N-Butyl Tetracaine as a Neuromytic Agent for Ultralong Sciatic Nerve Block

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Background: Neuromytic agents such as phenol (5% to 10%) and absolute alcohol have long been used clinically to destroy the pathogenic nerve regions that manifest pain. Both phenol and alcohol are highly destructive to nerve fibers. However, these agents exert only weak local anesthetic effects and therefore are difficult to administer to alert patients without pain. This report describes a tetracaine derivative that displays both local anesthetic and neurolytic properties. Studies with such a compound may lead to the design of neurolytic agents that are more effective and more easily administered than phenol and alcohol.

Methods: A tetracaine derivative, N-buty1 tetracaine quaternary ammonium bromide, was synthesized, and its ability to elicit sciatic nerve block of sensory and motor functions in vitro was tested in rats. A single dose of 0.1 ml N-buty1 tetracaine at 37 mM was injected into the sciatic nol. Transverse sections of treated sciatic nerves were subsequently examined to determine the neurolytic effect of this drug. Finally, the local anesthetic properties of N-buty 1 tetracaine were studied in vitro; both tonic inhibition and use-dependent inhibition of Na⁺ currents in neuronal G1H, cells were characterized under whole-cell ca)lage-clamp conditions.

Results: N-buty1 tetracaine at 37 mM (equivalent to 1.11% tetracaine–hydrochloric acid concentration) elicited prolonged sciatic nerve block of the withdrawal response to noxious pinch in rats for more than 2 weeks. The withdrawal response was fully restored after 9 weeks. Parallel to sensory block, motor functions of the hind legs were similarly blocked by this drug. Morphologic examinations 3 and 5 weeks after a single injection of drug revealed degeneration of many sciatic nerve fibers, consistent with the results of functional tests. Finally, N-buty1 tetracaine was found to be a potent Na⁺ channel blocker in vitro. It produced strong tonic and use-dependent inhibition of Na⁺ currents with a potency comparable to that of tetracaine.

Conclusions: A single injection of N-buty1 tetracaine produces ultralong sciatic nerve block in rats. This compound possesses both local anesthetic and neurolytic properties and may prove useful as a neurolytic agent in pain management. (Key words: Anesthetics; local. Anesthetic techniques: regional nerve block. Nerves; injury. Pain; chronic.)

LOCAL anesthetics (LAS) block voltage-gated Na⁺ channels and inhibit the propagation of action potentials in excitable membranes.1,2 Because the potency of individual LAS in blocking Na⁺ currents varies significantly, these compounds elicit sensory and motor block of different durations during regional anesthesia from hours to 10 h.3 No available ultralong-acting LAS can relieve pain for days or weeks when given as a single injection. Drugs that will fill this void may be useful for managing chronic and intractable cancer pain. Despite many attempts to identify an ultralong-acting LA that reversibly blocks Na⁺ channels, this goal has so far remained elusive.2,5,6

To alleviate chronic and intractable cancer pain, spinal opioid administration, surgical interventions, or both are often preferred. When these methods fail or provide insufficient relief, phenol (5% to 10%) or absolute alcohol may be used as a neurolytic agent to destroy the pathogenic nerve regions that manifest the pain.7-9 For some well-defined cases, neurolytic agents retain major clinical utility and may be the preferred treatment. For convenience, we herein define neurolytic agents as chemicals capable of irreversibly damaging the axonal membranes. These agents are locally neurotoxic by definition. At high concentrations, LAS (particularly tetracaine) are also neurolytic and can damage nerve fibers in vitro.8,10 However, tetracaine has not been used intentionally as a neurolytic agent, perhaps

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Fig. 1. Chemical structures of tetracaine and N-butyl tetracaine quaternary ammonium (QA) salt. The proton nuclear magnetic resonance spectrum (500 MHz) of N-butyl tetracaine was obtained in deuteriochloroform (CDCl3).

because at extremely high dosage it enters the blood stream rapidly and can cause severe central nervous system and cardiac damage.

This study examined the LA properties of a tetracaine derivative and assessed its use as a neurolytic agent. Because this compound contains a permanent positive charge, theoretically it should not enter the blood stream readily to cause central nervous system and cardiac damage. The availability of a neurolytic compound with strong LA properties would be advantageous in that conventional neurolytic agents exert only weak LA effects (phenol)¹¹,¹² or none at all (alcohol).⁷

Materials and Methods

Organic Synthesis of N-butyl Tetracaine Quaternary Ammonium Salt

N-butyl tetracaine quaternary ammonium (QA) was synthesized from tetracaine base (Sigma Chemical Co., St. Louis, MO) and 1-bromobutane (Aldrich, Milwaukee, WI) as described by Wang and associates.¹³ Analysis of the compound by mass spectrometry revealed a molecular mass of 321.4 daltons. Proton nuclear magnetic resonance spectrum (500 MHz) of N-butyl tetracaine was undertaken in deuteriochloroform (CDCl3) (fig. 1, bottom). Peak assignments were made by inspecting chemical shifts, peak intensities, and spin couplings. The following peaks were identified (δ = parts per million from tetramethylsilane):

0.94 (t, 6H); 1.43 (m, 4H); 1.73 (m, 4H); 3.18 (t, 2H); 3.49 (s, 6H); 3.65 (t, 2H); 4.15 (br, s, 2H); 4.72 (br, s, 2H); 6.92 (br, s, 2H); and 7.86 (d, 2H). The peak at 7.26 was from CDCl3. The results of NMR spectrum and mass spectrometry were consistent with the proposed structure of N-butyl tetracaine QA. Bromide was the counter ion for this positively charged QA compound. The structures for the neutral and protonated forms of tetracaine are included in figure 1 for comparison.

Sensory and Motor Block of Rat Sciatic Nerve

A neurologic evaluation of sciatic nerve block in the rat was conducted according to the method described by Thalhammer and colleagues.¹⁴ These protocols were approved by the Harvard Medical Area Standing Committee on Animals. All sciatic nerve functions were observed in rats handled under free-behavior conditions. Handling of rats, which took ~5 weeks before drug testing, reduced stress during neurologic examination without adverse behavior of biting, escaping, and vocalization. These procedures were taken to measure baseline behavior and to determine the influence of regional block on behavior in a reproducible manner. Drugs at a concentration of 37 μM in isotonic saline (equivalent to 1.11% tetracaine-hydrochloric acid concentration) were injected in a volume of 0.1 ml at the sciatic notch, as described previously.¹⁵ Functional impairment was measured by comparing values obtained before and at various intervals after injection. Changes of function were estimated and normalized as percentages of maximal possible effect (%MPE).¹⁴,¹⁵ Complete block of function was defined as 100% MPE and unchanged function as 0% MPE. This definition did not imply that our functional assays used a continuous variable but was applied to normalize the data for comparison. Throughout the experiment all animals were observed for abnormalities in behavior, such as alertness, responsiveness to environment, weight loss, motor activity, gait, and resting posture. This was to verify that rats were not under extreme stress induced by drug injection or by the researcher during behavior tests. Well-handled rats permitted a more precise neurologic examination and yielded reliable reproducible results. All behavior tests were not blinded and were performed by one person. Detailed descriptions of behavior tests can be found in Thalhammer and colleagues¹⁴ and are summarized briefly here.

Proprioception was evaluated based on the combined postural reactions (such as ‘hopping’ and ‘tactile placing’; see below) and was scored from 3 (normal postural
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Fig. 2. (A) Initial phase of functional impairment, shown as a percentage of maximal possible effect (%MPE; mean ± SE) after injection of 0.1 ml tetracaine (closed symbols, n = 8) or 0.1 ml N-butyl tetracaine (open symbols, n = 14), both at 37 mM in isotonic saline. The complete block induced by N-butyl tetracaine persisted for more than 24 h. Neurologic evaluation was performed before and at 5, 10, 30, 60, 120, and 180 min after injection of N-butyl tetracaine and before and at 5, 10, 30, 45, 60, 75, 90, 105, 120, and 180 min after injection of tetracaine. (B) The time course for recovery after N-butyl tetracaine injection, shown as %MPE (mean ± SE; n = 9 to 14). Some rats were killed for morphologic examination. Neurologic evaluation was performed both at the times listed for part A and 24, 168, 336, 504, 840, 1,008, 1,176, and 1,344 h after injection of N-butyl tetracaine.

reactions) to 0 (complete lack of postural reactions); a score of 0 was considered to represent 100% MPE.

To evaluate 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 (3 = normal reposition ability, 2 = slightly impaired, 1 = severely impaired, 0 = no attempt).

To assess hopping, the front half of the animal was lifted so that the body’s weight was supported by the hind limbs. One hind limb at a time was then lifted 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 (3 = normal hopping ability, 2 = slightly impaired, 1 = severely impaired, 0 = no hopping ability).

Motor function of the 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 foot. The force (measured in grams) necessary to bring the heel into contact with the platform of a balance was measured. The reduction in force resulting from reduced extensor muscle tone was considered the motor deficit. A force of <15 g was considered to represent an absence of extensor postural thrust or 100% motor block.

Nociception was evaluated by measurement of the different degree of the withdrawal response to noxious pinch. Care was taken to avoid tissue injury resulting in hyperalgesia by properly spacing the stimulations. The fifth toe was pinched (to 300 g) with a force-calibrated serrated forceps for 2 s, and the withdrawal response was graded as 4 (normal, brisk, generalized motor reaction; withdrawal of the stimulated hind limb; attempts to bite the forceps; and vocalization); 3 (the same as 4, but slower than on the control side); 2 (the same as 3, but with one of the responses lacking); 1 (only a weak attempt to withdraw); or 0 (no response).

Morphologic Changes in Treated Sciatic Nerves
At 21 and 35 days after N-butyl tetracaine injection, rats were killed by pentobarbital overdose. Sciatic nerves near the distal end of the injected site and the uninjected contralateral site were carefully dissected. Excess adipose tissue was trimmed, and the nerves were rinsed with saline solution. Each sample was placed in a separate vial containing 4% glutaraldehyde in 0.1 M cacodylate buffer (Poly Scientific Research & Development Corp., Bay Shore, NY). Histologic cross-sectioning and staining were performed as described by Anthony and coworkers.10

Voltage Clamp Experiments in GH3 Cells
Cell Culture. Rat clonal pituitary GH3 cells were purchased from the American Type Culture Collection (Rockville, MD) and maintained as described by Costa and Armstrong.20 Medium supplemented with 10% fetal bovine serum (HyClone) and 1000 U/ml penicillin and 500 μg/ml streptomycin was then used.

Whole cell voltage clamp experiments in a patch-clamp configuration were performed in 125 mM NaCl, 5 mM KCl, and 10 mM HEPES at pH 7.4. Pipettes were filled with KCl of 100 mM. 

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Fig. 3. Morphologic changes in transverse sections of sciatic nerve with and without the injection of 0.1 ml of 37 mM N-butyl tetracaine. Histologic cross sections of rat peripheral nerve were stained with hematoxylin and eosin. (A) Normal rat peripheral nerve contralateral to the site of injection 21 days after injection. The axoplasm forms a central eosinophilic region within each axonal fiber, with either a clear or a halo with radiating spokes corresponding to the myelin sheath. Large-caliber myelinated fibers are closely apposed to each other (magnification, ×720). (B) Acute stages of axonal degeneration show vaculation of most myelinated fibers. The increase in the number of nuclei is due to the proliferation of Schwann cells and an influx of macrophages (magnification, ×720). (C) Normal rat peripheral nerve contralateral to the site of injection 35 days after injection (magnification, ×720). (D) At 35 days, large vaculated fibers (which represent acute stages of axonal degeneration) are more prominent in the sciatic nerve from the site of injection, although an increase in cellularity is evident. Pale eosinophilic regions within the endoneurial space without discernable axonal profiles correspond to areas of axonal loss and fibrosis (magnification, ×720).

and Armstrong in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HyClone Labs, Logan, UT). For Na⁺ current recording, cells were grown in a 55-mm culture dish, which was then used as a recording chamber.

**Whole-cell Voltage Clamp.** The whole-cell variant of the patch-clamp method was used to measure Na⁺ currents in rat clonal GH₃ cells. The external solution contained 150 mM choline Cl, 0.2 mM CaCl₂, 2 mM MgCl₂, and 10 mM hydroxyethylpiperazine-ethane sulfonic acid adjusted to pH 7.4 with tetramethylammonium hydroxide. Micropipettes were fabricated and had a tip resistance of ~1 MΩ when filled with an Na⁺ solution containing 100 mM NaF, 30 mM NaCl, 10 mM EGTA, and 10 mM hydroxyethylpiperazine-ethane sulfonic acid adjusted to pH 7.2 with CsOH. The junction potential of electrodes was nulled before seal formation. After the patch membrane ruptured, the cell was allowed to equilibrate with the pipette solution for at least 15 min at a holding potential of ~100 mV. Tetracaine and N-butyl tetracaine QA at appropriate concentrations were applied to cells with a flow rate of about 0.12 ml/min through a series of narrow-bore capillary tubes positioned within 200 μm of the cell. Washout of drugs was performed using a tube containing the external solution without drug. Voltage clamp protocols were created with pClamp software (Axon Instruments, Foster City, CA). Leak and capacitance were subtracted by a leak and capacitance compensator, as described by Hille and Campbell. Additional compensation was achieved by the patch-clamp device (EPC7, List Electronic, Darmstadt/Eberstadt, Germany). All experiments were conducted at room temperature (25 ± 2°C). At the end of the experiments, the drift in the junction potential was generally less than 2 mV.

**Statistical Analysis**

An unpaired Student's t test (Sigmastat, Jandel Scientific Software, San Rafael, CA) was used to evaluate the significance of drug-induced changes in the rate and the steady state of tonic and use-dependent block. A Mann-Whitney rank sum test or a Kruskal-Wallis one-way analysis of variance on ranks (Sigmastat) was used to test the significance of differences in the magnitude and duration of functional changes detected by neurologic evaluation after tetracaine and N-butyl tetracaine injection. A probability value less than 0.05 was considered statistically significant.

**Results**

**Onset of Sciatic Nerve Block by N-butyl Tetracaine**

Within 5 min after its injection into the sciatic notch of rats, 0.1 ml of 37 mM N-butyl tetracaine elicited...
complete functional impairment of proprioception, motor function, and response to noxious pinch (fig. 2A, open symbols). This onset of the blocking of proprioception and motor function by N-buty1 tetrocaine was as fast as that by tertiary-amine tetrocaine (fig. 2A, closed symbols). Tetrocaine did not completely block the response to noxious pinch in all rats tested. Its effect was maximal (~95% MPE, fig. 2A, closed triangles) 10 min after tetrocaine injection. Thus, despite its permanent positive charge, N-buty1 tetrocaine elicited complete sciatic nerve block with a rapid onset comparable to or faster than that measured for tetrocaine.

**Duration of Complete Sciatic Nerve Block by N-buty1 Tetrocaine**

Complete functional impairment of the response to noxious pinch by N-buty1 tetrocaine in rats lasted longer than 2 weeks (336 h; fig. 2B, open triangles). This prolonged block of nociceptive function in the sciatic nerve has not been documented for any reversible LAs but resembles the long sensory analgesia produced by dodecyl triethyl QA ions on trigeminal nerve. Proprioception and motor function were also completely blocked for at least 24 h after N-buty1 tetrocaine injection (fig. 2B, open squares and circles); the block remained operative at up to 85% to 90% MPE for 2 weeks (336 h). In contrast, complete block of proprioceptive and motor function by tetrocaine lasted less than 60 min (fig. 2A, closed squares and circles). For each functional test, the duration of complete block by tetrocaine was significantly shorter than that of complete block by N-buty1 tetrocaine (P < 0.005). Thus N-buty1 tetrocaine elicited prolonged block of rat sciatic nerve functions after a single injection into the sciatic notch, whereas tetrocaine at the same concentration failed to do so.

**Recovery of Sciatic Nerve Functions**

With a single injection of 0.1 ml of 37 mM N-buty1 tetrocaine, impaired sciatic nerve functions recovered completely only after 8 weeks (1,344 h; fig. 2B, at which time the %MPE decreased to near 0 (with six of eight rats recovering completely). For the response to noxious pinch, partial recovery began between 3 (504 h) and 8 (1,344 h) weeks after injection of N-buty1 tetrocaine (fig. 2B, open triangles). For proprioceptive and motor functions, partial recovery began earlier. For example, 8 of 14 rats started to recover their motor function at 1 week, although 6 rats remained completely blocked (fig. 2B, open circles). Similarly, 4 of 14 rats had partially recovered their proprioceptive function at 1 week (fig. 2B, open square). Some of the early recovery in proprioceptive and motor functions might be due to accidental removal of LAs from the nerve root due to axons showed the LAs were not able to cross the root to reach and motor functions when the root was reinnervated over the time of recovery, complete function recovery was not complete after 8 weeks.

**Neurophylogenetic Fate of the N-buty1 Tetrocaine-Treated Sciatic Nerve**

Extensive degeneration of axons that was 24 h after injection with light microscopy of cross sections of the sciatic nerve at 2 and 8 weeks post-injection (fig. 3B). Degeneration of axons was detectable in the LAs, and tetrocaine-treated sciatic nerve (fig. 3B). Most axons had disappeared within 2 weeks, with the presence of axons at 8 and 16 weeks post-injection, but the axons were not apparent in cross sections. Additional elimination of axons was apparent at 16 weeks post-injection, although they were still evident at 24 weeks. Similar morphological changes were observed in the nerve 5 weeks post-injection.
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Fig. 5. Reversal of tonic block. With washing of the N-butyl tetracaine-treated cells with a drug-free external solution, the blocked Na⁺ current recovered slowly and only partially over a time course of 35 min (A). Reversal of tonic block after the administration of tetracaine at 100 μM was very fast, requiring no more than 2 min (B). The current traces were measured at 30-s intervals by the pulse protocol described in figure 4. Selected traces, including the trace before drug treatment (control), were superimposed for comparison. (C) The normalized peak current was plotted against time. The current amplitude blocked by N-butyl tetracaine recovered slowly. After 35 min of washing, cells exposed to N-butyl tetracaine regained only 57.8 ± 4.9% (n = 9) of the Na⁺ current amplitude. In contrast, the current blocked by tetracaine recovered rapidly within 2 min (103 ± 1.6%, n = 9) of washing with external solution. Solid lines drawn through data points are the best fit of single exponential functions. The estimated recovery-time constants were 36.7 ± 4.2 min (n = 9) and 0.21 ± 0.01 min (n = 9) for N-butyl tetracaine and tetracaine, respectively. The difference between these two time constants was statistically significant (P < 0.0001).

Due to adaptation of the rats. Nevertheless, this result showed that rats began to recover from proprioceptive and motor block at about 1 week after injection, but the time course could vary considerably in rats. The recovery of functions continued slowly until it was complete after 8 week (fig. 2B, open symbols).

Neurolytic Effects of N-butyl Tetracaine on Sciatic Nerve Fibers

Extensive degeneration of myelinated nerve fibers that was attributable to N-butyl tetracaine was evident with light microscopic examination of the transverse sections of rat sciatic nerve from the injected region. Figure 3B illustrates axonal loss and abundant axonal degeneration 3 weeks (504 h) after a single injection of N-butyl tetracaine. In sections of contralateral sciatic nerve (fig. 3A), a normal density of axons and an absence of axonal degeneration were evident. In addition, proliferation of Schwann cells and macrophages was apparent in the drug-treated nerve (fig. 3B). Some myelinated nerve fibers remained intact in the section, although their numbers varied widely in individual rats. Similar morphologic changes were found in sciatic nerve 5 weeks (840 h) after injection (fig. 3D). Morphologic changes were not evident by light microscopy in sciatic nerve 3 days after injection (data not shown) despite complete functional block. These results show that N-butyl tetracaine can elicit local acute axonal neuropathy and that its neurolytic activity causes ulrlong block of sciatic nerve functions.

Tonic Block of Na⁺ Channels by N-butyl Tetracaine

Typical tertiary amine LAs, including tetracaine, block voltage-gated Na⁺ channels in a complicated manner. Generally, they elicit tonic inhibition of Na⁺ currents when the nerve is stimulated infrequently. Figures 4A and B show the tonic inhibition of Na⁺ currents by N-butyl tetracaine and tetracaine, respectively. The rapid wash-in of tetracaine block at 100 μM was characteristic of tertiary amine LAs. Within 1 to 2 min, the block reached its steady-state level of about 80% of the control current. The wash-off of tetracaine was equally rapid, reaching its completion within 2 min (figs. 5B, C). N-butyl tetracaine at 100 μM had a significantly slower wash-in effect than did tetracaine (P < 0.0001); an interval of more than 20 min was required to reach the steady-state block of up to 80% to 90% of Na⁺ current.
measured; it was 10.0 ± 0.7% (n = 5) for A and 7.0 ± 1.2% (n = 6) for B, respectively. After drug treatment, the use-dependent block for each set of intervals was significantly greater than that for the control without drug. For example, at the 20-min interval, the use-dependent block elicited by N-butyl tetracaine was measured at 49 ± 2.6% (n = 5) on renormalization with respect to the first pulse of this data set. This use-dependent block by N-butyl tetracaine is significantly greater than the control value (P < 0.0001). For 20 μM tetracaine at the same interval, the use-dependent block was measured at 58 ± 3.9% (n = 6). This use-dependent block by tetracaine was also significantly greater than the control value (P < 0.0001).

Use-dependent Block of Na⁺ Channels by N-butyl Tetracaine

When a nerve was stimulated repetitively, additional use-dependent block occurred in the presence of tetracaine. This use-dependent block phenomenon was commonly noted when tertiary amine LAAs were used. Figure 6B shows that 20 μM tetracaine produced 40% to 50% tonic block of peak Na⁺ current. Repetitive depolarization of the cell at 2 Hz causes an additional 30% inhibition. At the end of 60 pulses, only about 50% of peak current remained. This use-dependent block by tetracaine is significantly greater than that measured for the control (=10%) in the absence of drug (P < 0.0001). For N-butyl tetracaine, a concentration of 100 μM is used because of its slow wash-in time course. Use-dependent block by this drug significantly exceeded the control value as early as 5 min after application (fig. 5A; P < 0.0001). Between 20 and 30 min, only about 20% of the peak Na⁺ current remains. However, approximately 50% of this remaining current is blocked after repetitive pulses. The rate of use-dependent block for N-butyl tetracaine is significantly slower than that for tetracaine (fig. 7; P < 0.0001). A similar rate difference has been found for lidocaine (a QA derivative of lidocaine) and for lidocaine. Clearly, N-butyl tetracaine QA retains the ability of its parent compound, tetracaine, to elicit tonic and use-dependent block.

Discussion

In Vivo Ultrasound Block of Sensory and Motor Functions in Rat Sciatic Nerve

This report shows that N-butyl tetracaine QA at 37 mM (equivalent to 1.11% tetracaine–hydrochloride) elicits neurolytic sensory block of rat sciatic nerve in vivo that generally lasts for more than 2 weeks. Scullock and Curtis19 reported that dodecyl triethyl ammonium ions at 14 mM elicit ultralong sensory block of the rat trigeminal nerve for 17 to 20 days. This interval is comparable to that of neurolytic sensory block elicited by N-butyl tetracaine QA ions.

Partial recovery of nociceptive function occurs as early as 21 to 60 days, with apparently full recovery after 60 days. Despite the paralysis of the drug-injected leg, all treated animals behaved normally. There was no apparent differential block between sensory and motor functions during the initial onset phase (fig. 2A). The onset of block in vivo for N-butyl tetracaine is as fast as that for tetracaine. Because N-butyl tetracaine has a permanent charge, it is not as rapidly membrane permeable as tetracaine, we surmise that N-butyl tetracaine has a high binding affinity toward Na⁺ channels, which could explain the rapid onset of block in vivo. The recovery time course of motor and proprioceptive functions after tetracaine injection is slower than that of the response to noxious pinch (fig. 2A). This difference in

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Fig. 7. Rate of use-dependent inhibition of Na⁺ current by N-butyl tetracaine and by tetracaine. Use-dependent inhibition of Na⁺ current 5 min after the application of 100 μM N-butyl tetracaine (A) and 20 μM tetracaine (B) was measured according to the protocol described in figure 6. Selected current traces were superimposed for comparison; the numbers in the figure correspond to the pulse applied. (C) The peak amplitudes of each data set were normalized with respect to the peak amplitude of the first pulse of the set. The normalized current was plotted against pulse number. Solid lines drawn through data points are the best fit of single exponential functions, with a time constant of 4.5 ± 0.3 pulse⁻¹ (n = 5) for N-butyl tetracaine and 1.00 ± 0.05 pulse⁻¹ (n = 6) for tetracaine. These two time constants are statistically different (P < 0.001).

In Vitro Block of Voltage-gated Na⁺ Channels by N-butyl Tetracaine Quaternary Ammonium

N-butyl tetracaine QA exhibits not only neurolytic but also LA characteristics. It blocks voltage-gated Na⁺ channels effectively when applied externally to GH, cells in vitro. At 100 μM, this drug produces profound tonic block of Na⁺ currents by more than 80%. Only 0.27% of the concentration is used in experiments conducted in vitro (37 mM versus 100 μM). The wash-in of this drug is relatively slow; it takes more than 30 min to reach steady state. The wash-out is equally slow; again, at least 30 min is required for partial removal of the block. These slow kinetics are to be expected because this compound has a permanent positive charge. A comparable slowness of the wash-in and wash-out of tetracaine, a permanently charged lidocaine derivative, was recently reported. In contrast, tetracaine, as a tertiary amine drug, can penetrate the membrane barrier rapidly and complete its blocking effect within 2 min. Similarly, tetracaine can be washed out of the cell within 2 to 5 min.

Like most tertiary amine LAs, N-butyl tetracaine elicits profound use-dependent block of Na⁺ current at 2 Hz. This is not surprising because QA compounds produce use-dependent block in the same manner as their tertiary amine LA counterparts. Without repetitive pulses, closed Na⁺ channels may not be able to interact with N-butyl tetracaine efficiently. For example, after 5 min of wash-in, the first single pulse elicits about 65%
block of Na⁺ current (fig. 6A), whereas the tenth pulse elicits about 50% block (with pulses applied every 30 s, fig. 4). These results can be explained if the LA receptor is not freely accessible to charged QA compounds when the Na⁺ channel is in its closed state. Similar results have been found for QX-314, a quaternary derivative of lidocaine. With as little as 5 min of incubation with external N-butyl tetracaine at 100 μM, more than 80% of Na⁺ currents can be blocked after 60 repetitive pulses at 2 Hz. This magnitude of use-dependent block is significantly greater than that elicited by tetracaine at 20 μM (P < 0.005). Because of the slow wash-in of N-butyl tetracaine in GH3 cells, the internal concentration of this drug after incubation for 5 min is probably near 20 μM. Thus N-butyl tetracaine at <100 μM can produce use-dependent block of Na⁺ current as effective as tetracaine. These results together demonstrate that N-butyl tetracaine QA at low concentrations (100 μM) is a LA that, like typical tertiary amine LAs, elicits both tonic and use-dependent block of Na⁺ currents.

N-Butyl Tetracaine Quaternary Ammonium as a Dual Local Anesthetic and Neurolytic Agent?

Our results show that the nociceptive functions of rat sciatic nerves are completely blocked for 14 days by 37 mM N-butyl tetracaine and that full functional recovery occurs after 60 days. In the central nervous system, where myelinated nerve fibers do not regenerate to the extent documented in the peripheral nervous system, N-butyl tetracaine may be used intrathecally as a neurolytic agent to substitute for currently used drugs such as phenol and absolute alcohol. N-Butyl tetracaine may relieve pain in the central nervous system for as long as phenol or absolute alcohol; in addition, it may display much stronger LA properties in vivo than these currently available drugs. Treatment with N-butyl tetracaine may also prove less distressing to patients than intrathecal injection of alcohols, which causes burning sharp pain. Such burning pain, usually lasting for several hours after alcohol injection, will not be a potential problem for N-butyl tetracaine injection because of its strong and fast LA effect.

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