Tonic and Phasic Block of Neuronal Sodium Currents by 5-Hydroxyhexano-2',6'-xylidide, a Neutral Lidocaine Homologue

DANIEL M. CHERNOFF and GARY R. STRICHARTZ

From the Anesthesia Research Laboratories, Brigham and Women's Hospital, Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology; and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT The effects of a neutral lidocaine homologue, 5-hydroxyhexano-2',6'-xylidide (5-HHX), on the kinetics and amplitude of sodium currents in voltage-clamped amphibian nerve fibers are described. 5-HHX produced two types of sodium current inhibition: (a) tonic block, in resting fibers (IC50 ~ 2 mM), and (b) phasic block, an additional, incremental inhibition, in repetitively depolarized fibers (frequency > 1 Hz). The kinetics of phasic block were characterized by a single-receptor, switched-affinity model, in which binding increases during a depolarizing pulse and decreases between pulses. In the presence of 4 mM 5-HHX, binding increased during pulses from -80 to 0 mV, with an apparent rate constant of 6.4 ± 1.4 s⁻¹. Binding decreased between pulses with an apparent rate constant of 1.1 ± 0.3 s⁻¹. There was little effect of extracellular pH on the kinetics of phasic block. These findings demonstrate that neither the presence of a terminal amine nor a net charge on a local anesthetic is required for phasic block of sodium channels.

INTRODUCTION

Local anesthetics (LA) comprise a broad class of compounds capable of blocking impulse transmission by reversibly inhibiting sodium channel function. Most clinically useful local anesthetics are tertiary amine compounds having pKₐ's in the range of 7-9, so that a mixture of the charged (protonated) and neutral forms exists at physiological pH. There has been considerable controversy concerning the relative potencies of the charged and neutral forms of drug for blockade of sodium channels, as well as their loci of action. Although quaternary amine (permanently charged) derivatives of the local anesthetic, lidocaine, block sodium channels from the cytoplasmic side of axonal membrane (Frazier et al., 1970; Strichartz, 1973), the neutral drug benzocaine (lacking the tertiary amine moiety) is also a potent inhibitor...
of sodium currents in nerve and muscle (Hille, 1977a; Neumcke et al., 1981). At least one of the binding sites for these tertiary amine anesthetics is likely to overlap that for benzocaine, as their blocking actions are competitive (Rimmel et al., 1978; Schmidtmaier and Ulbricht, 1980; but see Mrose and Ritchie, 1978), and both LAs competitively inhibit the binding of the specific sodium channel activator, veratridine (Ulbricht and Stoye-Herzog, 1984; Rando et al., 1986).

All LAs produce a dose-dependent inhibition of sodium currents ($I_{\text{Na}}$), as assessed with infrequent stimuli. This inhibition is known as "tonic block." When the stimuli are more frequent, many LAs cause a further inhibition of $I_{\text{Na}}$, so-called "phasic block" (Strichartz, 1973; Courtney, 1975). Phasic block is generally attributed to a specific interaction between the channel and the charged species of anesthetic. For example, the quaternary (permanently charged) lidocaine analogue QX-572 produces a profound phasic block of sodium current in frog nerve, while neutral benzocaine exhibits little if any phasic blocking behavior (Hille, 1977a). The phasic blocking property of tertiary amine anesthetics has, therefore, been associated with the protonated (charged) form of drug. Further support for this view comes from kinetic analysis of phasic block with lidocaine, where the proportion of charged/neutral forms is varied by adjusting extracellular pH ($pH_o$); the rate of recovery from block between depolarizations is slower at lower $pH_o$, where more of the lidocaine is in the charged form (Schwarz et al., 1977). The view that emerges from these studies is that exit from the binding site of closed channels is more difficult for the protonated drug molecule than for its neutral congener; one physical interpretation is that the greater lipophilicity of the neutral agent allows it to escape more easily into the surrounding lipid phase.

There are two main theories that can account for phasic block. Under the modulated receptor hypothesis (Hille, 1977b; Hondeghem and Katzung, 1977), the affinity of the binding site for LA is higher when the channel is open or inactivated than when in the resting state. Under the guarded receptor hypothesis (Starmer et al., 1984) the affinity is constant but access to the binding site is guarded by the activation and/or inactivation gates, such that the forward binding rate is faster when the channel is open or inactivated than under resting conditions.

There are two possibilities for the lack of phasic block by the neutral LAs so far examined. One is that a drug molecule must be charged for its binding to be enhanced by depolarization. A second possibility is that the binding of neutral LAs is also enhanced by depolarization, but that recovery from the extra block is comparable to, or more rapid than, the normal rate of recovery from inactivation, so no extra block is detectable.

In this study, we describe the effects of a neutral lidocaine homologue (lacking the tertiary amine), 5-hydroxyhexano-2',6'-xylidide, on sodium currents in amphibian nerve. We show that this compound can produce a phasic block similar to that produced by anesthetics bearing a net charge. These findings demonstrate that a charged (protonated) molecule is not required for phasic block of sodium channels.

**METHODS**

Single myelinated nerve fibers were dissected from the sciatic nerve of the toad, *Bufo marinus*, and a node of Ranvier was voltage clamped at 13°C (Dodge and Frankenhaeuser, 1958).
Except where noted, the holding potential was set at \( E_{\text{hold}} = -80 \text{ mV} \) (where 5–15% of the sodium channels were inactivated). The shunting artifact of Dodge-Frankenhaeuser (1958), which leads to an overestimate of the true applied membrane potential, was not measured and adjusted for in these experiments. Leak and capacity currents were subtracted using an analogue leak subtractor with two time constants. Membrane currents were estimated using an assumed internodal resistance of 10 MΩ.

Pulse protocols were generated by computer (IBM-PC/AT with Labmaster data acquisition board, Scientific Solutions, Inc., Cleveland OH, and pCLAMP Software, Axon Instruments, Inc., Burlingame CA) and the evoked currents were sampled with 12-bit resolution at 25,000 samples/s, after passing through a two-pole low-pass analogue filter with 5-kHz cutoff.

**Solutions**

The internodes were cut in a solution composed of (in millimolar): 120 CsCl, 5 NaCl, 10 3-(N-morpholino)propanesulfonic acid (MOPS), titrated to pH 7.3 with tetramethylammonium (TMA) hydroxide (~5 mM). This solution blocked >95% of the “delayed rectifier” K⁺ currents. The extracellular solution (“Ringer”) contained (in millimolar): 115 NaCl, 2.5 KCl, 2.0 CaCl₂, 10 MOPS, titrated to pH 7.3 with TMA·OH. In some experiments, 10 mM tetraethylammonium (TEA) chloride was added to the Ringer’s solution to further inhibit K⁺ currents, with no apparent effects on anesthetic block of sodium channels. In experiments at other levels of \( p{\text{H}}_\text{a} \), 10 mM of N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS: pH 8.5) or 2-(cyclohexylamino)ethanesulfonic acid (CHES: pH 9.4) was substituted for the MOPS.

The neutral lidocaine analogue, 5-hydroxyhexano-2',6'-xylidide (5-HHX or compound 13, Tenthorey et al., 1981) was supplied in HPLC-pure, crystallized form by Professors Paul D. McMaster and Eugene W. Byrnes (College of the Holy Cross and Assumption College, Worcester, MA, respectively). Stock solutions of 5-HHX (400 mM) were prepared in absolute ethanol (EtOH) and diluted as needed into the extracellular Ringer’s solution. Benzocaine (Eastman Kodak, Rochester, NY) was prepared as a 200-mM stock solution in EtOH. Drug structures are shown in Fig. 1. Stock solutions were stored at 6°C for up to 2 wk with no apparent loss in potency.

Solution changes were made by flushing 2 ml of the new solution through the pool bathing the node (volume, ~0.1 ml). In experiments where more than one concentration of drug was used, the lowest concentration was always applied first, since drug effects were difficult to reverse.
**Analysis**

The decline of sodium current during a step depolarization was fit by a double exponential function:

$$I = I_f e^{-t/\tau_f} + I_s e^{-t/\tau_s} + I_n$$  

where $I_f$ and $I_s$ are, respectively, the initial amplitudes of the fast and slow inactivating components (extrapolated to the start of the step), $\tau_f$ and $\tau_s$ are their respective time constants, $t$ is the time after the voltage step, and $I_n$ is the noninactivating component (small or absent in this preparation). Both exponentials were fit simultaneously to the declining phase of the sodium currents by a Levenberg-Marquardt algorithm (Marquardt, 1963).

The decline in peak $I_{Na}$ (proportional to the increase in blocked channels) during pulse trains was fit by the following equations (adapted from Starmer, 1986):

$$B_n = B_{n-1} e^{-\lambda_e} + E_e (1 - e^{-\lambda_e t_e}) e^{-\lambda_r t_r}$$

$$B_0 = 0$$  

where $B_n$ is the fraction of channels blocked just prior to the $n$th pulse in the train, $E_e$ is the equilibrium block associated with the "excited" state (i.e., during depolarization), and

$$\lambda = \lambda_e t_e + \lambda_r t_r$$

where $\lambda_e$ represents the net rate ($s^{-1}$) of binding during an excited interval (depolarization), $\lambda_r$ the net rate ($s^{-1}$) of unbinding during a recovery interval, and $t_e$ and $t_r$ represent the durations, respectively, of the excitation and recovery intervals. $B_0$, the equilibrium binding level for rested fibers, is assumed to be negligible for this analysis. Estimates of $E_e$, $\lambda_e$, and $\lambda_r$ were made using a least-squares "Pattern search" algorithm (Colquhoun, 1971).

**RESULTS**

**Tonic Block of Sodium Channels by 5-HHX**

As with other local anesthetic agents (Hille, 1966; Arhem and Frankenhaeuser, 1974), 5-HHX decreases the peak amplitude of depolarization-induced sodium currents without affecting the reversal potential (Fig. 2). The potency for this "tonic" block is rather low; $\sim 2$ mM 5-HHX was required to block 50% of the nodal sodium current compared with 0.2 mM lidocaine for 50% block at similar holding potentials (Chernoff and Strichartz, 1988). In two control experiments, the EtOH vehicle alone, up to 2% in Ringer's solution, had no significant effect on the size or kinetics of sodium currents (data not shown). In one experiment, 8 mM 5-HHX produced no more tonic block than seen with 4 mM drug ($\sim 60\%$). This observation does not appear to be related to the solubility of the drug in Ringer's, since the absorbance at 271 nm obeyed Beer's Law for aqueous concentrations up to 10 mM (data not shown).

**Steady-State Inactivation**

5-HHX produced changes in the apparent voltage dependence of fast inactivation, as measured using a conventional two-pulse protocol (Hodgkin and Huxley, 1952) (Fig. 3). Inactivation curves were fit by the empirical Boltzmann equation,

$$h = \frac{[1 + e^{(E_e - E_o)/k}]}^{-1}$$  

where $E_o$ is the reversal potential.
where $E$ is the prepulse potential, $E_{1/2}$ is the potential for which $h = 0.5$, and $k$ is a steepness factor. In Ringer's solution without drug, and using 50-ms prepulses, the data points were well fit by Eq. 5 (Fig. 3A: circles). In the presence of 2 mM (upward triangles) and 4 mM (inverted triangles) 5-HHX, Eq. 5 no longer fit the data well. On the basis of a least-squares fit, however, 5-HHX appeared to cause a concentration-dependent hyperpolarizing shift in $E_{1/2}$ and a decrease in the slope factor, $k$. Four of the five fibers tested showed this characteristic change in the voltage dependence of inactivation. In the fifth fiber, 4 mM 5-HHX produced <10% tonic block and little change in the voltage dependence of inactivation. Nonetheless, this fifth fiber, in common with the other fibers tested, showed altered kinetics of inactivation and phasic block was observed (see below).

**FIGURE 2.** Inhibition of sodium currents by 5-HHX. Sodium currents were evoked with a 50-ms, 30-mV hyperpolarizing prepulse (to remove resting Na channel inactivation), followed by a 16-ms test pulse to various potentials in 10-mV steps. (A) Unaveraged sodium current records from one node following leak and capacity current subtraction are shown before and after addition of 2 mM 5-HHX to the solution bathing the node. (B) I-V plot of peak $I_{Na}$ vs. test pulse potential. There is about a 60% decrease in peak $I_{Na}$ at all potentials in the presence of drug (○). The reversal potential did not change significantly. $E_{rev} = -80$ mV. pH$_o$ 7.3. Temperature, 13°C. Fiber 88412.

One possible reason for the alteration of the voltage dependence of inactivation in the presence of anesthetic (Fig. 3A) is that the binding of drug to sodium channels may be voltage dependent; if so, equilibrium might not be achieved within the 50-ms prepulse. To investigate this possibility, we increased the prepulse duration to 2.5 s (Fig. 3B). A hyperpolarizing 2.5-s prepulse in the presence of drug increased peak $I_{Na}$ to a greater extent than did a 50-ms prepulse (dotted lines are curves from Fig. 3A); similarly, depolarizing 2.5-s prepulses decreased peak $I_{Na}$ more than 50-ms depolarizing prepulses. Prepulses of 5 s produced no further changes. Eq. 5 fits the data obtained in the presence of drug with the 2.5-s prepulses (Fig. 3B) much better than it fits data obtained with 50-ms prepulses (Fig. 3A). The tonic effects of 5-HHX under steady-state conditions can thus be characterized as a simple voltage shift of the inactivation curve and a decrease in the maximum peak $I_{Na}$.
For comparison, we tested the effect of 1 mM benzocaine on the voltage dependence of inactivation (Fig. 3 C). Here, 50-ms prepulses reveal a large (20 mV) hyperpolarizing shift in $E_{1/2}$ with little change in the slope factor, similar to the findings of Hille (1977b) and Meeder and Ulbricht (1987). The binding reactions of benzocaine are evidently complete in 50 ms or less, as experiments using 2.5-s prepulses yielded results similar to those obtained with the shorter prepulses (data not shown).

**FIGURE 3.** Voltage dependence of inactivation. (A) The peak sodium current in response to a depolarization to 0 mV is plotted against prepulse potential for 50-ms prepulses (see inset for protocol), in the absence of drug (○) and in the presence of 2 mM (●) and 4 mM (▲) 5-HHX. Continuous curves are least-squares fits to Eq. 5, multiplied by $I_{\text{max}}$ (see Methods). $E_{1/2}$ was 63.2 mV (control), -70.1 mV (2 mM), and -70.2 mV (4 mM); $k$ was 6.59 mV (control), 8.68 mV (2 mM), and 10.24 mV (4 mM). Arrows mark $E_{1/2}$ on each curve. Fiber 88424. (B) Inactivation assessed using 2.5-s prepulses. Symbols as in A. $E_{1/2}$ was -64.2 mV (control), -72.1 mV (2 mM), and -73.3 mV (4 mM); $k$ was 6.18 mV (control), 6.28 mV (2 mM), and 6.62 mV (4 mM). Dotted lines recapitulate fits from A. The control, 2 mM, and 4 mM data were obtained 28, 39, and 68 min, respectively, after the fiber was placed in voltage clamp. Fiber 88424. (C) Steady-state inactivation (50-ms prepulses) assessed in the absence (○) and presence (▲) of 1 mM benzocaine. $E_{1/2}$ was -64.2 mV (control) and -86.1 mV (1 mM benzocaine); $k$ was 10.88 mV (control) and 10.11 mV (1 mM benzocaine). Fiber 88127B.

**Rates of Sodium Current Inactivation Are Slowed by 5-HHX**

5-HHX significantly altered the apparent kinetics of inactivation of $I_{\text{Na}}$. Fig. 4 shows sodium currents recorded during depolarization to 0 mV in the absence (A, solid trace) and presence (B) of 2 mM 5-HHX. The kinetics of activation could not be well resolved with the recording bandwidth used (5 kHz), but the time to peak was delayed and the rate of decay of $I_{\text{Na}}$ slowed in the presence of drug.
Further analysis of the depolarization-evoked $I_{Na}$ showed that the decay of $I_{Na}$ could be characterized by two exponential phases, fit by Eq. 1 (Fig. 4, C and D, and Table I). In control recordings, the relative amplitudes of the fast ($I_f$) and slow ($I_s$) components of inactivation and their time constants, are similar to those reported in frog node (Schwarz et al., 1983; Meeder and Ulbricht, 1987). The amplitudes of both the fast and slow components were reduced by 2 mM 5-HHX. In four of five fibers tested (see Table I), the fast component of decay appeared more sensitive to 5-HHX, so that the slow component comprised a greater fraction of the inactivating current (increase in $F_s$) after the addition of drug. The time constants of both exponential components increased in the presence of 5-HHX in roughly the same proportion (Table I). These findings are quite different from those reported for benzocaine in frog node, where benzocaine selectively reduced $I_s$ without a significant

### Table I

| Fiber   | Control | 4 mM 5-HHX/control |
|---------|---------|---------------------|
|         | $I_f$ (ms) | $I_s$ (ms) | $F_f$ | $F_s$ | $r_f$ | $r_s$ | $r_f$ | $r_s$ |
| 88127   | 0.25 | 0.59 | 2.33 | 0.85 | 1.09 | 2.07 | 0.92 | 1.63 | 1.75 |
| 88309A  | 0.16 | 0.50 | 2.33 | 0.69 | 0.98 | 1.32 | 0.92 | 1.75 | 1.34 |
| 88412   | 0.11 | 0.45 | 2.75 | 0.35 | 0.41 | 1.25 | 1.47 | 1.46 |
| 88420   | 0.16 | 0.51 | 2.63 | 0.35 | 0.79 | 2.02 | 1.65 | 1.26 |
| 88424   | 0.14 | 0.63 | 2.78 | 0.55 | 0.64 | 1.12 | 1.10 | 1.07 |
| Mean ± SEM | 0.16 | 0.54 | 2.56 | 0.55 | 0.75 | 1.37 | 1.55 | 1.38 |

*Parameter value after treatment with 4 mM 5-HHX.

*Drug effect is significant with $P > 0.95$, two-sided paired $t$ test.
change in τ₁ or τₑ (Neumcke et al., 1981; Schneider and Dubois, 1986; Meeder and Ulbricht, 1987).

**Phasic Block of Sodium Currents by 5-HHX**

Repetitive depolarization of nodal membrane in the presence of 4 mM 5-HHX produced frequency-dependent ("phasic") inhibition of sodium currents (Fig. 5). When pulses were applied at 10 s⁻¹, for example, each successive pulse produced an additional decrease in peak \( I_{Na} \) (Fig. 5 A). In Fig. 5 B, peak \( I_{Na} \) (normalized to the first pulse in the train) is plotted against pulse number for three stimulus frequencies. As with other agents producing phasic block of \( I_{Na} \), the response to periodic depolarization in the presence of 5-HHX can be modeled kinetically by assuming that net binding of drug (to a continuously available receptor) occurs during each depolarization.
zation and net unbinding occurs during each repolarized interval (Courtney et al., 1978; Starmer, 1986). The resulting equation (see Methods, Eq. 2) provided a good fit (by least-squares) to the data (Fig. 5B), yielding estimates for the binding parameters, $\lambda_1$ and $E_\infty$, and the unbinding parameter, $\lambda_2$ (Table II). In two control experiments, 2% EtOH (twice the concentration used for 4 mM 5-HHX) produced negligible phasic block of sodium channels (data not shown).

**Recovery from Phasic Block**

To observe the recovery of channels from phasic block more directly than in the preceding protocol, the following experiment was performed: a fiber was depolarized repetitively to produce phasic block, then repolarized for a variable duration, $t$, before applying a single test pulse to assess the extent of recovery of peak $I_{Na}$. The cycle was then repeated with a different recovery interval. As seen with quaternary and tertiary amine anesthetics (Yeh and Tanguy, 1985), sodium channels recovered in two distinct phases (Fig. 6). A double exponential equation,

$$I_{rec}(t) = 1 - A_1 e^{-t/\tau_1} + (1 - A_1)e^{-t/\tau_2}$$

was used to fit the recovery data. One fraction recovered quickly, at a rate similar to the rate of recovery from inactivation in control solution, while a second fraction recovered much more slowly. The slowly recovering fraction increased with higher doses of drug ($n = 4$). The time constant of the slowly recovering fraction, $\tau_2$, as estimated from Eq. 6, was (for this fiber) 0.93 s with 2 mM 5-HHX, 0.65 s with 4 mM (see Table II for comparison with other fibers), and 0.78 s with 8 mM; $A_1$, the fast recovering fraction was 0.83 with 2 mM, 0.74 with 4 mM, and 0.63 with 8 mM.

**Dependence of Phasic Block on Pulse Duration**

If phasic block represents binding to the open state alone, then one would expect no further binding for pulse durations longer than that required to inactivate all sodium channels. We tested this idea by varying the duration of the depolarizing step in a pulse train, holding the recovery interval constant at 100 ms (Fig. 7).
Although inactivation is substantially complete in <20 ms (see Figs. 2, 4, and 5), block was significantly enhanced with longer depolarizations. These data were fit by Eq. 2, with $\lambda_v = 4.17 \text{ s}^{-1}$, $\lambda_i = 1.30 \text{ s}^{-1}$, and $E_a = 0.57$. The fit was fair for the long duration pulses, but the equation predicts less block for the shortest duration pulses (<10 ms) than was observed. This provides one indication that binding occurs more rapidly early in a depolarization, when channels are open, than later, when channels are inactivated. Nevertheless, it seems clear that significant binding continues long after sodium channels have inactivated. The estimated binding rate during depolarization, $\lambda_v = 4.17 \text{ s}^{-1}$, primarily reflects the rate of binding to inactivated channels, as it is derived from long duration (>10 ms) pulses in which inactivation predominate.

**Kinetics of Phasic Block by 5-HHX Are Independent of pH**

As described in the Introduction, the kinetics of phasic block by tertiary amine anesthetics depend strongly on extracellular pH: the rate of recovery from phasic block is increased at alkaline pHo and slowed at acidic pHo, which is consistent with titra-
tion of the tertiary amine while the drug is bound to a receptor (Schwarz et al., 1977; Broughton et al., 1984; Starmer and Courtney, 1986). Phasic block with quaternary lidocaine derivatives is not pH$_a$-dependent (Schwarz et al., 1977), but the lack of demonstrable phasic block with neutral agents to date has precluded a similar analysis for neutral drugs. It was therefore of interest to examine the pH$_a$ dependence of phasic block with 5-HHX.

Phasic block was assessed at pH$_a$ 7.3, 8.5, and 9.4 in a single fiber, at three stimulus frequencies (Fig. 8). There were only modest effects of pH$_a$ on the kinetics of block with 5-HHX, compared with the dramatic effects of pH$_a$ on phasic block with lidocaine (Schwarz et al., 1977; Broughton et al., 1984; Chernoff and Strichartz, 1988). Control runs at these three pH$_a$ levels (in the absence of drug) revealed no systematic decline of peak $I_{Na}$ with pulse number for rates up to 20 Hz (data not shown). These findings are consistent with the conclusion of Schwarz et al., (1977) i.e., pH$_a$ has little direct effect on the binding site for local anesthetics.

**DISCUSSION**

We have made the somewhat arbitrary division of the actions of 5-HHX on sodium channels into tonic and phasic effects, where tonic refers to effects observed at low
rates of stimulation and phasic refers to cumulative effects at higher stimulation rates. If all the observed effects of 5-HHX can be attributed to binding to a single receptor, then the tonic/phasic division is merely operational. If, however, 5-HHX has more than one site of action, then the distinction between tonic and phasic block may reflect binding or perturbation at these different sites.

In the presence of the quaternary anesthetic QX-314, both tonic and phasic block of nodal sodium channels apparently require the open channel conformation (Strichartz, 1973). That is, the first depolarization after internal application of QX-314 reveals little decrease in \( I_{\text{Na}} \); subsequent pulses show a persistent block, even when applied infrequently. With the neutral anesthetic benzocaine, tonic block can occur without channel activation (Ulbricht and Stoye-Herzog, 1984). Hille (1977b) has suggested that these distinctions primarily reflect hydrophilic and hydrophobic access pathways to a common binding site: the channel must open to allow access of QX-314, while benzocaine has continuous access to open or closed channels through the membrane lipid. Under this hypothesis, the absence of phasic block by neutral agents in voltage clamp studies is due to their rapid dissociation from a receptor shared with charged agents. Our observation of phasic block in the presence of 5-HHX is consistent with the idea that 5-HHX binds to, and unbinds slowly from, a gated receptor like that for charged agents. However, some of the tonic effects of 5-HHX appear difficult to reconcile with a single locus of action (see below).

**Tonic Effects**

The tonic block of sodium channels by 5-HHX appears similar in most respects to that observed with other anesthetics; there is a decrease in sodium permeability without a change in the reversal potential, and a hyperpolarizing shift in the voltage dependence of inactivation. The low potency of 5-HHX for block of sodium channels may be related to its relatively low lipophilicity, as estimated from its octanol/water distribution coefficient \((Q)\) of \( \approx 9 \) at \( 22^\circ C \) (Chernoff and Strichartz; unpublished results). For comparison, \( Q \approx 65 \) for lidocaine at pH 7.3 and 25°C (Sanchez et al., 1987), and lidocaine is roughly ten times more potent under similar test conditions. The inability of 5-HHX to produce more complete inhibition of sodium currents when the concentration was raised to 4 and 8 mM in these experiments suggests, as one possibility, that there is more than one type of sodium channel in the nerve membrane, with different affinities for drug (e.g., see Benoit et al., 1985; Meeder and Ulbricht, 1987); 8 mM 5-HHX might then saturate one channel type while producing little inhibition of the second.

**Kinetics of \( I_{\text{Na}} \).** The slowing of the rate of decay of sodium currents in the presence of 5-HHX is unexpected, since benzocaine has been reported either to have no effect on (Schneider and Dubois, 1986; Meeder and Ulbricht, 1987) or to accelerate (Neumcke et al., 1981) the rate of \( I_{\text{Na}} \) inactivation. The slowing of the time-to-peak and the slower decay of \( I_{\text{Na}} \) may both be due to a selective effect of 5-HHX on either the microscopic activation or inactivation processes, depending on the model used for sodium channel gating. For example, if the drug slows the rate of delivery of channels to the open state, it will affect \( I_{\text{Na}} \) appropriately in models where activation is slow and inactivation is rapid. Conversely, if the drug slows the microscopic rate
of inactivation, it will produce the same macroscopic effects in models where activation is rapid and inactivation is slow (see Vandenberg and Horn, 1984, for a review). Therefore, mechanistic interpretation of this effect of 5-HHX requires further elucidation of the gating kinetics of sodium channels in this preparation, e.g., by gating current and single-channel measurements. The relationship of these effects on inactivation kinetics to phasic block, if any, is obscure.

**Phasic Effects**

Repetitive depolarization of nodal membrane in the presence of 5-HHX causes a frequency-dependent increment in the fraction of blocked channels. This excess block relaxes slowly enough at the holding potential to distinguish kinetically the bound channels from normally inactivated channels (Figs. 5 and 6). The estimated rate constant for recovery from block between pulses, \( \lambda \), is 1.01 s\(^{-1}\) (Fig. 5), is in fair agreement with the time constant (1/rate constant) of the slowly recovering sodium permeability measured directly, \( \tau_s = 1.69 \) s (Fig. 6 and Table II). The two components of recovery after phasic block in Fig. 6 are most simply interpreted as due to two populations of channels, one substantially unmodified (fast recovery) and the other anesthetic bound (slow recovery) (see Yeh and Tanguy, 1985).

We also calculated a predicted time constant for recovery based on the "size/solubility" hypothesis and regression equation relating recovery time constants to molecular weight and lipid solubility (Courtney, 1985). There is fair agreement (given the difference in experimental conditions) between the mean time constant for recovery in 4 mM 5-HHX in these experiments (1.08 or 1.65 s, depending on the method of measurement; see Table II), and the value predicted from Courtney's regression equation (0.80 s). Courtney's predicted recovery time constant for benzocaine, using an estimated partition coefficient of 65.9 (Froese, 1986), is 0.05 s; too fast, perhaps, to distinguish kinetically from inactivation recovery (\( \tau \approx 0.01 \) s). According to Courtney's hypothesis, the relatively slow rate of unbinding of 5-HHX, compared with benzocaine, is due in part to its lower lipid solubility and in part to its larger size. Modification of the structure of 5-HHX, by adding substituents, would either slow or accelerate unbinding, depending on the relative contributions of the substituents to the molecular weight and to the partition coefficient, permitting an experimental test of these ideas.

**Open vs. inactivated channel block.** The observation that block of channels continues to increase for durations longer than that required to inactivate all sodium channels (Fig. 7) strongly suggests that binding is not limited to the open state. Much of the block, in fact, can be accounted for by binding to an inactivated state. This is consistent with Hille's hypothesis (1977b) of hydrophilic and hydrophobic access pathways to a common receptor, i.e., neutral drugs have continuous access to the receptor via a hydrophobic path way.

**Binding affinities estimated by the modulated receptor and guarded receptor hypotheses.** If we assume that sodium channels exist in only two conformations at the holding potential, resting (R) and inactivated (I), then it becomes possible to estimate the relative affinities of drug for the resting and inactivated states from the dose dependence of the shift in the midpoint of the inactivation-potential curve (Bean et al., 1983). The shift in the midpoint, \( \Delta E_{1/2} \), is related to the relative affinities for the
resting and inactivated states by the following equation (from Bean et al., 1983):

\[
\Delta E_{1/2} = k \cdot \ln \left[ \frac{(1 + [D]/K_R)/(1 + [D]/K_I)}{1 + [D]/K_I} \right]
\]

where \( k \) is the slope factor, \( D \) is the drug concentration, and \( K_R \) and \( K_I \) are the apparent dissociation constants for the resting and inactivated states. If \( k \) is taken as 6.3 mV (the mean of the \( k \)’s from Fig. 3 B), \( \Delta E_{1/2} = -7.9 \) mV (2 mM 5-HHX), and \( \Delta E_{1/2} = -9.1 \) mV (4 mM 5-HHX), then \( K_R = 1.63 \) mM, and \( K_I = 0.29 \) mM. Although these estimates are based on only two concentrations, a relatively modest difference in the apparent affinities of drug for \( I \) and \( R \) is consistent with the smaller shifts in \( E_{1/2} \) with 5-HHX compared with benzocaine. Using these estimates, Eq. 8 predicts a maximum shift of \( E_{1/2} \) with 5-HHX equal to \( (\Delta E_{1/2})_{max} = k \cdot \ln (K_I/K_R) = -10.9 \) mV, much less than the \(-20\) mV shift seen with a nonsaturating dose (1 mM) of benzocaine.

The guarded receptor hypothesis can also explain the drug-induced shift in apparent steady-state inactivation (Starmer et al., 1984). The original formulation of this hypothesis, in which binding only occurs in open channels to a constant affinity receptor, predicts a decrease in the slope factor, \( k \), i.e., steeper voltage dependence, in the presence of drug (Fig. 3 of Starmer et al., 1984). This is not observed with 5-HHX or with benzocaine. However, the observed effects of these drugs can be reconciled with the guarded receptor hypothesis if neutral agents have access only to inactivated channels, but can unbind at any time and from any conformation (Starmer, personal communication). Under these restrictions, the midpoint shift in inactivation is:

\[
\Delta E_{1/2} = k \cdot \ln (1 + [D]/K_D)
\]

yielding \( K_D \) estimates of 0.80 mM (2 mM 5-HHX) and 1.23 mM (4 mM 5-HHX). There is not sufficient evidence in the present study to distinguish between these hypotheses.

We thank Drs. Bruce P. Bean, Stephen A. Raymond, Dinah W. Y. Sah, and Ging-Kuo Wang for helpful comments on the manuscript, Drs. Paul D. McMaster and Eugene W. Byrnes for their gift of 5-HHX, and Ms. Rachel Abrams and Mary Gioiosa for preparing the manuscript.

This work was supported by United States Public Health Services grants GM-15904 and GM-35647.

Original version received 11 July 1988 and accepted version received 14 November 1988.

REFERENCES

Arhem, P., and B. Frankenhaeuser. 1974. Local anesthetics: effects on permeability properties of nodal membrane in myelinated nerve fibres from Xenopus. Potential clamp experiments. Acta Physiologica Scandinavica. 91:11–21.

Bean, B. P., C. J. Cohen, and R. W. Tsien. 1983. Lidocaine block of cardiac sodium channels. Journal of General Physiology. 81:613–642.

Benoit, E., A. Corbier, and J.-M. Dubois. 1985. Evidence for two transient sodium currents in the frog node of Ranvier. Journal of Physiology. 361:339–360.
CHEROFF AND STRICHARTZ. Block of Na⁺ Currents by 5-HHX

Broughton, A., A. O. Grant, C. F. Starner, J. K. Klinger, B. S. Stambler, and H. C. Strauss. 1984. Lipid solubility modulates pH potentiation of local anesthetic block of Vmax reactivation in guinea pig myocardium. Circulation Research. 55:513–523.

Chernoff, D. M., and G. R. Strichartz. 1988. Lidocaine and bupivacaine block of sodium channels—potency correlates with rate of recovery from phasic block. Biophysical Journal. 55:537a. (Abstr.)

Colquhoun, D. C. 1971. Lectures in Biostatistics. Clarendon Press, Oxford. 263–266.

Courtney, K. R. 1975. Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. Journal of Pharmacology and Experimental Therapeutics. 195:225–236.

Courtney, K. R., J. J. Kendig, and E. N. Cohen. 1978. The rates of interaction of local anesthetics with sodium channels in nerve. Journal of Pharmacology and Experimental Therapeutics. 207:594–604.

Dodge, F. A., and B. Frankenhaeuser. 1958. Membrane currents in isolated frog nerve fibre under voltage clamp conditions. Journal of Physiology. 143:76-90.

Frazier, D. T., T. Narahashi, and M. Yamada. 1970. The site of action and active form of local anesthetics. II. Experiments with quaternary compounds. Journal of Pharmacology and Experimental Therapeutics. 171:45–51.

Froese, U. 1986. Untersuchungen zu Struktur-Wirkungs-Beziehungen von Aminobenzoesurederivaten am myelinisierten Nervenfasern. Thesis. University of Kiel.

Hille, B. 1966. Common mode of action of three agents that decrease the transient change in sodium permeability in nerves. Nature. 210:1220–1222.

Hille, B. 1977a. The pH-dependent rate of action of local anesthetics on the node of Ranvier. Journal of General Physiology. 69:475–496.

Hille, B. 1977b. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. Journal of General Physiology. 69:497–515.

Hodgkin, A. L., and A. F. Huxley. 1952. The dual effects of membrane potential on sodium conductance in the giant axon of Loligo. Journal of Physiology. 115:487–506.

Hondegem, L. M., and B. G. Katzung. 1977. Time and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. Biochimica et Biophysica Acta. 472:373–398.

Marquardt, D. W. 1963. An algorithm for least-squares estimation of nonlinear parameters. Journal of the Society of Industrial and Applied Mathematics. 11:431.

Meeder, T., and W. Ulbricht. 1987. Action of benzocaine on sodium channels of frog nodes of Ranvier treated with chloramidine-T. Pflügers Archiv. 409:265–273.

Mrose, H., and J. M. Ritchie. 1978. Local anesthetics: do benzocaine and lidocaine act at the same single site? Journal of General Physiology. 71:223–225.

Neumcke, B., W. Schwarz, and R. Stampfl. 1981. Block of Na channels in the membrane of myelinated nerve by benzocaine. Pflügers Archiv. 390:230–236.

Rando, T. A., G. R. Strichartz, and G. K. Wang. 1986. The interaction between the activator agents batrachotoxin and veratridine and the gating processes of neuronal sodium channels. Molecular Pharmacology. 29:467–477.

Rimmel, C., A. Walle, H. Kessler, and W. Ulbricht. 1978. Rates of interaction by procaine and benzocaine and the procaine-benzocaine interaction at the node of Ranvier. Pflügers Archiv. 376:105–118.

Sanchez, V., G. R. Arthur, and G. R. Strichartz. 1987. Fundamental properties of local anesthetics. I. The dependence of lidocaine’s ionization and octanol: buffer partitioning on solvent and temperature. Anesthesia and Analgesia. 66:159–165.
Schmidtmayer, J., and W. Ulbricht. 1980. Interaction of lidocaine and benzocaine in blocking sodium channels. Pfliigers Archiv. 387:47–54.

Schneider, M. F., and J.-M. Dubois. 1986. Effects of benzocaine on the kinetics of normal and batrachotoxin-modified Na channels in frog node of Ranvier. Biophysical Journal. 50:523–530.

Schwarz, J. R., B. Bromm, R. P. Spielmann, and J. L. F. Weytjens. 1983. Development of Na inactivation in motor and sensory myelinated nerve fibres of Rana esculenta. Pfliigers Archiv. 398:126–9.

Schwarz, W., P. T. Palade, and B. Hille. 1977. Local anesthetics: effects of pH on use-dependent block of sodium channels in frog. Biophysical Journal. 20:343–368.

Starmer, C. F. 1986. Theoretical characterization of ion channel blockade: ligand binding to periodically accessible receptors. Journal of Theoretical Biology. 119:235–249.

Starmer, C. F., and K. R. Courtney. 1986. Modeling ion channel blockade at guarded binding sites: application to tertiary drugs. American Journal of Physiology. 251:H848–H866.

Starmer, C. F., A. O. Grant, and H. C. Strauss. 1984. Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. Biophysical Journal. 46:15–27.

Strichartz, G. R. 1973. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. Journal of General Physiology. 62:37–57.

Tenthorey, P. A., A. J. Block, R. A. Ronfeld, P. D. McMaster, and E. W. Byrnes. 1981. New antiarrhythmic agents 6. Quantitative structure-activity relationships of aminoxylidides. Journal of Medicinal Chemistry. 24:798–806.

Ulbricht, W., and M. Stoye-Herzog. 1984. Distinctly different rates of benzocaine action on sodium channels of Ranvier nodes kept open by chloramine-T and veratridine. Pfliigers Archiv. 402:439–445.

Vandenberg, C. A., and R. Horn. 1984. Inactivation viewed through single sodium channels. Journal of General Physiology. 84:535–564.

Yeh, J. Z., and J. Tanguy. 1985. Na channel activation gate modulates slow recovery from use-dependent block by local anesthetics in squid giant axons. Biophysical Journal. 47:685–694.