Anesthesiology
83:1293–1301, 1995

Lippincott–Raven Publishers

Quaternary Ammonium Derivative of Lidocaine as a Long-acting Local Anesthetic

G. K. Wang, Ph.D., C. Quan, B.S.,† M. Vladimirov, M.D., Ph.D.,‡ W. M. Mok, Ph.D.,‡ J. G. Thalhammer, Dr.Med.Vet.§*

Background: **Use of long-acting local anesthetics that elicit complete neural blockade for more than 3 h often is desirable in pain management. Unfortunately, clinically available local anesthetics are in general not suitable for prolonged analgesia. This report describes the organic synthesis and functional testing of a lidocaine derivative that appears to fulfill the criteria of long-acting local anesthetics.**

Methods: **A lidocaine derivative, N-β-phenylethyl lidocaine quaternary ammonium bromide, was synthesized, and its ability to inhibit Na^+ currents in cultured rat neuronal GH_3 cells was tested *in vitro* under whole-cell voltage clamp conditions. Neurologic evaluation of sciatic nerve block of sensory and motor functions *in vivo* was subsequently performed in rats.**

Results: **N-β-phenylethyl lidocaine was found to be a potent Na^+ channel blocker *in vitro*. It produced both tonic and use-dependent blocks of Na^+ currents that exceeded lidocaine's effects by a factor of >2 ($P < 0.05$). *In vivo,* N-β-phenylethyl lidocaine elicited a prolonged and complete sciatic nerve block of the motor function and the withdrawal response to noxious pinch that was 3.6- and 9.3-fold longer than that of lidocaine ($P < 0.001$), respectively.**

Conclusions: **In an attempt to elicit prolonged local anesthesia, a quaternary ammonium derivative of lidocaine containing a permanent charge and an additional hydrophobic component was synthesized. Complete sciatic neural blockade of more than 3 h was achieved with this derivative. Of note, sensory blockade was prolonged to a greater extent than motor blockade. The approach used in this study may prove useful for developing new drugs applicable in pain management. (Key words: Anesthetics, local: N-β-phenylethyl lidocaine.)**

* Associate Professor of Anesthesia.

† Research Assistant.

‡ Instructor in Anesthesia.

§ Assistant Professor of Anesthesia.

Received from the Department of Anesthesia Research Laboratories, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts. Submitted for publication March 24, 1995. Accepted for publication August 29, 1995. Supported by National Institutes of Health grants GM35401 and GM48090 to Dr. Wang.

Address reprint requests to Dr. Wang: Department of Anesthesia, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

DURING the last two decades, the search has been on for new local anesthetics that produce analgesia of long duration, minimal impairment of autonomic function, and low toxicity.[1,2] Such drugs are highly desirable for pain management. Attempts to develop this new class of local anesthetics have so far yielded mixed results. For example, both bupivacaine and etidocaine are considered to be long-acting local anesthetics; the duration of the major nerve block by these two local anesthetics is about 3–12 h.[1] Unfortunately, both local anesthetics are also highly cardiotoxic.[2] Amino amide local anesthetic cyclization was invented in the early 1970s,[3] but this type of local anesthetic has not evolved for clinical use, probably because cyclization requires a haloalkyl amine component that is also a cancer-causing agent and because the chemical reaction of intramolecular cyclization often occurs before penetration of the nerve membrane. Finally, alkyl triethyl quaternary ammonium (QA) ions were reported to produce sensory block of rat infraorbital nerves for several days or weeks after injection.[4] Later studies, however, revealed that this type of compound gives rise to severe morphologic damage, loss of myelinated axons, and axonal edema within 4 weeks of treatment.[5]

What are the structural determinants of local anesthetics that cause reversible blockade of neural functions? Traditional local anesthetics, exemplified by lidocaine (fig. 1), contain a tertiary amine separated at a distance of 6–9 Å from a benzene ring by an intermediate chain. The intermediate chain is usually linked by either an ester (-COO-) or an amide (-CONH-) bond to the benzene ring. As a tertiary amine, lidocaine can be hydrophilic when it is protonated and can be hydrophobic when it is in its neutral form. If lidocaine is made permanently charged, such as its derivative QX-314 (fig. 1), the compound becomes inactive when applied externally.[6] Internal application of QX-314, however, elicits profound block of Na^+ channels, an indication that the charged form of lidocaine is an active form.[6] This result also suggests that external QX-

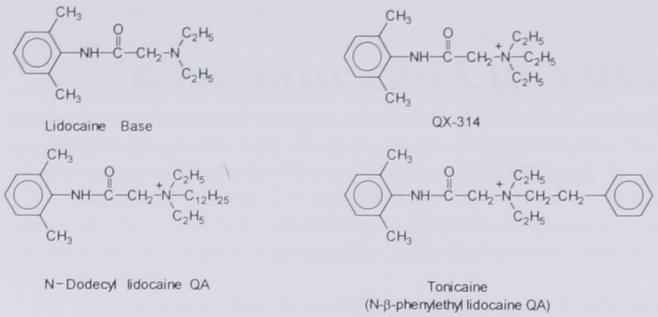

Fig. 1. Chemical structures of lidocaine and its derivatives.

314 cannot readily cross the cell membrane to reach its receptor site on the Na^+ channel.

Tertiary amine local anesthetics including lidocaine produce both tonic and use-dependent block of Na^+ channels. Tonic block is defined as the block of Na^+ channels in their resting state while the nerve is stimulated infrequently, whereas use-dependent block is the additional block of Na^+ channels in their activated states while the nerve is stimulated repetitively (usually $\geq$1 Hz). The underlying mechanism for these two types of block has been intensively studied under voltage clamp conditions. Hille[7] proposed a Modulated Receptor Hypothesis to explain these two types of Na^+ channel block elicited by local anesthetics. He hypothesized that the local anesthetic receptor site on the Na^+ channels is modulated by depolarization. The local anesthetic receptor site changes its configuration as functions of membrane potential and time (*i.e.*, voltage- and time-dependent conformational changes). At different states, the local anesthetic receptor is thought to have different affinity toward local anesthetics.

The ability of local anesthetics to block Na^+ currents therefore is relevant to the topology of the local anesthetic receptor in the Na^+ channel, because the higher the affinity of Na^+ channel blocker, the stronger the interaction with its receptor. Several additional findings on the structure-activity relationships of local anesthetics have now emerged from these studies.[8–12] First, there are two hydrophobic local anesthetic binding domains in the Na^+ channel.[8,10] Each binding domain can accommodate up to a 12-hydrocarbon chain. External amphipathic QAs apparently can cross the membrane barrier to reach their internal receptor site, albeit the rate of cross is slower than that of the traditional local anesthetics. Second, the neutral form of tertiary amine is in general far less potent.[12] Third, the permanently charged amphipathic local anesthetics, such as the products of cyclization of a haloalkyl amine local anesthetic compound, are easily "trapped" within the cell after external application.[9,11] These basic findings may permit us to develop potent Na^+ channel blockers. The purpose of this study was (1) to synthesize a long-acting lidocaine derivative, and (2) to test this drug both *in vivo* and *in vitro*.

Materials and Methods

Chemicals

Lidocaine base was purchased from Sigma Chemical Co. (St. Louis, MO); 1-bromododecane and (2-bromoethyl)benzene were from Aldrich, Chemical Company, Inc. (Milwaukee, WI). QX-314 chloride was donated by Astra Pharmaceutical Products (Worcester, MA). Silica gel G was obtained from Brinkmann Instruments, Inc. (Westbury, NY). All other chemicals were reagent grade from commercial sources.

Synthesis of Tonicaine

The conventional method for QA synthesis was used to modify the lidocaine structure. Tonicaine was synthesized from lidocaine (base) and (2-bromoethyl)benzene. A 2:1 molar ratio of lidocaine and (2-bromoethyl)benzene were refluxed at 80–90°C in absolute ethanol for 5 days. Excess ethanol was evaporated. The product was washed several times each with 25 ml warm hexane (60°C). Residual hexane was removed under vacuum. The product, N-β-phenylethyl lidocaine bromide [diethyl-(2,6-dimethylanilinocarbonyl)methyl-β-phenylethyl ammonium bromide], was purified by silica gel column chromatography. The mobile phases were chloroform/ethyl acetate (13/1, vol/vol) followed by chloroform/ethanol (80/20, vol/vol). Tonicaine was eluted in the chloroform/ethanol. The eluted fractions containing the product were pooled and the solvents were removed under vacuum. The product was >98% pure as judged by thin layer chromatography systems. Thin layer chromatography systems employed in the synthesis were normal phase thin layer chromatography plates (Fisher Scientific, PA) developed with ethanol, 96% ethanol/0.8 M NH_4Cl (80/20, vol/vol), or chloroform/ethyl acetate (13/1, vol/vol). The practical yield was 39%. Structural analysis of tonicaine by mass spectrometry yielded a molecular mass of 339.2, which is consistent with the structure of tonicaine cation shown in figure 1.

Synthesis of N-dodecyl Lidocaine Quaternary Ammonium

N-dodecyl lidocaine was synthesized from lidocaine (base) and 1-bromododecane by the method similar to that for tonicaine synthesis (see above). The product was >98% pure as judged by thin layer chromatography systems. Structural analysis of N-dodecyl lidocaine QA by mass spectrometry yielded a molecular mass of 403.2, which agreed with the structure of N-dodecyl lidocaine QA shown in figure 1.

Cell Culture

Rat clonal pituitary GH_3 cells were purchased from the American Type Culture Collection (Rockville, MD) and were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Hyclone Labs, Logan, UT), as described by Cota and Armstrong.[13] For Na^+ current recording, cells were grown in a 35-mm culture dish, which was then used as a recording chamber.

Whole-cell Voltage Clamp

The whole-cell variant of the patch-clamp method[14] was used to measure Na^+ currents in GH_3 cells. The external solution contained (in mM) 150 choline Cl, 0.2 $CdCl_2$, 2 $CaCl_2$, and 10 hydroxyethylpiperazine-ethane sulfonic acid adjusted to pH 7.4 with tetramethyl hydroxide. Micropipettes were fabricated and had a tip resistance of ~1 MΩ when filled with an Na^+ solution containing (in mM) 100 NaF, 30 NaCl, 10 EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid), and 10 hydroxyethylpiperazineethane sulfonic acid adjusted to pH 7.2 with CsOH. The junction potential of electrodes was nulled before seal formation. A liquid junction potential of 1.6 mV for the 0-mM-Na^+ external solutions[15] was not corrected in this report. After the rupture of the patch membrane, the cell was allowed to equilibrate with the pipette solution for at least 15 min at the holding potential of −100 mV. Under these reversed Na^+ gradient conditions, outward Na^+ currents were activated at approximately −30 mV. The advantages of using the reversed Na^+ gradient have been discussed by Cota and Armstrong.[13] Tonicaine, N-dodecyl lidocaine QA, and QX-314 at appropriate concentrations, were applied to cells with a flow rate of about 0.12 ml/min *via* a series of narrow-bored capillary tubes positioned within 200 μm of the cell. Washout of drugs was performed *via* a tube containing the external solution without drug present. Voltage-clamp protocols were created with pClamp software (Axon Instruments, Inc., Foster City, CA). Leak and capacitance were subtracted by a leak and capacity compensator as described by Hille and Campbell.[16] Additional compensation was achieved by the patch clamp device (EPC7, List-Electronic, Darmstadt/Eberstadt, Germany). All experiments were performed at room temperature (23 ± 2°C). At the end of the experiments, the drift in the junction potential was generally <2 mV.

Neurologic Evaluation of Sciatic Nerve Block in the Rat

The following protocols have received approval from the Harvard Medical Area Standing Committee on Animals. The observations of all sciatic nerve functions were made under free behavior conditions. The integrity of neurologic functions was examined in handled rats. Handling reduces stress during neurologic examination. Measurements of functional impairment were performed by comparison of functions before and after injection. Suitable anatomic landmarks (greater trochanter and ischial tuberosity[17]) easily located by palpation make it possible to administer the studied drug with precision. Changes of function were estimated as percentage of maximal possible effect (% MPE) before and at different times after drug administration. Complete block of function was considered 100% MPE, no change in function 0% MPE. Motor function, proprioception, and nociception were evaluated at 10 min before, at 5, 10, 30, and at every 30 min until full recovery after injection of 0.1 ml tonicaine (n = 14, over a period of 9–12 h) and at 10 min before and at 1, 5, 10, 20, 30, and every 15 minutes until full recovery after injection of 0.1 ml lidocaine (n = 6, over a period of 3 h) at the sciatic notch. Six of the rats were injected with lidocaine (1%) and three with isotonic saline at the contralateral side and 24–36 h before the injection of tonicaine. The molar concentration was identical for tonicaine and lidocaine at 42.67 mM in saline. Throughout the experiment all animals were observed for abnormalities in mental status and free motor behavior, *e.g.*, alertness, responsiveness to environment, motor activity, gait, and resting posture. The observer was blinded to the type of drug or saline solution injected. Details of the functional evaluation can be found in Thalhammer *et al.*[18]

Proprioception. The evaluation was based on resting posture, gait, and postural reactions such as "hopping" and "tactile placing"[18,19] and measured by scores from

3 (normal posture and gait) to 0 (full absence of postural reactions). Full absence of postural reactions was considered 100% MPE.

Tactile Placing. The toes of one foot were ventroflexed and their dorsi were placed onto the supporting surface, while the animal was kept in normal resting posture. The ability to reposition the toes was evaluated.

Hopping. The front half of the animal was lifted off the ground to allow the body's weight to be supported by their hind limbs. Then one hind limb at the time was lifted off the ground surface and the animal's body was moved laterally. The ability of the animal to follow the lateral movement of the body by hopping with the weight-supporting limb was evaluated.

Motor function of hind limbs was evaluated by the "extensor postural thrust." The rat was held upright so that the hind limbs were extended and the body's weight was supported by the distal foot. The force necessary to bring the heel in contact with the platform of a balance was measured (in grams). The reduction in force, resulting from reduced extensor muscle tone, was considered motor deficit. A force <15 g was considered absence of extensor postural thrust or 100% motor block.[18]

Nociception was evaluated by measuring the latency (heat) or amplitude (pinch) of withdrawal response to noxious heat and noxious mechanical stimulation. Care was taken to avoid tissue injury resulting in hyperanalgesia by properly spacing the stimulations and by a 10-s cutoff time for heat stimulation.[18]

Withdrawal Response to the Heat Stimulation (Heat WR). Latency measurement of the withdrawal response to the application of a hot (51.0 ± 0.5°C) handheld metal probe through which hot water was circulated to the dorsolateral surface of the metatarsus. Absence of withdrawal to 10-s stimulation was considered 100% MPE.

Withdrawal Response to Pinch (Pinch WR). The fifth toe was pinched (to 300 g) with a force-calibrated serrated forceps for 2 s[18] and the WR was graded as 4 (normal, brisk generalized motor reaction, withdrawal of the stimulated hind limb, attempts to bite forceps, and vocalization); 3 (like 4, but slower than on the control side); 2 (like 3, but with one of the responses lacking, *e.g.*, no vocalization or no general motor reaction, only turning of the head); 1 (only weak attempt to withdraw); or 0 (no response).

Statistical Analysis

Results of analyses from voltage clamp experiments are presented as mean ± SE. An unpaired Student's *t* test and a one-way analysis of variance (SigmaStat, Jandel Scientific Software, San Rafael, CA) were used to evaluate the significance of changes produced by the drugs on the tonic and the use-dependent block, respectively. $P < 0.05$ was considered statistically significant.

Magnitude of functional changes from neurologic evaluations is expressed in % MPE, mean ± SE. Duration of functional changes is expressed in minutes, mean ± SE. Validity of differences was tested by an unpaired Student's *t* test.

Results

Tonicaine as a Potent Na^+ Channel Blocker

Like lidocaine, tonicaine is a Na^+ channel blocker but with a potency far greater than that of its parent compound. Tonicaine when applied externally to cultured neuronal GH_3 cells tonically inhibited Na^+ currents by 55.0 ± 7.0% (n = 5) at the concentration of 100 μM for 30 min, whereas lidocaine usually inhibited currents by 27.1 ± 6.8% (n = 7, fig. 2A). As for most local anesthetics, the block of Na^+ currents by lidocaine reached its steady state within 1.5 min. In contrast, the time course of the block by tonicaine was much slower than that by lidocaine at 100 μM (figs. 2B and 2C). Because of its permanently positive charge, it is likely that tonicaine does not cross the cell membrane as easily as lidocaine, which can be in a neutral form at physiologic pH.[7] Tonicaine continued to inhibit Na^+ currents by 80–90% after 1 h of treatment. Due to this slow on-rate of tonicaine, we were unable to perform the conventional dose-response study, because the time required for tonicaine to reach steady state effect at lower concentrations would be prohibitively long for the voltage clamp experiments. Preincubation of cells with this drug, unfortunately, would prevent us from determining the current amplitude without drug.

Trapping the Charged Compound within the Cell

The tonic block elicited by tonicaine was only partially and slowly reversed after continuous perfusion of a drug-free external solution. Figures 3B and 3C shows that Na^+ current was inhibited by about 80% by 100 μM tonicaine for over 45 min and recovered to about 55% of the control value after a 30-min wash. In contrast, the blocking effect of lidocaine at 100 μM was quickly and often completely reversed by washing (figs. 3A and 3C). The half times of washout for lidocaine and tonicaine were <30 s and ~30 min, respectively.

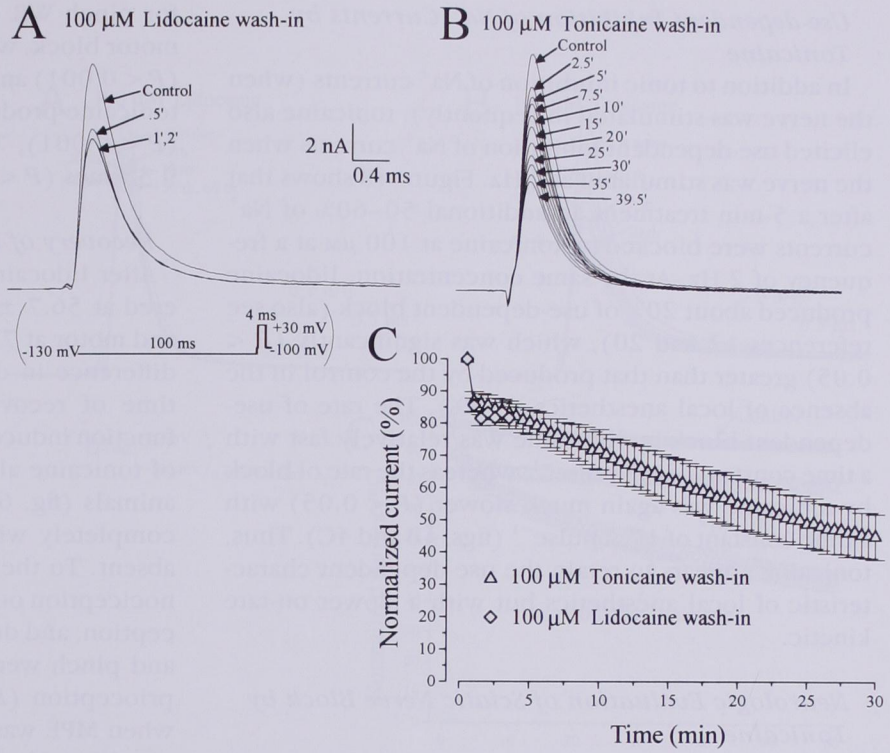

Fig. 2. Tonic inhibition of Na^+ currents by lidocaine and tonicaine. The tonic effects of lidocaine (100 μM) and tonicaine (100 μM) on Na^+ currents were studied using reverse Na^+ gradient. (*A*) The rate of onset for lidocaine was relatively fast. Steady-state concentration was achieved less than 1.5 min from administration of the drug. The holding potential was set at −100 mV. Prepulse was set at −130 mV for 100 ms, followed by an interpulse of −100 mV for 0.2 ms, and then a test pulse of +30 mV for 4 ms. This pulse protocol is illustrated at the bottom. (*B*) Tonicaine, conversely, had a slower rate of onset and did not reach steady-state readily, even after 40 min of continuous perfusion (100 μM). (*C*) The time course of tonicaine block. The Na^+ currents before and during administration of tonicaine (100 μM) were measured at 30-s intervals. The peak currents were normalized and plotted against time. The peak current was inhibited by 55.0 ± 7.0% (n = 5) after 30 min of drug administration. This magnitude of inhibition by tonicaine is significantly different from that produced by lidocaine (27.1 ± 6.8%, n = 7; $P < 0.05$).

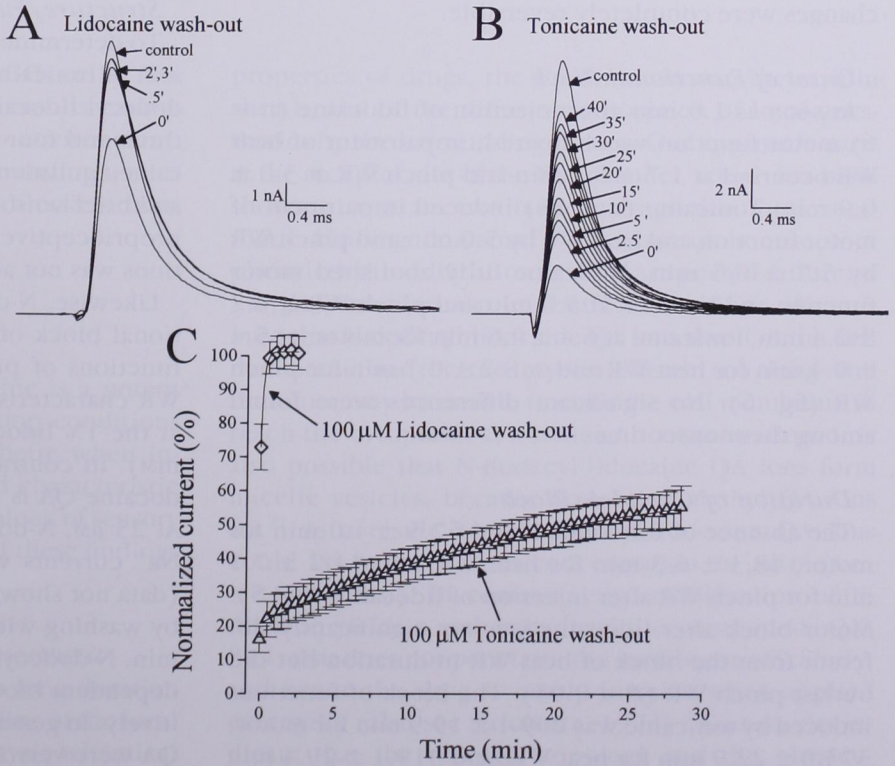

Fig. 3. Reversal of tonic block. (*A*) Upon washing of the lidocaine-treated (100 μM) cell with a drug-free external solution, tonic block was completely reversed in approximately 1.5 min, and the peak Na^+ current was fully recovered. (*B*) Reversal of tonic block after administration of tonicaine (100 μM for longer than 45 min) was slow and incomplete. As shown in this set of traces, the current was still recovering after 40 min of washing with a drug-free external solution. The currents were measured at 30-s intervals by the same pulse protocol as described in figure 2. (*C*) The normalized percentage of peak current recovered is plotted against time. The current amplitude blocked by lidocaine recovered rapidly (103.3 ± 5.0%; n = 7) within 1 min of washing with external solution. After 28.5 min of washing, cells exposed to tonicaine regained only 46.7 ± 6.2% (n = 5) of the blocked current amplitude. Solid lines drawn through data points are best-fit of single exponential functions. The estimated recovery time constants were 0.18 ± 0.01 min and 20.8 ± 1.2 min for lidocaine and tonicaine, respectively. The difference in these two time constants is statistically significant ($P < 0.05$).

Use-dependent Inhibition of Na^+ Currents by Tonicaine

In addition to tonic inhibition of Na^+ currents (when the nerve was stimulated infrequently), tonicaine also elicited use-dependent inhibition of Na^+ currents when the nerve was stimulated at 2 Hz. Figure 4B shows that after a 5-min treatment an additional 50–60% of Na^+ currents were blocked by tonicaine at 100 μM at a frequency of 2 Hz. At the same concentration, lidocaine produced about 20% of use-dependent block (also see references 12 and 20), which was significantly ($P < 0.05$) greater than that produced by the control in the absence of local anesthetics (~8%). The rate of use-dependent block by lidocaine was relatively fast with a time constant of 1.7 $pulse^{-1}$, whereas the rate of block by tonicaine was again much slower ($P < 0.05$) with a time constant of 17.2 $pulse^{-1}$ (figs. 4B and 4C). Thus, tonicaine appears to retain the use-dependent characteristic of local anesthetics but with a slower on-rate kinetic.

Neurologic Evaluation of Sciatic Nerve Block by Tonicaine

In no instance was death or any visible impairment of mental status, ataxia, or posture abnormality in the noninjected limbs observed. All neurobehavioral changes were completely reversible.

Onset of Functional Block

As soon as 1.0 min after injection of lidocaine (n = 6) motor function was impaired; impairment of heat WR occurred at 1.7 ± 0.7 min and pinch WR at 3.0 ± 0.9 min. Tonicaine (n = 14) induced impairment of motor function and heat WR by 5.0 min and pinch WR by 5.7 ± 0.5 min. Lidocaine fully abolished motor function and heat WR at 5.0 min and pinch WR at 9.2 ± 2.4 min, tonicaine at 6.4 ± 0.6 min for motor, at 5.4 ± 0.4 min for heat WR and at 8.2 ± 0.7 min for pinch WR (fig. 6). No significant differences were found among these onset times.

Duration of Complete Block

The absence of functions lasted 57.5 ± 4.0 min for motor, 48.3 ± 6.3 min for heat WR, and 34.2 ± 7.1 min for pinch WR after injection of lidocaine (fig. 5). Motor block after lidocaine was not significantly different from the block of heat WR in duration but did outlast pinch WR ($P < 0.05$). The block of functions induced by tonicaine was 209.1 ± 19.9 min for motor, 373.0 ± 22.9 min for heat WR, and 319.1 ± 29.4 min for pinch WR (fig. 5). The duration was shortest for motor block, which was shorter than block of heat WR ($P < 0.001$) and block of pinch WR ($P < 0.005$). Thus, tonicaine produced blocks 3.6 times longer for motor ($P < 0.001$), 7.7 times for heat WR ($P < 0.001$) and 9.3 times ($P < 0.001$) for pinch WR than lidocaine.

Recovery of Functions

After lidocaine injection pinch WR was fully recovered at 56.7 ± 6.4 min, heat WR at 59.3 ± 9.8 min and motor at 77.5 ± 4.6 min. There was no significant difference in duration of partial impairment (*i.e.*, in time of recovery) between motor and nociceptive function induced by lidocaine ($P > 0.1$). After injection of tonicaine all functions recovered in 9–24 h in all animals (fig. 6). Whenever nociception was blocked completely with lidocaine, all other functions were absent. To the contrary, with tonicaine, full block of nociception outlasted full block of motor and proprioception, and during recovery the % MPEs of WR to heat and pinch were higher than those of motor and proprioception ($P < 0.05$). At 300 min after injection, when MPE was 100% for heat WR, MPE of pinch WR was 86 ± 9%, motor 70 ± 7%, and proprioception 65 ± 7%.

Structure/Function Relationship.

To determine the role of the additional hydrophobic arm of tonicaine, *in vivo* injection of QX-314 and N-dodecyl lidocaine QA was subsequently performed in three and four rats, respectively. QX-314 at 1% lidocaine equivalent concentration elicits only incomplete and brief sensory and motor block. Complete block of proprioceptive, motor, and heat and pinch WR functions was not achieved.

Likewise, N-dodecyl lidocaine QA elicited no functional block of the sciatic nerve in rats. None of the functions of proprioceptive, motor, heat, and pinch WR characteristics were impaired by this compound at the 1% lidocaine equivalent concentration (42.67 mM). In contrast to this *in vivo* result, N-dodecyl lidocaine QA is a strong Na^+ channel blocker *in vitro*. At 25 μM, N-dodecyl lidocaine QA slowly blocked all Na^+ currents within 40 min of external application (data not shown). This tonic block was not reversible by washing with an external drug-free solution for 30 min. N-dodecyl lidocaine QA also elicited strong use-dependent block when the cell was stimulated repetitively. In general, the results from N-dodecyl lidocaine QA were very similar to those from the trimethyl C_{14}-

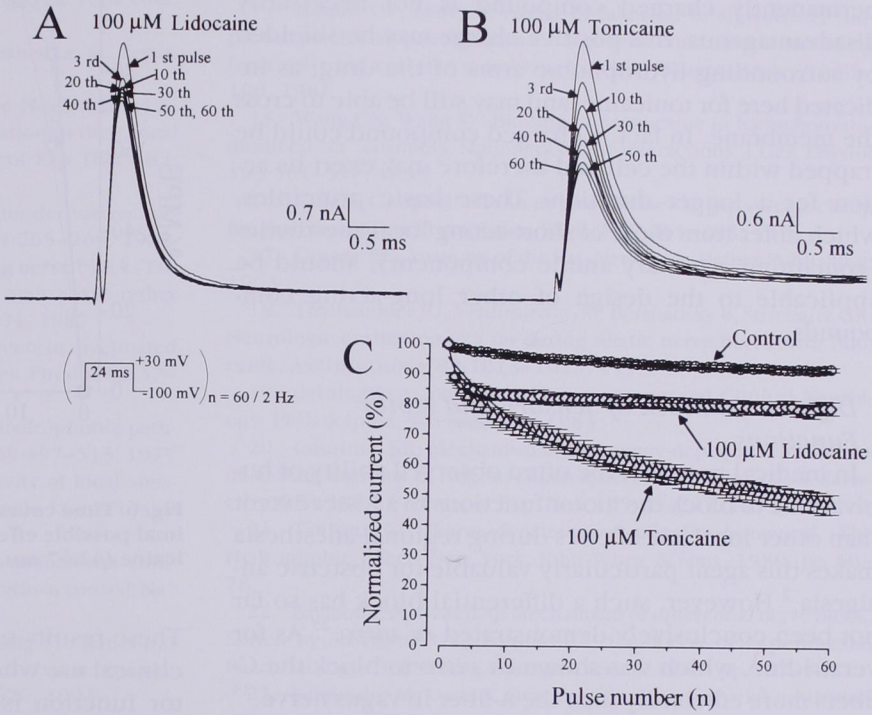

Fig. 4. Use-dependent inhibition of Na^+ current. Holding potential was set at −100 mV and the test pulse, repeated 60 times, was set at +30 mV for 24 ms at 2 Hz (see inset). Representative Na^+ current traces with repetitive pulsing at 2 Hz are shown in (*A*), after the cell was allowed to reach steady state with lidocaine (100 μM). Representative Na^+ current traces at 2 Hz, 5 min after the start of tonicaine (100 μM) administration, are shown in (*B*). Note that after 60 pulses the peak amplitude is still decreasing. (*C*) Before use-dependent inhibition in the presence of either drug was recorded, a control set of peak amplitudes were taken. The peak amplitudes of each data set were normalized with respect to the amplitude of the first pulse of the set. The normalized current was then plotted against pulse number. Under control condition there was 7.4 ± 0.7% reduction in peak amplitude with 60 pulses at 2 Hz (n = 18). There was 20.4 ± 2.2% reduction in peak amplitude in the presence of lidocaine (n = 11), while in the presence of tonicaine the reduction in peak amplitude was 52.4 ± 3.3% (n = 5). The differences among these three groups are statistically significant ($P < 0.05$). Unlike the curve for tonicaine, the ones representing the control and lidocaine indicate that the cells were able to reach steady-state inhibition well before the 60th pulse. Solid lines drawn through data points are best-fit of single exponential functions.

QA compound described previously in GH_3 cells.[9] Together they demonstrate that *in vitro* results cannot always be extrapolated to the *in vivo* environment. Neural sheaths, myelin sheath, and surrounding tissues may hinder some potent Na^+ channel blockers from reaching target sites *in vivo*.

Discussion

This report demonstrates that tonicaine is a potent Na^+ channel blocker under voltage-clamp conditions *in vitro* and a long-acting local anesthetic when injected into rats *in vivo*. An unexpected characteristic of tonicaine is that it elicits differential block of sensory and motor functions. The importance of these findings is discussed later.

Tonicaine as a Long-acting Local Anesthetic

The design of local anesthetics with a long duration of analgesia requires detailed understanding of the local anesthetic receptor topology; of the physicochemical properties of drugs, the lipid membrane, the myelin sheath, and of the diffusion/adsorption of local anesthetics by the surrounding tissues. Our approach starts at the receptor site of the Na^+ channel, which has been shown to contain two large hydrophobic local anesthetic binding domains.[8,10] The reason to use N-β-phenylethyl modification is the unexpected failure of $-C_{12}H_{25}$ modification in sciatic nerve block. We surmise that N-dodecyl-lidocaine QA ions dwell in the cell membrane and/or the myelin sheath too long and therefore may be unable to cross the cell membrane to reach the cytoplasm at a sufficient concentration. It is also possible that N-dodecyl-lidocaine QA ions form micelle vesicles, because dodecyl trimethyl QA ions do.[21] As a result, free N-dodecyl-lidocaine QA ions could be rather low in concentration *in vivo* (*i.e.*, $<<42.67$ mM). In contrast, ethyl-modification of lidocaine yields a rather hydrophilic compound, QX-314, that does not penetrate the membrane efficiently, and hence the drug is nearly inactive when applied externally (also see Strichartz, 1973).[6] Our results indicate that the receptor topology and the physico-

chemical properties of the drug are both important in determining the local anesthetic potency *in vivo*. A permanently charged compound is not necessarily disadvantageous; this positive charge may be shielded by surrounding hydrophobic arms of the drug, as indicated here for tonicaine, and may still be able to cross the membrane. In fact, a charged compound could be trapped within the cell and therefore may exert its action for a longer duration. These basic principles, which differ from those of short-acting local anesthetics (containing a tertiary amine component), should be applicable to the design of other long-acting compounds.

Differential Block of Sensory and Motor Functions

In medical practice, the often observed ability of bupivacaine to block the motor functions to a lesser extent than other local anesthetics during regional anesthesia makes this agent particularly valuable for obstetric analgesia.[2] However, such a differential block has so far not been conclusively demonstrated *in vitro*.[22] As for veratridine, which was shown *in vitro* to block the C-fibers more efficiently than the A-fiber in vagus nerve,[23] the *in vivo* experiments show no such differential block of sensory and motor functions in the sciatic nerve.[24] It appears that tonicaine can elicit a significant differential block of sensory and motor functions *in vivo* during recovery (with motor function and proprioception recovering to a greater extent than nociception).

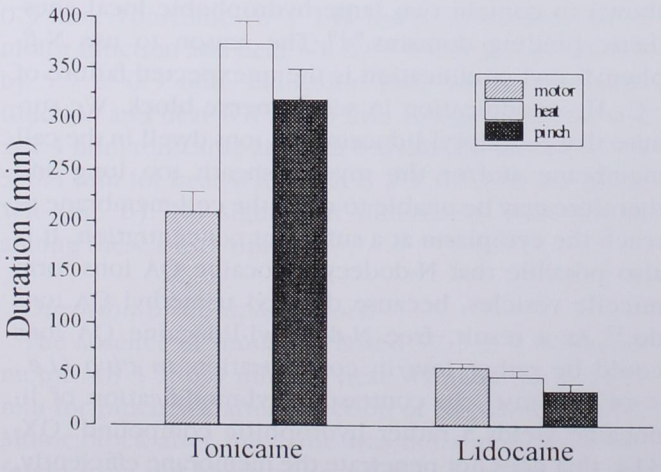

Fig. 5. Block duration in minutes (mean ± SE) of withdrawal response to noxious heat and noxious pinch and of motor function after injection of 0.1 ml lidocaine (42.67 mM, n = 6) or tonicaine (42.67 mM, n = 14).

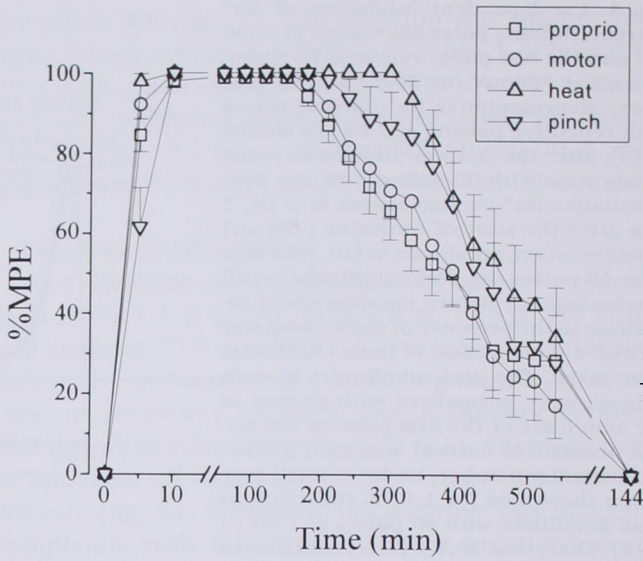

Fig. 6. Time course of functional impairment in percent maximal possible effect (mean ± SE) after injection of 0.1 ml tonicaine (42.67 mM, n = 14).

These results suggest that tonicaine may be of greater clinical use when differential block of sensory and motor function is desirable during regional anesthesia. Furthermore, tonicaine may provide a tool to dissect the underlying mechanisms of differential block, which until now remain elusive.

In Vivo Versus In Vitro *Block*

We have shown that tonicaine acts more slowly than lidocaine at 100 μM in blocking Na^+ currents in GH$_3$ cells (it takes longer than 1 h to reach steady-state concentration), possibly because of its permanent charge, which may hinder its ability to cross the cell membrane quickly. *In vivo* injection of tonicaine at 1% lidocaine equivalent concentration (42.67 mM), however, elicits complete functional block for pinch WR within 10 min. Perhaps the difference in effect between the two preparations is the result of differences in the effective concentration of tonicaine present in neuronal tissue between the *in vivo* and *in vitro* models. Other factors such as the permeability of the neural sheath by these compounds and the adsorption/diffusion of these compounds in the surrounding tissues are not clear and remain to be examined.

The authors thank Dr. G. Richard Arthur for his assistance on statistical analysis, Dr. Lin-Er Lin for her initial screening of lidocaine derivatives, Ms. Rachel Abrams for secretarial services, and Ms. Jaylyn Olivo for editorial services.

References

1. Covino BG, Vassallo HG: Preclinical aspects of local anesthesia, Local Anesthetics: Mechanisms of Action and Clinical Use. New York, Grune & Stratton, 1976, pp 44–53

2. deJong RH: On the horizon, in Local Anesthetics. St. Louis, Mosby Yearbook, 1994, pp 381–401

3. Ross SB, Akerman SBA: Cyclization of three N-ω-haloalkyl-N-methylaminoaceto-2,6-xylidide derivatives in relation to their local anesthetic effect *in vitro* and *in vivo*. J Pharmacol Exp Ther 182: 351–361, 1972

4. Scurlock JE, Curtis BM: Tetraethylammonium derivatives: ultralong-acting local anesthetics. ANETHESIOLOGY 54:265–269, 1981

5. Lipfert P, Seitz RJ, Arndt JO: Ultralong-lasting nerve block: Triethyldodecyl ammonium bromide is probably a neurotoxin rather than a local anesthetic. ANETHESIOLOGY 67:896–904, 1987

6. Strichartz GR: The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. J Gen Physiol 62:37–57, 1973

7. Hille B: Local anesthetics: Hydrophilic and hydrophobic pathways for the drug receptor reaction. J Gen Physiol 69:497–515, 1977

8. Wang GK: Binding affinity and stereoselectivity of local anesthetics in single batrachotoxin activated Na^+ channels. J Gen Physiol 96:1105–1127, 1990

9. Wang GK, Simon R, Wang SY: Quaternary ammonium compounds as structural probes of single batrachotoxin-activated Na^+ channels. J Gen Physiol 99:1–20, 1991

10. Wang GK, Simon R, Bell D, Mok WM, Wang SY: Structural determinants of quaternary ammonium blockers in batrachotoxin-modified Na^+ channels. Mol Pharmacol 44:667–676, 1993

11. Wang GK, Mok, WM, Wang SY: Charged tetracaine as an inactivation enhancer in batrachotoxin-modified Na^+ channels. Biophys J 67:1851–1860, 1994

12. Schwarz W, Palade PT, Hille B. Local anesthetics: Effect of pH on use-dependent block of sodium channels in frog muscle. Biophys J 20:343–368, 1977

13. Cota G, Armstrong CM: Sodium channel gating in clonal pituitary cells: The inactivation step is not voltage dependent. J Gen Physiol 94:213–232, 1989

14. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 391:85–100, 1981

15. Wang GK, Wang SY: Binding of benzocaine in batrachotoxin-modified Na^+ channels: State-dependent interactions. J Gen Physiol 103:501–518, 1994

16. Hille B, Campbell DT: An improved vaseline gap voltage clamp for skeletal muscle fibers. J Gen Physiol 67:265–293, 1976

17. Greene EC: Anatomy of the Rat. New York, Hafner Publishing, 1963

18. Thalhammer JG, Vladimirova, M, Bershadsky B, Strichartz GR: Neurologic evaluation of a rat during sciatic nerve block with lidocaine. ANETHESIOLOGY 82:1013–1025, 1995

19. deLahunta A: Veterinary Neuroanatomy and Clinical Neurology. Philadelphia, WB Saunders, 1983

20. Courtney KR: Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA-968. J Pharmacol Exp Ther 195:25–263, 1975

21. Tanford C: Thermodynamics of micelle formation, The Hydrophobic Effect. New York, John Wiley & Sons, 1980, pp 60–78

22. Raymond SA, Gissen AJ: Mechanisms of differential nerve block. Edited by Strichartz GR, Volume 81, Handbook of Experimental Pharmacology. Heidelberg, Springer-Verlag, 1987, pp 327–347

23. Schneider M, Datta S, Strichartz G: A preferential inhibition of impulses in C-fibers of the rabbit vagus nerve by veratridine, an activator of sodium channels. ANETHESIOLOGY 74:270–280, 1991

24. Vladimirova M, Thalhammer JG, Strichartz GR: Effects of veratridine on sciatic nerve-mediated functions in rat (abstract). Reg Anesth 20 (suppl 2):129, 1995