Local Anesthetics: Hydrophilic and Hydrophobic Pathways for the Drug-Receptor Reaction

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ABSTRACT The properties of Na channels of the node of Ranvier are altered by neutral, amine, and quaternary local anesthetic compounds. The kinetics of the Na currents are governed by a composite of voltage- and time-dependent gating processes with voltage- and time-dependent block of channels by drug. Conventional measurements of steady-state sodium inactivation by use of 50-ms prepulses show a large negative voltage shift of the inactivation curve with neutral benzocaine and with some ionizable amines like lidocaine and tetracaine, but no shift is seen with quaternary QX-572. However, when the experiment is done with repetitive application of a prepulse-testpulse waveform, a shift with the quaternary cations (applied internally) is seen as well. 1-min hyperpolarizations of lidocaine- or tetracaine-treated fibers restore two to four times as many channels to the conducting pool as 50-ms hyperpolarizations. Raising the external Ca^{++} concentration also has a strong unblocking effect. These manipulations do not relieve block in fibers treated with internal quaternary drugs. The results are interpreted in terms of a single receptor in Na channels for the different drug types. Lipid-soluble drug forms are thought to come and go from the receptor via a hydrophobic region of the membrane, while charged and less lipid-soluble forms pass via a hydrophilic region (the inner channel mouth). The hydrophilic pathway is open only when the gates of the channel are open. Any drug form in the channel increases the probability of closing the inactivation gate which, in effect, is equivalent to a negative shift of the voltage dependence of inactivation.

INTRODUCTION

This paper continues the analysis of two major questions concerning local anesthetic action: where on the Na channel is the receptor, and how do the anesthetic molecules get there? In this work the anesthetic solution is applied long enough for the distribution of drug to approach an equilibrium. Nonetheless the degree of block is shown to be strongly dependent on the rate and waveform of the testing voltage clamp pulses, on the holding potential, and on the external Ca^{++} concentration. The persistence of after effects of recent stimulation is quite analogous to the rate-dependent block of Na channels seen in myocardial fibers treated with antiarrhythmic drugs (see references in Gettes and Reuter, 1974). As has already been found in previous work on myelinated nerve (Strichartz, 1973; Khodorov et al., 1974; Courtney, 1975; Khodorov et al.,

1976), the reactivity of the local anesthetic receptor is a sensitive function of the state of the voltage-dependent gates of the Na channel. Factors that affect gating affect the susceptibility to block with local anesthetics, and block by anesthetics affects gating. As in the preceding paper (Hille, 1977), measurements are made by using neutral, ionizable, and permanently cationic drug molecules with a range of lipid solubilities in order to examine the effect of charge and hydrophobicity on the drug-receptor interaction.

MATERIALS AND METHODS

Single myelinated nerve fibers were studied under voltage clamp as described in the preceding paper. All experiments were done at 15°C with an external solution containing NaCl 100–115 mM, $CaCl_2$ 2 mM, and tris(hydroxymethyl) aminomethane-HCl buffer 1–6 mM. The pH was always 7.4 in these experiments. In most cases 1–7 mM tetraethylammonium ion (TEA) was added to block currents in K channels. The ends of the fibers were cut in unbuffered 120 mM KCl, and drugs were added to the "internal" or external solutions as before. The structures of the drugs are given in Fig. 1 of the preceding paper.

RESULTS

Fig. 1 shows a family of voltage clamp currents from a fiber before and during exposure to 1 mM lidocaine at pH 7.4. In the untreated fiber (Fig. 1 A), the test pulses elicit the usual transient increase of sodium permeability P_{Na} that reaches a peak in the 1st ms and then a slower rise of potassium permeability P_K that takes more than 10 ms to develop fully (not shown in its entirety). With a 50-ms prepulse to −143 mV (E_{pp}) to remove resting sodium inactivation, the peak inward sodium current I_{Na} reaches −33.2 nA in the step to −23 mV, and the outward potassium current rises to 19.1 nA at 67 mV (at 10 ms but not drawn in the figure). When the same fiber is treated with 1.0 mM lidocaine and tested with the same pulses (Fig. 1 B), peak P_{Na} is reduced to 15% of the control and peak P_K is reduced to 80%. This observation confirms the general finding with myelinated fibers that local anesthetics block Na channels more than K channels (Hille, 1966; Århem and Frankenhaeuser, 1974). From now on Results focus on comparing the block of Na channels with amine, neutral, and quaternary anesthetics, and in most experiments P_K is deliberately depressed by adding tetraethylammonium ion to the solutions. The results are divided into two sections. The first concerns the effect of prepulses, holding potential, and Ca^{++} on steady-state block with low rates of stimulation. These factors are modulators of the resting state of channels. The second section concerns the cumulative effect of higher rates of stimulation on block. The depolarizing stimulus pulses activate Na channels and modify their probability of becoming blocked.

Prepulse, Holding Potential, and Ca^{++}

SODIUM INACTIVATION SEEN WITH SHORT PREPULSES. In normal myelinated fibers roughly 30–50% of the Na channels are inactivated at the resting potential, and their resting inactivation may be removed by a 30–45-mV hyperpolarizing prepulse lasting tens of milliseconds (Frankenhaeuser, 1960). These phenomena are well described in normal fibers by the conventional inactivation

variable h of Hodgkin and Huxley (1952). After local anesthetic treatment, however, the extent of resting sodium inactivation is increased in a manner which may involve the development of new inactivated states. (Khodorov et al., 1974, 1976; Courtney, 1974, 1975).

For some but not all local anesthetics changes of inactivation may be seen in experiments with 50-ms prepulses preceding the test pulse. An increase of resting inactivation with 1 mM lidocaine is shown in Fig. 1. In the untreated fiber (Fig. 1 A and C), removing the 50-ms prepulse (E_{pp}) decreases peak P_{Na} by 35%, while in the treated fiber (Fig. 1 B and D) removing the prepulse decreases P_{Na} by 90%. The voltage dependence of sodium inactivation measured with 50-ms prepulses from a holding potential of −83 mV is shown in more detail in Fig. 2.

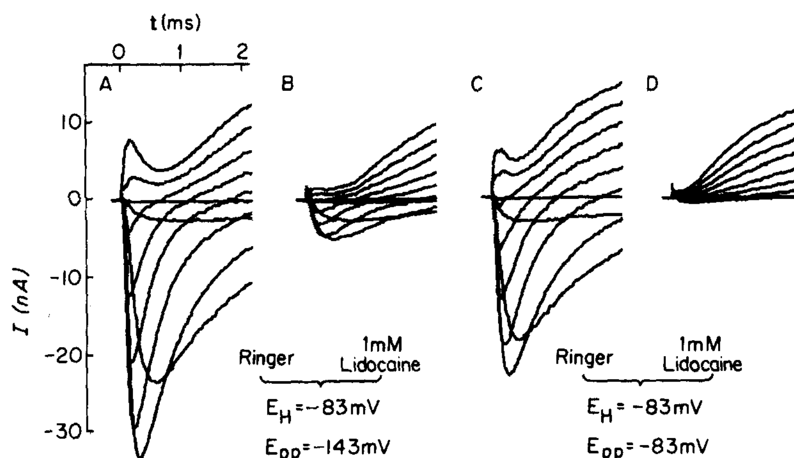

FIGURE 1. Families of voltage clamp currents before and during treatment with 1 mM lidocaine. Solutions contain no TEA. The node is held at a holding potential E_H = −83 mV and depolarized by test pulses ranging in 15 mV steps from −68 to +67 mV. In (A) and (B) the test pulse is preceded by a 50-ms conditioning prepulse to −143 mV. In (C) and (D) there is no prepulse. Test pulses applied at intervals of 1.0 s. Same node as in Figs. 3 and 5.

In the control these measurements define a conventional h_∞ curve, but with anesthetic the situation is more complicated and the curve will be called simply the inactivation curve. The changes with 1 mM lidocaine may be described as a 30-mV shift to more negative potentials of the midpoint of the curve accompanied by a reduction of slope. Inactivation curves from the same fiber are also shown during partial block with the uncharged molecule benzocaine and the quaternary cation QX-572. A 23-mV shift to more negative potentials is found with 1 mM benzocaine, but no change is found after external application of 0.6 mM QX-572. In other experiments with internal application of quaternary QX-572 or RAD 250 I, there was at most a 5-mV shift of the inactivation curve at low rates of stimulation, although P_{Na} was reduced by the drugs to 25–40% of the control value. Some amine anesthetics shift much less than lidocaine does. For instance, in two experiments I found that 2 μM tetracaine reduces P_{Na} to 8.5% of control and shifts the inactivation curve by only 7 mV, and Courtney (1975)

showed that GEA 968 gives no shift. It is important to note that the inactivation referred to here is defined by the effects of 50-ms prepulses on currents measured at stimulation rates too slow to give the kind of cumulative block described later.

MODULATION BY HOLDING POTENTIAL. In addition to the rapid effects from short conditioning prepulses, there are slower inactivation-like processes modulating the available P_{Na} in myelinated nerve (Peganov et al., 1973; Fox, 1976; Neumcke et al., 1976). A local anesthetic-dependent inactivation with 200–900-ms time constants at room temperature was discovered and analyzed extensively by Khodorov et al. (1974, 1976). These authors used the name slow sodium inactivation and designated the time constant τ_s and the fraction of available channels s_∞. In the experiments to be described, removal of such slow components of inhibition was accomplished by making the holding potential more negative in steps of 15 mV and holding at the new level for about 1 min before measurements were made.

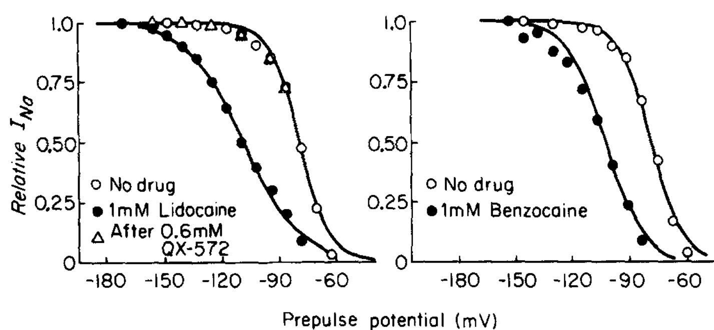

FIGURE 2. Effect of 50-ms conditioning prepulses on peak Na currents with neutral, amine, and quaternary anesthetics. All observations from the same node held at $E_H = -90$ mV and tested with test pulses to -15 mV. For each treatment, the current at the most negative prepulse potential shown is normalized to 1.0. Curves are drawn according to the formula $1/(1 + \exp((E - E_0)/k))$ where from left to right in the figure $E_0 = -109, -79, -102, -79$ mV, and $k = 14.4, 7.5, 9, 7.5$ mV.

Fig. 3 shows families of currents measured on the same fiber as was used for Fig. 1. The external solution contains 1 mM lidocaine and 3.6 mM TEA. In A the peak I_{Na} is only -0.5 nA at the holding potential E_H of -83 mV without a prepulse. In B a 60-mV hyperpolarizing prepulse lasting 50 ms increases peak P_{Na} 10-fold. According to the inactivation curve of Fig. 2, P_{Na} should now be within 15% of its maximum value. Nevertheless, as is shown in Fig. 3 C and D, peak P_{Na} can be increased a further 70% or 250% over that in Fig. 3 B by stepping the holding potential to -98 or -143 mV for 1 min. Long hyperpolarization relieves anesthetic block. The voltage dependence of this slow effect of holding potential is shown by triangles in Fig. 4 for the lidocaine experiments of Fig. 3 and for a different experiment with 2 μM tetracaine. For comparison the current amplitudes (circles) found in inactivation measurements with 50 ms prepulses starting from $E_H = -83$ mV are shown as well. With 1 mM lidocaine

less than 5% of the Na channels are available when holding at −83 mV without prepulses, and more than 50% of the channels are restored to the conducting pool by long hyperpolarizations. The situation with 2 μM tetracaine is similar. By contrast in experiments with the quaternary molecules QX-572 and RAD 250 I (*N*-

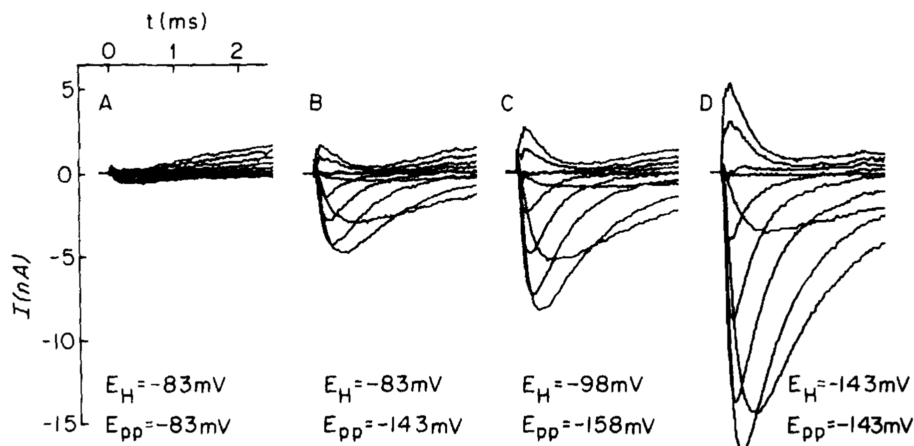

FIGURE 3. Relief of lidocaine block by more negative holding potentials. Families of voltage clamp currents in the presence of 1 mM lidocaine and 3.6 mM TEA. Same node and same test pulse potentials as in Figs. 1 and 5. Voltage of holding potential E_H and of the 50 ms prepulse E_{pp} are indicated below.

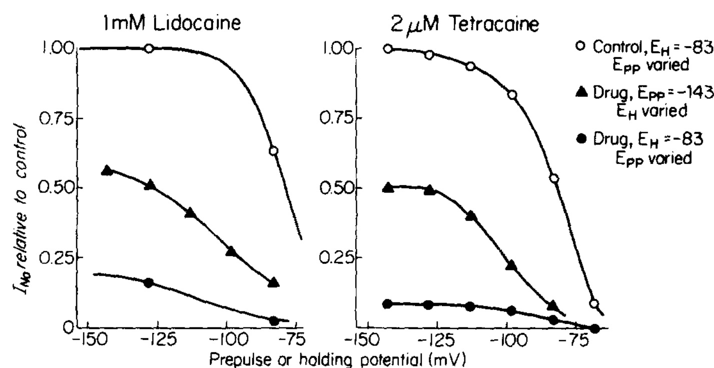

FIGURE 4. Slow and fast inactivation in fibers treated with lidocaine and tetracaine. The relative peak I_{Na} is shown (filled circles) as a function of the prepulse potential with a constant holding potential ($E_H = -83$ mV) or (filled triangles) as a function of the holding potential with a constant prepulse potential ($E_{pp} = -143$ mV). Unlike in Fig. 2, all currents are normalized with respect to the largest current in the control measurement before drug treatment. Smooth curves for controls are like those in Fig. 2 with $E_o = -80$ mV for the left curve and $E_o = -82$ mV for the right, and $k = 7.5$ mV. Curve through filled circles for lidocaine has $E_o = -110$ mV, $k = 14.4$ mV, and an asymptote of 0.2. Other curves arbitrary.

methyl mepivacaine) and with neutral benzocaine, changing the holding potential for 1 min had no apparent effect on the available P_{Na}. The holding potential also had no significant effect on fibers in the drug-free control solution.

The time course of the effects of holding potential was measured by stepping

E_H for varying periods of time before testing with a standard prepulse to ca. -125 mV followed by a test pulse to -5 mV. With 1 mM lidocaine, changes of E_H from -80 to -125 mV or back again gave nearly full effects in less than 200 ms. In this case it was difficult to separate the "slow" process from removal or development of normal inactivation since their time courses overlapped considerably. With 2 μM tetracaine the separation was clear. Unblock on stepping E_H from -80 to -125 mV developed with a time constant τ_s of 10.2 s, and block on stepping back to -80 mV developed with a τ_s of 2.8 s. These time constants at 13°C may be compared with values of 10 s for 0.6 mM GEA 968 at 10°C and -74 mV (Courtney, 1975) and 220–370 ms for 0.18 mM trimecaine and 640–700 ms for 0.9 mM procaine both at 20°C and -100 mV (Khodorov et al., 1976).

MODULATION BY EXTERNAL CALCIUM. The effects of hyperpolarizing holding potentials may be imitated by raising the external Ca^{++} concentration. Fig. 5 shows measurements on the same fiber as was used for Figs. 1 and 3. The top line shows currents recorded in the standard 2 mM Ca^{++} Ringer with 1 mM lidocaine as before. The lower line shows currents in 12.8 mM Ca^{++} with 1 mM lidocaine. Comparing frame A with frame D shows that elevating Ca^{++} dramatically increases the available P_{Na} when the fiber is held near the resting potential. Comparing B with D and C with E shows that hyperpolarizing the fiber in 2 mM Ca by 15 mV for 1 min restores the peak sodium current in a similar manner to raising Ca^{++} to 12.8 mM. According to earlier studies on the node of Ranvier (Hille, 1968; Vogel, 1974; Hille et al., 1975), increasing Ca^{++} from 2 mM to 12.8 mM would be equivalent to adding a 16.1-mV bias to the voltage dependence of activation of Na channels. Indeed, in Fig. 5 the maximum inward current occurs at -8 mV in 12.8 mM Ca^{++} and at -23 mV in 2 mM, and the entire P_{Na}-E relation is shifted about 15 mV. Hence, at least to a first approximation, steady hyperpolarization and elevated Ca^{++} probably modulate lidocaine effects through a common mechanism involving the electric field in the membrane. In parallel with the lack of effect of holding potential on block with quaternary molecules inside, raising the external Ca^{++} concentration did not increase peak P_{Na} with QX-572 or RAD 250 I inside. Indeed, in two experiments with RAD 250 I, elevating Ca^{++} actually decreased peak P_{Na} measured at a stimulation rate of 1 s^{-1}.

VOLTAGE- AND FREQUENCY-DEPENDENT INHIBITION. Strichartz (1973) first described what he called voltage-sensitive inhibition with quaternary derivatives of lidocaine (especially QX-314) inside myelinated fibers. The degree and rate of block of Na channels depended on the amplitudes and durations of prepulses and test pulses, and the blocking or unblocking effects accumulated with successive pulses given at rates down to 0.1 s^{-1}. He suggested that the gates of Na channels have to be open for the cationic drug molecules to enter or leave the internal receptor site and that in addition the externally applied electric field acting on the cationic drug alters the rates of entering and leaving open channels. Courtney (1974, 1975) described a related phenomenon that he called frequency- or use-dependent inhibition with amine local anesthetics including lidocaine, procaine, procaine amide, and most especially the lidocaine derivative GEA 968. The faster the rate of stimulation, the more the block depended on

pulse amplitudes and durations. Courtney added to Strichartz's hypothesis the suggestion that channels blocked by amine anesthetics also have their h_∞ curve shifted strongly to the left. A test for voltage- and frequency-dependent inhibition with the three charge classes of anesthetic molecules is now described.

Fig. 6 A shows four pulse waveforms which were applied repetitively to observe voltage- and frequency-dependent inhibition in my experiments. In waveform 1 the membrane is hyperpolarized by 45 mV for 50 ms and then depolarized by 60 mV for 1 ms to measure the peak sodium current I_{Na}. In waveform 2 the hyperpolarization is deleted. In waveforms 3 and 4 an extra 5 ms depolarization is added after the test pulse. The amplitude is 150 mV in no. 3

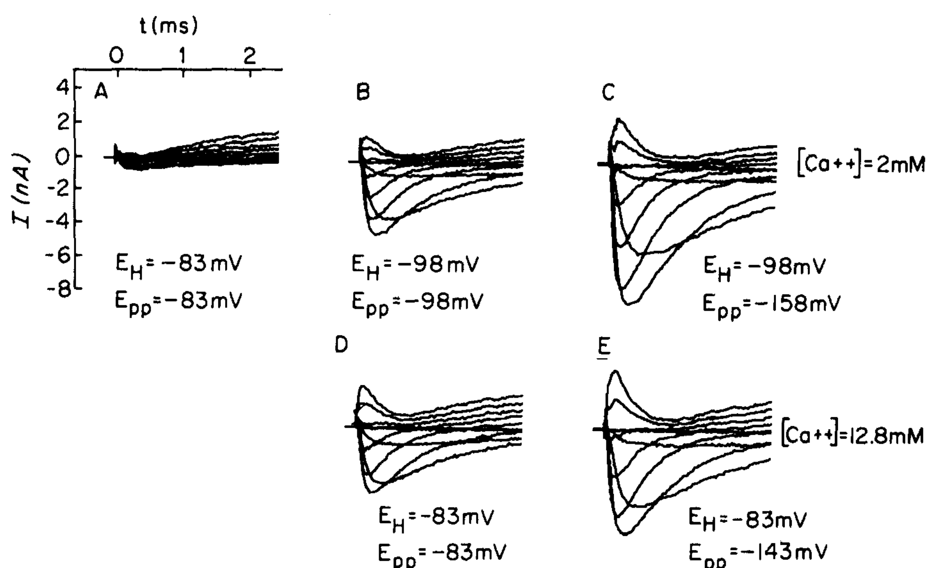

FIGURE 5. Parallelism between relief of block by elevated external [Ca^{++}] and relief by hyperpolarizing holding potentials with 1 mM lidocaine. Same fiber and test pulses as in Figs. 1 and 3. For (A), (B), and (C) [Ca^{++}] = 2 mM and for (D) and (E) [Ca^{++}] = 12.8 mM. Holding potential (E_H) and prepulse (E_{pp}) given below.

and 60 mV in no. 4. Frames B–F show the relative peak I_{Na} measured during the test pulse when the pulse waveforms are applied repetitively at rates of 3.3–5 s^{-1}. Where a waveform different from no. 1 is used, the number of the waveform is given above the data points. In each case the fiber was rested for more than 1 min before the test sequence began.

In the control measurement without drug treatment (Fig. 6 B) the relative I_{Na} does not vary from the first to the last stimulus of waveform 1, and it drops to 0.67 when the prepulse is removed, as expected from conventional sodium inactivation. Adding a 150 mV pulse after the measurement has no effect on subsequent measurements of I_{Na}. With 1 mM benzocaine (Fig. 6 C) the situation is nearly the same except that the resting sodium inactivation is far larger than without drug. By contrast after external application of lidocaine or QX-572 or internal application of RAD 250 I (Fig. 6 D, E, F), there is clear evidence of

voltage- and frequency-dependent inhibition. On starting the pulse program and at each change of waveform, the relative I_{Na} drifts toward a new steady level in a manner demonstrating the cumulative effect of closely spaced stimuli. Additional depolarization (waveform 3 or 4) after the test pulse significantly decreases the available P_{Na} in the next test pulse. In summary, amine and quaternary molecules give strong voltage- and frequency-dependent inhibition while the neutral molecule benzocaine gives none. Surprisingly, when the same series of stimuli was applied at 5 s^{-1} in the presence of 2 μM tetracaine (not shown), there was little drift after each change of waveform and no extra block on switching to waveforms 3 and 4.

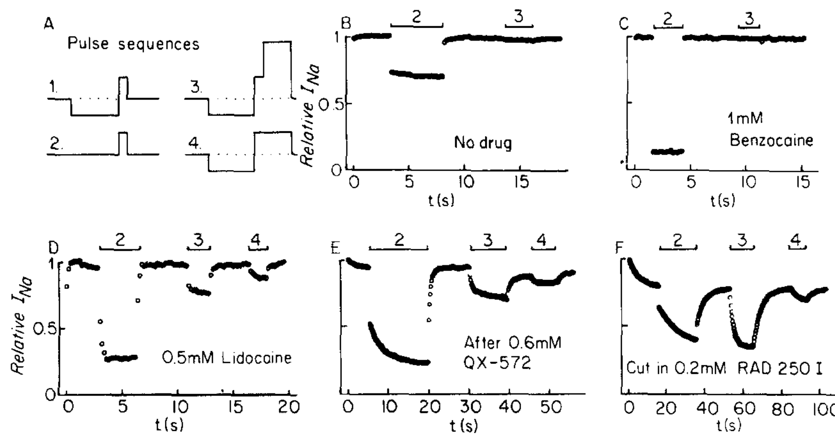

FIGURE 6. Modulation of anesthetic block by repetitive stimulation with four different voltage clamp waveforms. Holding potential −90 mV. Stimulus interval of 200 ms except in (F) where the interval was 300 ms. (A) shows the four pulse waveforms composed of a 50-ms prepulse to −90 or −135 mV, a 1-ms test pulse to −30 mV, and a 5-ms post pulse to −90, −30, or +60 mV. The fibers were resting until 0 s and then waveform 1 was started. Other waveforms were applied during the periods indicated by a bar. (B), (D), and (E) are done with the same fiber. (E) was measured after a solution containing 0.6 mM QX-572 had been applied externally for 3 min and then briefly washed off. (F) was measured 40 min after cutting the current-pool internode in 0.2 mM RAD 250.

The drifts of peak I_{Na} for each pulse waveform are qualitatively similar with lidocaine, QX-572, and RAD 250 I, but the rates of reaching the final value are obviously different. For example, in Fig. 6, on switching to waveform 2 four pulses suffice with lidocaine to get to the new level of I_{Na}, while the 75 pulses shown for QX-572 only just suffice and the 66 pulses for RAD 250 I are far from enough. The rate difference shows up another way. At stimulation rates below 1 s^{-1} lidocaine- or procaine-treated fibers show no cumulative effect of repetitive stimulation (Courtney, 1974, 1975; Hille et al., 1975) while fibers treated with the quaternary compounds QX-314 (Strichartz, 1973), QX-572, and RAD 250 I show cumulative effects even at 0.2 s^{-1}.

Fig. 6 reveals another aspect of sodium inactivation with quaternary and amine molecules. As was already discussed, switching from waveform 1 to

waveform 2 reduces I_{Na} at once by permitting resting inactivation to remain and then depresses I_{Na} further by some cumulative effect of repetitive stimulation. Courtney (1974, 1975) found a similar effect with the amine anesthetic GEA 968. He noted that the inactivation curve with GEA 968 is unchanged from the control provided that the fiber starts from a constant level of conditioning or a long rest and provided that inactivation is calculated from the relative I_{Na} on the first pulse after a change to a new prepulse level. On the other hand, he also found that if the inactivation curve with GEA 968 is calculated from the steady level of peak current reached after a train of impulses at each prepulse level, the curve appears shifted to the left by more than 20 mV and has a much smaller slope than the control. In these same terms the present results (Fig. 2 vs. Fig. 6) may be described by saying that benzocaine gives its full shift on the first test pulse and shows no cumulative effect of stimulation, lidocaine shows some shift on the first pulse and a further shift on fast repetitive testing, and the quaternary molecules tested show almost no shift on the first pulse and a considerable but slowly developing shift on repetitive testing.

TABLE I

BLOCKING PROPERTIES OF THE THREE CHARGE CLASSES OF LOCAL ANESTHETICS AND ANALOGS

Property	Molecular type		
	Quaternary	Amine	Neutral
Modulation by holding potential	No	Yes	No
Modulation by [Ca^{++}]	No	Yes	–
h_∞ shift on first pulse	No	Yes or No	Yes
Further h_∞ shift with many pulses	Yes	Yes	No
Block enhanced by large depolarizations	Yes	Yes	No
Persistence time of memory in seconds	>20	0.4–12	<0.2

The various blocking properties described here for the three charge classes of local anesthetics are summarized in Table I.

DISCUSSION

The degree of block of Na channels measured in voltage clamp experiments with local anesthetics varies over a wide range. The block depends on whether the membrane is held at rest or is hyperpolarized electrically or pharmacologically for longer or shorter periods, and it depends on the frequency and type of recent stimulation (Strichartz, 1973; Courtney, 1974, 1975; Hille et al., 1975; Khodorov et al., 1974, 1976). These complexities create a problem of even defining what should be meant by the potency of a local anesthetic drug in such measurements and suggest that reported parameters like "half-blocking concentrations" will vary from laboratory to laboratory. As already pointed out by Courtney (1974, 1975), frequency- and use-dependent inhibitions limit the maximum rate of firing or the minimum interspike interval of a nerve or muscle cell and may play a role in specific antiarrhythmic and analgesic actions. The remainder of the Discussion is devoted to developing a model to explain frequency- and use-dependent inhibition.

Previous Work on Sodium Inactivation and Anesthetics

SLOW SODIUM INACTIVATION. Block of Na channels by local anesthetic analogs and inactivation of channels by potential changes seem to be interacting processes. Block changes inactivation and inactivation changes block. The early literature reports a relief of block by hyperpolarization in myelinated fibers treated with procaine, urethane, or pentothal (Takeuchi and Tasaki, 1942; Posternak and Arnold, 1954; Schoepfle, 1957; Khodorov and Beljaev, 1964*a*; Khodorov, 1974); however, without voltage clamp measurements these results were difficult to interpret. The relief could have been due to the simple removal of resting inactivation that happens even in normal axons rather than to the reversal of some special drug-induced effect.

Subsequent voltage clamp work in Khodorov's laboratory in particular has demonstrated that the changes of inactivation are actually profound. Khodorov et al. (1974, 1976) described what they called slow sodium inactivation during treatment with procaine or trimecaine. The extra inactivation was produced by 1.0 s depolarizations and could be removed only very slowly upon hyperpolarization. The recovery time constants τ_s were 200–900 ms at room temperature. Similarly, with GEA 968 Courtney (1974, 1975) found an extra block of Na channels produced by repetitive depolarization with short pulses which reversed with time constants as long as 10 s (10°C) when the fiber was held at rest. Khodorov et al. found that the steady-state voltage dependence of the extra inactivation (described by their s_∞ parameter) is qualitatively similar to that of ordinary inactivation (described by the h_∞ parameter) and that it is shifted towards more positive potentials by adding extra Ca^{++}, Ni^{++}, or H^+ ions to the external solution. Adding H^+ ions but not Ca^{++} ions also appreciably lengthened the time constant τ_s of recovery. Khodorov et al. envisioned the new inactivated state as being reached from the normal inactivated state by a slow transition involving the binding of an anesthetic molecule to an "inactivation gating particle" of the Na channel. This receptor causing slow sodium inactivation was placed on the extracellular side of the membrane to account for the greater potency of amine anesthetic agents applied externally than of those applied at the cut end. However, the previous paper (Hille, 1977) shows that the potency difference is only a consequence of the relative thickness of internal and external unstirred layers, so the receptor for amine molecules could well be identical to that for quaternary molecules which is accessible only from the inside.

ORDINARY INACTIVATION. Weidmann (1955) found a negative shift of the inactivation curve in cardiac Purkinje fibers treated with cocaine. However, similar shifts were not considered to be likely in axons after Taylor (1959) found none in squid giant axons treated with procaine. Recently, however, Courtney (1974, 1975) reported an apparent negative voltage shift in axons with local anesthetics. As was already described, he found that inactivation is not shifted by GEA 968 when measured from the first pulse at each conditioning level but then, during trains of conditioning pulses combined with test pulses, a shift develops. I have found a shift of the inactivation curve on the first 50-ms prepulse with lidocaine, tetracaine, and benzocaine (Fig. 2, Table I) and further apparent shifts after trains of pulses with both amine and quaternary drugs. In agreement

with my observations, Strichartz (1973) found no shift on the first test pulse with quaternary analogs, but because his experiments did not include protocols like that of Fig. 6, the need to postulate shifts with multiple pulses was not so evident. He did, however, study the influence of prepulses on the rate of block or unblock caused by depolarizing test pulses and found a prepulse dependence resembling the normal h_∞ curve for block and resembling a 15 mV shifted h_∞ curve for unblock. Khodorov et al. (1974, 1976) report inactivation measurements on procaine- and trimecaine-treated fibers, but as there was no comparison to normal fibers, it is not clear whether they find a shift. Their "h_∞" and "s_∞" curves might in fact be incomplete since the most negative pulses they show (−80 to −90 mV) would not have removed all the inactivation seen in my work (Figs. 3 and 4).

CALCIUM AND ANESTHETIC BLOCK. The literature is confusing on the question of whether Ca^{++} ions alter the blocking effects of local anesthetics. Several studies show recovery of excitability in single blocked vertebrate cells upon elevation of the external divalent cation concentration (Aceves and Machne, 1963; Khodorov and Beljaev, 1964*b*; Seeman et al., 1974). The drugs tested include procaine, lidocaine, chlorpromazine, heptanol, and barbital, but again without voltage clamp methods those results were not easily interpreted. Four previous voltage clamp studies looked for relief of procaine block by added Ca^{++} ions. In two studies with myelinated fibers (Khodorov et al., 1974, 1976; Århem and Frankenhaeuser, 1974) no variation of P_{Na} was found on changing the Ca^{++} concentration in the range 0.74–20 mM. The nodes were held hyperpolarized in the range −90 to −110 mV between test pulses in an effort to avoid complications from resting sodium inactivation. However, Khodorov et al. (1974, 1976) did observe that Ca^{++} ions shift the voltage dependence of their drug-dependent slow blocking process, giving a relief of block at the normal resting potential comparable to that shown in Fig. 4A and D of this paper. In summary, Ca^{++} ions unblock Na channels of drug-treated myelinated fibers held at the resting potential but not of fibers that are already strongly hyperpolarized. With squid giant axons held at −70 mV, Narahashi et al. (1976) found no recovery of Na channel block upon raising the Ca^{++} concentration from 10 to 100 mM but there was a small enhancement of block on lowering the Ca^{++} concentration to 5 or 2 mM. By contrast, with lobster giant axons held at −90 to −130 mV Blaustein and Goldman (1966) obtained a large decrease of block on raising the Ca^{++} from 50 to 183 mM. Blaustein and Goldman (1966) and Khodorov et al. (1974) conclude that Ca^{++} and procaine compete directly for an external site important in gating, while Århem and Frankenhaeuser (1974) and Narahashi et al. (1976) conclude that the actions of Ca^{++} and procaine are independent. In a model described in the next section, I conclude that the binding sites for the two agents are on opposite sides of the membrane but that the anesthetic receptor is strongly coupled to the sodium inactivation mechanism which is in turn sensitive to the membrane potential and to external Ca^{++} ions.

Modulated Receptor Model

A major question that led to the experiments described in this paper is whether local anesthetics and their neutral and quaternary analogs act by common or by

different mechanisms. The blocking properties summarized in Table I for each of the charge classes are not identical, and for no criterion listed do the three molecular types show exactly the same behavior. Nevertheless, a single hypothesis building on those of Strichartz (1973), Courtney (1974, 1975), and Khodorov et al. (1974, 1976) seems to reconcile most differences. As a first approximation, all inhibitory actions of the drugs are attributed to binding to a single receptor. Slow sodium inactivation, cumulative frequency-dependent block, and voltage-dependent block are all seen as manifestations of a single inhibitory mechanism. The special features of the model include that the receptor has a voltage- and

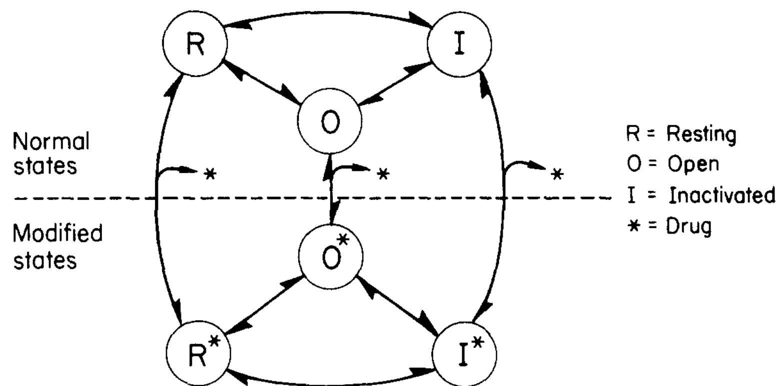

FIGURE 7. Modulated receptor hypothesis represented as a kinetic scheme with interconversions among normal states of the channel (R, O, and I) and drug-modified states (R*, O*, and I*). All interconversions may in principle be voltage dependent and the equilibria and rates for the R-O-I-R pathway may be different from those for the R*-O*-I*-R* pathway. When this scheme is applied to local anesthetics, the O* state is not electrically conducting, the R-R* and I-I* pathways are accessible primarily to hydrophobic drug forms and the O-O* pathway is accessible to hydrophilic as well as hydrophobic forms.

time-dependent conformation and that there may be several physical pathways for the drug to reach the receptor.

THE GENERAL MODEL. A schematic diagram of a quite general type of drug-receptor hypotheses is given in Fig. 7. The normal Na channel is represented as making transitions between several states: resting, open, and inactivated. The exact number of normal states is certainly larger than three but the three shown here embody the important features needed for this discussion. In principle each channel form may react with a drug molecule (*) to give modified forms which themselves can mark transitions between resting, open, and inactivated states. These transitions of the modified channels may have rates and equilibria different from those for normal channels. Consequently, the reaction of drug with one form may also have different rates and equilibria from the

reaction with another form. This last statement is equivalent to saying that the receptor for drug changes state when the channel changes state and is sufficiently general that it will be given the name "modulated receptor" model. The concepts of modulated receptors and of allosteric sites seem closely related.

The first modulated receptor model for ionic channels was developed by Armstrong (1966, 1974) to explain block of K channels by internal quaternary ammonium ions. Such models were subsequently used to explain the Na channel-opening effects of DDT (Hille, 1968) and a scorpion venom (Cahalan, 1975) and the Na channel-blocking effects of quaternary (Strichartz, 1973) and amine (Courtney, 1974, 1975) local anesthetic analogs.

The action of local anesthetic analogs is now discussed in terms of Fig. 7 and the modulated receptor model. Throughout the Discussion the conversion of a normal form of the channel (R, O, or I) into a modified form (R*, O*, or I*) is called the drug-receptor reaction. As a general principle, voltage- or frequency-dependent inhibition will arise in the scheme of Fig. 7 only if at least one of the drug-receptor reactions has rate constants that can be varied by stimulation or if the voltage dependence of some of the modified R*-O*-I* transitions differs from that of the normal R-O-I transitions. Examples of both cases are given below. The R*, O*, and I* states are all considered to be nonconducting, drug-blocked states.

MODELS OF STRICHARTZ AND COURTNEY. In a normal (untreated) fiber at rest, roughly 60% of the Na channels are in state R and the remainder in state I. If the membrane is then depolarized, most of the channels in state R proceed to the transient open state O and then inactivate to state I. Strichartz (1973) found that Na channels are most quickly blocked by internally applied quaternary QX-314 when the channels are cycled through transient state O by repetitive depolarizing pulses. Presumably, axoplasmic drug has access to the receptor only when the channel is open. In addition, channels recover most quickly from block when repetitive pulses are applied that are thought to cycle them through transient state O*. Without such pulses recovery is much slower, taking minutes. Hence with quaternary anesthetic analogs, the fast rate constants for the drug-receptor reaction are associated with open channels reacting via the O-O* pathways, while the reaction with closed channels (I-I* and R-R* pathways) has comparatively slow or possibly negligible rate constants. According to Strichartz the entire modulation of block by stimulation (as in Fig. 6 F with quaternary RAD 250 I) is attributed to voltage dependence of the O-O* and O*-O reactions thought to arise from the electrostatic potential energy changes of the cationic drug within the electric potential profile of the membrane. This voltage dependence is seen in Fig. 6 F as the sizable extra steady-state block obtained with pulse waveform 3 over that with waveform 4. Strichartz did not realize that in addition the inactivation mechanism in blocked channels is modified, meaning that the R*-O*-I* transitions of blocked channels are changed from the corresponding transitions of unblocked channels.

Courtney (1974, 1975) studied the exceptionally hydrophilic amine lidocaine analog GEA 968. As with Strichartz's work on QX-314, the fastest drug-receptor reactions of GEA 968 required depolarizing pulses that open channels and favor

the O-O* pathway. However, Courtney also found several differences. With GEA 968 the blocked forms recover at the resting potential with a time constant near 10 s, so pulses need to be applied at rates higher than 0.1 s^{-1} for cumulative block to be studied. In terms of Fig. 7, the recovery implies that the channel need not be open for reaction to occur, i.e. the R*-R or I*-I rate constants are appreciable. Also, with GEA 968 the O-O* reaction is not strongly voltage dependent. Finally, Courtney concluded that the h_∞ curve of modified (blocked) channels is shifted to more negative potentials, making the I* state relatively more stable than the I state. Then, unless strong hyperpolarizing prepulses are given, Na channels eventually become trapped in state I* during repetitive stimulation.

The changes of inactivation might be physically interpreted as in Fig. 8. The

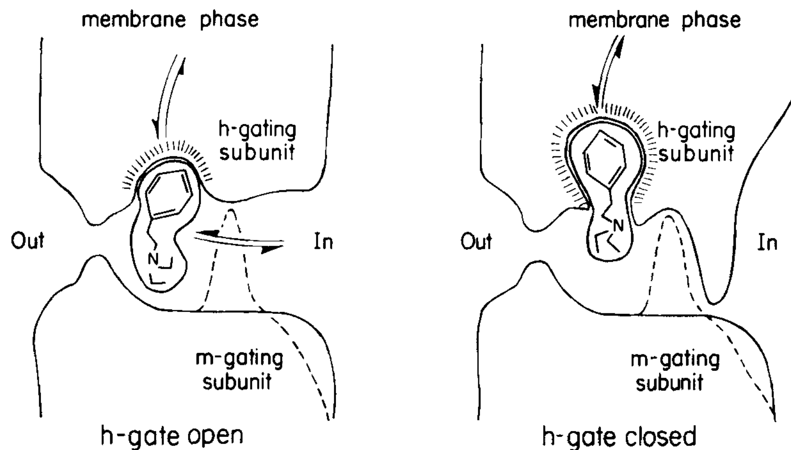

FIGURE 8. Diagram of a local anesthetic molecule binding in the pore of an Na channel in a manner that promotes Na inactivation. The molecule can reach its binding site from the intracellular solution if activation and inactivation gates are both open. It can also reach the site from the membrane phase even if one or both of the gates are closed. The binding site has an important hydrophobic component (shading) and closure of the inactivation gate enhances the hydrophobic interaction.

anesthetic receptor is in the channel near the inactivation gating subunit. When the h gate is open, binding to the receptor is not very firm, but when the gate is closed the receptor is modified and the binding is stronger. This mutual interdependence of drug binding and sodium inactivation is equivalent to saying that anesthetic binding increases the probability of the inactivated state I*. In the drawing the surface area of drug-receptor interactions is shown to increase as the channel inactivates. The interactions must include an important hydrophobic component required to account for the Overton-Meyer rule plus, very probably, some polar component. However, since permanently cationic and permanently neutral drug molecules both appreciably shift the inactivation curve, neither the drug-receptor reaction nor the extra interaction upon inactivation has an important charge-charge component.

RECONCILING THE ACTIONS OF THREE CHARGE TYPES. My observations seem best explained by supplementing the ideas of Strichartz and Courtney with the concept that while hydrophilic molecules in their charged form are obliged to bind and unbind from open channels (O-O* pathway), neutral molecules or hydrophobic cations may also use the R-R* or I-I* pathways. The more hydrophobic the molecule, the more rapid are the transitions in these alternative recovery pathways and the shorter is the persistence of the influence of previous stimulation. A kinetic diagram of this concept is shown in Fig. 9. Lipid-soluble molecules are shown exchanging between the inner and outer solutions and the hydrophobic membrane. Since they are amphipathic, drug molecules accumulate preferentially at the two boundaries of the nonpolar membrane phase and the polar medium (e.g. McLaughlin, 1975). The drug has access to the receptor both from the membrane pool and from the internal solution. The dashed

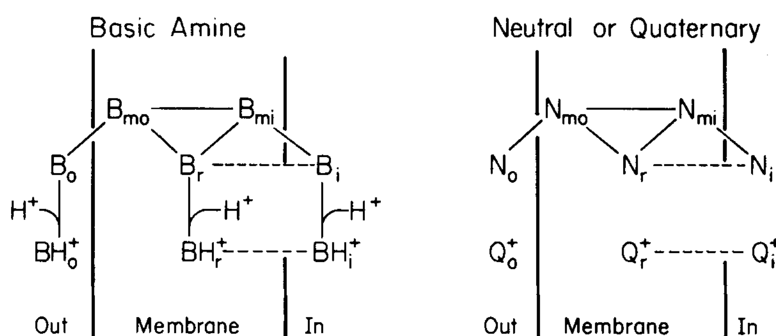

FIGURE 9. Diagram of the movements of local anesthetic molecules through the membrane phase and to and from the receptor. Dashed lines are transitions requiring open gates in the Na channel. Solid lines are transitions possible any time. Charged forms are drawn as unable to cross the membrane. Amine molecules can protonate or deprotonate while in the channel or in the aqueous medium. Meaning of subscripts: *o*, outside medium; *mo*, outer surface of membrane; *r*, receptor; *mi*, inner surface of membrane; *i*, inside medium.

pathway is the direct route for axoplasmic anesthetic to pass by the open gates and through the channel to the receptor. It will be called the hydrophilic pathway to distinguish it from the hydrophobic pathways which bypass the gates by diffusion through the membrane. The dashed hydrophilic pathway is closed when gating closes the channel and is the only route for quaternary compounds. The hydrophobic pathways are available at all times to lipid-soluble molecules.

The time constants for recovery from drug-induced slow sodium inactivation show strong parallels with the half-block times for sudden application of drug described in the previous paper (Hille, 1977), probably because both processes require diffusion of the drug through part of the membrane. According to this hypothesis different time constants for removal of slow sodium inactivation reflect differing rates of departure of drug molecules via the hydrophobic pathway during the maintained hyperpolarization. A permanently neutral molecule such as benzocaine can leave in times well under 50 ms so that one finds

only a shifted inactivation curve but no long time constants of inactivation. However, amine molecules are in the lipid-diffusible neutral form for only a small fraction of the time at neutral pH, so they leave less quickly than benzocaine and show slower recovery during hyperpolarization. Indeed, Khodorov et al. (1976) showed that τ_s, the time constant of recovery from slow inactivation, is lengthened further by acidifying the external medium, as expected if the drug cation formed in acid media does not escape through the hydrophobic pathway. Again, the pH effects on rate of recovery from inactivation correlate well with the pH effects on half-times of block in the previous paper (Hille, 1977). Probably, the pK_a of the drug, which determines the lifetime of the protonated form, and the difference between the pK_a and the local pH, which determines the average fraction of molecules in the protonated form, will both be important determinants of the time constants τ_s. Finally, quaternary molecules show no recovery with a 1-min hyperpolarization presumably because the permanent cation is trapped in the channel by closed activation gates. The same arguments explain why a relief of block by high calcium occurs with amine but not with quaternary molecules. Namely, when external Ca^{++} ions relieve some of the resting inactivation through their effect on the electric field within the membrane (Frankenhaeuser and Hodgkin, 1957; Hille et al., 1975), amine molecules on the receptor can escape gradually via the R*-R pathway while quaternary molecules cannot and remain trapped behind closed activation gates.

All of the compounds tested give apparent shifts of the inactivation curve (Table I). With the more hydrophobic molecules the shift is evident on the first test pulse, while with the hydrophilic molecules the shift requires several pulses. The number of pulses required to obtain the shift increases in the sequence: benzocaine < lidocaine < GEA 968 < QX-572 < RAD 250 I. These observations seem well explained by Courtney's idea that blocked channels have their h_∞ curve shifted by the bound drug, while the channels without drug are normal. The more hydrophobic molecules reveal the shift on the first prepulse since with them the blocked channels in the I* state can be restored to the conducting pool rapidly via the hydrophobic pathway. With benzocaine, for example, during a single 50-ms hyperpolarizing prepulse, all the channels in state I* might revert first to R* and then lose drug fast enough to make R. With more hydrophilic drugs, however, less R is formed from R* in 50 ms of hyperpolarization because they move less well in the hydrophobic pathway and must wait for the conversion of R* to O* and thence partly to O during each depolarizing test pulse. Therefore, the channels opening after a single short prepulse are mostly those that were unaffected by drug in the first place.

To summarize, then, in my view there is a single specific receptor in the Na channel for all local anesthetics. Occupancy per se may be equated with block of the channel, and occupancy also has a profound influence on sodium inactivation. The drug-receptor interactions change as the channel changes among its various voltage-dependent states, and the physical pathways used for the drug-receptor reaction change as well. While an axon is being stimulated, the drug never reaches equilibrium with the receptor but is constantly being driven on and off as Na channels are cycled through their changes of state. In this

hypothesis the apparent differences in the actions of neutral, amine, and quaternary molecules are largely explained by the differences in the rates of various allowed on and off reactions. The recovery time constant τ_s defined by Khodorov et al. (1974, 1976) would then describe the time required for a sequence of events including reversal of the inactivation of blocked channels at the holding potential and departure of the drug via the hydrophobic pathway into the membrane phase. To the old question of inside vs. outside action, the answer is within the membrane, and to the old question of cation vs. free base the answer is both, but with different pathways and hence with different phenomenology.

The idea proposed here of a modulated receptor with alternate hydrophobic and hydrophilic pathways is probably applicable to other cases. The rate-dependent action and the inactivation shift with various antiarrhythmic drugs applied to the myocardium (Weidmann, 1955; and references in Gettes and Reuter, 1974) are so similar to the actions of local anesthetics on Na channels of nerve that the underlying mechanisms may be very similar. It would therefore be interesting to study the drug-treated myocardium again by using different repetitive waveforms as in Fig. 6 and comparing drugs of different lipid solubility and pK_a in order to test if the memory of previous stimulation is again related to the difficulty of escape through the membrane phase. Another example of a drug-receptor interaction varying with the state of a channel and probably having a hydrophilic and hydrophobic reaction pathway is the block of K channels by the aminopyridine isomers (Meves and Pichon, 1975; Yeh et al., 1976*a*, *b*). The analogies are extensive. Repetitive or long depolarization modulates the blocking effect with time constants which are longer for compounds of higher pK_a and for drug solutions of low pH. The lipid-soluble molecules may be applied from the outside, but are quite possibly acting from the insde or from the membrane itself. The major difference is that aminopyridines bind more firmly to resting K channels than to activated ones, but the analogy is nonetheless striking.

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