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Review Article

Molecular Mechanisms of Nerve Block by Local Anesthetics

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LOCAL ANESTHETICS are drugs that block impulse conduction in nerves. They have been used clinically for almost a hundred years,[1,2] but the exact mechanism of their action on nerves remains unknown. Several significant details about the molecular mechanisms of local anesthesia have appeared during the past five years. This information has come from neurophysiologic experiments on isolated, single nerve fibers, from physicochemical studies of nerve membranes during anesthesia, and from investigations of the effects of local anesthetics on artificial, lipid-bilayer membranes. The object of this review is to summarize critically these results as they relate to current hypotheses of the molecular basis of anesthetic action. Before delving into the recent experimental evidence, I briefly review the current concept of the ionic basis of nervous conduction. (A previous review of anesthetic mechanisms appeared in 1973[3].)

I. Excitability and ionic permeability of membranes

1. Conduction requires ionic flow through selective "channels" in nerve membranes

The conduction of impulses in almost all nerves requires a flow of sodium ions into the nerve in response to depolarization of the nerve membrane.[4] At rest, the electrical potential inside the nerve is negative relative to the outside and sodium ions are at a higher concentration outside than inside the nerve. During the passage of a nerve impulse, or "action potential," the permeability of the membrane to sodium ions increases transiently (fig. 1) and sodium flows into the nerve, passing though the membrane via sodium-selective ionic channels that open and close in response to changes in the electrical potential across the membrane. The number of

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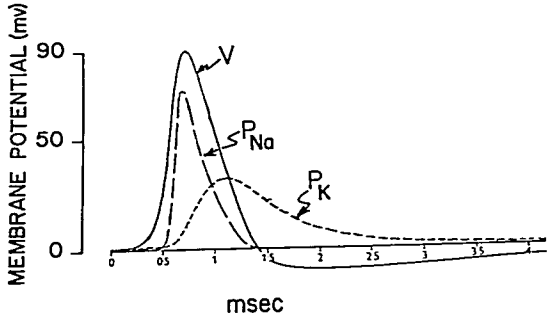

FIG. 1. Time courses of the changes in membrane potential (V) and concomitant changes in sodium permeability (P_{Na}) and potassium permeability (P_K) during an action potential in a squid giant axon (after Hodgkin and Huxley[3]).

sodium ions transported during one action potential is too small to modify measurably the concentration difference across the membrane. Sodium channels are very sparsely distributed in the membrane[6,7]; in some small non-myelinated C-fibers, for example, the density may be as low as ten channels μm^{-2} or one channel for about every 10^5 lipid molecules in the membrane.[8]

Excitable membranes also contain potassium channels that allow the efflux of K^+ ions during the repolarization phase of the action potential[1,7] (fig. 1). The nerve-blocking action of local anesthetics is on the Na^+ channels,[9–11] hence the K^+ channels are not discussed here.

2. Sodium channels are lipoproteins

Sodium channels are probably composed of lipoproteins that span the thickness of the nerve membrane.[12] The outer opening of the channel appears to be a relatively static structure. It is the site where drugs like tetrodotoxin† bind and block the sodium ion influx.[12,13] Tetrodotoxin binds to the channels whether they are open or closed, *i.e.*, whether the nerve membrane is at rest or stimulated,[14] and even binds to this site after the membrane has been dissolved by detergents.[15–17] Exposure of nerves to proteolytic enzymes inhibits the binding of tetrodotoxin, suggesting a destruction of the outer opening of the sodium channel.[12] This enzymatic digestion is facilitated by removal of membrane phospholipids prior to proteolytic digestion[17]; thus, the channel protein appears to be embedded in the hydrophobic matrix of the membrane.

The inner end of the sodium channel is probably the location of structures that open and close the channel, the so-called "gating functions." Gating properties can be selectively affected by exposure of the inner surface of the nerve membrane to specific agents. For example, in certain large nerve fibers like the squid giant axon (0.5 mm diameter) the cytoplasm can be replaced by a continuously flowing artificial solution containing a known concentration of drugs, enzymes, or ions. Such agents can also be infiltrated into a smaller, myelinated nerve fiber (0.015 mm diameter) by allowing diffusion from a cut across the myelinated internodal region of the axon to the node of Ranvier (fig. 2). A remarkable result occurs when the inside of a squid axon is exposed to pronase, a mixture of several proteolytic enzymes. Normally the sodium channels respond to a maintained membrane depolarization by rapidly opening, then spontaneously closing at a slower rate (fig. 3). This slower closing is called sodium "inactivation" and partially accounts for the phenomena of accommodation and for the refractory behavior

† Tetrodotoxin is a small (mw ~ 320) organic molecule that is synthesized by and stored in both the Pacific pufferfish family, or fugu, and American newts of the genus *Taricha*. Tetrodotoxin blocks nerve conduction by very specifically abolishing the sodium permeability of the nerve membrane.

of nerves. Following pronase treatment the inactivation function disappears, and sodium channels respond to maintained depolarizations by opening and remaining open.[18] This experiment implies that the inactivation part of the gating structure is made at least partly of protein.

3. Local anesthetics block sodium currents

Local anesthetics block nerve impulse conduction by preventing the influx of sodium ions.[9–11] (fig. 3). They do this by altering the normal increase in sodium permeability associated with the impulse and not by altering the ionic gradients or the resting membrane potential of the nerve. Local anesthetic molecules are characteristically composed of tertiary amines linked to lipophilic, hydrocarbon groups by amine or ester bonds (fig. 4). Near physiologic *p*H such molecules exist in both protonated and uncharged forms; the latter are able to penetrate and cross the hydrocarbon region of the cell membrane. Two questions often asked are: 1) which of the two anesthetic forms is the more active, the charged cation or the neutral base, and 2) where are the sites of action on the cell membrane?

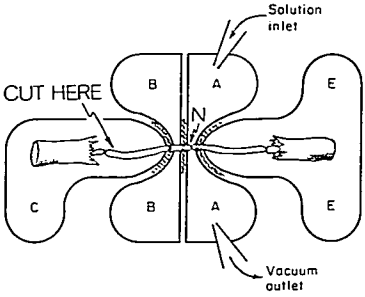

FIG. 2. Schematic of the chamber used in the study of single myelinated nerves. The excitable membrane at the node of Ranvier, *N*, can be externally exposed to drugs that are dissolved in the Ringer's solution in *A*, or the drugs can reach the inside of the node by diffusing down the axoplasm from pool *C* after sectioning of the internode, as shown. Membrane current is injected via the internode in pool *E* and pool *B* is at ground. The pools of solutions are isolated from each other by Lucite partitions topped by Vaseline seals (crosshatched areas). (From Hille[57]).

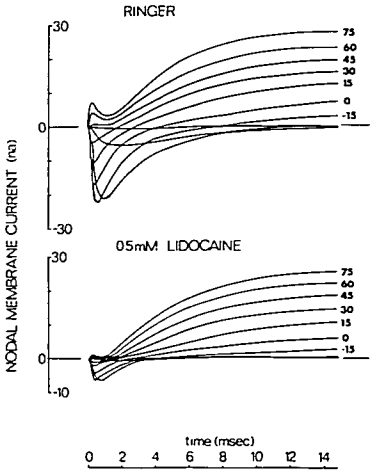

FIG. 3. Ionic currents during voltage-clamp in a node of Ranvier from the frog. Each trace is the total ionic current that flows during a 15-msec depolarization to the membrane potential at the right (traces for −30 and −45 mV depolarizations are not labelled). Inward currents are negative, outward positive. The early inward current is carried predominantly by sodium ions; it reaches maximum values within the first 2 m/sec of the depolarization and then "inactivates." The later, outward currents, which do not diminish with time, are carried by potassium ions. In this preparation the sodium current is selectively blocked by 0.5 mM lidocaine. Resting potential −75 mV, pulse frequency 1 sec^{-1}, temperature 6–7 C and *p*H 7.0 (from Strichartz,[54] by permission of Raven Press).

4. Cationic anesthetics inside nerves are potent blocking agents

The first question has been explored using many different nerve preparations. The experiments easiest to interpret are those on single, desheathed nerve fibers. Single action potentials can be measured unambigously on isolated fibers. The maximum rate at which the membrane potential changes during the

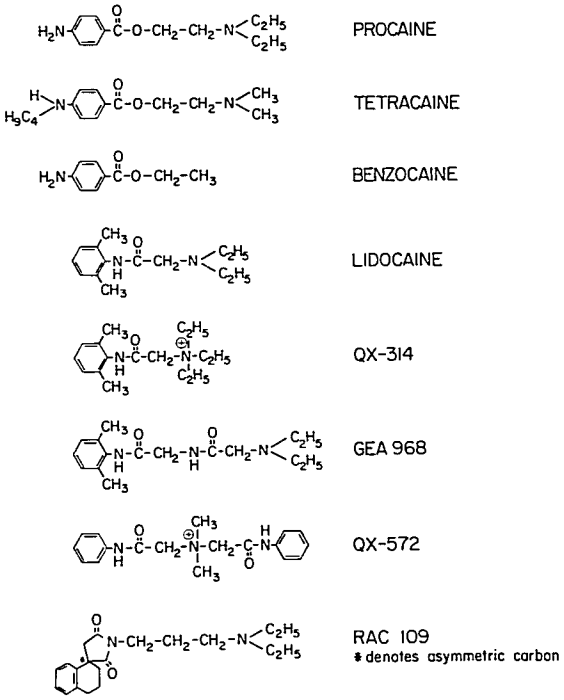

FIG. 4. Structures of several local anesthetics and quaternary derivatives. QX-314 is a quaternary derivative of lidocaine but is active only from inside the nerve. GEA-968, an experimental lidocaine derivative, produces block that depends strongly on the frequency of nerve impulses. QX-572 is a relatively membrane-permeant quaternary derivative of lidocaine that produces block in several minutes when diminished in Ringer's solution bathing the outside of the nerve. RAC 109 blocks sodium permeability stereospecifically, one enantiomer being about twice as potent as the other. Its quaternary derivative, RAC 421, is not shown, but differs only in having a third ethyl group on the tertiary alkyl amine.

rising phase of the action potential, $\dot{V}$, is an indication of the sodium current flowing into the fiber at that time[19] (fig. 1). Alternatively, by using the special technique of voltage-clamping, the sodium current can be measured directly. In a voltage-clamp experiment the membrane potential is externally controlled and therefore the voltage-dependent sodium permeability, P_{Na}, can be modified in a controlled way (fig. 3). The early sodium current that flows during depolarization of voltage-clamped nerves is very similar to the early influx of sodium ions during an action potential[5,19] (compare figs. 1 and 3). Consequently, measuring the extent to which the sodium current is inhibited by drugs during a voltage-clamp experiment provides a good measure of the conduction-blocking potency of a particular agent. The direct observation of membrane currents removes several

ambiguities encountered when only action potentials are measured. For example, the contribution of potassium currents to the rising phase of partially blocked action potentials can be determined directly.

The actions of many local anesthetics appear to be closely related to the "inactivation" aspect of sodium permeability. Membrane sodium permeability increases transiently during a depolarization under voltage-clamp (fig. 3). As mentioned above, the spontaneous decrease in sodium permeability that results from a maintained depolarization is called "inactivation." The extent of inactivation can be tested by presenting a long conditioning "prepulse" before a brief depolarizing test pulse. If the prepulse is a depolarization inactivation is increased, so the sodium currents flowing during the test pulse are smaller than those observed normally. If the prepulse is a hyperpolarization, inactivation is lessened and the sodium currents are larger than normal. (The dependence of the sodium current on prepulse potentials is illustrated in figure 7A, and the dependence of P_{Na} on this potential in figure 7C.) The role of inactivation in local anesthetic mechanisms is discussed in detail in Sections II.3 and II.4.c.

Experiments have been performed on single-fiber preparations where both the rate of appearance and the extent of the steady-state inhibition induced by local anesthetics were measured as a function of the external pH.[10,20-22] Both rate and extent of inhibition of sodium permeability produced by anesthetics bathing the fiber were greater when the anesthetic solutions were at high pH rather than neutral pH. However, when a local anesthetic was perfused directly to the inside of the fiber without having to cross the plasma membrane, then an increase of internal pH lowered the anesthetic potency.[10] A high external pH would increase the relative concentration of neutral anesthetic in the solution bathing the nerve as well as the concentration of neutral anesthetic in the nerve membrane; it would also increase the concentrations of both neutral and charged species in the nerve cytoplasm (fig. 5), but see section II.4.c. and reference 23. Internal application clearly exposes only the cytoplasm and membrane phases to anesthetic (the exterior volume is large and is continuously

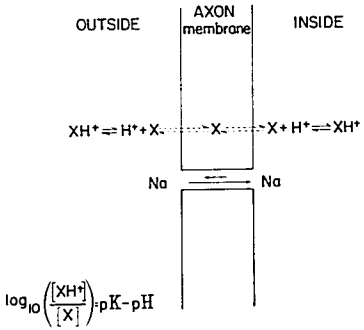

FIG. 5. Distribution of local anesthetic molecules around a nerve membrane. The relative amounts of cationic (XH^+) and neutral (X) anesthetic depend on pH of the medium and pK of the individual anesthetic, according to the Henderson-Hasselbalch equation, lower left. The neutral form is usually significantly more soluble in the membrane's hydrocarbon interior than is the charged form, but some lipophilic, quaternary derivatives (*e.g.*, QX-572, see fig. 4) apparently do pass across the membrane in minutes. Anesthetics may also bind at the interface between the membrane and the aqueous solutions (see fig. 6).

washed out), and increasing internal pH would decrease the concentrations of charged species at these sites. The observations from these experiments are consistent with the hypothesis that tertiary local anesthetics are most potent in the cationic form acting from the inside of the nerve.

This conclusion is supported by other data. Quaternary derivatives of the tertiary local anesthetics (*e.g.*, QX-314, see fig. 4) have been applied selectively to the inside or the outside of single nerve fibers. These molecules have nitrogen atoms covalently bound to four alkyl groups and are always positively charged. Because of this charge quaternary derivatives cross membranes very slowly and during a given experiment are usually restricted to one side or the other of the nerve membrane. The sodium permeability of an axon is unchanged by external application of quaternary derivatives (≤10 mM),[23-25] but is reduced at least 50 per cent by quaternary QX-314 (<1 mM) applied internally.[24,25] Finally, if an extremely lipid-soluble tertiary

TABLE 1. Potencies of Local Anesthetics

	Effective* Concentration (mM)	Assay			Reference
		Measured	Tissue	Axon Diameter	
External application					
Procaine	3 (*p*H 7.9)	P_{Na}	Squid stellate	Giant axon: 500 μm	11
Procaine	8 (*p*H 8.3)	C_M	Lobster	Giant axon: 200 μm	103
Procaine	0.8	AP_c	Rabbit vagus	C-fibers: 0.75 μm	27
Procaine	0.4–0.8	C_M	Cat spinal roots	C-fibers: ~1 μm	99
Procaine	0.25	P_{Na}	Frog sciatic	Node of Ranvier, of Aα myelinated§: 15–20 μm	87
Procaine	1	$\dot{V}$	Frog sciatic	Node of Ranvier, of Aα myelinated§: 15–20 μm	20
Procaine	5	AP_c	Frog sciatic	All fast myelinated	56
Procaine	0.21	P_{Na}	Toad sciatic	Node: 25 μm	22
Procaine	41	C_M	Cat saphenous	Single Aγ-Aδ: 2–6 μm	100
Procaine	2	C_M	Cat lumbosacral	Aδ ≤ 6 μm	99
Procaine	>2.2	C_M	Cat lumbosacral	Aα, Aβ: 8–20 μm	99
Procaine	>41	C_M	Cat saphenous	A_α, Aβ: 8–20 μm	100
Lidocaine	0.2	P_{Na}	Frog sciatic	Node: 15–20 μm	Strichartz (unpublished observation)
Lidocaine	0.25	P_{Na}	Frog sciatic	Node: 15–20 μm	87
Lidocaine	0.44	$\dot{V}$	Frog sciatic	Node: 15–20 μm	20
Lidocaine	1.2	P_{Na}	Toad sciatic	Node: 25 μm	22
Lidocaine	0.3	AP_c	Rabbit vagus	C-fibers: 0.75 μm	28
Cocaine	0.7	$\dot{V}$	Frog sciatic	Node: 15–20 μm	20
Cocaine	2.6	AP_c	Frog sciatic	All fast myelinated	56
Cocaine	~3.3	C_M	Cat spinal roots	Aδ: 6 μm	99
Dibucaine	0.2 (*p*H 8)	P_{Na}	Squid stellate	Giant axon: 500 μm	104
Dibucaine	0.33	AP_c	Rabbit vagus	C-fibers: 0.75 μm	26
Dibucaine	0.005	C_M	Frog sciatic	All fast myelinated	56
Benzocaine	0.5	P_{Na}	Toad sciatic	Node: 25 μm	22
Benzocaine	0.8	AP_c	Rabbit vagus	C-fibers: 0.75 μm	28
RAC 109(I)	0.22	P_{Na}	Frog sciatic	Node: 15–20 μm	21
QX-572	1 (*p*H 8.0)	$\dot{V}$	Squid stellate	Giant axon: 500 μm	24
Internal applications of anesthetics and derivatives					
RAC 421(I)	0.034	P_{Na}	Frog sciatic	Node: 15–20 μm	21

TABLE 1 (*Continued*)

	Effective* Concentration (mM)	Assay			Reference
		Measured†	Tissue	Axon Diameter	
QX-314	0.2	P_{Na}	Frog sciatic	Node: 15–20 μm	25
QX-314	2	$\dot{V}$	Squid stellate	Giant axon: 500 μm	24
QX-572	0.05	$\dot{V}$	Squid stellate	Giant axon: 500 μm	24
QX-572	0.13	P_{Na}	Frog sciatic	Node: 15–20 μm	87

* All measurements were made at *p*H 7.0–7.5, unless otherwise noted in parentheses.

† Measurements of anesthetic efficacy, listed in order of decreasing sensitivity: P_{Na}, sodium permeability under voltage-clamp reduced by 50 per cent; $\dot{V}$, rate of rise of action potential in single nerve reduced by 50 per cent; AP_c, height of compound action potential in nerve bundle bathed by anesthetic reduced 50 per cent; C_B, propagated action potential blocked by minimum anesthetic concentration. As elaborated in the text, anesthetic effectiveness depends on *p*H of bathing medium and on stimulation frequency.

‡ Nerve still in sheath during anesthetic application.

§ Subsequently referred to as "node."

local anesthetic, such as dibucaine, is equilibrated in a nerve bundle, it will be washed out only after many hours in anesthetic-free solution. Ritchie and Greengard[26] showed that by alternating the *p*H of an anesthetic-free bathing solution from 7 to 9, the impulses in a nerve bundle previously treated with dibucaine could be alternately blocked and reactivated, respectively. Thus, by titrating the anesthetic molecules sequestered in the nerve they were able to block the action potential reversibly. Again, the cationic form was more active.

5. Neutral anesthetics also block sodium currents

Evidence from several sources indicates that the uncharged form of some local anesthetics also has blocking activity. First, the drug benzocaine can produce a nerve block,[27] even though it has no tertiary amino group and is negligibly charged at *p*H 7.0 (fig. 4). Both the rate of onset and the extent of the steady-state conduction block by benzocaine are independent of the external *p*H and are about equal to the corresponding values for procaine block at *p*H 9.[28] Second, quantitative analysis of the results of the internal perfusion experiments described in I.4, where the effects of internal and external *p*H on anesthetic potency were examined, indicates that *some* anesthetic potency must be assigned to neutral species. (In the original analysis it was assumed that the *p*H inside the axon responded rapidly and sensitively to changes in external *p*H,[10] although many experiments on nerve and muscle cells have shown that the internal *p*H responds slowly and little to external *p*H changes.[29–31]) The sodium permeability of the nerve membrane is also selectively inhibited by aliphatic alcohols[32] and the neutral form of barbiturates.[33] Whatever the molecular mechanism of these neutral compounds may be, their potency supports the notion that active anesthetic molecules need not be electrically charged.

In summary, both cationic and neutral species of anesthetics are active in blocking conduction. Their relative potencies probably depend on a number of variables, including the anesthetic being tested, the assay methods, and the particular nerve tissue used in the assay (see table 1). The relative anesthetic sensitivities of large and small nerve fibers are discussed at the end of this review.

II. Specific models of anesthetic block

By what mechanism can local anesthetics affect sodium permeability? There are currently three general hypotheses to explain the mechanism of local anesthetic block. Anesthetics may interfere with the membrane permeability by expanding the plasma membrane, by binding to the membrane surface in the charged form and changing its properties through an alteration of the electrical potential, or by combining with a specific receptor in the membrane. There is evidence

that all of these phenomena do occur, and the critical problem is to determine which are important as anesthetic mechanisms. A fourth hypothesis is that local anesthetics displace calcium ions from some site on the membrane where calcium regulates sodium permeability. The calcium-interference mechanism is usually ascribed to an electrostatic competition between Ca^{2+} and cationic local anesthetics for negatively charged membrane sites. Therefore, it is discussed after the section on surface potential changes.

1. Membrane expansion

The membrane expansion theory of anesthesia states that anesthetics absorb to hydrophobic regions of excitable membranes, expanding some critical region(s) in the membrane and thus preventing the sodium permeability increase.[34] This theory paraphrases on a molecular scale the Meyer-Overton law of anesthesia: anesthesia occurs when the concentration of a chemically inert substance reaches some critical value in the membrane.[35,36] The Meyer-Overton law describes the thermodynamic state required for general anesthesia, usually in terms of concentration of gaseous or volatile anesthetic, and has been modified by Ferguson[37] and by Mullins[38] to make the chemical activity and the molecular volume, respectively, the critical modalities for anesthesia. Gaseous anesthetics can produce local anesthesia, but the conduction block requires ten times the concentration necessary to produce general anesthesia.[39] The extensive literature on general anesthesia and membrane interaction has been reviewed by Mullins.[40]

When local anesthetics are absorbed, there are increases in the volume of nerve membrane[41] and in the fluidity of the membrane interior.[42] The membrane volume changes by at least 4–6 per cent in biological samples[43] (the real value may be as high as 10–15 per cent) exposed to local anesthetics in nerve-blocking concentrations, but the volume changes of phospholipid–cholesterol membranes are much smaller.[44] Apparently the membranes must contain protein to show large volume changes. Since the van der Waals volume occupied by the absorbed anesthetic molecules is only about 10 per cent of the total increase in membrane volumes, the drugs must be perturbing the membrane structure by more than just a space-filling process.[39,45]

Where do local anesthetics interact to expand the volume? Since pure lipid membranes are expanded little compared with biological membranes, some investigators have suggested that proteins are the targets of anesthetic action.[44] The membrane phase in which anesthetics dissolve may be reflected in measurements of the free energy of absorption of anesthetics by membranes. The free energy of alcohol anesthetic absorption by erythrocyte membranes agrees better with the free energy of uptake into hydrocarbon solvents than with that of uptake into proteins.[46,47] But the proteins used in these thermodynamic measurements may have lacked the hydrophobic surface of membrane proteins. Furthermore, thermodynamic measurements of anesthetic uptake into the bulk of the membrane may obscure the interaction of the drug at a critical site present in relatively low density. Indeed, anesthesia produced by the neutral molecule TEMPO is enhanced by an increase of the atmospheric pressure around the nerve, which also increases the TEMPO population in relatively polar sites in nerve membranes.[48,49] Of course, anesthetics are probably dissolved throughout the membrane as well as at the ionic channels; at blocking concentration there may be as many as 10^4 local anesthetic molecules in the membrane for each sodium channel.[39]

The dynamic behavior of membrane lipids is modified by local anesthetics. During anesthesia the lipid hydrocarbon tails in the membrane can rotate and bend more easily and the membrane interior becomes less ordered.[42,50,51] The membrane also appears more elastic to lateral stress since the anesthetics stabilize it against osmotic lysis.[41] The involvement of lipids in the dynamics of sodium channels is not known, but while the bulk of the membrane interior becomes more fluid during anesthesia, the rate of opening of sodium channels becomes a little slower.[11]

At least one appealing feature of the membrane-expansion theory is its universal application. It would explain the actions of general and local anesthetics by a single mechanism.[42] However, there is no reason to expect these drugs to act similarly, es-

pecially when they are so different chemically. General anesthesia probably results from the alteration of synapses in the CNS,[52] where the ionic conductance mechanisms are quite unlike those in axons.

Both local and general anesthetics block axonal conduction by inhibiting sodium permeability selectively.[39] Are the mechanisms of action similar? We do not know. Changing the atmospheric pressure has different effects on local and general anesthetics. Conduction block by gaseous anesthetics can be reversed by atmospheric pressures of 100–200 atm, conduction block produced by volatile liquid anesthetics is only partially reversible by pressures of these magnitudes, and block by nonvolatile anesthetics is unrelieved by pressure[19]; sometimes it is even enhanced.[48,49] We might speculate that conduction block results when anesthetics occupy membrane sites at sodium channels, causing some reversible conformational change in channel protein. The sites for general anesthetics may differ from those for local anesthetics, but they could also be the same, in which case the different effects of increased pressure that are observed may be explained by postulating different changes in volume when different types of anesthetics bind at the site of action. Atmospheric pressures of less than 250 atm do not affect action potentials,[49,53] so it is most likely that the binding of gaseous and volatile anesthetics is reversed by pressure, rather than that pressure exerts a direct effect on the conformation of sodium channels.

In summary, nerve membranes do expand and become more "fluid" during local anesthesia. Expansion may correspond to conformational changes in protein resulting from anesthetic binding, but this has not been proven. At present there is no direct evidence that conduction is blocked by the membrane expansion *per se*, and the theory remains difficult to test experimentally.

2. Changes in membrane surface charge

The second hypothesis proposes that local anesthetics act by binding to the membrane and changing the electrical potential at the membrane surface.[54,55] According to this hypothesis, cationic drug molecules are aligned at the membrane–water interface with their lipophilic portions in the hydrocarbon core of the membrane and their charged polar ends at the polar, aqueous surface (fig. 6*B*). Since some of the anesthetic molecules carry a net positive charge, they will make the electrical potential at the membrane surface more positive and thus decrease excitability. This hypothesis is consistent with the observation that positively charged anesthetics inside axons are more potent blockers than neutral ones; it requires that the dominant change in electrical potential occur at the inside surface of the nerve membrane. Local anesthetics are known to absorb to lipid bilayers and change their surface potentials.[54] These potential changes depend on the anesthetic structure in the same order as do the blocking potency,[56] surface tension changes,[57] and monolayer penetration.[58]

The electrical potential at the surfaces of membranes may be measured by several methods. The one most frequently used relies on changes in the cation conductance induced by certain antibiotics in protein-free, artificial, lipid-bilayer membranes. These membranes are very similar to the lipid bilayers of biological membranes in structure and composition, except for the absence of protein. When the membranes are doped with certain antibiotics, which act as cation "carriers," the membrane cation conductance increases by several orders of magnitude.[59,60] This cation conductance is very sensitive to electrical potentials at the membrane surface because the conductance depends on the cation concentration at the membrane–water interface, and this concentration is a function of the surface potential. The conductance acts as a voltmeter to measure surface potential changes at a molecular scale.[61] Following addition of charged local anesthetics to these bilayer membranes the carrier-mediated ion conductance decreases, indicating that the surface potential has become more positive.[54]

There are several objections to the surface-charge hypothesis as a unique explanation of anesthetic action. It cannot explain the ability of uncharged anesthetic molecules to block nerve impulses. (The carrier-mediated conductances in bilayers, mentioned above, are not inhibited by the neutral anesthetics benzocaine and benzyl alcohol, or the uncharged forms of dibucaine and tetracaine,[54] from which we conclude that alkali metal *carriers* are poor models for the sodium-permeability

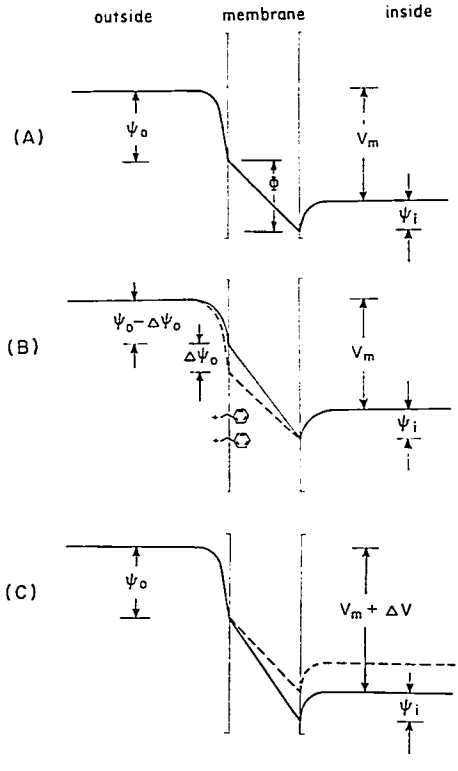

FIG. 6. Profiles of electrical potentials in nerve membranes. *A*, the solid line shows the electrical potential profile near a sodium channel. The potential measured by electrodes in the bulk solution, V_m, is the sum of the potential change across the insulating region of the membrane, Φ, and the difference in the potentials at the outside and inside surfaces of the membrane, ψ_o and ψ_i, respectively. The surface potentials, ψ, are due to negative charges on molecules fixed to the membrane (from McLaughlin and Harary[105]). *B*, hypothetical adsorption of charged local anesthetic molecules to the outside surface of the membrane would make the surface potential less negative by an amount $\Delta\psi_o$. Dashed line is the original profile from *A*. The potential profile then becomes steeper because the decrease of ψ_o appears as an increase of Φ. Raising the external Ca^{2+} concentration would have the same effect. *C*, the transmembrane potential, Φ, can also be increased by increasing V_m while keeping ψ_o and ψ_i constant. The permeability of the sodium channel responds only to the electrical potential across the membrane, Φ. Thus the states of sodium permeability are the same in examples *B* and *C*.

mechanism in nerve.) Still, the cationic, protonated species of amine anesthetics could block conduction by altering surface potentials.

Changes in surface potential could alter the sodium conductance of the membrane by three mechanisms. The local potential difference across the membrane at the sodium channel could be changed, thus shifting the apparent transmembrane potential (measured between bulk solutions) needed to "turn on" the sodium channels (fig. 6). Such an effect would appear as a shift in the relationship between conductance and membrane potential along the potential scale (see fig. 7*B*, *C*).[62,63] In fact, shifts like this do occur in some anesthetized axons where the "inactivation" aspect of sodium conductance is shifted dramatically (see II.4.c). But large shifts of inactivation *vs.* voltage also occur when uncharged benzocaine is the anesthetic (B. Hille, personal communication), so they cannot be explained uniquely by a surface-charge mechanism.

A second mechanism involving surface charges occurs by a lowering of the conduct-

ance of open sodium channels. The charged anesthetic, acting presumably at the inner surface, would bind at or near the sodium channel and thus prevent ionic flow by electrostatic screening. Channels could be blocked by anesthetic molecules that introduce a positive charge sufficiently near the normal pathway for ions, thus increasing the free energy needed for ionic translocation far above the available thermal energy.[64] Recent evidence shows that charged anesthetics must not be binding near the *external* openings of sodium channels because they do not displace positively charged toxins, which are thought to bind reversibly at this site.[8,12,16] The physiologic action of these toxins, measured in voltage-clamp experiments, is also independent of the presence of local anesthetics.[65] The second hypothetical mechanism must then only occur when cationic anesthetics bind at the inner openings of sodium channels. There is increasing evidence that such binding does occur (See Section II.4).

The third mechanism hypothesizes that changes in surface potential affect general membrane structure (*e.g.*, cause expansion) and thereby interfere with the normal operation of sodium channels. This mechanism is one step removed from the primary membrane-expansion hypothesis and is similarly difficult to test by direct experimentation.

While changes in surface potential may not explain the mechanism of nerve block by local anesthetics, such changes are probably very important in explaining the effects of local anesthetics on a variety of biological phenomena, as well as in understanding the binding of any charged molecule to a membrane surface.

The electrical surface potential will be altered by charged anesthetics adsorbing to *any* membrane.[54] Furthermore, the adsorption of the drug depends on its concentration in the solution adjacent to the membrane,[66] and that concentration itself depends on the membrane surface potential. This follows because the anesthetic is partially charged and therefore its free energy, and equilibrium distribution, will depend on the electrical potentials in the different phases of the system. Thus, the more anesthetic that is adsorbed, the more positive the membrane potential becomes and the lower the concentration of charged anesthetic in the local solution at the membrane surface. Since the local surface potential near the sodium channel in axons has been estimated to be as negative a value as −60 mV,[63] the initial concentration of the cationic species of anesthetic at this locale will be ten times the concentration in the bulk solution.[61] Analogously, the proton concentration at the membrane surface will be increased by the same factor by the negative surface potential. Hence, the proton-association constant (and the pK) of anesthetics is not changed by surface

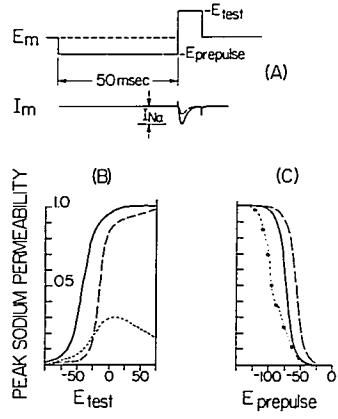

FIG. 7. Sodium permeability is affected by local anesthetics in two ways. *A*, the patterns of membrane potential (E_m) and membrane sodium current (I_m) during a voltage-clamp experiment. Peak sodium permeability can be calculated by measuring peak current (I_{Na}) as a function of E_{test}. Sodium permeability is maximized when E_{test} is a large membrane depolarization (solid line in *B*) that is preceded by a longer, hyperpolarizing pulse, $E_{prepulse}$ (solid line in *C*). E_{test} depolarizations "active" sodium permeability; $E_{prepulse}$ hyperpolarizations "remove inactivation," and in the absence of a hyperpolarizing prepulse I_{Na} is smaller (dashed line in *A*). *B*, The effects of 0.5 mM lidocaine on sodium activation are illustrated as dotted lines; *C*, the effects of 0.6 mM GEA-968 on sodium inactivation are shown as open circles connected by dashes. For comparison, the effects of increasing external Ca^{2+} from 2 to 20 mM are shown as a broken line (control data from Hille[6]; lidocaine data from Strichartz, unpublished observations; GEA 968 data from Courtney[62], and Ca^{2+} data from Woodhull[71] and Hille, Woodhull and Shapiro[63]).

potentials. This restriction holds for any titratable molecule that is not fixed to the membrane.

3. Calcium does not interfere directly with local anesthetic activity.

One frequently mentioned mechanism for anesthetic block is the displacement of Ca^{2+} from some membrane site that controls sodium permeability.[35,67,68] This mechanism is very improbable for three reasons. First, while Ca^{2+} is known to shift the voltage-dependence of sodium-permeability variables[69,70] (see fig. 7*B*, *C*), such shifts arise from alterations of surface potential through diffuse, nonspecific mechanisms.[61,63] The specific binding of Ca^{2+} to sodium channels has been ruled out as a regulating mechanism.[69,71] Local anesthetics are quite different from Ca^{2+} in their effect upon nerve membranes; local anesthetics depress sodium permeability but have only a marginal effect on the voltage-dependence of sodium permeability.[10,11,23,72] The sodium-inactivation modality is shifted significantly by some local anesthetics, but this shift is produced by drug molecules inside the axon. These molecules need not be charged (Section II.4.c), so the mechanism must differ from that of Ca^{2+}. While there is some evidence that increased internal Ca^{2+} may enhance potassium permeability,[73,74] no such effect on sodium permeability has been observed.

Second, the block by local anesthetics can be enhanced by stimulating nerve fibers.[25] However, inhibition by external Ca^{2+} does not behave this way. If anything, depolarization of the axon would relieve a calcium block of sodium channels.[71]

The most critical evidence against the Ca^{2+} displacement mechanism is that varying the concentration of Ca^{2+} bathing a myelinated nerve fiber or squid axon has no effect on the potencies of applied local anesthetics.[20,22,72] Whether the fiber is blocked by external procaine[20,22] or lidocaine,[22] the relative block does not vary with Ca^{2+} concentration. While these results differ from earlier findings in a study of lobster axons,[67,68] the discrepancy may arise from morphologic differences. Lobster axons are tightly bounded by a glial sheath, and the access route of local anesthetics must be *through* the glial cells. Calcium might conceivably hinder the passage of local anesthetics across glial membranes, since it is known that Ca^{2+} and anesthetics bind antagonistically to some membrane sites.[56,75,76] In a myelinated nerve fiber, however, the diffusion barriers to the nodal membrane are insignificant for small molecules, so an analogous interaction between Ca^{2+} and local anesthetics would not exist.

Note added in proof: The preceding evidence (II.3.) has been cited to support the argument that there is no direct interaction between Ca^{2+} ions and local anesthetics. However, there is evidence for an indirect antagonism between Ca^{2+} and local anesthetics in nerve membranes. Local anesthetics alter the voltage-dependence of the sodium inactivation function (see section II.4.c.); in the presence of externally applied anesthetics larger hyperpolarizations are required to "remove inactivation." Both the normally-present, fast inactivation,[8] and, particularly, the slow inactivation induced by local anesthetics can be removed by hyperpolarizing the membrane potential by 30–40 mV for a few minutes (ref. 23 and B. Hille, personal communication). Another way of changing the voltage-dependence of sodium inactivation is to increase the external Ca^{2+} concentration; elevated Ca^{2+} changes the inactivation functions in the same way as hyperpolarizing the membrane. Thus, certain local anesthetics (*e.g.*, procaine, lidocaine) produce a decrease of P_{Na} (slow inactivation) that can be removed either by holding membrane potentials at very negative values (−110 mV) or by raising the external Ca^{2+} concentration (from 2 to 20 mM). Under these conditions it appears that external Ca^{2+} ions antagonize the blocking action of local anesthetics, but the apparent antagonism may occur because the two agents have opposite effects on sodium inactivation and still need not interact directly with each other. (The reason this was not seen by investigators in refs. 20, 22, and 72 is that either they did not induce slow inactivation by long depolarizations, or they were already holding the membrane potential at ~ −100 mV and removing any slow inactivation.)

4. The specific-receptor hypothesis

The third hypothesis proposes that local anesthetics act by complexing with specific receptors in the nerve membrane. The action of the drug is direct, not mediated by some change in general membrane properties. The idea of specific receptors was developed to explain the effects of intra-axonal quaternary lidocaine derivatives upon sodium per-

meability,[25] and it has remained consonant with observations on several tertiary local anesthetics. The hypothesis stems from the following observations.

a. Quaternary local anesthetic derivatives interact directly with sodium channels

When quaternary lidocaine derivatives (QX) are present inside an axon, the extent to which action potentials are inhibited is a function of the frequency of stimulation.[25,77] (Recall that QX compounds are inactive when outside the nerve membrane.) The faster the rate of stimulation, the greater the inhibition. This phenomenon is called "use-dependent inhibition" and has been observed for a variety of local anesthetics and antiarrhythmic agents, *e.g.*, procaine on nerves,[78–80] procaine and procainamide on atrial muscle,[81] and lidocaine on single nerve fibers[77,82] and isolated papillary muscles.[83]

The mechanism of use-dependent inhibition was clarified by voltage-clamp experiments. A brief description of the response of normal sodium permeability under voltage-clamp will facilitate an understanding of the results of the QX study.

There are two aspects to sodium permeability, and both can be directly controlled by the voltage-clamp. These aspects are illustrated in figures 3 and 7A. When the membrane is subjected to depolarizing "test" pulses, sodium permeability undergoes a transient increase ("activation"). Test pulses of increasing depolarization amplitude produce increasing permeability changes, up to some limiting value (fig. 7*B*). In other words, the number of sodium channels that open depends on the size of the membrane depolarization, but there are a finite number of channels and above some membrane potential all of them will have opened. If the test pulse is preceded by a long hyperpolarizing "prepulse" then the subsequent permeability change during depolarization will be larger. But if the prepulse is depolarizing, the permeability during the test pulse will be smaller (fig. 7*C*). The prepulse, therefore, determines the extent to which sodium permeability can be activated. The recruitment of more sodium channels by a hyperpolarizing prepulse is called the "removal of inactivation" (see Section I.4). These two aspects, removal of inactivation by prepulses and activation by test pulses, must be maximized for all the sodium channels to be opened.

A "use-dependent" type of inhibition is observed in voltage-clamp experiments on nerve fibers with QX inside. The QX inhibition is enhanced when the nerve is depolarized by pulses that would normally open the sodium channels; the block is minimal when the nerve is at rest and grows as the nerve is stimulated with a train of brief depolarizing pulses, finally reaching a new steady-state inhibition. When such depolarizing pulses are preceded by hyperpolarizing prepulses the *rate* at which inhibition grows is enhanced.[25] In normal, drug-free nerve fibers, this is a pattern of stimulation that both increases the activation of sodium permeability and removes sodium inactivation. The onset rate of the use-dependent block and the removal of sodium inactivation depend upon the prepulse voltage in almost exactly the same way (fig. 7*C*), *i.e.*, the block by internal QX both becomes larger and increases at a faster rate as more sodium channels are opened. This suggests that the sodium channels themselves are directly involved in this type of anesthetic block.

The use-dependent QX block will persist for minutes if the nerve is returned to rest and the sodium channels remain closed. But the block is reversed faster by brief, small depolarizing pulses that open the channels. The reversal rate is proportional to the frequency of application of the pulses, that is, to the fractional time that the sodium channels are open. It also depends on the prepulse voltage, as did the onset rate of the block, although the relationship between reversal rate and prepulse voltage is not the same as the relationship between normal sodium inactivation and prepulse voltage. Specifically, larger-than-normal hyperpolarizing prepulses are necessary to remove sodium inactivation in QX-blocked channels. In other words, the work necessary to make sodium channels available for activation increases when the channels are blocked by quaternary local anesthetics.

All these results strongly support the idea that anesthetic molecules combine selectively

with "open" sodium channels, and that channels bound by drug molecules can close and prevent the drugs from dissociating. The inactivation mechanism of sodium channels appears to be explicitly involved in QX binding. When channels are inactivated QX does not bind to them and, reciprocally, the inactivation of QX-blocked channels is harder to remove.[25,84]

b. Tertiary amine local anesthetics block like their quaternary derivatives

To what extent does the normal action of tertiary amine local anesthetics resemble that of their quaternary derivatives? They resemble each other in several aspects. One already mentioned is that conditions of *p*H that favor the presence of the protonated, cationic form of tertiary anesthetic inside nerves enhance anesthetic potency. However, this result might be merely fortuitous, and it would be particularly gratifying if some similar receptor specificity could be shown for tertiary local anesthetics and their quaternary derivatives. This has, in fact, been shown using the tertiary amine anesthetics RAC 109 I and II (fig. 4). Structurally, they differ only in being enantiomers, but their anesthetic potencies differ by a factor of 2 or more.[85] The two enantiomers are taken up equally by nerves, and their partition coefficients between solvents are identical, so the potency difference must result from differential binding to the receptors.[86]

The potencies of RAC 109 I and II on voltage-clamped, single myelinated nerve fibers have been compared with the potencies of the respective quaternary derivatives, RAC 421 I and II, applied inside the axons.[21] In both cases, with tertiary compounds outside or quaternary compounds inside, enantiomer I blocked more than enantiomer II, even when enantiomer II was applied at twice the concentration. Similar results held when RAC 109 I and its quaternary derivative, RAC 421 I, were compared with lidocaine and its quaternary derivative, QX-314; RAC 109 I was several times more potent than lidocaine, at *p*H 8.3, and RAC 421 I was severalfold more potent than QX-314.[21] Hence, the receptors have similar stereospecificities.

c. Sodium inactivation is modified by local anesthetics

A second similarity between tertiary and quaternary compounds is that the inhibitions produced by both are dependent on stimulation frequency. Use-dependent inhibition induced by a quaternary derivative of lidocaine (QX-314) was described above. The same effect appears with internal RAC–421 and with a slightly lipid-soluble quaternary compound, QX-572, applied internally or externally.[21] Several minutes elapse before external QX-572 produces block, probably because it must traverse the membrane to reach its site of action.[87–89] Tertiary amine local anesthetics also produce use-dependent block. An experimental lidocaine derivative, GEA-968, showed dramatic frequency dependence even when voltage-clamp pulses were applied at a frequency of 1/sec.[82] The blockages produced by lidocaine, procaine and procainamide are also enhanced by depolarizing pulses.[21,77] Higher stimulation frequencies are necessary to develop use-dependence with these anesthetics, probably because their "affinities" for open sodium channels are lower than the affinities of QX compounds and of GEA 968. Thus, many tertiary local anesthetics manifest the use-dependent behavior seen with internal quaternary anesthetics.

The sodium-inactivation gate is affected by tertiary as well as quaternary compounds. The voltage-dependence of inactivation is shifted similarly by both types of compounds; larger hyperpolarizations are necessary to remove inactivation in anesthetized nerve fibers (fig. 7*C*).[25,82,84] Weidmann has observed similar results on mammalian Purkinje fibers treated with cocaine, and was the first to attribute shifts of inactivation to the influence of local anesthetics;[90] Aceves and Machne found that the action potentials of frog spinal ganglion cells treated with procaine are affected by hyperpolarizing prepulses in a manner consistent with this inactivation shift.[91] Furthermore, after the inhibition of sodium currents in tertiary drug (GEA 968)-treated fibers is increased by a train of brief depolarizing pulses the inactivation functions are shifted even further. Again, as with quaternary drugs,

the onset of use-dependent block is enhanced by the removal of sodium inactivation. Last, with both external GEA 968 and internal QX-314, the times needed for a hyperpolarizing prepulse to remove inactivation are lengthened.[25,77]

Despite the strong parallels between the effects of tertiary and quaternary anesthetics, there is a signal difference. Recent experiments by Khodorov and colleagues on frog node of Ranvier have revealed another aspect of anesthetic block called "slow inactivation."[23] Slow inactivation refers to a decrease in sodium permeability brought about by long (tens to hundreds of milliseconds) depolarizations in the presence of externally applied tertiary-amine anesthetics. The phenomenon is not observed in the presence of internally applied tertiary or quaternary molecules, or benzocaine. From studies of the kinetics of slow inactivation and its dependence on membrane potential, Khodorov *et al.* conclude that it results from the binding of anesthetic molecules to *inactivated* sodium channels. To explain the several different effects of local anesthetics and derivatives inside and outside nerve membranes, they propose a variety of anesthetic receptors on the macromolecular complex of the sodium channel. The extent to which slow inactivation actually reduces P_{Na} depends on the resting membrane potential, the pulse frequency, and the pulse duration; therefore, its contribution to conduction block cannot be casually assigned. What is clear from the work of Khodorov *et al.* is that certain receptors for anesthetics are accessible only from the external membrane surface and their occupancy produces effects different from those of the occupancy of "internal" receptors.

In summary, there is good evidence that both tertiary and quaternary local anesthetics act by binding to sodium channels. Under some conditions (internal quaternary, tertiary drugs), the channels must be "opened" in the normal, ion-conducting sense for the drugs to bind, and the binding is weakly stereospecific; in particular, the inactivation of the channels must be removed before the appearance of a use-dependent anesthetic block. Under other conditions (external tertiary amines) channels must be inactivated by long-lasting depolarizations to produce slow inactivation. In either case, once the channels are blocked the normal inactivation response becomes significantly constrained.

The interaction between open sodium channels and local anesthetic molecules may occur with neutral as well as charged species. Preliminary studies with benzocaine reveal that this neutral anesthetic probably blocks by a similar mechanism. During benzocaine block the inactivation modality is shifted along the voltage axis in the hyperpolarizing direction by tens of millivolts (B. Hille, personal communication).

d. The nature of the anesthetic binding site

Initial studies of the effects of internally applied QX-314 suggested that the drug molecules were bound in the aqueous pores of the channels at some point about halfway through the membrane.[25,34] This reasoning followed the observation that the block by QX-314 continued to be enhanced by depolarizations of increasing amplitude, even at voltages above those that normally opened all the sodium channels. Apparently the actual binding reaction of drugs with fully opened sodium channels was voltage-dependent. The simplest model for a voltage-dependent binding reaction envisaged the cationic QX molecules being "electrophoresed" down the sodium channel under the influence of membrane depolarizations (see refs. 7,71). These observations on QX-314 have now been extended to the tertiary local anesthetics by Courtney,[82] who has reached similar conclusions based on his thorough study of GEA-968.

It appears that the sodium channel may have one internal binding site for different local anesthetics, and the results of a few experiments reported here suggest some properties of that site. First, hydrophobic interactions appear to account for a significant part of the binding energy. Not only are the most lipophilic tertiary drugs the most potent, but these parallel properties hold also for the internal quaternary compounds (table 1). Since the internal quaternary compounds do not cross the membrane to produce block, their potency differences cannot arise from differences in drug permeability through the membrane.

The weak stereospecificity of the drug receptor discriminates among local anesthetic enantiomers around an asymmetric carbon located in the bulky, lipophilic regions of the anesthetics (fig. 4). This portion of the drug molecule is not indiscriminately "dissolved" in the hydrocarbon interior of the membrane, but neither is it enveloped by some rigid, tightly-fitting receptor. If it were, the stereospecificity would be more pronounced.

The tertiary or quaternary amine group has always seemed important for anesthetic function.[57,92] Yet two observations question that assumption: 1) benzocaine lacks the terminal alkyl amine, yet is a potent anesthetic, acting quite similarly to amine-containing molecules; 2) quaternary ammonium compounds containing tri-ethyl ammonium groups identical to those in quaternary lidocaine and a lipophilic "tail" do not block sodium channels (at 20 times the concentration of internal QX).[93,94] These quaternary ammonium compounds lack the ester or amide linkage common to the local anesthetics and their derivatives. Evidently these linkages are important for local anesthetic action. The polar nature of the oxygen- and/or nitrogen-containing bonds may be important, since procaine derivatives containing the more electronegative *Se* or *S* atoms in place of oxygen at the ester bond are more potent than procaine itself.[95] And GEA 968, which is derived from lidocaine by adding another amide bond in the alkyl chain to the terminal amino group (fig. 4), is more potent than lidocaine, and appears to bind more tightly to the sodium channel, since it produces a use-dependent block at relatively low action-potential frequencies.[82] GEA is a larger molecule, and larger, bulkier anesthetic molecules tend to be more potent (table 1); such molecules are almost always more lipophilic, so the effect of size alone is not clear. However, it does not appear that the anesthetics must fit tightly within a site to block sodium permeability.

We must be cautious in interpreting these results as a literal picture of a local anesthetic binding site or of a sodium channel. In particular, mechanical analogies should be viewed suspiciously. At a molecular level, drugs may exert their effects on membrane components through the electrostatic forces, at distances of as much as 50 Å as well as by van der Waals occupancy at 0.5-Å separations. Moreover, the physical properties that determine clinical anesthetic potency may guide the drug molecules to their site of action without being necessary for binding at the site. The structural requirements for anesthetic function should become clearer as the techniques of voltage-clamping and internal perfusion of drugs are applied systematically to a variety of anesthetic molecules.

III. Differential anesthetic block of large and small nerve fibers

One of the oldest observations about local anesthetic block is that sensation is lost before motor function.[96] There are two pertinent questions concerning such a differential block: is the selective loss of sensation a result of the *rate* at which sensory fibers are blocked compared with motor fibers, or is there a differential sensitivity at the *steady state*, so that different concentrations of anesthetic produce an *absolute* differential block? Certainly nerve fibers of smaller diameter are blocked at lower concentrations of procaine and cocaine than fibers of larger diameter.[97–99] Thus, large, myelinated Aα fibers continue to conduct impulses after the smaller Aγ and Aδ fibers have been completely blocked.[98,99] The blockade of conduction in nonmyelinated C-fibers shows an anesthetic concentration dependence like that of Aδ fibers,[100] but some C-fibers often conduct impulses, although somewhat slowed, after all the Aδ and even some of the Aγ fibers have been blocked.[99,100] Reports that small fibers were blocked more rapidly than large fibers by supra-critical anesthetic concentrations have not been substantiated in studies of individual nerve fibers.[100]

Of the several explanations for differential block, the one proposed by Franz and Perry[100] seems most tenable. These authors examined the extent of differential block by monitoring single nerve fibers contained in a nerve that was exposed to anesthetic over different lengths. When the length of the bundle exposed to anesthetic solutions was 4 mm or more, all fibers were blocked by 0.2 per cent procaine, but when the exposed length was

only 2 mm, Aδ fibers could be blocked completely with no block of Aα fibers. Based on these results, Franz and Perry proposed that differential block results from the variation in *critical lengths* of fibers of different diameters that must be exposed to anesthetic. Accordingly, differential block is a manifestation of the electrical differences among the nerves arising from their geometric differences.

Consider, for example, a large-diameter myelinated nerve fiber and a small-diameter myelinated nerve fiber. In myelinated nerve fibers action potentials are propagated from node to node by a flow of current down the axoplasm of the internodal region; "active," net inward current is not generated at places other than the nodes.[78,101] The extent of depolarization that occurs at a resting node due to an action potential at an adjacent node depends on the amount of current flowing from the adjacent node. Usually the current is sufficient to depolarize the resting nodal membrane far above its threshold potential and the generation of the action potential in the adjacent node is ensured. One or even two nodes along the fiber can be blocked, and sufficient current will reach the third node to cause depolarization to threshold, but if three nodes in a row are blocked, then propagation of the impulse will fail.[102] Since the numbers of nodes along a given length of nerve vary inversely with diameter, smaller-diameter myelinated fibers will have more nodes exposed along a uniformly anesthetized nerve tract than will larger-diameter fibers. Thus, for some *critical length* the small-diameter fibers can be blocked absolutely while the large-diameter fibers still conduct. For example, within the 2 mm of nerve tract exposed to anesthetic in the study of Franz and Perry, most of the delta fibers (0.3–0.7 mm internodal length) will have at least three nodes blocked, but few of the alpha fibers (0.8–1.4 mm internodal length) will have more than one node blocked.[100] The critical-length hypothesis also explains the differential *rate* of blocking that has often been reported. As the anesthetic diffuses through the nerve bundle it first reaches a blocking concentration over a length sufficient to block small axons before spreading over lengths sufficient to block large axons also.

Differential anesthetic susceptibility between myelinated and nonmyelinated axons requires a more detailed explanation. In principle, the critical-length argument of Franz and Perry is also applicable to nonmyelinated fibers, because the length that must be exposed to anesthetic to cause conduction failure will also be shorter for smaller-diameter nonmyelinated fibers. However, it is very difficult to make predictions of the relative conduction-blocking potencies of anesthetics on myelinated vs. non-myelinated axons since such predictions require a knowledge of the specific membrane properties, such as the sodium and potassium currents flowing during small depolarizations and the resting membrane resistances. These data are not currently available.[103]

Differential block of sensory over motor function is a subjective experience as well as an experimental observation. The selective loss of sensation may depend on more factors than just fiber diameter. Although sensory impulses are transmitted via nerve fibers that are, on the average, smaller than motor nerve fibers, they also are often transmitted as bursts or trains of impulses. Under clinical conditions, where the patient is often lying down, the relative activity in motor fibers may be abnormally low. It was previously reported that anesthetic effectiveness increases at higher stimulation frequencies, so we would expect sensory signals to be preferentially blocked, especially during transition into the anesthetized state.[96,100] In fact, transition (Wedenski) block is characterized by a selective failure of high-frequency impulse transmission and, furthermore, this impairment appears to be more severe in small axons than in large axons.[100]

Summary

Local anesthetics block nerve conduction by preventing the increase in membrane permeability to sodium ions that normally leads to a nerve impulse. Among anesthetics containing tertiary amine groups, the cationic, protonated form appears to be more active than the neutral form. However, the neutral forms, as well as uncharged molecules like benzocaine and the aliphatic alcohols, also depress sodium permeability.

Studies of single myelinated nerves and squid axons show no direct interaction between calcium ions and local anesthetics, thus disproving theories based on competition between these two agents. Likewise, hypotheses attributing local anesthesia to changes in electrical potentials at the membrane–water interface are disproven by the demonstrated potencies of electrically uncharged anesthetics. Hypotheses that propose that local anesthetics act by expanding the nerve membrane and causing a change in protein conformation that blocks sodium permeability are vague in conception and difficult to test experimentally.

Evidence from voltage-clamp studies of single nerve fibers indicates that anesthetic molecules interact with the sodium channels directly, from the inner side of the nerve membrane. Anesthetics bind within sodium channels which have opened during membrane depolarization, preventing the normal sodium ion flux. Anesthetic molecules can dissociate from open channels, but not from channels that remain closed when the nerve is kept at rest. The "gating" properties that regulate the opening and closing of sodium channels are reversibly modified during anesthesia. Specifically, the *inactivation* function responds more slowly and requires more negative membrane potential changes to reach the same values as in unanesthetized nerves. A second, slow inactivation is observed following external application of tertiary amine anesthetics.

The selective binding of anesthetics to open sodium channels provides a simple explanation for Wedenski inhibition, in which the block increases with the frequency of nerve impulses. When impulses occur at higher frequencies more sodium channels are open over a period of time comparable to the time necessary for the anesthetic binding reaction, thus more channels are blocked. In addition, the changes of the inactivation function result in a longer refractory period and, thus, a decrease of impulse height at higher frequencies.

Charged anesthetic molecules may bind in the pore of the sodium channel. Their binding can be modulated by the electrical field in the membrane. The channel has a higher affinity for larger anesthetic molecules, but this may result from their greater hydrophobicity as well as from their size. The binding site favors molecules that contain more polar linkages between the amine group and the aromatic residue. Binding of amine anesthetics is weakly stereospecific and, surprisingly, shows no absolute requirement for the terminal alkyl ammonium moiety present in most local anesthetics.

The greater anesthetic sensitivity of smaller-diameter nerve fibers probably arises from the differences in critical lengths of axons that must be exposed to anesthetics in order to block impulse propagation. Smaller-diameter fibers have shorter length constants, whether they are myelinated or nonmyelinated, so that the potential excitability decreases proportionately more over the same anesthetized length of a small axon than a large one. To some extent the selective block of sensory impulses may be accounted for by the facts that sensory information is often transmitted as bursts or trains of high-frequency impulses and that anesthetic effectiveness often increases demonstrably with increasing impulse frequency.

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