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The pH-Dependent Local Anesthetic Activity of Diethylaminoethanol, a Procaine Metabolite

John F. Butterworth IV, M.D., Philip A. Lief, M.D.,† Gary R. Strichartz, Ph.D.‡*

To test whether the products of procaine hydrolysis have local anesthetic actions resembling those of procaine, the authors compared the ability of procaine and its metabolites diethylaminoethanol (DEAE) and *para*-aminobenzoic acid (PABA) to block compound action potentials in excised, desheathed frog and rat sciatic nerves. Studies were performed in solutions of impermeant buffers at *p*H 7.4 (corresponding to mammalian physiologic *p*H) and at *p*H 9.2 (close to the pK_a of procaine and DEAE) to test for extracellular *p*H-dependent increases in drug permeation and potency. Both procaine and DEAE inhibited compound action potentials at *p*H 7.4 and 9.2 in a reversible and dose-dependent manner, and both were approximately ten-fold more potent at *p*H 9.2 than at *p*H 7.4, procaine inhibiting the action potential height by 50% at 0.15 mM (*p*H 9.2) and 1.1 mM (*p*H 7.4), DEAE at 4 mM (*p*H 9.2) and 70 mM (*p*H 7.4). In contrast, PABA at concentrations up to 25 mM and at either *p*H failed to inhibit compound action potentials, and did not modify the effects of DEAE when both drugs were given together. Procaine produced greater use-dependent block at the higher *p*H and at higher stimulation rates (100 Hz *vs.* 40 Hz); DEAE produced almost no use-dependent block. These observations suggest: 1) that DEAE might account for some of the neuropharmacologic activity of procaine in techniques that favor the accumulation of metabolites (such as those requiring large doses or prolonged infusions); and 2) that alkalinization of procaine and DEAE solutions appears to increase their potency for both resting and use-dependent block of action potentials. (Key words: Anesthetics, local: metabolism; procaine. Diethylaminoethanol: *para*-aminobenzoic acid.

NEARLY 40 YEARS AGO, Brodie and his colleagues determined that procaine was metabolized in serum to equal amounts of diethylaminoethanol (DEAE) and *para*-aminobenzoic acid (PABA) (fig. 1).[1] They demonstrated that DEAE, following intravenous injection, had many of procaine's actions, including antiarrhythmicity and analgesia.[2,3] Thus, it is possible that procaine metabolites interact with neuronal voltage-gated Na^+ channels in a manner similar to that of procaine and other local anesthetics, thereby explaining DEAE's ability to mimic procaine after intravenous injection. Were DEAE or PABA to have such an effect on nerves, procaine metabolism and metabolite accumulation might explain the prolonged efficacy of procaine infusions identified by Brodie *et al.* and others.[2,4,5] We, therefore, tested that possibility by comparing the ability of procaine, DEAE, and PABA to block compound action potentials in frog and rat sciatic nerves.

Methods and Materials

PREPARATION OF NERVES

Adult frogs (*Rana pipiens*, purchased from Connecticut Valley Farms, Northampton, MA) of either sex and measuring 8–10 cm from snout to rump were pithed and their sciatic nerves excised. After the nerve sheaths were removed with jeweler's forceps and sharpened sewing needles, the nerves were halved and kept for 1–2 h in frog Ringer solution before drug testing.

Male retired-breeder Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 0.3–0.5 kg were anesthetized with 25 $mg \cdot kg^{-1}$ of intraperitoneal pentobarbital. The sciatic nerves, exposed by lateral reflection of the skin and gluteal musculature, were removed and desheathed using jeweler's forceps, sharpened sewing needles, and Castroviejo scissors (Roboz Surgical Instruments, Washington, DC). After both sciatic nerves had been removed, the rats were killed with 50 $mg \cdot kg^{-1}$ intraperitoneal pentobarbital.

Nerves were mounted in a four-pool sucrose gap chamber as described by Strong *et al.*,[6] and as illustrated in figure 2. For the amphibian nerve experiments, pools A, B, C, and D were filled with frog Ringer solution containing (in mM) NaCl 115, KCl 2.5, $CaCl_2$ 2, 3-(N-morpholino) propanesulfonic acid (MOPS) 5, and tris(hydroxymethyl)-methylaminopropanesulfonic acid (TAPS) 5 titrated with concentrated NaOH to *p*H 7.4 or 9.2; for the mammalian nerve experiments, the pools were filled with rat Tyrode solution containing (in mM): NaCl 140, KCl, 4, $CaCl_2$ 2, $NaHCO_3$ 12, $MgCl_2$ 2, NaH_2PO_4 0.4, glucose 5, and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) 5 titrated with concentrated NaOH to *p*H 7.4.

STIMULATION PROTOCOLS AND RECORDING TECHNIQUES

A model S44A stimulator (Grass Instrument Co., Quincy, MA) or a Model 1800 isolated generator

* Instructor of Anesthesia.
† Assistant Professor of Anesthesia.
‡ Associate Professor of Pharmacology in Anesthesia.

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Address reprint requests to Dr. Butterworth: Department of Anesthesia, Wake Forest University Medical Center, 300 South Hawthorne Road, Winston-Salem, North Carolina 27103.

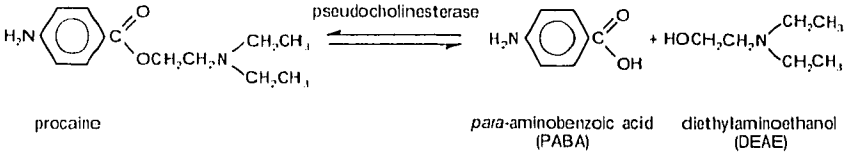

FIG. 1. Hydrolysis of procaine by serum esterases yields PABA and DEAE.

(World Precision Instruments, New Haven, CT) delivered supramaximal stimuli through Ag/AgCl electrodes in pools A and B. The stimulus duration was maintained at 50 μsec in the amphibian study, and at various durations in the mammalian study, depending on whether the A- or the C-fiber elevations were being tested. Stimulus conditions for the A fibers resembled those of frog nerves (typically, 50–100 μsec stimulus duration); C fibers, on the other hand, required longer (typically, 100–1000 μsec) stimuli at higher voltages. A differential electrometer (Model AK47UU, MetaMetrics, Carlisle, MA) amplified the compound action potentials that were displayed on an analog storage oscilloscope (Model PM3234, Phillips, Inc., The Netherlands). Traces were photographed on Polaroid® film for permanent records.

The procaine (Sigma Chemical Company, St. Louis, MO), the DEAE and PABA (Aldrich Chemical Company, St. Louis, MO), and all other chemicals used in this study were reagent grade or better. The pK_a for 10 mM DEAE (at 25° C in 150 mM NaCl) was determined potentiometrically to be near 10.2. The pK_a for procaine determined under similar conditions is 9.06 (V. Sanchez, G. R. Arthur, and G. Strichartz, unpublished observation).

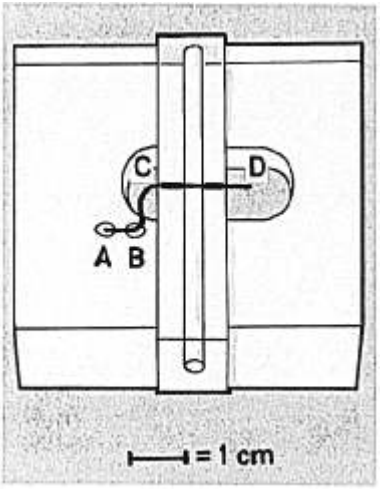

FIG. 2. Schematic of sucrose gap nerve chamber. The nerve fiber is the submerged black strand running from A to D. Current pulses passed between Ag/AgCl electrodes in pools A and B stimulated the nerve. Compound action potentials and resting potentials were measured by the voltage difference between pools C and D. Isotonic sucrose flowed through the cylindrical passage in the center block and over the short segment of nerve located between pools C and D. Petroleum jelly (Vaseline®) sealed and electrically isolated the pools. Initially, all aqueous pools (A–D) contained only the appropriate Ringer solution. Test solutions were added to pool C, in which a length of nerve of ≃1 cm was submerged.

The drugs were dissolved in the appropriate Ringer or Tyrode solution, and then applied to the nerve in pool C of the sucrose gap chamber (fig. 2). Drugs were diluted to their final concentration in the appropriate solution, and that solution was applied to nerves that had been equilibrated previously with the same solution, but free of any drugs. After the nerves were tested with increasing doses of a drug, allowances were made for nerve recovery in a drug-free solution. Complete recovery to steady-state sometimes required longer than 40 min; hence, many nerves were tested with only one drug. The effects of DEAE were less reversible than those of procaine, particularly when recovery from nearly complete impulse blockade was attempted.

Data Collection and Analysis

The term resting impulse block refers to the ratio of amplitudes of the steady-state compound action potential evoked by infrequent stimulation (<0.1 Hz) before and after drug application. During repetitive stimulation, Na^+ currents and action potentials in nerves partially inhibited by local anesthetics are transiently inhibited even further (*i.e.*, further reduced in amplitude). The term use-dependent block defines that additional decrease in action potential amplitude (compared to the control signal) produced by high-frequency (approximately 40 Hz) stimulation at a given drug concentration.[7] (For a more extensive explanation of use-dependent block, the reader is referred to Hille.[8])

Results

Frog Nerve Fibers

Procaine reduced the height of compound action potentials in a dose-dependent, reversible manner at both *p*H 7.4 and *p*H 9.2. A typical time course for the onset and reversal of procaine block at *p*H 7.4 is illustrated in figure 3. Baseline action potential height and stimulus requirements remained unaffected by changes in *p*H of drug-free solutions between 7.4 and 9.2. Procaine concentrations of approximately 1.1 mM and 0.15 mM (*p*H 7.4 and 9.2, respectively) reduced the action potential height by 50% at steady-state (fig. 4). The procaine-induced resting block reversed rapidly, but not always

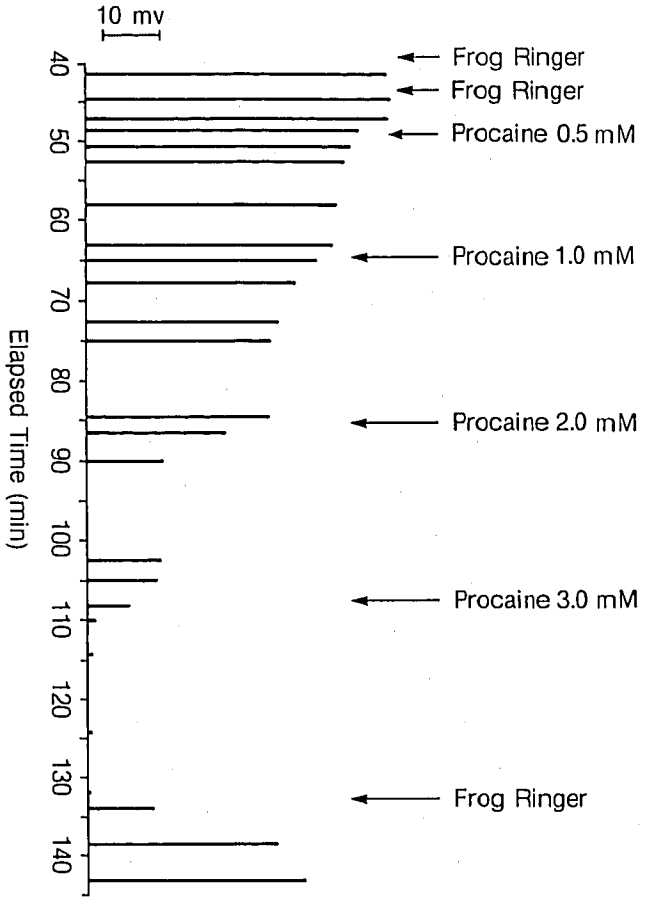

FIG. 3. Typical kinetics of onset and reversal of block of action potentials by procaine (pH 7.4). The horizontal bars show the peak amplitudes of compound action potentials. Numbers on the vertical axis list experimental time in minutes. Solution changes in pool C (see fig. 2) are indicated by arrows.

completely, when the nerves were washed with drug-free Ringer solution. The rate and degree of reversibility of block declined as the duration of drug exposure and degree of blockade increased.

DEAE was less potent than procaine at both pH 7.4 and pH 9.2. The concentrations reducing the action potential height by 50% at steady-state were approximately 70 mM and 4 mM, respectively, as illustrated in figure 5.

At concentrations up to 25 mM, PABA showed no measurable block. Nor did 12.5 mM PABA modify the block induced by 50 mM DEAE when both drugs were applied at pH 7.4.

Procaine produced greater, more rapidly developing use-dependent block at pH 9.2 than at pH 7.4, as illustrated in figure 6. In contrast, for DEAE no detectable use-dependent block occurred at either pH under conditions of an equivalent resting block (fig. 6).

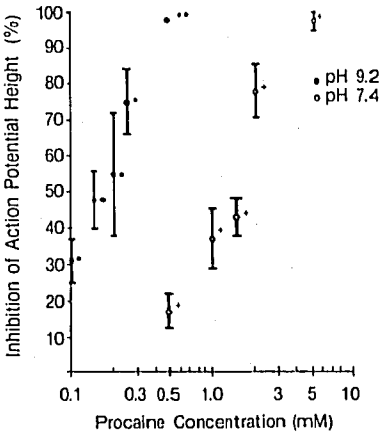

FIG. 4. The dose dependence of resting action potential blockade by procaine at steady state in frog sciatic nerves, compared at pH 7.4 and 9.2. The data points show the means and the vertical bars ± SEM. Error bars are drawn only if the standard error exceeded the size of the symbol (*n = 3, $^{+}$n = 4, **n = 5).

RAT NERVE FIBERS

Mammalian nerves required levels of procaine and DEAE similar to those required by amphibian nerves to inhibit their action potentials (figs. 7, 8). The mammalian C-fiber elevation of the compound action potential was inhibited by anesthetic concentrations similar to those that blocked both the amphibian and mammalian A-fiber elevation; however, the precise relationship between the drug sensitivities of these two populations could not be determined due to the limited number of nerves in which satisfactory C-fiber elevations could be detected.

Both drugs consistently produced greater use-dependent block of A-fiber action potentials of rat nerve at higher stimulation frequencies or a pH of 9.2 than at lower stimulation frequencies or a pH of 7.4. Figure 9 illustrates the effect of stimulation frequency on use-dependent block by DEAE. Mammalian nerves showed a pronounced use-dependent block by DEAE, even at pH 7.4, in contrast to the absence of this phenomenon in the A fibers of amphibian nerves.

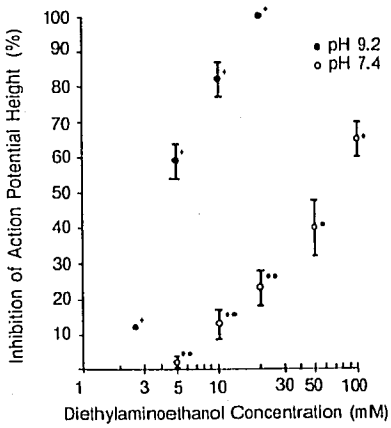

FIG. 5. The dose dependence of resting impulse blockade by DEAE in frog sciatic axons at steady state, compared at pH 7.4 and 9.2. Symbols as in figure 4.

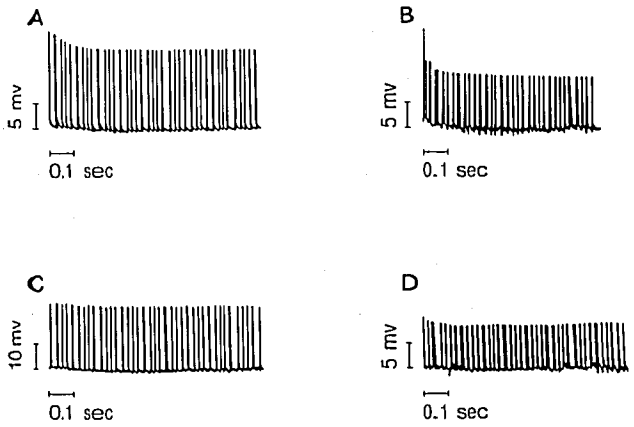

FIG. 6. Effect of *p*H on use-dependent block of compound action potentials by procaine and DEAE in four different frog sciatic nerves. *A.* After incubation with 3.0 mM procaine at *p*H 7.4, the action potential height of an infrequently stimulated (<0.5 Hz) nerve has been reduced by approximately 65% (compared to its control height before exposure to the local anesthetic). During a 40-Hz stimulus train, additional use-dependent block is produced. *B.* Procaine 0.25 mM at *p*H 9.2 reduced the action potential height by 40% before the 40-Hz stimulus train. *C.* DEAE 100 mM at *p*H 7.4 reduced the action potential height by 40% before the 40-Hz stimulus train. *D.* DEAE 20 mM at *p*H 9.2 reduced the action potential height by 40% before the 40-Hz stimulus train. No detectable use-dependent block is seen in *C* or *D*.

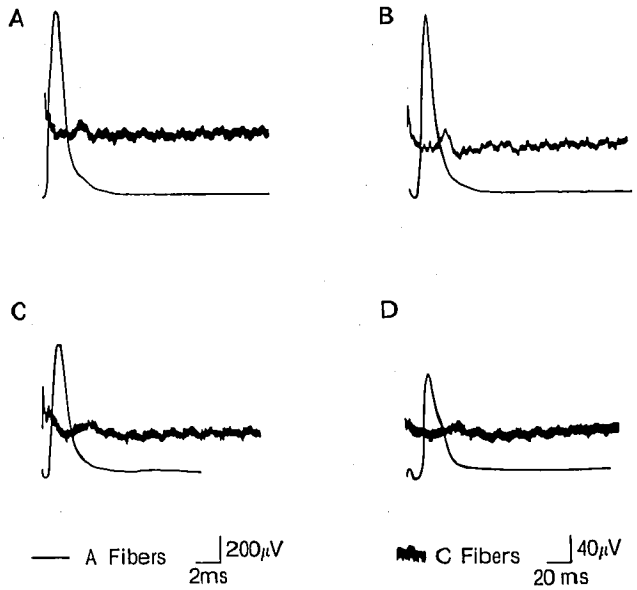

FIG. 7. Block of A and C fiber elevations of rat sciatic nerve action potentials by DEAE at *p*H 7.4. The larger, smoother tracing reflects currents generated in A fibers; the smaller, coarser tracing reflects currents generated in C fibers. *A.* Control action potentials in Tyrode solution. *B.* Effect of 10 mM DEAE. *C.* Effect of 50 mM DEAE. *D.* Effect of 100 mM DEAE. Note the nearly complete loss of the C-fiber elevation when the A-fiber deflection was decreased to less than 50%.

Discussion

We have demonstrated that blockade of impulses in isolated, desheathed amphibian and mammalian nerve fibers by DEAE resembles that by procaine, particularly at alkaline *p*H or high drug concentration. The close correlation between anesthetic blocking concentrations for A fibers in amphibian and mammalian species confirms our previous findings.[9] A selective susceptibility of C-fibers to local anesthetics was not unequivocally identified in the few experiments in which we could directly compare blocking concentrations for A and C fibers; moreover, it has not been found in any of the studies done on single C-fibers.[10,11] For resting block, procaine was an order of magnitude more potent than DEAE at both neutral and alkaline *p*H. This difference probably is due in large degree to the greater lipophilicity (related to procaine's aromatic substituent and greater size) as well as the lower pK_a of procaine, both factors that increase its membrane permeation over that of DEAE.[12] Procaine produced greater use-dependent block at higher than lower *p*H; DEAE produced considerably less use-dependent block than procaine at any given degree of resting block.

The potency of both procaine and DEAE increased ten-fold as the *p*H was raised from 7.4 to 9.2, in agreement with voltage-clamp studies showing that Na^+ currents are blocked to a greater degree by tertiary amine local anesthetics at alkaline *p*H.[13] The essential role of the un-ionized species of drug is implied by this result; this form permeates the axonal membrane and delivers anesthetic to the axoplasmic compartment. If one assumes, with admitted simplification, that all effects of *p*H on tertiary amine anesthetic potency are related directly to the drug fraction in the un-ionized membrane permeant form, which thereby determines the equilibrium axoplasmic and membrane anesthetic concentrations of both neutral and protonated species, and that extracellular drug is without direct effect,[14-16] then one can predict a dependence of potency on *p*H by the equation:

$$R = K_a/(K_a + [H^+]_o), \quad (1)$$

where R is the fraction of un-ionized drug, K_a is the ionization constant for the drug, and $[H^+]_o$ is the hydrogen ion concentration outside the axolemma. At alkaline *p*H more uncharged anesthetic is available to cross the membrane and accumulate within the axoplasm. Potency will then appear to correlate directly with the extracellular un-ionized anesthetic concentration.

The previous studies of Dettbarn *et al.*[17] rigorously support this conclusion. When squid axons were bathed with extracellular anesthetic-laden solutions of differing

pH, the ratio of procaine in the axoplasm to that in the bathing solution followed almost exactly the ionization behavior of the drug in solution. Comparable axoplasmic procaine concentrations correlated with a comparable degree of resting block, regardless of the extracellular pH and procaine concentration, showing that axoplasmic (or intramembranous) drug was acting to produce inhibition. Narahashi *et al.*[18] made similar observations, but they also varied internal pH and noted a block of squid axon sodium currents by local anesthetics that required an attribution of blocking potency to both protonated and uncharged species. A recent review of the literature indicates a general consensus that both protonated and neutral species of local anesthetics can inhibit Na^+ channels, but, perhaps, at different sites and by different molecular mechanisms.[19]

According to equation 1, the fraction of unprotonated, permeant procaine will rise about 30-fold when pH_0 is raised from 7.4 to 9.2; the change we observed in EC_{50} was 70-fold. The approximately 20-fold rise in DEAE potency correlates with the calculated approximately 60-fold rise in concentration of the permeant, uncharged form of this drug. These two- to three-fold discrepancies between calculated and measured pH-dependent potencies may be due to factors that underlie changes in potency aside from pH_0-related changes in the permeant drug species. First, any elevation of axoplasmic pH in response to increased pH_0 will lower the total protonated internal species, decreasing the effective internal drug concentration in a direction consistent with the response of DEAE but opposite to that of procaine. Second, lowering $[H^+]_0$ increases the external negative surface electrical charge, which is equivalent to depolarizing the membrane.[20,21] This maneuver inactivates Na^+ channels but activates K^+ channels, thereby directly increasing the affinity of local anesthetics and indirectly increasing their potency for impulse blockade.[22] If procaine's differential affinity for inactivated over resting channels exceeded that of DEAE, it would explain the discrepancy between their predicted and observed pH-dependent potencies. Third, further complications in the analysis involve the inhibition by anesthetics of K^+, as well as Na^+ channels,[23] which will modify the impulse blocking potency[22] and the possible nonspecific effects of the necessarily high concentrations of DEAE, particularly at neutral pH.

Our finding that procaine and DEAE produce greater use-dependent block at alkaline pH supports the recommendation of DiFazio *et al.* to alkalinize local anesthetics before their clinical use.[24] During the onset of nerve block, prior trauma or surgical stimulation produces continuous impulse traffic through the region of incipient block, leading to use-dependent block. Our data suggest that, with procaine and DEAE, use-dependent block would be augmented by the use of alkaline local anesthetic solutions.

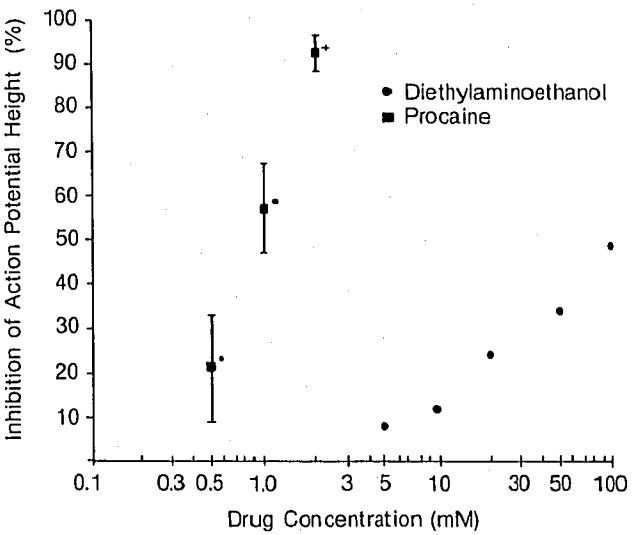

FIG. 8. Impulse blockade of A-fiber elevation of rat sciatic nerve action potentials by procaine and DEAE at pH 7.4. Procaine data expressed as means ± SEM (*n = 3, +n = 4). DEAE data plotted as means of two determinations.

The prolonged actions of systemic procaine may be due to persistence of DEAE in serum. One would anticipate finding elevated serum levels of DEAE long after

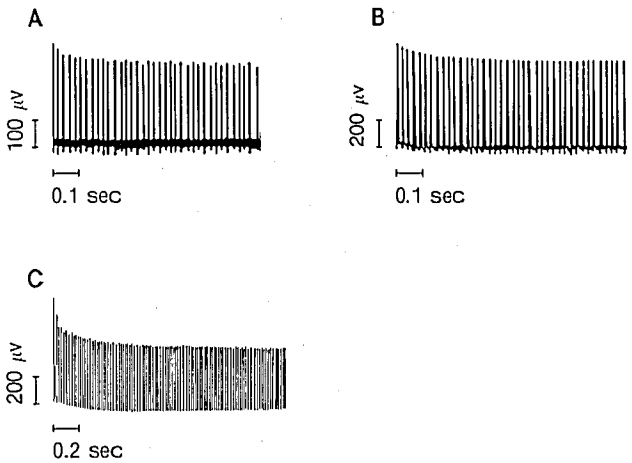

FIG. 9. Use-dependent block of compound action potentials from A fibers of rat sciatic nerves. *A*. After incubation with procaine 1 mM at pH 7.4, the steady-state resting block is approximately 50%, (*i.e.*, with infrequent stimuli, the action potential amplitude has been reduced by 50% relative to its control amplitude before the local anesthetic was added). A 40-Hz train of stimuli further reduces the amplitude of the action potential as shown here. *B*. DEAE 100 mM, pH 7.4; steady-state resting block is approximately 50% preceding the 40-Hz train. *C*. Same nerve and same conditions as in B, except that stimulus frequency is 100 Hz.

procaine concentrations had fallen due to enzymatic hydrolysis, and this has been demonstrated by Brodie *et al.*[1] The presence of cytoplasmic esterases along with the high pK_a of DEAE could lead to "trapping" of protonated DEAE molecules within the relatively acidic axoplasm, and the prolonged availability of "active" local anesthetic for binding to Na^+ channels.

We conclude that DEAE blocks compound action potentials of excised, desheathed amphibian and mammalian sciatic nerve fibers, and does so more effectively at an alkaline *p*H. DEAE could gradually accumulate in circumstances where procaine has shown efficacy beyond its serum lifetime—*e.g.*, following large intravenous bolus injections and after prolonged intravenous infusions, such as are used to produce general anesthesia and to treat debility and depression in the aged.[4,5] Hence, DEAE metabolism and accumulation may underlie the prolonged systemic actions of procaine in those settings, and may contribute to the cardiovascular and central nervous system toxicity seen following accidental intravenous injections of procaine during attempted regional anesthesia.

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