in humans.[26–29] Whether transient neurologic symptoms represent the lower end of a spectrum of toxicity is speculative, and the therapeutic index of lidocaine is undergoing much discussion.[30]

The cause of this prolonged partial blockade is unclear. Because partial sensory blockade of the tail recovered in animals of the tonicaine group within 5 days and in those of the bupivacaine group within 3 days, it is unlikely that it was achieved by axonal regeneration, because this would necessitate a much longer time period.[31] In clinical practice it is well-known that patients experience a prolonged sensation of numbness in the sacral area after otherwise complete resolution of lumbar subarachnoid block. The nerves that innervate this distal part of the tail may be more sensitive to the effects of tonicaine, provided that tonicaine is more easily trapped in some nerve fibers than in others. Alternatively, our injection method may deliver a higher concentration of drug more closely to the nerve fibers innervating the tail.

Tonicaine at 0.5 mM and also bupivacaine at 28.8 mM may cause degeneration of a small number of nerve fibers, as suggested by the finding of infrequent degenerating fibers in peripheral nerve roots of these rats. Similar histologic findings were found with 4% lidocaine with intrathecal injections in rabbits without any detectable neurologic deficit.[32] Such a low frequency of nerve fiber degeneration, distributed evenly over the intrathecal nerve roots, may have a greater effect on sacral roots than on lumbar roots, which have the largest intrathecal segments. The nerve fiber degeneration may be part of a neuronopathy, implying that the cause of the axon degeneration is a direct injury to the neuronal cell body by at least partial injection into the spinal cord.[33] This injury probably is not caused by the needle trauma itself, but rather is the effect of a relatively undiluted highly potent local anesthetic (tonicaine or bupivacaine) because this result was not seen with the control group (glucose 5%) injection. It is feasible that a subclinical injury is unmasked by a potent local anesthetic.

Half the rats in the tonicaine group developed temporary hyperreflexia to pinching, which was most prominent during the beginning of blockade regression, an effect also seen in the bupivacaine group but not in the lidocaine group. In rats, it is possible that this phase is detectable only with tonicaine and bupivacaine because their greater potency and prolonged block duration allow such behavior changes to be observed more readily. The anatomy of rats, *i.e.*, the relation of the injection site and the spinal cord,[25] may be the primary reason for this observed hyperreflexia. The drug may spread to the spinal cord and affect interneurons, which serve important integrative functions as part of multisynaptic spinal reflexes and feedback circuits.[34,35]

The concentration of 0.5 mM tonicaine was chosen because higher concentrations (1, 2.5, and 5.0 mM; data not included) often have been associated with permanent complete block to midthoracic levels. This finding may be related to pathologic spinal cord damage seen in animals receiving high doses. Although these animals showed block regression, it is unclear whether they would have fully recovered because some animals with a persistent high block developed hematuria at day 3 and therefore were killed using an overdose of sevoflurane no more than 3 days after spinal injection. This observed hematuria most likely did not result from direct kidney toxicity because it did not occur after rat sciatic nerve block, for which much higher doses (> 70-fold) were used.

In general, it appears quite difficult to correlate histopathologic changes with clinically detectable neurologic deficit because rats receiving spinal lidocaine injections were shown to develop conduction block and no clinical nerve damage, but histopathologic changes already were detectable at a concentration of 0.5%,[36] and residual paralysis at 1.5% was shown without correlation of histologic and clinical findings.[37] In addition, persistent sacral sensory deficits can be associated with variable degrees of motor impairment in the tails at a concentration of 5%.[38] Similarly, with intrathecal injections in rabbits it was found that 2% lidocaine caused no histologic lesions, but two of five animals showed lesions of the spinal cord already at a 4% concentration of lidocaine, and some neurologically normal animals showed marked histologic changes in the cauda equina.[32]

Tonicaine at a concentration of 0.5 mM caused neither neurologic deficit nor significant nerve fiber degeneration, as shown by morphologic evaluations. It does appear, however, to have a relatively small therapeutic range if injected at the L4–L5 intervertebral space,

which may reflect the fact that the injection site is above the level of the cauda equina.[25]

We conclude that tonicaine is a very potent long-acting local anesthetic that significantly differentially prolongs sensory blockade over motor blockade in rats. The narrow therapeutic range seems to be a major problem in our rat model, perhaps because of injection directly to the spinal cord rather than into the cerebrospinal fluid. Our added *in vivo* studies suggest that tonicaine has substantial neurotoxicity and may have limited clinical value. Further investigation in different animal models, in which the possibility that the findings result from direct spinal cord injection is diminished because of better anatomic correlation with humans, *e.g.*, sheep, is necessary to clarify the therapeutic range of this drug.

The authors thank Marina Vladimirov, M.D., Ph.D., and Klaus Auktor for technical assistance, and Gary Strichartz, Ph.D., for helpful discussions.

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