The pH-Dependent Rate of Action of Local Anesthetics on the Node of Ranvier

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ABSTRACT Local anesthetic solutions were applied suddenly to the outside of single myelinated nerve fibers to measure the time course of development of block of sodium channels. Sodium currents were measured under voltage clamp with test pulses applied several times per second during the solution change. The rate of block was studied by using drugs of different lipid solubility and of different charge type, and the external pH was varied from pH 8.3 to pH 6 to change the degree of ionization of the amine compounds. At pH 8.3 the half-time of action of amine anesthetics such as lidocaine, procaine, tetracaine, and others was always less than 2 s and usually less than 1 s. Lowering the pH to 6.0 decreased the apparent potency and slowed the rate of action of these drugs. The rate of action of neutral benzocaine was fast (1 s) and pH independent. The rate of action of cationic quaternary QX-572 was slow (>200 s) and also pH independent. Other quaternary anesthetic derivatives showed no action when applied outside. The result is that neutral drug forms act much more rapidly than charged ones, suggesting that externally applied local anesthetics must cross a hydrophobic barrier to reach their receptor. A model representing diffusion of drug into the nerve fiber gives reasonable time courses of action and reasonable membrane permeability coefficients on the assumption that the hydrophobic barrier is the nodal membrane. Arguments are given that there may be a need for reinterpretation of many published experiments on the location of the anesthetic receptor and on which charge form of the drug is active to take into account the effects of unstirred layers, high membrane permeability, and high lipid solubility.

INTRODUCTION

This paper and the following one (Hille, 1977) concern the interaction of local anesthetics with their membrane receptor. Most clinical local anesthetics are tertiary amines which are constantly interconverting between an uncharged free amine form and a cationic protonated form at neutral pH. The free amine form which predominates at high pH is usually quite lipid soluble and can readily cross tissue and cell membranes. The cationic protonated form which predominates at neutral and low pH is largely confined to the aqueous phase and is expected to cross membranes far less readily.

Local anesthetics block the excitability of nerve by blocking Na channels (Taylor, 1959). The major questions considered in these two papers are where on the Na channel the receptor is and how do the anesthetic molecules get there. In the most widely accepted theory, local anesthetic molecules diffuse in the amine form from the extracellular site of application to the inside of excitable cells, but once inside, the active form is the cation. The original evidence that the internal cation is the active form came from experiments with various values of external or internal pH (Ritchie and Greengard, 1961; Ritchie et al., 1965*a, b;* Narahashi et al., 1969; Frazier et al., 1970; Narahashi et al., 1970; Strobel and Bianchi, 1970*a, b;* Narahashi et al., 1972; Narahashi and Frazier, 1975). Newer direct evidence comes from experiments with the charged *N*-ethyl or *N*-methyl derivatives of lidocaine and trimecaine. Such permanently cationic molecules block Na channels well when applied inside squid giant axons (Narahashi et al., 1969, 1972; Frazier et al., 1970) or inside myelinated nerve fibers (Strichartz, 1973; Khodorov et al., 1976) but not when applied outside. Structure-activity correlations suggest with certain molecules that the quaternary derivatives act on the same receptor as the conventional amine parent compound (Hille et al., 1975). The evidence demonstrating activity of anesthetic cations must at the same time be reconciled with the local anesthetic activity of benzocaine, a permanently neutral analogue of procaine (Ritchie and Ritchie, 1968). The effective half-blocking concentrations for benzocaine, procaine, lidocaine, and its *N*-ethyl derivative QX-314 (applied internally) are all in the neighborhood of 0.1–1.0 mM. Evidently, the receptor, if it is one single type, does not discriminate completely between neutral and cationic molecules. Alternatively, there could be several different local anesthetic receptors, each with its individual binding specificities and resulting effects on the channel (Khodorov et al., 1976). These questions are considered further in this paper.

The approach of this paper is to measure the time course of development of block when solutions containing local anesthetic at high or low pH are applied rapidly to the outside of a single myelinated nerve fiber. A special advantage of this preparation is that the external unstirred layers can be made small and that solutions can be changed in a small fraction of a second while the fiber is being studied under voltage clamp. Nevertheless, the rate of block of channels by some anesthetics is so fast at high pH that the measurement may be limited by the rate of solution change. At lower pH where the protonated molecular form dominates, the rate of block is appreciably slower as if external protonated anesthetic molecules are inactive and cannot reach the receptor without first losing a proton. The results are interpretable in terms of a model for diffusion of neutral molecules into the axon. The following paper considers the drug-receptor interaction after longer exposure to drug, when internal and external concentrations have presumably equilibrated. A preliminary report of some of this work has been published (Hille et al., 1975).

MATERIALS AND METHODS

Most of the experiments were done in the period December 1973 to January 1975. Single large myelinated nerve fibers dissected from the sciatic nerve of the frog *Rana pipiens* were studied under voltage clamp (Dodge and Frankenhaeuser, 1959; Hille, 1971) at 15°C. The membrane was normally held at potentials near −80 mV (inside minus outside) where ~60–70% of the Na channels are not inactivated (i.e., h_∞ = 0.6–0.7). For measurements the membrane potential was changed in three successive steps. First, ordinary resting sodium inactivation was removed by a 50-ms hyperpolarizing prepulse to

−125 mV. Then the peak inward Na current was measured in a 1-ms depolarizing test pulse to −20 mV, and finally the maximum outward K current was measured in a further 5-ms step to +70 mV. The currents were recorded and corrected for leakage and capacity by a computer (Hille, 1971).

To permit rapid changing of the external solution, the volume of the pool bathing the node of Ranvier was reduced to 0.1 ml. The design of the measurements was typically to equilibrate the fiber in a solution strongly buffered at pH 8.3 for at least 3 min. Then measurements of Na and K currents were begun by repetitive application of the three-step waveform at a frequency of 0.2–5 s^{-1}, and 3 ml of test solution containing anesthetic and some tetraethylammonium ion (TEA) at pH 8.3 was rapidly perfused through the chamber. Solutions were injected with a 5-ml syringe at about 0.7 ml/s at one end of the pool and removed by suction from the other end. The solution flowed past the node with an estimated velocity of 25 cm/s during the solution change. Usually, the test solution was washed off again within 20 s to avoid accumulation of anesthetic in the fiber. The whole procedure of equilibration, test, and wash was repeated at pH 6.0 and again at pH 8.3 to ensure full reversibility of any pH effects seen. The TEA was included with the anesthetic as an independent control for rapid solution changes and to mark reproducibly the time of the solution change. According to Vierhaus and Ulbricht (1971*a*, *b*) TEA blocks K channels in less than 100 ms, so the time course of K current block is a good reflection of the solution change in these experiments. Zero time in the figures given later is chosen as the last clamp pulse before a significant decrease in K current.

The external solutions contained 90 mM NaCl, 2 mM $CaCl_2$, and a strong buffer mixture. For pH 6.0 the final buffer concentrations were 10 mM 2(*N*-morpholino)ethane sulfonic acid (MES, pK_a = 6.2) and 10 mM *bis* (2-hydroxyethyl)-imino-tris(hydroxymethyl)methane (*bis*-Tris, pK_a = 6.5), and for pH 8.3 they were 28 mM glycylglycine HCl (pK_a = 3.1, 8.1) and 55 mM tris(hydroxymethyl)aminomethane (Tris, pK_a = 8.1). For the few experiments at pH 7.4, Tris buffer (2 mM) was used alone. Concentrated stocks of local anesthetics were diluted in double-strength stocks of the external solutions to make the final test solution. TEA was also added to a final concentration of 0.5–1.7 mM.

The ends of the isolated nerve fiber were cut in unbuffered 120 mM KCl solution. In a few cases quaternary drugs in KCl were applied at one cut end of the fiber (in the current pool) to allow them to diffuse down the axoplasm to the inside of the node (Koppenhöfer and Vogel, 1969; Armstrong and Hille, 1972). Theoretically and also in practice, the major part of the diffusion from the cut internode to the node should be complete within 15 min. There is, however, no independent measure of internal drug concentration in these experiments. Normally, the internode extending into the current pool where the drug was applied was cut about two-thirds as long as the other internode. Therefore, if the cut ends do not heal over, the final intranodal quaternary drug concentration is expected to be about 60% of the concentration applied in the current pool.

The structures of the compounds used are shown in Fig. 1 or described in the figure legend. The following compounds were kind gifts from Drs. Bertil Takman and Rune Sandberg of Astra Pharmaceutical Products, Worcester, Mass., and Södertälje, Sweden: Lidocaine·HCl, its quaternary ethyl derivative QX-314, its double-ended quaternary relative QX-572, its glycine adduct GEA 968, optical isomer I of tertiary RAC 109, its quaternary ethyl derivative RAC 421 I, optical isomer D − (−) of tertiary mepivacaine, and its quaternary methyl derivative RAD 250 B.

In this and the following paper the lipid solubility and the charge form of the molecules will be important. The lipid solubility gives an idea of how readily the molecules may diffuse across membranes and also how well they might bind to a hydrophobic receptor. The oil:water or oleyl alcohol:water partition coefficients F for the free amine forms and the pK_as of ionization of some of the molecules are given in Table I. Because the experiments are done at pH values where some anesthetics are partially ionized, a calculated effective distribution coefficient q_{pH} is also given relating the amount of anesthetic base in oleyl alcohol or oil to the total base plus cation in the aqueous phase at pH 6, 7.4, and 8.3. Indeed, the compounds are listed in Table I in order of decreasing $q_{6.0}$. Partition coefficients for the permanent cations QX-314, RAC 421, and RAD 250 have not been measured, but would be expected to be extremely low. The cation QX-572 is far more hydrophobic than the above three and has a measured oleyl alcohol:0.25 M phosphate buffer partition coefficient as high as 0.9 (Astra Pharmaceutical Products). However this must represent phosphate salt extraction into the bulk hydrophobic phase and not partition of the cation in the usual sense. Despite their permanent charge, such large and hydrophobic monovalent ions actually can cross lipid bilayer membranes, although with an effective permeability much lower than for an isosteric neutral species (Liberman and Topaly, 1969).

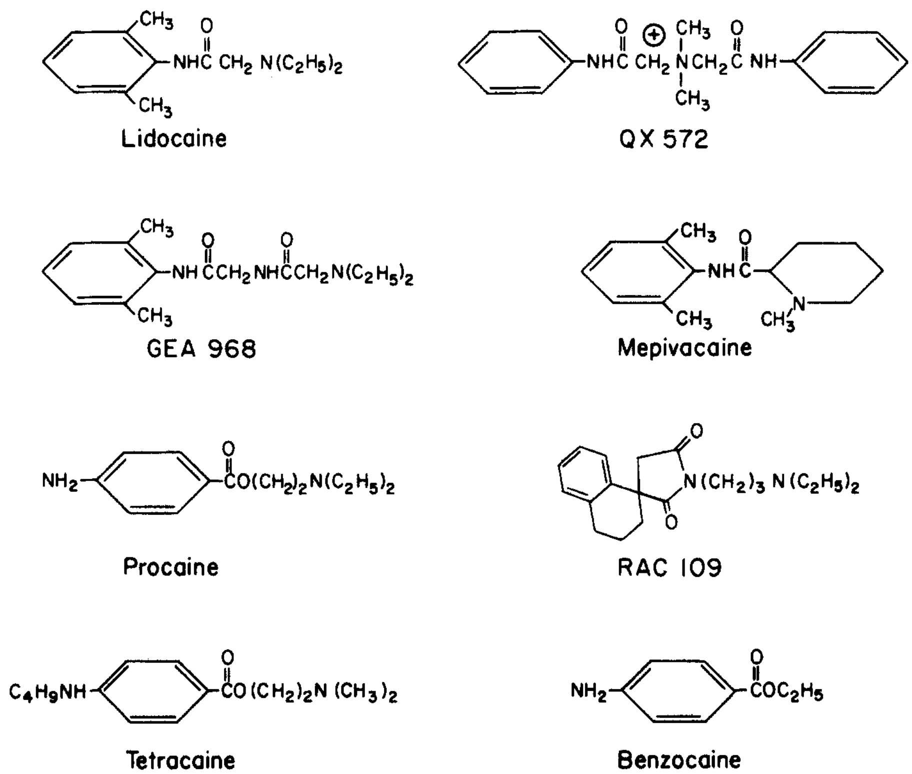

FIGURE 1. Chemical structures of the local anesthetic drugs. Three quaternary compounds not shown are: QX-314, the *N*-ethyl derivative of lidocaine; RAD 250, the *N*-methyl derivative of mepivacaine; and RAC 421, the *N*-ethyl derivative of RAC 109.

RESULTS

Rate of Block with Amine Local Anesthetics

All the molecules shown in Fig. 1 block Na channels. Of the amine compounds, lidocaine had the fastest and the least pH-dependent action between pH 6 and pH 8.3. Fig. 2 shows the fall of I_{Na} and I_K with three successive applications of lidocaine-TEA solutions to the same fiber. Between runs the fiber was rinsed for several minutes in drug-free solution to allow recovery. As was already dis-

TABLE I

pK$_a$ AND PARTITION COEFFICIENT OF LOCAL ANESTHETICS

Drug	pK$_a$	Hydrophobic partition coefficient F	$q_{6.0}$	$q_{7.4}$	$q_{8.3}$	Medium	Reference
Benzocaine	2.6	41	41	41	41	oleyl alcohol	Büchi et al., 1966
Lidocaine	7.9	225	2.8	54	161	oleyl alcohol	Löfgren, 1948
Tetracaine	8.5	273	0.86	20	106	cod liver oil	Brandström, 1964
Mepivacaine	7.8	46	0.72	13	35	oleyl alcohol	Friberger and Åberg, 1971
RAC 109	9.4	260	0.10	2.6	19	cod liver oil	Astra Pharmaceuticals
Procaine	8.9	45	0.057	1.4	9.0	oleyl alcohol	Löfgren, 1948
GEA 968	7.7	1.3	0.025	0.43	1.0	cod liver oil	Astra Pharmaceuticals

F = oil or alcohol-buffer partition coefficient of the free base form.

$q_{6.0}$ = estimated effective distribution coefficient of base in hydrophobic phase vs. base plus cation in buffer at pH = 6.0, 7.4, or 8.3. Calculated from $q_{pH} = F/(1 + 10^{pK-pH})$.

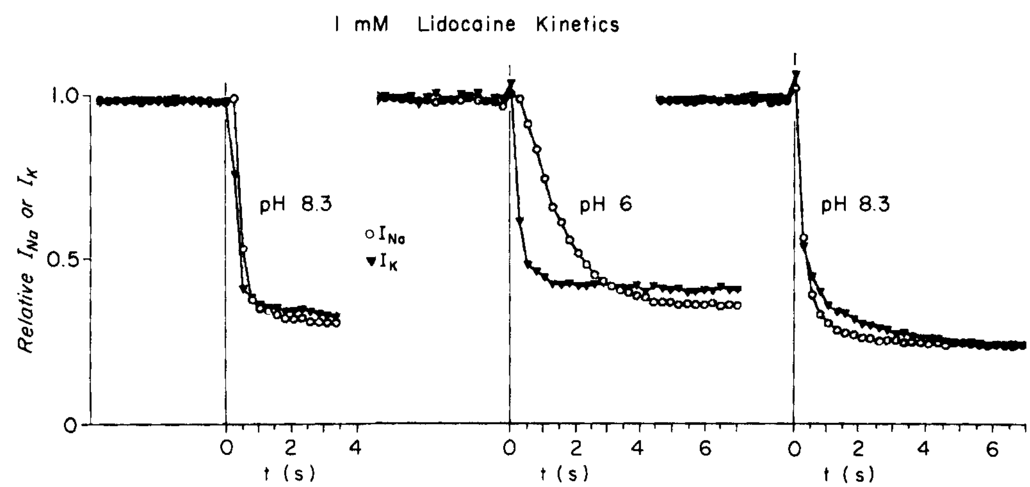

FIGURE 2. The kinetics of Na and K channel block with lidocaine-TEA mixtures at pH 8.3 and 6.0. Circles are normalized peak Na currents at −20 mV in a fiber stimulated every 260 ms and triangles are normalized K currents after 5 ms at +70 mV. Solutions containing 1 mM anesthetic and 0.85 mM TEA (pH 8.3) or 1.7 mM TEA (pH 6.0) were injected at zero time. The three records are from the same fiber. The control solution present before each application of drug has the same pH as the drug solution.

cussed, the fall of I_K is taken as a measure of the speed of solution change. In both runs at pH 8.3 the fall of I_{Na} lags the fall of I_K by less than one 260-ms sample interval. Therefore, the rate of action of lidocaine at pH 8.3 can only be said to be too fast to resolve well by this method. On the other hand, the rate of action at pH 6 is obviously slower, taking >1 s, and can be resolved. In this paper the rate of action of drugs is specified by a half-time of action t_{on}. This is defined as the time taken to block half the sodium current that will be blocked by the drug, minus the time taken to block half the potassium current that will be blocked by TEA in the same run. While this empirical measure is supposed to correct for delays in solution changes, it still is only roughly related to the rate of arrival of drug at its receptor since block is not linearly related to the effective drug concentration. Values of t_{on} are summarized in Table II. According to Table II, t_{on} for lidocaine is in the range 0–0.75 s at pH 8.3 and lengthens to 1.1–1.5 s at pH 6.

Change of pH seems to affect the depth of block as well as the rate of block. In Fig. 2 the block after 15–20 s exposure to drug was slightly larger at pH 8.3 than at pH 6. However, as is shown in the following paper (Hille, 1977), the depth of block of Na channels measured even after long drug exposures can be varied widely by changing the holding potential, the Ca^{++} concentration, or the rate of stimulation. Therefore, further experiments would be needed to decide what factors contribute to the small change in depth of block of I_{Na} in Fig. 2. The small

TABLE II

ON AND OFF HALF-TIMES FOR LOCAL ANESTHETIC ACTION

Drug	pH = 6			pH = 7.4			pH = 8.3		
	t_{on}	t_{off}	concn	t_{on}	t_{off}	concn	t_{on}	t_{off}	concn
	s	*s*	*mM*	*s*	*s*	*mM*	*s*	*s*	*mM*
Benzocaine	1.0(1)	–	1	0.5–0.7(2)	0.6–1.2(2)	1–2	1.0(2)	–	1
Lidocaine	1.1–1.5(3)	2.5–2.6(2)	1	–	–	–	0–0.7(6)	0.5(1)	1
Tetracaine	>15(1)	>15(1)	0.01	–	–	–	1.3(1)	3	0.005
Mepivacaine	–	–	–	1(3)	–	0.25	–	–	–
RAC 109	2.0(1)	5.0(1)	1	–	–	–	0.9–1.1(2)	2.0–4.8(2)	0.08–0.25
Procaine	3.3–>4.5(2)	–	1	–	–	–	0.5(2)	–	1
GEA 968	>200(1)	–	2	–	–	–	1.1–2.0(3)	2.0–2.5(3)	1–2
QX-572	160(1)	≫300(1)	0.14	58–280(3)	–	0.5	130–>200(2)	>300(1)	0.14
RAC 421	–	–	–	>2000(1)	–	2	–	–	–
QX 314	–	–	–	>4500(2)	–	2	–	–	–

Numbers in parentheses indicate the number of fibers studied.

differences in block of K channels by TEA at low and high pH in Fig. 2 are reproducible and have been successfully described as an external surface-potential effect by Mozhayeva and Naumov (1972). In Fig. 2 the apparent potency of TEA is reduced more than twofold by lowering the pH since the concentration of TEA used at low pH is twice as high as at high pH and yet the block is less.

The actions of the other amine anesthetics are more obviously pH dependent than the action of lidocaine. The measured half-times are summarized in Table II, and individual examples are discussed below. Fig. 3 A shows that 1 mM GEA 968 at pH 8.3 acts with a t_{on} of 1.1 s to give a steady block of 70%, while 2 mM drug at pH 6 takes hundreds of seconds to act and gives only 22% block in 300 s. The rate and depth of block with 1.0 mM procaine are affected by pH in a similar manner (Fig. 4 B). The effect of lowered pH can be approximately compensated by increasing the drug concentration. For example with RAC 109 I (Fig. 3 B), 0.09 mM drug at pH 8.3 acts with a t_{on} of 1.1 s and 1.1 mM drug at pH 6 acts with a t_{on} of 2.0 s. The ratio of drug concentrations used was 1:11. As judged from the residual differences a concentration ratio of 1:20 or 1:25 might compensate the pH effect more completely. With tetracaine the pH effects are slightly different (Fig. 4 A). The rate of block is strongly depressed by lowering the pH, but the depth of block is not so much affected. In summary, all ionizable amine anesthetics studied act with half-times of less than 1.5 s at pH 8.3 and more slowly at pH 6. The pH dependence of the depth of block is considerable for procaine, GEA 968, and RAC 109 I and much less for lidocaine and tetracaine.

The blocking potency of amine anesthetics is reduced by elevating the Ca^{++} concentration (see Hille, 1977), but the rate of action is not changed. In one experiment with 20 mM Ca^{++} and pH 8.3, 0.076 mM RAC 109 I acted with a t_{on} of

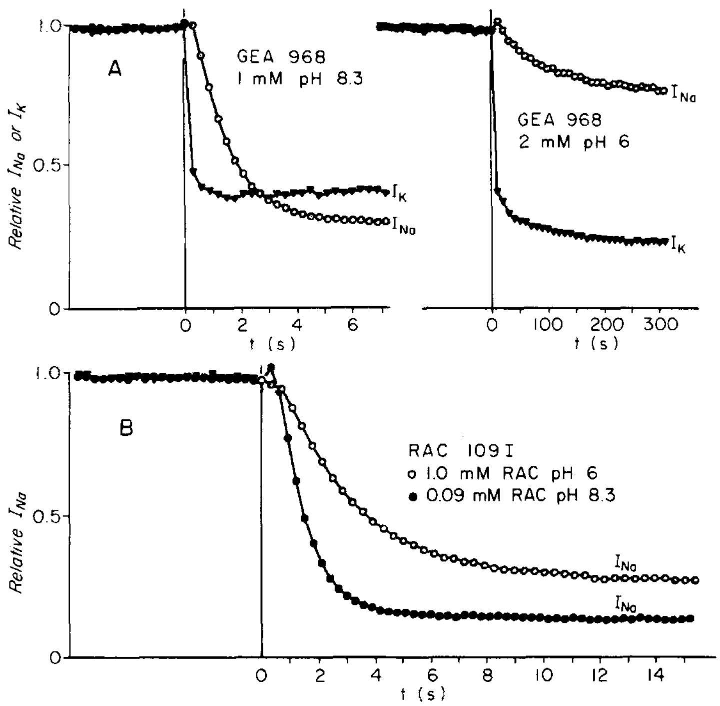

FIGURE 3. The pH-dependent action of GEA 968 and RAC 109 I. Method as in Fig. 2. (A) Relative I_{Na} (circles) and I_K (triangles) in a fiber stimulated every 300 ms and exposed to 1 mM GEA 968 with 0.85 mM TEA at pH 8.3 and in a different fiber stimulated every 10 s and exposed to 2 mM GEA 968 with 1.8 mM TEA at pH 6.0. Note different time scales. (B) Relative I_{Na} in a fiber stimulated every 300 ms and exposed to 0.09 mM RAC 109 I with 0.9 mM TEA at pH 8.3 (filled circles) and in a different fiber stimulated every 360 ms and exposed to 1.0 mM RAC 109 I with no TEA at pH 6.0 (open circles).

0.9 s to give a final block of 63% compared with values of 1.1 s and 87% block for the experiment of Fig. 3 B with the normal 2 mM Ca^{++} and 0.09 mM drug.

Rate of Block with Neutral Local Anesthetic

Benzocaine rapidly blocks Na channels when applied outside the fiber (Fig. 4 C). The apparent pH dependence is very slight. At high and low pH the blocking half-time t_{on} is 1.0 s or less (Table II), and unlike the amine anesthetics, the final block is apparently stronger at low pH.

Rate of Block with Quaternary Compounds

Quaternary derivatives of local anesthetics are known to block Na channels from the inside at concentrations comparable to those needed for equilibrium block with the parent amine compounds. For example, 90% block is obtained 30 min after cutting one end of a fiber in 0.5 mM QX-314 (Strichartz, 1973) and 65% block 60 min after cutting in 0.035 mM RAC 421 I (Hille et al., 1975). On external application for 30 min these same compounds have almost no effect, indicating a very low rate of penetration to the inside of the fiber. An example with external application of 2 mM RAC 421 I is given in Fig. 4D. Note that 2 mM is about 80

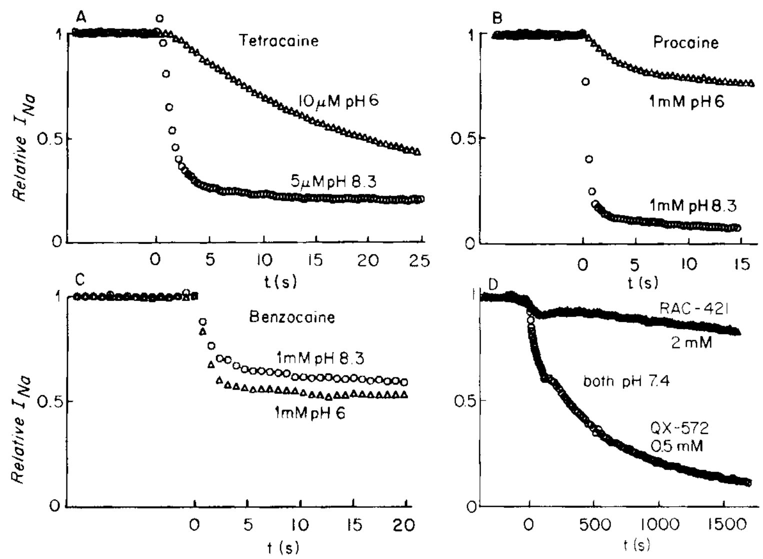

FIGURE 4. The kinetics of block of Na channels with neutral, amine, and quaternary local anesthetics. Method as in Fig. 2. The two records with tetracaine are from the same fiber and similarly for the records with procaine and with benzocaine. Stimulus intervals: (A) 300 ms at pH 8.3 and 500 ms at pH 6.0. (B) 300 ms at pH 8.3 and 500 ms at pH 6.0. (C) 800 ms at pH 8.3 and 6.0. (D) 10 s with RAC 421 and 5 s changing to 20 s at 115 s with QX-572.

times the half-blocking concentration for internal application of this compound. The rate of decline of I_{Na} is hardly distinguishable from the expected normal rundown. Similar results were obtained with 2 mM QX-314. Attempts to study 15 and 60 mM QX-314 were inconclusive, because the nodal membrane broke quickly in these solutions.

The more hydrophobic cation QX-572 does give an appreciable block of Na channels with external application. An example with 0.5 mM QX-572 outside is given in Fig. 4D. The half-time of block is 280 s. The wiggle in the record at 115 s occurred when the interval between test pulses was lengthened from 5 to 20 s (see Hille, 1977). Other experiments are summarized in Table II. Unfortunately, each experiment had to be done on a different fiber since there was virtually no recovery, so the effects of pH on quaternary drug action could not be studied as reliably as with other drugs. In one pair of runs the depth and rate of action of 0.15 mM QX-572 applied externally were found to be similar at pH 8.3 and 6.

Block with internal application of drug was studied in an experiment with 0.24 mM QX-572 and 18 mM TEA in KCl applied to one cut end of a fiber. A decrease of I_K by internal TEA was evident within 2 min, finally leveling off at 50% block before 10 min (cf. Armstrong and Hille, 1972). A decrease of I_{Na} by internal QX-572 also began early but did not level off in the 20-min measuring period. At 10 min the block of I_{Na} was 45% and at 20 min, 65%. Comparison of the time course of block of I_{Na} and I_K suggests that TEA diffuses down the internode at least three times faster than QX-572. Some of this difference may be due to a difference in free diffusion constant but there could also be some slowing of QX-572 diffusion by partitioning into or binding to hydrophobic phases during transit down the internode.

Recovery from Block

The experiments were not designed to investigate recovery from block, and the drug-free recovery solution was often injected slowly to avoid damage to the fiber. Nevertheless, judging from the rate of recovery of I_K from block upon returning to control solution, the wash-off was sometimes good and recovery half-times for I_{Na} could be measured. The half-time t_{off} is defined as the time for half-recovery of the sodium current minus the time for half-recovery of potassium current. The following conclusions may be drawn from the observations and the values of t_{off} summarized in Table II. With exposure to drug for as little as 15 s, the recovery is only a little slower than the previous rate of block, but with longer exposure the recovery also takes longer, as if some store has built up in the tissue. In addition, for equal exposure time in the range of 15–30 s and for conditions giving equal depth of block, the recovery is slower for drugs and pH values where the onset is also slower. Thus, recovery is slower at pH 6 than at pH 8.3 with lidocaine, tetracaine, RAC 109, and procaine, and recovery at pH 8.3 is slower with GEA 968, tetracaine, and RAC 109 than with procaine, lidocaine, or benzocaine. Finally, fibers treated with QX-572 for 3–10 min do not show appreciable recovery in the next 10 min, although some reversal can occur in much longer times.

DISCUSSION

Previous studies of the pH dependence of local anesthetic action have focused on the steady state of block with nerve bundles or single fibers or on the rate of block with nerve bundles. This paper is the first systematic study of the pH dependent of rate of block with single fibers. Although the results are superficially at variance with observations on nerve bundles (see below), there probably are no contradictions and an interpretation consistent with all work can be given. The present results are interpreted first and then earlier observations are discussed. It is shown that all results with the various forms of anesthetic molecules are consistent with an internal site of action.

At high pH where the neutral free base predominates, the action of amine anesthetics on the node is very rapid, often taking less than 1 s, as has been reported before (Tasaki, cited in Kato, 1936; Tasaki, 1953; Hille et al., 1975; Wagner and Ulbricht, 1976). At low pH, however, the protonated form predominates and the rate of action may be very much slower, sometimes taking hundreds of seconds. Assuming that the drug-receptor reaction is intrinsically rapid, the extreme slowing at low pH (Figs. 3 and 4) implies that protonated forms of these amine anesthetics have no external receptor. It is already known that quaternary cationic derivatives of the same molecules have no external receptor (Fig. 4 D; Narahashi et al., 1969, 1972; Frazier et al., 1970; Strichartz, 1973; Hille et al., 1975; Khodorov et al., 1976). Now the possibility of an external receptor for the neutral form of local anesthetic molecules must be considered. Two arguments advanced in favor of this possibility are the very rapid rate of action of externally applied neutral forms (benzocaine and amine molecules at high pH) and the relatively weak action of amine anesthetic (trimecaine) applied to the cut ends of a fiber (Khodorov et al., 1976). However, as is shown from the theoretical discussion below, both of these observations are equally consistent with an internal anesthetic receptor and therefore there is no requirement to consider external receptors for any form of local anesthetic molecules.

Simplified Diffusion Model

The time course of changes in drug concentration in the vicinity of a receptor may be calculated from an appropriate form of the diffusion equation. In the case of the single fiber, the geometry includes a large external bath and a membrane of limited permeability with external and internal unstirred layers. The movement of drug was calculated for a highly simplified model including three diffusion processes shown diagrammatically in Fig. 5 A: (*a*) radial diffusion in the unstirred layer of thickness δ outside the node; (*b*) radial diffusion across the nodal membrane of permeability P_N; and (*c*) longitudinal diffusion down the internodal axoplasm extending a distance l on either side of the node. The diffusing molecule is assumed to be a single molecular species able to permeate only at the node and with no special affinity for specific parts of the fiber. Thus the model ignores all possible protonations and deprotonations, binding or accumulation in hydrophobic regions, and alternative diffusion paths directly through myelin or the paranodal region. Each of these omitted factors may be significant in some real cases but probably does not disturb the major conclusion that drugs acting internally could act as rapidly after external application as was found in these experiments.

For further computational simplicity the problem was approximated by the one-dimensional geometry represented in Fig. 5 B and divided into small compartments of thickness Δx for simulation by standard multicompartment kinetics. The nth compartment could be specified by a volume V_n, the drug concentration c_n, and the effective permeability P_n and area A_n of the imaginary barrier separating it from the $n+1$th compartment. The flux from the nth compartment to the $n+1$th was taken as

$$m = (c_n - c_{n+1})P_nA_n, \tag{1}$$

where P_n is related to the diffusion coefficient D by

$$P_n = \frac{D}{\Delta x}, \tag{2}$$

except for the barrier that includes the nodal membrane as well, where

$$P_n = \frac{1}{\frac{\Delta x}{D} + \frac{1}{P_N}}. \tag{3}$$

The rate of change of concentration is given by

$$\frac{dc_n}{dt} = \frac{m_{n-1,n} - m_{n,n+1}}{V_n}. \tag{4}$$

Typically, Δx was chosen in the range 0.5–6 μm and the progress of the diffusing molecules could be followed by integrating Eq. (4) by the simple Euler method in time steps of 0.2–20 ms. As is suggested in Fig. 5 A and B, the areas A_n and volumes V_n decrease continuously in the extracellular radial diffusion regime

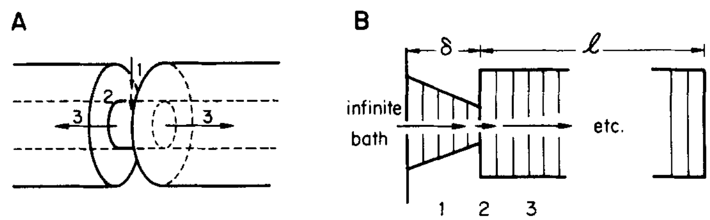

FIGURE 5. Two simplified representations of the diffusion path for externally applied drugs to enter a myelinated nerve fiber. (A) A schematic node of Ranvier showing the three components of the drug diffusion regime: (1) extracellular radial unstirred layer, (2) membrane, (3) unstirred layer extending down the axis cylinder. (B) A further simplification of the diffusion problem in terms of multicompartment kinetics. The extracellular unstirred layer has thickness δ and tapers towards the nodal membrane. The axis cylinder is closed at a distance l from the node and has a cross-sectional area twice that of a standard axon to imitate the effect of two internodes.

until the node is reached and then take new steady values in the intracellular regime. At time zero the drug concentration in an infinite bath bathing the outermost compartment was stepped from 0 to 1.0 arbitrary units and the subsequent time course of drug concentration changes in all compartments was calculated.

Fig. 6 shows the calculated time course of drug concentration in the first intracellular compartment, where drug molecules would be in position to bind to a receptor on the inside of the nodal membrane. The different curves are for different values of the assumed membrane permeability coefficient P_N in a fiber with an extracellular unstirred layer thickness $\delta = 6$ μm, nodal gap width gap 1.5 μm, axonal radius 5 μm, and internal and external drug diffusion coefficient $D = 0.5 \times 10^{-5}$ cm^2 s^{-1}. The internodes on each side are assumed to be closed at a distance of $l = 768$ μm from the node. All time courses depend on these five fixed (and undetermined) parameters of the model, so the curves should be regarded more as a rough guide to the behavior of such a diffusion system rather than as an exact calculation. The half-time for the intranodal concentration rise ranges from about 275 ms for high membrane permeability P_N to many tens of minutes for the lowest P_N used. As is characteristic of diffusion processes, the time courses look like the sum of many exponentials, and even for the lowest value of P_N a part of the internal concentration increase occurs rapidly, although most of it is slow.

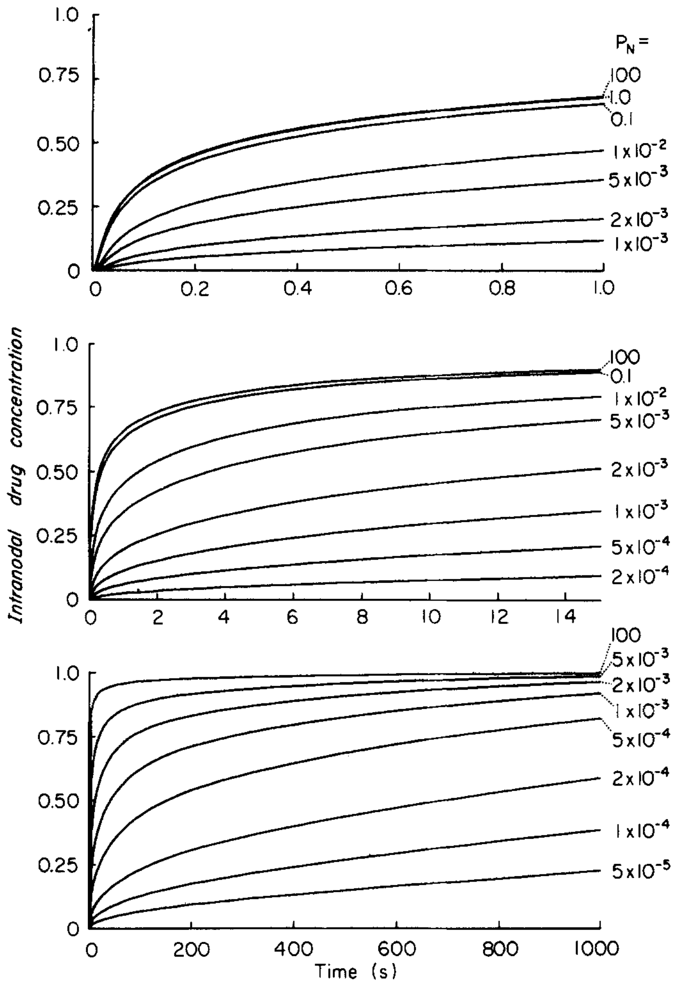

FIGURE 6. Solutions of Eqs. (1)-(4) representing diffusion of drug following the scheme of Fig. 5 B. Permeability of the nodal membrane is given in units of cm s^{-1}. Assumed parameters: δ = 6 μm, D = 0.5×10^{-5} cm^2 s^{-1}, l = 768 μm, nodal gap width 1.5 μm, axonal radius 5 μm. The curves plot the drug concentration in the compartment immediately inside the nodal membrane after a step concentration increase from 0 to 1.0 in the infinite bath.

Further understanding of the rate-limiting steps for drug entry may be obtained by order-of-magnitude arguments. In the steady state, the extracellular unstirred layer acts as a diffusion barrier with an effective permeability of roughly D/δ or 8×10^{-3} cm s^{-1}. Therefore, if the permeability P_N of the nodal membrane is much larger than this value, the drug diffuses as if there were no nodal membrane (cf. the similarity of curves for $P_N = 0.1$, 1.0, and 100 cm s^{-1}) and the minimum calculated half-time of concentration rise just inside the node is 275 ms. Although the intranodal concentration rises rapidly with high values of P_N, it should be noted that most of the more distal compartments are still empty and the total amount of drug in all intracellular compartments equilibrates with a long characteristic time of $(2l/\pi)^2D^{-1}$ or 500 s. At the other extreme for values of P_N much lower than 8×10^{-3} cm s^{-1}, the outer unstirred layer becomes negligible and membrane permeation is the rate-limiting step. It may seem surprising that the theoretical minimum half-time of intranodal concentration rise for a very permeant molecule is as long as 275 ms when the concentration of Na^+ ions in contact with the outer membrane surface can be changed experimentally with half-times of 19–23 ms as judged by changes in the rate of rise of action potentials (Vierhaus and Ulbricht, 1971*a*). Indeed, in the model here, the calculated half-time at the outside of the membrane is only 19 ms for an impermeant molecule, but it increases by more than an order of magnitude as the membrane permeability increases, since molecules arriving at the outer surface can then diffuse right on into the axoplasm. Thus, elevating P_N slows the time course of the external concentration rise.

The calculations of Fig. 6 assume that the cut ends of the fiber are sealed and prevent loss of drug from the internode. Since the ends are closed, all curves rise asymptotically to an equilibrium value of 1.0. The present experimental situation is possibly better imitated by assuming the cut ends of the internode to be open instead. Then the curves will be asymptotic to 1.0 only when the permeability-area product of the node is much larger than the corresponding product of the two internodes, or for the geometry assumed here, when P_N is much larger than 4×10^{-4} cm s^{-1}. For smaller P_N the steady-state concentration inside the node would be less than 1.0 and the curves in Fig. 5 would overestimate the drug concentration after exposure times on the order of 500 s. Thus, relatively impermeant drugs cannot be applied effectively at the node if the ends of the fiber are open and permit the drug to leave. A reversed form of the same argument shows that relatively membrane-permeant drugs would not be very effective if applied at the cut ends rather than externally to the node. In this case, diffusion out from the intranodal compartment predominates over diffusion to the node from the cut end. The limiting case occurs when P_N is much higher than the effective permeability D/δ (0.008 cm s^{-1}) of the external unstirred layer. Then the steady-state intranodal drug concentration would be no more than (0.0004)/(0.0004 + 0.008) or $^1/_{21}$ of the concentration applied at the cut end.

Application of the Diffusion Model

PREDICTION OF THE PERMEABILITY COEFFICIENTS. Before the calculated curves of Fig. 6 are compared with the experimental observations, it is necessary to find some basis for estimating the permeability P_N of the nodal membrane to different anesthetic molecules. This is difficult because for technical reasons nonelectrolyte permeability studies on biological and model membranes have been limited to molecules with permeabilities and lipid solubilities more than 10^4

times smaller than those expected for the neutral form of local anesthetics. Special methods to determine the permeability of the neutral form of weak acids have been applied to two molecules remotely similar in structure to local anesthetics. Salicylic acid was found to have permeability of 0.7 cm s^{-1} in egg lecithin-decane lipid bilayer membranes (Gutknecht and Tosteson, 1973) and carbonylcyanide *m*-chlorophenylhydrazone (CCCP), a permeability of 11 cm s^{-1} in similar egg lecithin-cholesterol-decane membranes (LeBlanc, 1971). The permeability of algal cells to less permeant nonelectrolytes was studied in the classical work of Collander and Bärlund (1933) and others. An empirical equation (Collander, 1954) for calculating the permeability of cells of *Nitella* to nonelectrolytes is

$$P = 6.5\, F^{1.35} M^{-1.5}, \tag{5}$$

where F is the olive oil:water partition coefficient and M is the molecular weight of the nonelectrolyte. For the permeability of lecithin-decane lipid bilayers to poorly permeant nonelectrolytes, Finkelstein (1976) has used the empirical equation

$$P = 6 \times 10^5\, K_{hc} D, \tag{6}$$

where K_{hc} is the hydrocarbon:water partition coefficient and D is the diffusion coefficient of the nonelectrolyte in water. Applying Eq. (5) and (6) to the permeation of salicylic acid gives predicted permeabilities of 0.08 and 0.09 cm s^{-1} with the values $F = 10$, $K_{hc} = 0.03$ (Leo et al., 1971), and $D = 0.5 \times 10^{-5}$ $cm^2\, s^{-1}$. The similarity of the predictions says that biological membranes are expected to have permeabilities like those of lecithin bilayers, and the eightfold difference between the measured (0.7 cm s^{-1}) and the predicted value for bilayers suggests that Eq. (6) may underestimate P when applied to very permeable molecules. If $P = 0.7$ cm s^{-1} is correct for salicylic acid ($F = 10$, olive oil) on biological membranes, then P for benzocaine and the neutral forms of mepivacaine and procaine ($F = 41$–46, oleyl alcohol) would be expected to be about 3 cm s^{-1}. The expected P for the neutral forms of lidocaine, tetracaine, and RAC 109 ($F = 225$–273, oleyl alcohol and cod liver oil) would be higher, and that for GEA 968 ($F = 1.3$, cod liver oil) lower. Little experimental basis exists for predicting P for quaternary anesthetic derivatives. A value of 2×10^{-8} cm s^{-1} may be calculated for dimethyldibenzylammonium from a single steady-state conductance measurement on bull brain phospholipid-decane bilayers (Liberman and Topaly, 1969), but that system has not been studied in detail. An apparent value of 2–5×10^{-8} cm s^{-1} may be calculated for tetracaine cations from the steady-state conductance of phosphatidylethanolamine-decane bilayers in 10 μM tetracaine (McLaughlin, 1975), but here the current-carrying species is a dimer of a neutral with a protonated molecule and the concept of permeability is not readily applied. In any case, charged anesthetic molecules are expected to have permeability coefficients much smaller than those of the neutral molecules.

TIME COURSE OF DRUG ACTION. The model curves of Fig. 6 may now be compared with the observations in Figs. 2-4 and Table II. According to the preceding discussion, the neutral forms of all molecules in Table I may have a membrane permeability higher than 0.05 cm s^{-1}. At high pH the penetration of such highly permeant molecules should be limited primarily by the thickness of the external unstirred layer, and the half-times for their concentration rise within the node would be shorter than 500 ms. The membrane permeability is so high that the membrane might as well not be there. These predictions are in good agreement with the observations on the rate of block of Na channels, showing that the rapid action of local anesthetics at high pH is easily reconciled with an initial requirement to penetrate the nodal membrane. If anything, the rate of block is slower than the calculated rate of entry, a discrepancy which could be due to errors in the parameters of the model or to a finite rate of reaction of the drug with its receptor.

At low pH, the whole diffusion process becomes more complicated because the effects of buffers and drug dissociations must be explicitly considered (Gutknecht and Tosteson, 1973; Neumcke and Bamberg, 1975; McLaughlin, 1975). Although this explicit calculation is not done here, it should be evident that reducing the external pH would decrease the rate of arrival of anesthetic inside the node by decreasing the fraction of molecules in the permeant neutral form. A rough indication of the effects of low pH is obtained from the values of the effective distribution coefficients $q_{6.0}$ in Table II. By this criterion, the rate of action at pH 6.0 should follow the sequence: benzocaine > lidocaine > tetracaine > RAC 109, procaine, and GEA 968; and the rate of action of lidocaine at pH 6 should not be slower than the rate for GEA 968 at pH 8.3. All of these predictions are in good agreement with the observations and show that, at least when the external pH is low, anesthetic molecules must cross a hydrophobic barrier before reaching their receptor site. Indeed, all the observations at high and low pH are completely consistent with diffusion of the neutral form of molecules into the node as a first step in reaching an internal receptor. They also do not rule out the possibility of an external receptor for neutral molecules, although an external receptor for cations is definitely ruled out.

Quaternary derivatives cross the nodal membrane far more slowly than neutral molecules, so the external unstirred layer is no longer rate limiting. Comparison of the experiment of Fig. 4 D with the theoretical time courses of Fig. 6 suggests that the membrane permeability for QX-572 is well below 10^{-3} cm s^{-1}. Since internal application via one end pool leads to about as much block as external application, P_N for QX-572 may be close to 10^{-4} cm s^{-1}. With RAC 421, on the other hand, the drug is much more than 80-fold more potent by internal application, so P_N must be far less than 10^{-6} cm s^{-1}. Qualitatively, these results are in the expected direction. However, it is surprising that QX-572 has a permeability as high as 10^{-4} cm s^{-1} since the compound dimethyldibenzyl ammonium, which is far less polar, has an estimated permeability several orders of magnitude lower in lipid bilayers. The alternative hypothesis that external QX-572 is reacting slowly with an external receptor is untenable because: (*a*) the other quaternary drugs demonstrate that there is no external receptor; (*b*) attempts to reverse the block by external washing after a 10 min exposure to QX-572 are ineffectual; and (*c*) the block with external application has the same complex "use-dependence" (Hille, 1977) as is found with internal application of QX-572.

In conclusion, the diffusion model used here gives a basis for understanding the different rates of action of the different local anesthetic charge classes and the influence of external pH. The essential feature of the model is that externally applied anesthetic molecules must cross a hydrophobic barrier before reacting with their receptor. For the calculation, the nodal membrane was assumed to be the barrier and the inner surface of the membrane, the receptor-containing compartment. However, in the following paper (Hille, 1977) a slightly different conclusion is reached, namely, the receptor is located within the Na channel in a position that can be reached by hydrophobic molecules directly from the hydrophobic bulk of the membrane and by hydrophobic and hydrophilic molecules from the axoplasmic compartment. This hypothesis still requires external molecules to diffuse through a hydrophobic barrier and does not substantially alter the conclusions drawn from the simpler model used in the calculations.

Critique and Comparison with Earlier Work

UNSTIRRED LAYERS AND THE INTERNAL VS. EXTERNAL RECEPTOR QUESTION. The high membrane permeability of typical amine anesthetics has important implications for interpreting experiments involving supposed selective application of anesthetics to the inside or outside. In such experiments, the drug concentration at the inner and outer surfaces of the membrane will actually be nearly equal unless the total internal and external unstirred layer thickness is less than D/P. For a molecule with $P = 1$ cm s^{-1} and $D = 0.5 \times 10^{-5}$ cm^2 s^{-1}, the combined unstirred layer thickness would need to be reduced below 0.05 μm, a clearly impossible goal. Thus, the location of the receptor for highly permeable neutral drugs is not determinable by such methods.

This limitation of the method has been overlooked in many studies. For example, Khodorov et al. (1976) found that procaine and trimecaine are much less potent when applied to the cut ends of myelinated fiber than when applied externally. They conclude, therefore, that there is an external receptor. However, the result is merely a consequence of the relative shortness of the external diffusion path compared with the internodal one and does not bear on the sidedness of the receptor at the membrane. Århem and Frankenhaeuser (1974) take the same observation that there is some block with anesthetic applied at the cut ends to mean that there must be an internal receptor in addition to an external receptor which they had already inferred from the rapid action (<10 s in their work) of lidocaine, procaine, and benzocaine applied externally. Neither of these conclusions is justified by the experiments. Just the opposite arrangement of unstirred layers is encountered with the internally perfused squid giant axons used in the experiments of Narahashi's laboratory. There the internal unstirred layer is relatively thin because the axon is small (radius 200 μm) and the internal flow fast, while the external unstirred layer is thicker (>50 μm) because the axons have a glial sheath and a fairly thick external layer of other adhering nerve fibers and because the flow is slower. In this situation with a larger unstirred layer outside than inside, an externally applied amine anesthetic such as procaine will necessarily seem less potent than an internally applied one as has been found (Narahashi and Frazier, 1975), but, again, a conclusion regarding sidedness is not justified. With compounds of low membrane permeability such as quaternary derivatives, these problems should be nowhere near as severe.

EXTERNAL PH AND RATE OF ACTION ON NERVE TRUNKS. There is no other comparable study on single nerve fibers. A decrease in potency and a slowing of the rate of action of local anesthetics by lowering the pH is already familiar and universally accepted in experiments on various nerve trunks with intact connective tissue sheath (Skou, 1954; Rud, 1961; Ritchie et al., 1965*b;* Strobel and Bianchi, 1970*a, b;* see many earlier references in Ritchie and Greengard, 1966). The explanation seems obvious that the epineurial sheath is a hydrophobic barrier allowing primarily the neutral form of anesthetic to cross. Thus, there are formal similarities between pH effects on single fibers and on intact nerve, although in one case the time scale of drug action is on the order of 1 s and in the other, 2–50 min.

Paradoxically, experiments on nerve trunks with the epineurium removed give results apparently opposite to those with intact epineurium and to those on single myelinated fibers. The most striking difference is that the block is faster at low pH with dibucaine or lidocaine (but not procaine) applied to desheathed rabbit vagus and frog sciatic (Ritchie et al., 1965*a;* Ritchie and Ritchie, 1968; Strobel and Bianchi, 1970*a, b*). This result has been considered as evidence that the cationic form of the drug is the active form. I believe instead that the effect is the result of a system combining uptake with diffusion. Highly lipid-soluble substances may penetrate slowly into the depths of a desheathed nerve bundle because they partition so strongly into myelin and other lipid regions before reaching the middle of the bundle. The free concentration near the middle stays low until all superficial stores become loaded. On the other hand, at low pH amine molecules in the cationic form could diffuse more quickly to the center of the bundle without becoming lost in the lipid on the way. According to this idea, the most lipid-soluble drugs should be the slowest to act at high external pH because they are taken up the most in lipid. Indeed, dibucaine ($F_{\text{oleyl alcohol}}$ = 5,000) takes more than 50 min to act on desheathed vagus at pH 9.2 (Ritchie and Ritchie, 1968). In addition, the same idea suggests that drugs of sufficiently low lipid solubility should act faster at high pH than at low pH (as all local anesthetics do on single fibers) because their partitioning into lipid is too small to disturb their diffusion. Presumably, this is why procaine (F = 45) acts faster at high pH while lidocaine (F = 225) and dibucaine (F = 5,000) act faster at low pH on the desheathed vagus (Ritchie and Ritchie, 1968). GEA 968 (F = 1.3) should show this effect even more clearly than procaine. The conclusion is, therefore, that existing studies of the pH dependence of the rate of action reflect primarily the requirement for a form of the drug which can diffuse to the receptor and are not suitable for determining the form of the molecule which is active at the receptor.

PH AND DEPTH OF BLOCK WITH VAGUS NERVE. There are many published experiments on the effect of bathing pH on the depth of block with vertebrate nerve (e.g., Skou, 1954; Rud, 1961; Strobel and Bianchi, 1971*a, b;* and see references in Ritchie and Greengard, 1966, and Ritchie, 1975). The most influential of the recent experiments are those of Ritchie's laboratory on desheathed rabbit vagus nerve. Ritchie and Greengard (1961; Ritchie et al., 1965*a, b*) found an enhancement of conduction block within minutes of lowering the external pH from 9.6 to 7.2 around a vagus nerve pretreated with a long-lasting anesthetic agent such as dibucaine or chlorpromazine. With shorter acting agents such as lidocaine or procaine there is also a small enhancement, but it is only transient (Strobel and Bianchi, 1970*a*). The enhanced block at low pH was interpreted to mean that the drug cation is the active form (Ritchie et al., 1965*a*, *b*). Then Narahashi et al. (1970) added the further suggestion that lowering the external pH might have lowered the internal pH of the axons of the vagus nerve, thus increasing the proportion of intracellular drug cation (the form which they regarded as the active one). No estimate has been made of how large the intracellular pH change is expected to be, but it might be acceptable to calculate this by using information from other tissues. Izutsu (1972) has measured intracellular pH changes in bullfrog toe muscle with mean fiber diameters of 40 μm. 2 h after dropping the extracellular pH from 7.3 to 6.6 with a 5 mM bicarbonate buffer, the intracellular pH had fallen by 0.35 units. For cells with similar membrane properties and intracellular buffer values, the speed of these pH changes should vary directly as the surface-to-volume ratio. On this basis, the same 0.35 pH unit decrease would occur in only 1.5 min in 0.5-μm unmyelinated nerve fibers and even faster in the very thin glial cells surrounding them. The intracellular pH of myelin sheath Schwann cells should also change rapidly, but that of the large myelinated axons might change far more slowly because of the limited area available for entry of protons. The conclusion is that the pH in most intracellular compartments of a desheathed vagus nerve should change considerably within the first few minutes after the extracellular pH has been changed.

The surprising point, in retrospect, is that the experiments with vertebrate nerve seem to point toward nearly exclusive activity of the drug cation when at the same time neutral benzocaine is found to be roughly equipotent with procaine (Ritchie and Ritchie, 1968; Århem and Frankenhaeuser, 1974; Khodorov et al., 1967). However, the experiments may need reinterpretation in terms of the strong accumulation of a drug such as dibucaine in the lipid regions (especially glia and Schwann cells) of nerve. For example, to make an extreme case, assume that the intracellular pH exactly follows the extracellular, that the highly lipid-soluble drug involved has a pK_a of 8.0, and that the product of the lipid:water partition coefficient of the neutral form times the volume fraction of lipid in the nerve is 100. A nerve is equilibrated in 10 μM drug at pH 10. The aqueous phases contain 9.9 μM-free base and 0.1 μM cation, and the total nerve, including lipid, contains 1 mM drug, 99% of which is in the lipid. The nerve still conducts action potentials. Now the pH is lowered to 6, and throughout the nerve a large quantity of drug is released as cations from the lipid stores. Before diffusion removes drug from the nerve, the total 1 mM drug redistributes, leaving 49.7% in the lipid and 4.97 μM-free base and 497 μM cation in the aqueous phases (neglecting any adsorption of the cationic form to membranes). Conduction of action potentials fails. Gradually, diffusion removes drug from the nerve until the concentrations are again in equilibrium with the 10 μM solution applied, and conduction returns. This experiment would prove that cations have at least 1% of the activity of the free base rather than support the theory that cations are the most active form. The example is deliberately extreme but shows that the experiments of Ritchie laboratory might be reconciled with more equal blocking potencies for neutral forms and cations than has previously been thought. The essential feature of the calculation is that lowering the pH causes drug molecules to be transferred from lipid stores into the medium. Such a transfer of local anesthetics is confirmed by studies on monolayers of frog sciatic nerve lipids, on red blood cell ghosts, and on whole desheathed nerve (Skou, 1954*b;* Kwant and Seeman, 1969; Strobel and Bianchi, 1970*b*). Because of adsorption of drug cations to the membrane (McLaughlin, 1975), the actual release of drug is probably significantly less than calculated in the above example.

PH AND DEPTH OF BLOCK WITH SQUID GIANT AXONS. The importance of the internal drug cation was proven by the discovery of a strong blocking effect with quaternary lidocaine analogs like QX-314 and QX-572 perfused inside squid giant axons (Frazier et al., 1970). In the same series of experiments amine anesthetics perfused internally were found to block less strongly when the internal perfusate had a high pH than when it had a low pH (Narahashi et al., 1970, 1972). The obvious conclusion was that the protonated form of amine molecules blocks much better than the neutral form; however, there remains the alternative possibility that part of the stronger block arises from an unstirred layer problem. It is conceivable that the internal perfusion cannot control the surface concentration of highly permeant drugs which will be constantly escaping to the outside. Since raising the pH increases the percentage of permeant neutral drug, it could decrease the total drug concentration at the axoplasmic layer closest to the membrane by allowing more molecules to escape. If that is true, then a lack of activity of neutral amine forms has not been proved. Unfortunately, this question of an unstirred layer is a quantitative problem whose relative importance could not be estimated without considerable calculation and perhaps some new experiments.

SUMMARY OF CRITIQUE. The extremely high membrane permeabilities and lipid solubilities of local anesthetic molecules complicate the interpretation of most of the published experiments concerning the rate of action, site of action, and active form of these drugs. Thus, many of these sophisticated experiments cannot be interpreted uniquely without a further detailed quantitative analysis. For the moment, it may be necessary to place more emphasis on other kinds of experiments, such as those where the molecule is guaranteed to be cationic or neutral by its chemical structure or those where all conditions are adjusted to keep an equilibrium distribution of drug between internal and external compartments. Then transient phenomena or steady states dominated by unstirred layer effects are less likely to be misinterpreted by equilibrium theories.

A major result of this paper is the confirmation that quaternary cationic molecules and cationic protonated amine molecules have no external receptor on Na channels and the demonstration that, even with a naked single fiber, externally applied drug molecules must cross a hydrophobic barrier before they can block Na channels. The following paper (Hille, 1977) considers the questions of active form and location of the receptor in further detail.

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