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Molecular Mechanisms of Local Anesthesia: A Review

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CONTENTS

LOCAL ANESTHETICS (LAs) are a class of similar chemicals that reversibly block peripheral and central nerve pathways following regional administration. Despite the continuous clinical use of local anesthesia since the phenomenon was first described by Koller and Freud[1] more than a century ago, an electrophysiologic basis for the action of LAs on nerve has been established only within the past 20 yr.

Since the last review of this subject appeared in ANESTHESIOLOGY in 1976,[2] our understanding of LA mechanisms has been refined and extended, principally through measurements of Na^+ channel "gating" currents, recordings of current passing through single Na^+ channels, and through clarification of the competitive antagonism between LAs and the "activator" class of drugs. In this review we first discuss the electrophysiology and biochemistry of the Na^+ channel, then highlight recent discoveries that have refined our understanding of LA mechanisms. Four key questions address the electrophysiology and chemistry of local anesthesia: 1) Which microscopic events regulate the ion permeability changes that underly the nerve impulse? 2) What is the structure of the Na^+ channel? 3) Where and how do LA bind to the Na^+ channel? 4) What are the fundamental microphysiological actions of LAs? Finally, we speculate whether inhibition of Na^+ currents is the sole mechanism by which a LA produces spinal or epidural anesthesia.

Cellular Electrophysiology

In all excitable cells, ionic disequilibria across semipermeable membranes provide the potential energy for impulse conduction.[3] For nerve cells the most important ionic disequilibria are created and maintained by the electrogenic, energy-requiring, membrane-bound enzyme, $Na^+ - K^+$ ATPase, which pumps Na^+ ions out of the cell and K^+ ions in. The membrane potential of normal resting cells is near the K^+ equilibrium potential (−50–−90 mV with the interior of the cell negative to the exterior). This steady-state resting potential is due to the combined effects of $Na^+ - K^+$ ATPase (three Na^+ ions are extruded for every two K^+ ions that are absorbed), which results in a hyperpolarizing, outward current, plus an inward "leak"‡ current. Changes in either of these current components

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‡ "Leak" currents have no apparent voltage dependence. In contrast to the clearly voltage-dependent Na^+ or K^+ conductances of excitable cells, "leak" conductances are of a constant value at all membrane potentials but are still ion selective. Ion selectivity of "leak" conductance differs among tissues; in some it may be K^+ selective, in others, Cl^- selective.

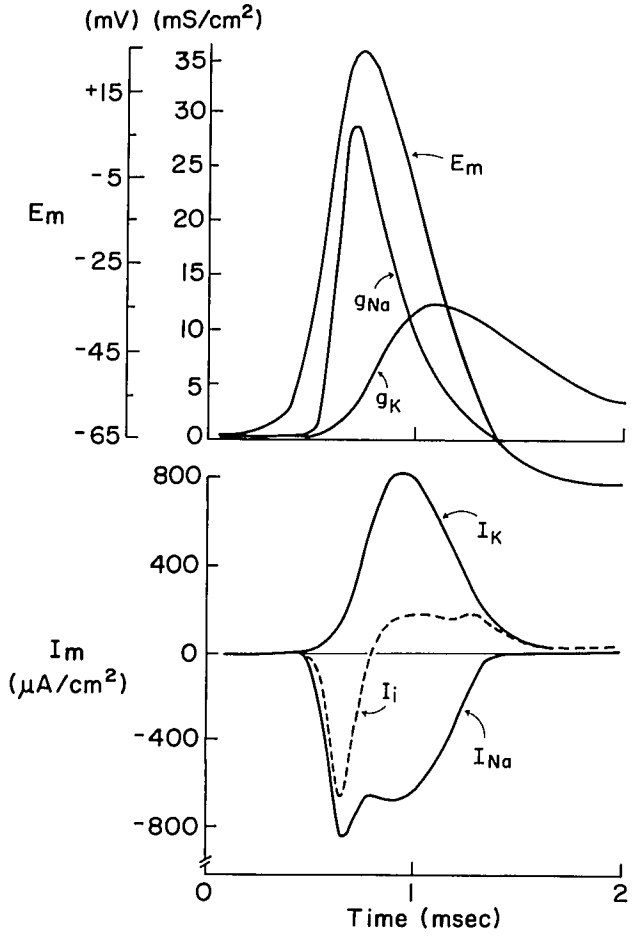


FIG. 1. The course of changes in membrane potential (E_m), membrane conductances of Na^+ (g_{Na}), and K^+ (g_K), (*top*), and the related Na^+ currents (I_{Na}), K^+ currents (I_K), and total membrane ionic current (I_i), (*bottom*), during propagation of an action potential in squid giant axon.[6,7] The early, inward I_{Na} drives the regenerative depolarizing phase of the impulse, whereas the more slowly developing I_K underlies the rapid phase of repolarization.

as well as the minimal activation of voltage-gated§ conductances will alter the resting potential.

During an action potential, voltage-gated Na^+ channels open briefly, allowing a small quantity of extracellular Na^+ ions to flow into the cell, thus depolarizing the plasma membrane.[4,¶] Sodium channels close spontaneously (inactivate), even if the depolarization could be maintained artificially, and thus the duration of the depolarizing, inward-Na^+ current is limited. A more slowly developing outward current, often of K^+ ions flowing through voltage-gated K^+ channels, helps to repolarize the membrane rapidly and restore electrical neutrality[5] (fig. 1). A local ionic current flowing through cytoplasm helps propagate the "regenerative" wave of depolarization throughout the cell's excitable membrane.

Electrophysiologic Techniques

There are a number of ways to use electrical responses of excitable cells to study LAs. We will consider four techniques: extracellular measurement of action potentials, voltage clamp, gating currents, and single-channel observations by patch clamp. The simplest technique is to measure action potentials in excised nerves using extracellular electrodes. Local anesthetics reduce the amplitude and the conduction velocity of action potentials in a reversible, concentration-dependent manner. However, due to the complicated relationship between LA binding and inhibition of nerve conduction, only limited conclusions can be drawn from studies employing extracellular electrodes.

A more complicated but more informative technique, voltage clamping of excitable membranes, has been used extensively to define the roles of Na^+ and K^+ currents in generating action potentials.[6-9] The voltage clamp can rapidly change and then maintain the membrane potential at a "command" value by supplying sufficient current from a laboratory source to offset the induced ionic currents that would otherwise change the membrane potential. The monitored "output" of the voltage clamp represents, with a change of sign, the membrane ionic currents. The voltage clamp method therefore permits quantitative analysis of specific ion conductances and of anesthetic actions.

In addition to ionic currents, which can be measured only when channels open, it is also possible to measure small, nonionic currents associated with channel gating.[10-12] Gating refers to those movements of the channel molecule that underly the transitions between conducting and nonconducting forms. Conceptually, channels are enzymes that, in their open form, catalyze the passage of ions through otherwise high-resistance membranes. A channel is open when the ion-conducting pathway presents a sufficiently low energy barrier that ions flow through the channel. Rearrangements of the "lining" of the channel pore more rigorously depict molecular events during gating than mechanical models of swinging doors or plugged tubes. Gating currents are caused by movements of electrically charged regions of the channel macromolecule. Ordinarily, the corresponding ionic currents are so large that they obscure the miniscule gating currents. But ionic Na^+ and K^+ currents may be blocked pharma-

§ Ion channels may be either voltage gated (*e.g.*, Na^+ and Ca^{2+} channels) implying that current is gated (or regulated) by membrane potential (voltage), or chemically gated (*e.g.*, acetylcholine receptors and γ-aminobutyric acid receptors) implying that current is gated primarily by binding of a chemical rather than by the membrane potential.

¶ The kinetic scheme by which Na^+ channels undergo transitions between ion-conducting states ("open") and nonconducting states (either "resting" or "inactivated") will be considered in detail in later sections.

cologically or nulled electronically without altering gating phenomena. Under these circumstances channel gating currents can be measured (fig. 2). The "on" gating current, a transient outward current appearing at the start of depolarization, disappears as the sodium conductance reaches its peak. The "off" gating current immediately follows repolarization, is inward, and continuously diminishes during persisting depolarization. Consequently, the ratio of "off" gating charge to "on" gating charge falls from 1:1, for very short depolarizations, to zero for relatively long ones. The present view is that the "activation" of the Na^+ channel, a sequence of channel transitions between the resting (nonconducting) and open (conducting) states, accounts for the "on" gating current. Open Na^+ channels may spontaneously convert to a nonconducting, nonactivatable, "inactivated" form that effectively "immobilizes" gating charge. Longer depolarizations favor inactivation, thereby reducing the "off" gating current, which represents the reversal of the activation reactions.

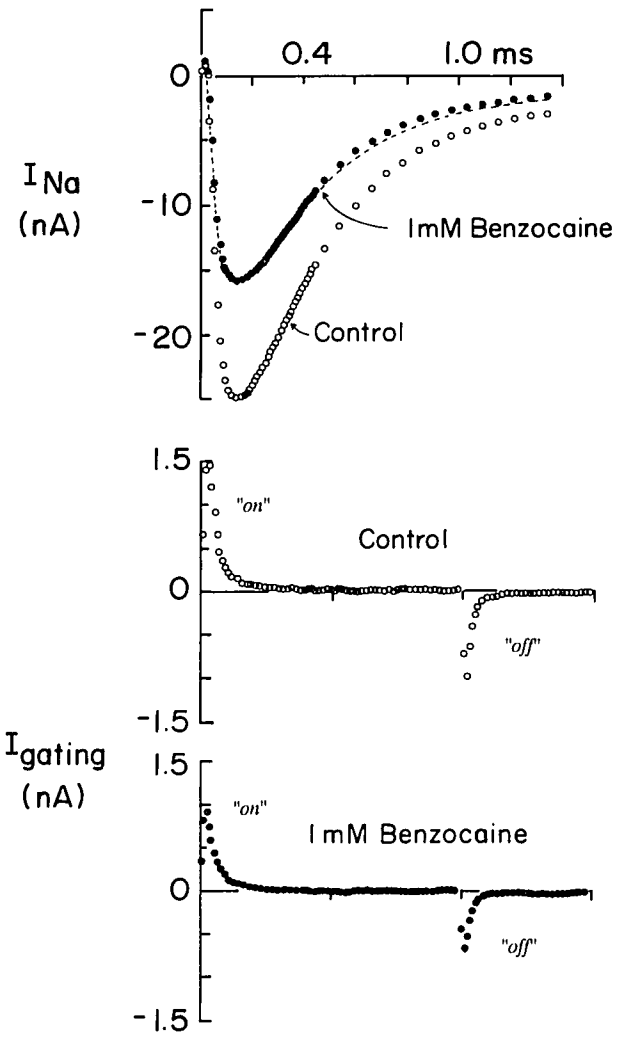


FIG. 2. Ionic Na^+ current (I_{Na}, *upper panel*) and gating current (I_{gating}, *lower panels*) in a frog node of Ranvier. The "on" gating current (upward, equivalent to "outward") is greatest during the rapid activation phase of I_{Na} and falls to zero as activation is complete. Benzocaine inhibits the ionic current with little change in kinetics, as the dashed line, a scaled-down trace of the "Control" record, indicates. The integral of the "on" gating current, equal to the total gating charge and proportional to the degree of total channel activation, is reduced proportionately to I_{Na} by benzocaine. E_m = 100 mV; T = 15° C. (Reprinted from reference 54, with permission.)

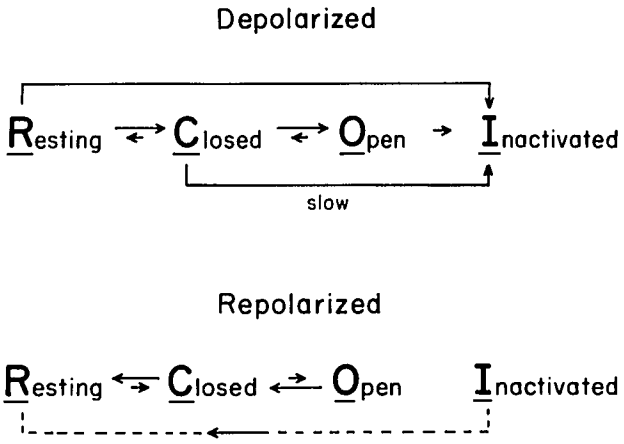


FIG. 3. A simple kinetic scheme for conformational transitions of Na^+ channels in depolarized (*top*) and repolarized membrane (*bottom*). Depolarization induces transitions from resting states (R), through a sequence of closed states (C) to an open (O) (conducting). Open channels eventually "inactivate" to the nonconducting state (I). Resting channels, or intermediate closed ones may also inactivate without opening. Subsequent repolarization reverses activation, converting open states to closed and resting forms; inactivated channels do not open directly but return instead to the resting state by an alternative, parallel pathway.

To recapitulate, there are at least two nonconducting states of the Na^+ channel: the resting form and the inactivated form. Resting channels can activate, thereby producing an "on" gating current. Inactivated channels, on the other hand, have "immobilized" gates that cannot activate without first returning to the resting conformation. A general scheme for these kinetic relationships is shown in figure 3.

Sodium channel kinetics may differ depending upon the duration of depolarization. Prolonged depolarization (lasting seconds) reveals "slow" inactivation, recovery from which requires prolonged repolarization. Conventional, "fast" inactivation follows brief periods (tens of milliseconds) of depolarization. Similarly, short repolarization reverses "fast" inactivations. First described in lobster giant axons,[13] "slow" inactivation has now been identified in vertebrate skeletal muscle[14] and myelinated nerve.[15] Some drugs may selectively alter either "fast" or "slow" inactivation.[16,17] The role of "slow" inactivation in LA action is a topic of current research and dispute.[17]

Finally, currents flowing through individual ion chan-

nels can be measured by "patch clamping," a technique consisting of voltage clamping a small patch of excitable membrane containing only one or a few ion channels.[18] Single ion channels are characterized by their conductance, their ionic selectivity, their gating behavior, and their response to pharmacologic agents.[19] An example of current flowing through single Na^+ channels in the absence of LA is shown in figure 4 A.

Currents carried through different ion-selective channels can be separated by the use of specific inhibitors (*e.g.*, tetraethylammonium [TEA] ions block many but not all types of K^+ channels,[20,21] and tetrodotoxin [TTX] blocks almost all types of Na^+ channels[22,23]). The ion selectivity of the separate channels can be tested by replacing the "normal" permeant ion by another in the solution bathing the membrane (*e.g.*, Li^+ ions replacing Na^+, or Cs^+ replacing K^+).[5,24,25] A combination of electrophysiologic, chemical, and pharmacologic methods permits the separation of individual ion currents that contribute to the nerve impulse.

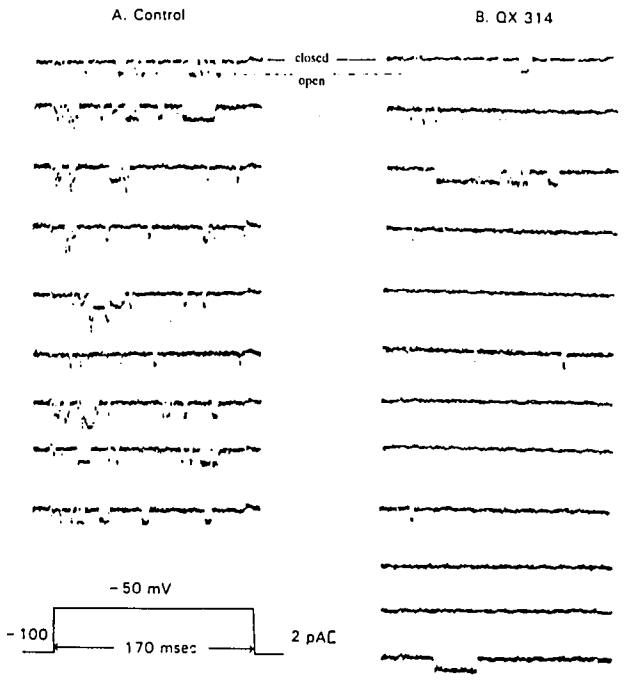


FIG. 4. Currents through single Na^+ channels (from cultured cells) recorded under patch clamp conditions in drug-free solution (A: Control) and in solution containing a quaternary LA (B: QX314). The time course of membrane potential during both experiments is indicated at the bottom of the left panel. The membrane patch, ripped away from the cell and exposed, cytoplasmic side, to the drug, was held at −100 mV resting potential, depolarized to −50 mV for 170 ms, then returned to −100 mV. At −50 mV, inactivation is not irreversible and channels continue to open and close throughout the depolarization. In the presence of QX314 individual opening events are far less frequent but the current through open channels is the same as in Control records. (Reprinted from reference 61, with permission.)

Tonic and Phasic LA Actions

Local anesthetics block impulses by inhibiting individual Na^+ channels and thereby reducing the aggregate inward sodium current[26,27] of the nerve fiber. Inhibition of other channels (*e.g.*, K^+, leak) may offset inhibition of Na^+ currents. The interplay between these competing actions determines the relative impulse-blocking potency among LAs.[28] An individual LA's repertoire of pharmacologic effects depends also on the conditions of testing (*e.g.*, temperature, *p*H, resting potential). In this review we will focus on the LA actions on Na^+ channels. To guide the reader through the many drugs discussed here, we show their structures and some physicochemical properties in table 1.

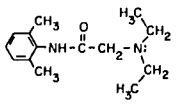
Tertiary and quaternary amine LAs, as well as neutral LA homologues, inhibit Na^+ current in two modes: tonic and phasic[29,30] (fig. 5). Tonic inhibition is measured during infrequent stimulation (<0.5 Hz in most cases, but this depends on the particular LA). Phasic inhibition results when the frequency of depolarizations is increased. (Phasic inhibition has often been termed "use-dependent" block, but we avoid that nomenclature here because of its mechanistic implications.) The distinction between tonic and phasic inhibition may arise from differences in LA binding kinetics at a single site or it may represent LA binding to separate sites, perhaps with different actions on the Na^+ channel. In the following sections we discuss the experimental evidence for several mechanisms proposed to explain LA inhibition of Na^+ currents.

Three general mechanisms of channel inhibition are schematized in figure 6. Figure 6 A shows the normal conductance pattern of a single channel that in this case, opens, closes towards rest, reopens, and finally inactivates.[31,32] In figure 6 B, "open channel block" occurs when the channel first activates, then rapidly and reversibly binds a LA molecule (L). Drug binding "blocks" the channel that may or may not continue in its kinetic transit to an inactivated, drug-bound form. The next two schemes show LAs inhibiting the activation process. In figure 6 C, binding of LAs by resting channels converts them to an inactivated form. These bound, inactivated channels must release bound LA and revert to the resting state before they can be activated again. In figure 6 D, the channel activates partially to an intermediate, nonconducting form (denoted by C) that binds LA and thus cannot activate further or conduct ionic current. The latter two mechanisms (C and D) require an abolished or abbreviated activation process and commensurately smaller gating currents.

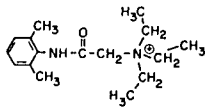
Tonic and phasic modes of inhibition are implicit in all three mechanisms. Tonic inhibition occurs in figure 6 D when LAs bind to channels that have spontaneously activated at rest; such activation is substantially diminished

TABLE 1. Properties of Local Anesthetics

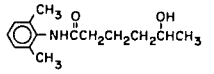
Lidocaine



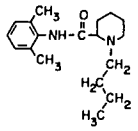
QX314



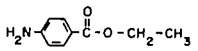
5-HHX



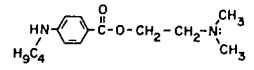
Bupivacaine



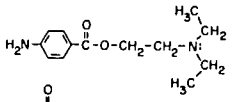
Benzocaine



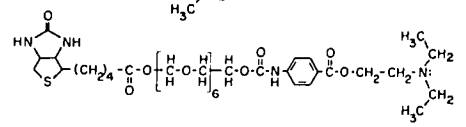
Tetracaine



Procaine



P-6-Bio



Drug	m.w.	pK$_a$ (25°/36°)*	P^0†	P^+†	$Q_{7.4}$‡
Lidocaine	234	8.19/7.77	300/370	0.060/0.085	43/110
QX314	263	U.P.	U.P.	0.034/0.053	0.034/0.053
Bupivacaine	288	8.21/8.10	2500/3400	1.5/2.0	350/560
Benzocaine	165	U.P.	78/130	U.P.	78/130
Tetracaine	264	8.59/8.38	3600/5800	0.46/0.79	221/541
Procaine	236	9.06/8.89	80/100	0.002/0.004	1.7/3.1
P-6-Bio§	800	N.M.	N.M.	N.M.	N.M.

U.P. = Undefined parameter.

N.M. = Parameter not measured.

* Measured spectrophotometrically in the same aqueous solution used for the partition coefficients: 150 mM NaCl and 5 mM each of morpholino ethane sulfonic acid (MES); morpholinopropane sulfonic acid (MOPS); and cyclohexylaminosulfonic acid (CHES).

† Partition coefficients for neutral (P^0) and protonated (P^+) species between octanol and the aqueous medium described in * (above). Values are expressed as the ratio of concentrations (mol/l) in the two solvents and listed for equilibration at both 25° C (left of /) and 36° C (right of /).

‡ Relative concentrations of total drug in octanol compared with aqueous medium at pH 7.4; values for 25° C and 36° C as described in † (above).

§ Procaine conjugated to biotin by the linear hexamer of ethylene glycol. The arrow shows the depth to which this molecule would be buried in the biotin binding cleft of avidin, a large protein that binds biotin with $K_a \sim 10^{15}$ M^{-1}.

All data from Sanchez V, Arthur GR, Strichartz GR: unpublished results. See reference 129 for detailed methodology.

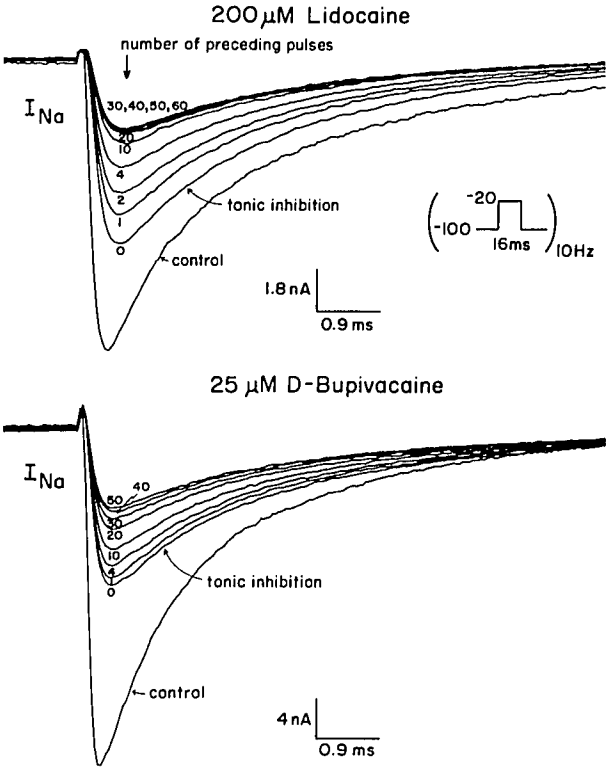


FIG. 5. Tonic- and phasic-block of Na^+ currents in two nodes of Ranvier. The time-course of I_{Na} during depolarizing pulses applied at 10 Hz is shown for two nodes exposed to lidocaine (*top*) or bupivacaine (*bottom*). Separate traces show I_{Na} before the drug (control), for the first depolarization (labelled "O") in a train applied 5 min after LA application (tonic block), and for subsequent depolarizations as noted in the figure (phasic block). Although the same pulse frequency was used and the same phasic inhibition (75%) was achieved at steady-state for both drugs, the development of the block with bupivacaine is about four times slower than with lidocaine; pH = 7.3; T = 13° C. (Reprinted from Chernoff DM: Kinetics of local anesthetic binding to sodium channels: Role of pK_a. Ph.D. Dissertation, Mass. Inst. Technology, 1988. Used with permission.)

by membrane hyperpolarization, which is known to reduce tonic LA inhibition. Tonic inhibition in other schemes results from LA binding to nonactivated, closed channels (denoted by R or I in fig. 6).

Phasic inhibition may be accomplished by selective binding of LA to either activated (but not yet conducting), open (conducting), or inactivated (nonconducting) states of the channel depending on the particular scheme. Selective binding of LAs to a particular channel conformation may occur for either of two reasons. The first is that the true binding affinity of a LA molecule could vary among the different channel states. This "modulated receptor" hypothesis presumes that the LA binding site is altered during the channel's conformational transitions, resulting in tighter binding to open and especially to inactivated states.[33,34] The second hypothesis proposes that

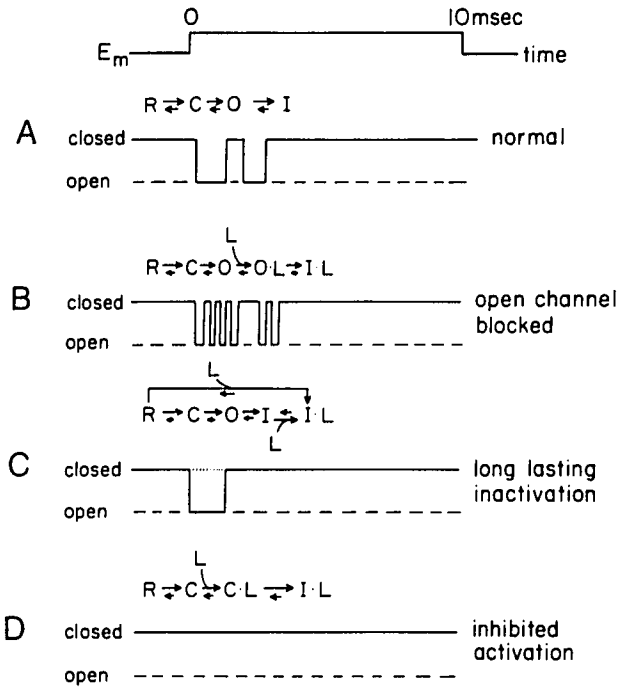


FIG. 6. A schematic diagram showing the binding schemes for different models of LA block and the resulting idealized patterns expected for single channel behavior. R, C, O, and I denote resting, closed, open, and inactivated channels, respectively. L represents one LA molecule. (*A*) Normal channels may open and close (deactivate) several times before converting almost irreversibly to the inactivated state. (*B*) Anesthetic may bind to the open state forming a complex (O · L) that will reversibly dissociate to give the "flickering block" behavior shown and may also accelerate inactivation. As an alternative (not shown), open channels bound by LA may actually inactivate more slowly producing periods of "flicker" longer than the normal open times of control channels. (*C*) Resting and Inactivated channels may selectively bind LAs thereby limiting the open period to a single event and promoting a long-lasting form of inactivation (I · L). (*D*) LA may bind to intermediately activated closed channels to subvert the activation process, reduce the gating current, and reduce the number of openings that occur (inhibited activation).

the binding affinity of the site for the LA is unchanged but that the site is a "guarded receptor," access to which depends on the channel's conformation.[35,36] The distinction between these hypotheses is difficult to resolve experimentally. Both hypotheses require that an increasing fraction of the total channels become drug bound (and thus nonconducting) with repeated depolarizations to produce phasic block.

Local anesthetics modify channel gating, producing an increase in the probability of channel inactivation.[30,33,34] This could result if LAs promote a different LA-specific channel form, the kinetics of which resemble inactivated channels, as well as through LA binding to normal inactivated channels *per se*.[37] Indeed, LAs produce tonic and phasic effects on chemically modified Na^+ channels that lack normal inactivation.[38–42] Such chemically oxidized channels are slightly less susceptible to LAs than unmodified channels,[39,42] but direct chemical modification of a LA binding site may contribute to this change in potency.

Before we discuss the experimental evidence relevant to the microphysiologic actions of LAs, several terms need explicit characterization. The first is inhibition. A broad variety of agents (opiate analgesics,[43] barbiturates,[44] alcohols,[45] inhalational anesthetics,[46] nonpeptide neurotoxins,[47] as well as traditional LAs) inhibit normal channel operation in ways that resemble LAs. Among these chemical classes and even among different LAs, several different mechanisms of inhibition may exist. Therefore, only the most general models of channel inhibition may be universally valid.

The second caveat concerns tonic and phasic inhibition as previously noted: these two phenomena may arise from one or several binding reactions, depending on the particular drug under study. For example, some LAs produce little tonic and much phasic block; others act oppositely. For some LAs, full phasic block develops during bursts of very brief depolarizations (0.1 ms), too short to open more than a small fraction of all Na^+ channels; for others, phasic block continually increases with depolarizations lasting 50 ms and longer.[39,48] The overall kinetics of inhibition are therefore a product of both channel kinetics and "state-dependent" rate constants (*i.e.*, rate constants that depend upon the form of the Na^+ channel).

For many readers, the term "use-dependent block" suggests inhibition of open Na^+ channels or their subsequently inactivated conformations. Because we now know that this form of inhibition often requires neither open nor inactivated channels, we prefer the broader term "phasic block" to provide a fresher, less mechanistic name from which to describe possible mechanisms.

We are also concerned that results observed in one tissue may be erroneously assumed to occur in another. Almost all of the results reported in this review derive from experiments on nerve and skeletal muscle. Current evidence suggests that Na^+ channels in cardiac tissue have a distinctly different LA pharmacology, both with respect to state-dependent kinetics and the location of binding sites.[49,50] Therefore, direct extrapolations from actions on neuronal channels to the antiarrhythmic effects of LAs should be avoided.[51]

Finally, we worry that false conclusions may be drawn from words and images such as the verb "to block." For many readers, a drug "blocks" a channel as a cork stoppers a bottle. This image incorrectly depicts LA inhibition of Na^+ channels as static and uniform. At the molecular level, small ligands, as well as the more mobile regions of macromolecules, continuously and randomly move about as a consequence of thermal energy. Local anesthetics may not move directly to one site, occupy it, and then unidi-

rectionally depart. Furthermore, even when our intuitions can perceive kinetics at the molecular scale, a model for "open-channel block" does not require that a LA molecule enter and occlude a channel's pore, but only that the "open" (conducting) conformation be more susceptible than other conformations to LA binding and inhibition by whatever process.

Microphysiologic Analysis of Local Anesthetic Action

Through what microscopic mechanism are channels inhibited by LAs? There appear to be three distinctly different mechanisms: 1) LAs might lower the fraction of activatable channels, *e.g.*, by increasing the fraction of "inactivated" channels; 2) LAs might interfere with activation directly, inhibiting some or all of the conformational steps through which resting channels convert to an open form; or 3) LA might reduce the ionic current flowing through individual open channels. The physiologic consequence of the first two "gating" mechanisms is identical, although the kinetics might differ.[52] As a measure of the number of activatable channels, and of the extent and speed of channel activation, gating currents are an ideal assay for mechanisms that alter channel gating. The third possibility is best tested through studies of single Na^+ channels.

Gating and Gating Currents

Like ionic currents, gating currents may be inhibited by LAs in tonic and phasic modes.[53] Inhibition of gating currents parallels that of ionic currents. Comparison of the behavior of permanently charged or neutral LAs to that of ionizable LAs is particularly informative. Both a neutral LA (benzocaine)[54–56] and a permanently cationic quaternary LA (QX314)[53,57] reduce tonically the amplitude of the Na^+ channel gating current (figs. 2 and 7). Reduction of this gating current is often proportional to reduction of the ionic Na^+ current. Quaternary LAs produce an additional phasic reduction of both gating and Na^+ current[53,57] (fig. 7), while the uncharged benzocaine has almost no phasic actions on either gating or ionic currents.[53–55] Tertiary amine LAs (*e.g.*, lidocaine) reduce gating currents phasically with a potency intermediate between that of quaternary and neutral LAs.[58] Similarly, tertiary amine LAs inhibit ionic Na^+ currents phasically with less potency than their quaternary derivatives.

Some LAs appear to inhibit ionic currents exclusively by interfering with channel activation. That is, the gating current decreases with increasing LA concentration exactly as does the ionic current. However, other LAs (*e.g.*, procaine) appear to have an additional inhibitory effect, for the ionic currents are reduced proportionately more than the gating currents.[58] Some other type of channel "block" may account for this additional inhibition of ionic current (see below), and this extra action must, therefore,

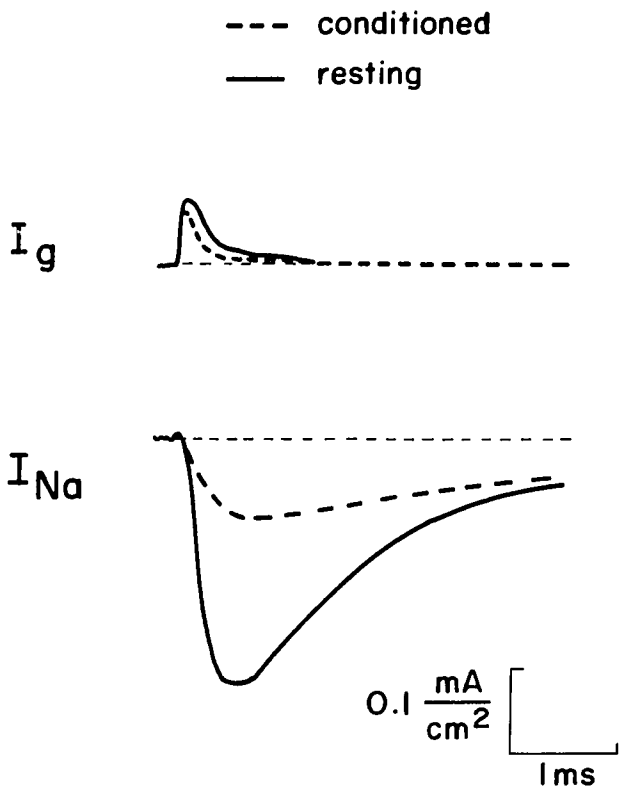


FIG. 7. Gating currents (upper traces) and ionic Na^+ currents (lower traces) in a squid giant axon perfused internally with quaternary LA (QX314, 1 mM). Tonically inhibited currents in "resting" axons (solid lines, potential held at −70 mV) are further reduced by phasic "conditioning" with a train of depolarizations (21 pulses to +70 mV) to the steady-state phasic level (dashed line). E_{test} = +55 mV for gating currents, +30 mV for ionic current. (Adapted from reference 57, used with permission.)

occur by mechanisms separate from those that inhibit channel activation.

Local anesthetic-bound channels may not undergo the full sequence of activation-related conformational changes, for LAs appear to change the time course of the gating current. Later components of gating current are reduced more than earlier ones[57] (fig. 7). This alteration in kinetics could result if intermediates in the activation sequence were selectively removed, retaining normal initial transitions but eliminating the later ones.[59] Studies of phasic block of Na^+ currents following brief depolarizations support this explanation. For several LAs, depolarizations far too short to open all Na^+ channels nevertheless produce maximal phasic block, as if activated, but nonconducting channel conformations had especially high LA affinity.[60]

Open Channel Block

The available experimental evidence suggests that LAs do not inhibit current flow through open Na^+ channels.

Under patch-clamp conditions, addition of the quaternary LA QX314 on the cytoplasmic side of the membrane patch dramatically decreases the fraction of activatable channels[61] (figs. 4 B and 8). Neither QX314 nor a much larger quaternary compound (9-aminoacridine) lowers the conductance of individual Na^+ channels, although both reduce macroscopic Na^+ currents.[61] Analysis of single channel openings in the presence of QX314 shows that, while opening frequency is greatly reduced, occasional longer-lasting openings appear (fig. 8). But while the channels responsible for these longer-lasting openings appear to have inactivation processes inhibited by LAs, they show no reduction in their conductance.

The short duration of the open state of Na^+ channels hinders measurement of any open channel block, so experiments have been performed on channels with open times prolonged by the steroidal activator batrachotoxin (BTX).[62] Such experiments are usually conducted on Na^+ channels incorporated in planar lipid bilayers (artificial "black" lipid membranes, or BLMs). Such channels show voltage-dependent gating, toxin-binding, and ion-selectivity properties identical to those of BTX-activated channels studied *in situ*.[63-66] Openings and closings briefer than 10 ms cannot be distinguished in this system (a serious limitation), but some LA-mediated inhibitions are slower than this. Faster binding and unbinding of LA to an open channel would be "time averaged" and would appear as a reduced single channel conductance in these measuring systems. In fact, an apparent conductance decrease is observed when BTX-modified Na^+ channels in BLM are treated with tertiary amine LAs.[67] Single Na^+ channels incorporated in BLMs but modified by a different activator, veratridine (VTD), are also inhibited by LAs in a complex way. Bursts of channel openings induced by VTD are shortened by LAs, nonconducting periods are lengthened, but the apparent single-channel conductance is not affected.**

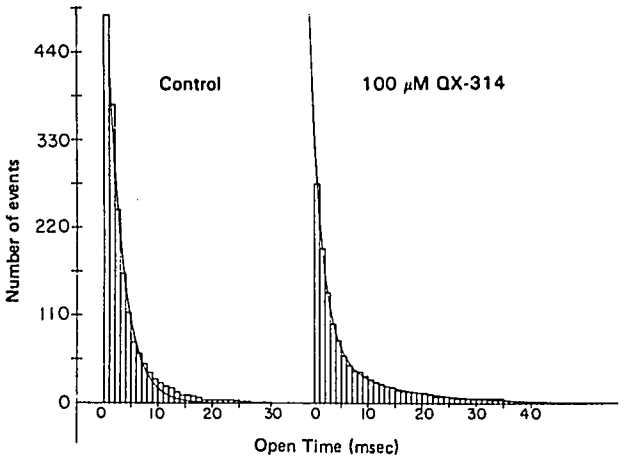


FIG. 8. Open time histogram for single Na^+ channels in control recordings and in the presence of quaternary LA. This cumulative histogram shows the number of events of duration shorter than the period shown on the abscissa. Data are from experiments like that shown in figure 4.[61] The LA reduces dramatically the total number of events but has insignificant effects on the mean open time of conducting channels.

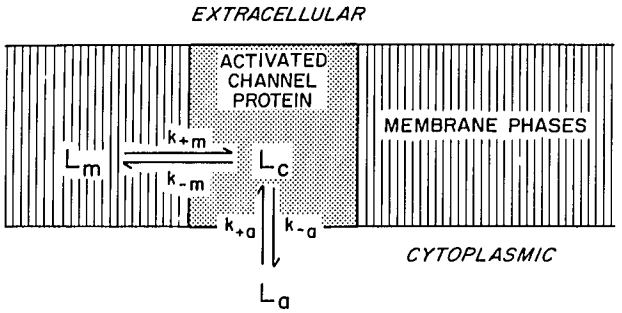


FIG. 9. A diagram schematizing the hydrophilic and hydrophobic binding reactions for a single LA binding site on the activated (depolarized) Na^+ channel. Drug in the cytoplasm (L_a) reaches the site by the "hydrophilic" route and binds with rate constant k_{+a}, dissociates with rate constant k_{-a}; drug in the membrane (at concentration $[L_m]$, equal to the product of $[L_a]$ and the membrane:buffer partition coefficient) reaches the site by a "hydrophobic" route and binds and dissociates with rate constants k_{+m} and k_{-m}, respectively. The plural designation of membrane phases emphasizes that the membrane is heterogeneous and that the interfacial region (polar, charged), the zone where fatty acids are esterified to the glycerol moiety of phospholipids (dipolar), and the acyl core (hydrocarbon, low dielectric constant) markedly differ in their physicochemical properties. The equilibrium distribution of LAs will vary within these separate regions as will their orientation and dynamic motion.

Some aspects of LA-activator interactions are presented briefly here; a more extensive description follows (see Antagonisms by Lipophilic Activators). Part of the actions of LAs on pharmacologically activated Na^+ channels may arise from changes in activator binding. Local anesthetics displace radiolabelled BTX analogs from Na^+ channels, either through direct competition or by an allosteric†† mechanism.[68,69] Activation by veratridine (as well as by other activator ligands) is competitively antagonized by LAs[70] (fig. 9).

An additional factor that complicates comparison of LA actions on BTX-activated channels with LA actions on normal unmodified Na^+ channels is the bias in data collected under conditions where the channel is almost always open. The opening process itself cannot be assayed. Local anesthetic actions on the modified activation process have not been examined in single channels even though LAs reduce gating currents of both normal and BTX-modified channels, strongly implicating inhibition of ac-

** Montal M: personal communication.

†† Allosteric competition occurs between ligands acting at two different binding sites. Ligand binding to either site will alter the binding affinity of the other ligand at its site.

tivation as a major mechanism. The LA-induced closing of activator-modified open channels, the inhibitory process best described in BLM studies, may be a secondary and unimportant phenomenon. Its relevance to normal inhibitory mechanisms in activator-free Na^+ channels remains to be demonstrated.

Before beginning a discussion of the molecular pharmacology of LA action we present a description of the biochemistry and structure of the Na^+ channel. The kinetic and molecular details of LA inhibition can then be set against this structural-chemical background.

Structure of the Na^+ Channel

Sodium channels have been isolated and purified by biochemical methods.[71-73] In these studies, the tissue to be examined is mechanically homogenized and the membranes separated and then solubilized with nonionic detergents. Density gradient centrifugation and lectin-affinity chromatography are used to isolate a channel-rich fraction from the remaining solubilized proteins. The presence of Na^+ channels is confirmed by the binding of the specific, radiolabelled ligands TTX, and an analogous toxin, saxitoxin (STX). These small cationic toxins inhibit Na^+ channels by binding at the extracellular surface, occluding the channel by a mechanism separate from that of LAs. The extent of channel purification is measured by comparing the number of toxin-binding sites per mass of protein to the same value in the starting material. Purification continues until the density of toxin-binding sites reaches a constant value.

The amino acid sequence of the Na^+ channel found in eel electroplax has been deduced from its gene sequence.[74] The channel has large hydrophobic regions, probably in α-helical conformations that span the membrane, interspersed with hydrophilic regions that presumably either "line" the Na^+-conducting "pore" of the channel or the aqueous, polar interfaces of the membrane.

Sodium channels possess, in addition to one major glycoprotein with molecular weight of roughly 200,000, differing numbers of other subunits, depending on the species and tissue of origin. Channels isolated from the eel's electroplax organ include only a single large glycoprotein;[71] those from rabbit muscle have an additional smaller subunit;[75,76] and those from rat brain have two additional smaller subunits, each having a mass of about 40,000 d.[73] The electroplax Na^+ channel protein is densely glycosylated and contains an unusually large fraction of acidic groups, totalling about 100 negative charges per channel.[77] It is assumed that the Na^+ channel, in common with other large integral membrane proteins, is oriented with its glycosylated groups on the outside surface of the cellular membrane.

Purified Na^+ channels can be dissolved in micelles of nonionic detergent stabilized with phospholipids. The unusually hydrophobic Na^+ channel macromolecule has an anomalously high detergent-binding capacity, due in part to more than a dozen long-chain fatty acids associated with each channel molecule.[78] Bound to the protein by covalent or noncovalent bonds, these acyl chains may anchor and orient the channel in the membrane, stabilizing the channel's three-dimensional structure. Long-chain fatty acids also may participate in binding of lipophilic drugs such as LAs.

Voltage-gated Na^+ channels from excitable tissues of all organisms share fundamental biophysical properties. For example, all Na^+ channels exhibit a similar selectivity among cations.[24,25,79,80] Similarly, channels from amphibian nerve, rat muscle and brain, and rabbit brain have the same rank order of affinity for the various saxitoxin-like toxins (produced by the "red tides" responsible for shellfish poisoning), although the absolute values of the binding constants among these toxins range over three orders of magnitude.[81-84] Interestingly, some Na^+ channels found in immature vertebrate skeletal muscle and brain, in certain adult invertebrates, and in the adult mammalian myocardium have a much lower absolute affinity for TTX and STX.[23,85-87] Despite their relative insensitivity to STX and TTX, these channels appear to be normally susceptible to other drugs, including LAs.[50,88]

Voltage-gated Na^+ channels from various species and tissues exhibit similar "gating" and kinetic properties.[8,9,89,90] When compared at the same membrane potential, all Na^+ channels seem to undergo similar conformational changes, first activating to open state(s) and then inactivating;[31,32,52,91] however, the rates and extent of inactivation of Na^+ channels may vary at different stages of development,[92] among different species,[93] tissues,[94] and even within the same cell.[95,96]

A variety of protein and nonprotein toxins modify the physiology of Na^+ channels.[97] At present six different binding sites for toxins have been postulated. These include extracellular surface sites for TTX/STX and for two different classes of peptide toxins (α and β, usually isolated from scorpion venom), intramembranous sites for two classes of lipophilic organic molecules (brevetoxin/ciguatoxin and the classical activators described below), and the site(s) of LA action. Each of these sites appears to be linked to at least one other site, accounting for conformationally coupled interactions among drug classes that often are dependent on the membrane potential.

Nature and Locus of the Local Anesthetic Binding Site

There is no direct biochemical evidence identifying the location of any LA binding site on Na^+ channels. Instead, physiologic and biochemical data provide indirect clues

about the site. The relevant experiments take three forms: studies on LAs with different structures and under different conditions, examinations of LA inhibition of chemically modified Na^+ channels, and studies of interactions between LAs and other drugs. By integrating these findings, we can deduce many properties of the routes to and properties of a LA site on the Na^+ channel.

Questions regarding the number of LA binding sites on a single Na^+ channel fall into two broad categories: Are there separate binding sites for neutral and charged LAs? Do tonic and phasic block result from LAs binding to the same site or to different sites? If multiple sites are hypothesized, a third question arises: May multiple sites be occupied simultaneously, *i.e.*, does binding at one site prevent binding at another? We review the evidence in the next section.

Manipulations of Drug Structure and Conditions

Based on the chemical structures known for LAs and implied for Na^+ channels, there are several loci on the channel where LAs are likely to bind. These possibilities are compounded further by the ionizable character of most LAs because channels might be inhibited by either the protonated or the neutral form of LAs. The problem has been approached by two strategies: altering LA structure to produce permanently neutral or permanently charged molecules (permanently charged molecules having limited permeation of membranes), and controlling the *p*H to set the level of ionization of tertiary amine LAs.

Manipulation of external *p*H affects both tonic and phasic inhibition. Tertiary amine LAs act to inhibit Na^+ channels of single isolated axons much faster in alkaline solution than when the same drug is applied at neutral *p*H. In contrast, the rate of onset of inhibition by permanently charged (slow) or permanently neutral (fast) LAs is independent of external *p*H. Thus, the major effect of *p*H is on LA ionization, not on the channel protein. Most investigators believe that these results show that LA molecules must pass into and/or through the nerve membrane to reach their site of action, and that the neutral species penetrates much faster than the protonated one.

By controlling *p*H inside or outside single squid giant axons during the application of tertiary amine LAs, Narahashi *et al.* showed that the protonated (charged) form in the axoplasm was the most potent species.[98] This finding is consistent with the relatively weak Na^+ channel blockade produced by a permanently neutral lidocaine homologue, 5-HHX (table 1), which is 10–20 times less potent than lidocaine at *p*H 7.3.[99] However, quantitative potency ratios for charged and neutral species depend on the particular LA. For example, at alkaline external *p*H (8–9), procaine is five to seven times more potent tonically as it is at neutral *p*H,[100] but four to five times more potent than its neutral homologue, benzocaine.[101–104] Curiously, Ritchie *et al.* found that impulse conduction in nerves pretreated with dibucaine, a highly lipophilic LA, could be either blocked or relieved by setting the *p*H of anesthetic-free bathing solutions at neutral (*p*H 7) or alkaline (*p*H 9) values, respectively.[104,105] As will be shown below, *p*H has effects on the distribution of LA between aqueous phases and the membrane as well as on the charge of membrane-associated LAs. More hydrophilic LAs will be drawn out of the membrane by acid *p*H and their potency thereby reduced. In contrast to the findings of Narahashi *et al.*,[98] with dibucaine the extracellular *p*H regulates block by drug molecules already within the nerve. This is an example where more hydrophobic LAs, which tend to stay within the nerve membrane, may be potentiated by aqueous acidification.

For most LAs, lower cytoplasmic *p*H should favor protonated over neutral tertiary amine LAs, and should thus increase the apparent LA potency. This effect was observed for tonic inhibition when the axoplasmic compartment of the squid giant axon was acidified during extracellular LA application.[98] In contrast, phasic block by LA applied externally to frog muscle was not potentiated when cytoplasmic *p*H was lowered.[106] Protons by themselves can block Na^+ channels[47] as well as alter their gating, and the effects of altered *p*H on LA action may be complicated by the separate actions of the LAs on the channel.[33,100,106] The effect of internal *p*H on the different interactions between LAs and the Na^+ channel is unclear.

Cationic LA derivatives are potent Na^+ channel inhibitors, much like protonated tertiary amine LAs. These small quaternary ammonium LAs, permanently charged due to an additional alkyl substituent on their terminal amine nitrogen, are relatively lipid insoluble and membrane impermeant (*e.g.*, QX314; table 1). When applied outside the cell membrane, small quaternary LAs do not block Na^+ currents. However, when applied on the cytoplasmic side of a membrane, quaternary LAs strongly block Na^+ currents.[29,107] These results have suggested an "internal binding site" for charged LAs accessible *via* a hydrophilic pathway from the cytoplasm.[29,33,108]

Chemically modified procaine and tetracaine block neuronal action potentials upon external application, even when covalently bound to biotin at the LA's aromatic region (P-6-Bio, table 1). But when these LA derivatives are bound by the large biotin-binding protein, avidin (molecular weight: 60,000 d), they do not block impulses when applied externally even though the unmodified tertiary-amine portion of the conjugated drug can, in theory, still penetrate 10–15 Å into the membrane.[109] In light of the relative potency of externally *versus* internally applied quaternary LAs, it appears that LA permeation and distribution in the membrane, and not the chemical reactivity

of the terminal amine nitrogen, are the factors controlling LA action.

Phasic block with internal quaternary LAs is profound, even at low depolarization frequencies, and appears to require an activated conformation of the Na^+ channel.[29,110,111] In contrast, the neutral LA benzocaine produces an insignificant phasic inhibition of Na^+ currents[33,53,56] and the neutral lidocaine homologue, 5-HHX, produces a weak phasic block.[99] Ionizable, tertiary amine LAs (*e.g.*, lidocaine) also produce phasic inhibition that is maximized by neutral or mildly acidic external solutions (pH 6.5–7) but is minimized by alkaline external solutions (pH 9).[17,33,106] External pH has only small effects on the kinetics of phasic inhibition by nonionizable LAs, showing that the primary role of protons is on the drug itself and not on the channel.

As mentioned previously, phasic inhibition may result from differential LA binding affinities among various channel conformations (the "modulated receptor"[33]) or from differential access to and from a binding site of constant LA affinity (the "guarded receptor"[112]). Either LA access or affinity might be modulated by the channel's conformation and by the ionization of the LA. Examination of phasic block by tertiary amine LAs during channel activation as well as recovery from phasic block between pulses has revealed some surprising results. The on-rate for binding of tertiary amine LAs to the activated channel that occurs during brief depolarizations, increased with alkaline pH, consistent with a much faster binding by the neutral species than by the protonated form.[48] The pH-dependence of the on-rate for binding was quite similar to that for the fraction of the nonionized species of LA. The rate of LA dissociation from the activated channel was independent of external pH, as if extracellular protons could not change the ionization of drug bound to activated channels. In contrast, the dissociation of tertiary amine LA from closed Na^+ channels in repolarized membranes depended strongly on external pH, being ten to 50 times slower in mildly acidic (pH 6.2) than in alkaline (pH 9–10) solutions. The dependence on external pH of this off-rate was described by a LA ionization with pK_a 0.3–0.4 pH units higher than that measured in aqueous solution.[113]

A physical model for phasic inhibition by tertiary amine LAs emerges from these studies. LA molecules dissolved in the membrane or in the cytoplasm bind to a site on the activated channel; membrane-dissolved drug approaches the binding site through a "hydrophobic" pathway whereas LA in the cytoplasm has access to the site *via* a "hydrophilic" pathway.[33] Either protonated and neutral species of LA may dissociate equally rapidly from the activated channel or external protons cannot reach the activated LA binding site. Conversely, when the channel closes, extracellular protons can reach the bound LA; dissociation of protonated LA from the closed channel is slow but finite and a charged LA can dissociate without first losing its proton. Whether this dissociation follows the same "hydrophobic" pathway as that of the neutral species remains to be shown. At low external pH, tertiary amine LAs behave more like their charged homologues, whereas at high pH they behave like the uncharged compounds.[106]

Charge is not the only factor influencing the kinetics of phasic LA binding. Recent results reveal marked phasic inhibition by a neutral lidocaine homologue in which an hydroxyl group replaces the tertiary amine.[99] The rate constant for dissociation of this drug from closed channels, roughly 1 s, lies between the estimated values for the protonated and neutral forms of lidocaine (0.1 s and 7 s, respectively), demonstrating that even uncharged LAs can dwell for relatively long times on the binding site. The slow dissociation rate may result from larger size, lower lipophilicity (limiting hydrophobic escape), or hydrogen bonding of this LA to the phasically activated channel.

Pathways to the LA Binding Site

Indirect evidence implicates the channel's ion-conducting pore as the hydrophilic pathway. For example, Na^+ flow through the channel alters LA action. Phasic block by quaternary drugs is enhanced when impermeant cations are substituted for external Na^+.[111,114] Similarly, application of TTX (which occludes the channel on its external surface and prevents entry of Na^+ ions) to an axon simultaneously exposed to internal quaternary LA reduces the gating current measured with infrequent stimulation to its most phasically reduced level.[115] In both of these studies, reducing the Na^+ flux potentiates LA actions, implying that influx of external Na^+ ions inhibits LA binding to its receptor. Direct, competitive antagonism would require either that the LA receptor lie within an aqueous region of the Na^+ channel (where it could be reached easily by Na^+ ions), or that there be allosteric antagonism between Na^+ and LAs at two distinct by interacting sites.

The hydrophobic pathway is difficult to define. "Hydrophobicity" is usually defined by comparing the relative concentration of a substance in a hydrophobic solvent with its concentration in an immiscible aqueous phase at steady-state. Hydrophobicity may be quantified by the partition coefficients for each of the separate forms of that substance. For example, partition coefficients for base (P^0) and protonated (P^+) forms of LAs are often measured between oils or octanol and aqueous solution (table 1). Octanol is assumed to model the membrane and "hydrophobicity," so defined, is often and erroneously equated with "lipophilicity." An example of such thinking is the erroneous notion that only the neutral species of LAs

partition into membranes, based on studies of organic solvents, not membranes. Octanol:buffer partition coefficients for tetracaine, *e.g.*, are 3×10^4 and 4, for neutral and protonated species, respectively, whereas the same parameters for membrane:buffer partitioning are 10^4 and 3×10^3.[116] Clearly, the membrane does not exclude protonated drugs; in fact, approximately equimolar membrane uptake of the two tetracaine species occurs at physiologic *p*H.

Where are LAs absorbed in membranes? Membranes composed of phospholipids have at least three separate regions: 1) a charged or zwitterionic (*i.e.*, containing both positive and negative charges) interface with the aqueous solvent; 2) a region of high-dipole intensity (near the ester bonds that join fatty acyl groups to the glycerol or ceramide moieties); and 3) an apolar core containing only the acyl hydrocarbon tails. LAs interact with the lipid molecules differently in each of these different zones, none of which behaves identically to octanol. From studies of LA and lipid behavior in model membranes during LA binding it appears that LAs are bound primarily near the membrane interface.[116,117] Protonated LAs extend further towards the polar head groups of phospholipids while the unprotonated species dwells a bit deeper in the membrane.[118] The drug molecules shuttle relatively rapidly between deeper and more superficial sites;[119] much of the binding energy arises from hydrophobic interactions,[120] some from stabilization of the LA's dipole (ester or amide bond) in the membrane's dipole field, and some from interactions of tertiary amines with polar regions of the lipids.[116]

Ionization of LAs will also be altered by membrane adsorption. Negative charges on the membrane surface will concentrate protons from the bulk solution in the "double layer"‡‡ adjacent to the membrane.[120] This localized acidity may raise the apparent pK_a for *p*H can only be measured in the bulk solution. The opposite effect will result from immersing the tertiary LA's amine group in a medium with a dielectric constant lower than that of water (*i.e.*, a medium in which the charged LA is less soluble); the protonated species will be destabilized relative to the neutral species, leading to a drop in the apparent pK_a.[121] Both LA adsorption and changes in ionization will be altered by the uptake of large amounts of LA. The complex overall effects may depend on the particular LA and on the membrane in question.

Experiments measuring rates of Na^+ channel inhibition by LAs applied at different *p*H cannot discriminate the relative importance of the hydrophilic *versus* hydrophobic pathways for phasic inhibition. The faster onset of tonic block by tertiary amine LAs at more alkaline *p*H could result from higher concentrations of either neutral or protonated drug species in both the membrane and the cytoplasm.[102,103,122] Neutral drug molecules diffuse so rapidly through membranes that their concentration differs insignificantly from one side to the other, being only a small fraction of the concentration gradient across the internal and external "unstirred layers."§§ For protonated LAs, the membrane is the major diffusion barrier, but LA distribution in the unstirred layers will depend on buffer strength and the relative mobility of the buffering species.[123]

The relationship between hydrophobicity and rate of LA inhibition is complex. At equipotent doses, small LAs with modest hydrophobicity ($P^0 < 10^2$) have slower rates of inhibition than larger LAs with intermediate hydrophobicity ($10^2 < P^0 < 10^3$). But LA molecules that are very hydrophobic ($P^0 > 10^4$) do not inhibit more rapidly; rather their rate of tonic block and their dissociation from closed channels, both assumed to depend on "hydrophobic" partitioning, are slower than those of intermediately hydrophobic LAs. There are three explanations for this. The first, proposed by Courtney, is that molecular size as well as hydrophobicity is a factor in LA kinetics and that values of octanol:buffer partition coefficients must be modified by a molecular weight correction to account for the slow actions of hydrophobic, relatively large LAs.[124]

The second explanation is that membranes are nonhomogeneous compartments. Even if octanol-based hydrophobicity accurately models the membrane's hydrocarbon core, it cannot account for LA adsorption at the dipolar region or near the phospholipid head groups. Such adsorption will not only concentrate amphiphilic drugs at certain intramembranous zones, but will also orient these molecules, restricting their motion and diffusion within and across the membrane.[125]

The third explanation questions whether one may use equilibrium measurements to make kinetic predictions. The partition coefficient expresses the relative distribution of a drug between two phases at equilibrium, a value that is the ratio of rate constants for adsorption and desorption but is otherwise unrelated to the absolute rates. One would conclude from the second and third explanations that until we know how fast LAs move into and out of the various phases of a membrane we cannot correctly attribute physiologic effects to a particular pathway. But those dynamic parameters are not available, and present efforts at modelling LA mechanisms must rely on simple physicochem-

‡‡ Double layers form boundaries of electrical potential extending 10–20 Å from the membrane surface.

§§ The unstirred layer is an immoveable slab of solution adjacent to the membrane surface.[120,123] Estimates of the thickness of unstirred layers range up to 10^2 μm, whereas the membrane is only 10^{-2} μm thick.

ical properties, even though any correlation may be merely fortuitous.

Drug lipophilicity also has implications for clinical anesthesia. LAs with low lipophilicity will pass very slowly through the membranes that ensheath nerves. Conversely, those of unusually high lipophilicity will be of limited aqueous solubility and also will partition so strongly into tissue near the site of application that their bulk diffusion through that tissue is severely limited. Such extreme behavior has important consequences for a drug's kinetics in clinical situations. For example, the faster membrane penetration and greater potency of more lipid-soluble agents may be counterbalanced by the enhanced level of nonspecific binding to adventitia, perineurium, and superficial nerve fascicles. This phenomenon correlates clinically with the relatively long onset time but high potency for peripheral nerve block by bupivacaine in comparison to lidocaine, compounds with similar pK$_a$s and diffusion coefficients but very different hydrophobicities[126] (table 1).

Properties of LA Binding Sites

The LA binding on the sodium channel remains undefined. It is possible that LA binding to any one of several sites may inhibit Na^+ currents. In this section we will summarize the relationship between the physicochemical properties of LAs and their pharmacologic actions, as these determine the general characteristics of a putative binding site. Courtney has studied Na^+ channels in a variety of tissues using structurally diverse agents that vary in their aromatic residues, hydrocarbon, or amine regions.[124] He found correlations between LA hydrophobicity tonic potency and the LA dissociation rate from closed channels, but features of the LAs other than hydrophobicity also varied, including pK$_a$, size, and the region of the LA molecule that contained the altered hydrophobicity.[127]

Physicochemical qualities may also be addressed by examining a homologous series of drugs or by comparing the kinetics of quaternary compounds in which differences in hydrophobicity exist independent of effects on ionization. The more hydrophobic LAs in a homologous series are more potent Na^+ channel inhibitors. Both tonic and phasic inhibition increase with hydrophobicity, although these two modes of action are characterized by different kinetic parameters. Tonic inhibition reflects the equilibrium LA occupancy of binding sites. Phasic inhibition, an intrinsically transient process, depends on the interplay between increased binding of LA during depolarizing pulses and dissociation from the site between pulses. For example, as shown in figure 5, similar levels of steady-state phasic block result from two quite different drugs (lidocaine [200 μM] and (+) bupivacaine (25 μM]) acting at different on and off rates. Structural changes that alter hydrophobicity inevitably produce changes in pK$_a$, but when these are factored into an estimation of the degree of ionization, the important interrelationship of hydrophobicity and potency emerges. For a series of lidocaine homologues, both tonic impulse inhibition and the steady-state phasic inhibition have potencies proportional to the calculated octanol:buffer distribution coefficient.[48,128] (The distribution coefficient is the ratio of total LA, neutral plus protonated species, in octanol compared with water.) The potencies of the LAs in these series were thus proportional to their relative tendencies to distribute into a hydrophobic medium. Similar findings were reported by Hille[102] for a more diverse collection of LAs inhibiting Na^+ currents tonically over a range of pH values.

One interpretation of the dependence of LA potency on hydrophobicity views the membrane concentration of a LA as proportional to its hydrophobicity. If this "membrane concentrating" hypothesis were true then the effective concentration of free LA in the membrane would be equal for all LAs at equipotent doses (identical to the Meyer-Overton hypothesis for general anesthesia) and the rates of onset of inhibition would be comparable despite large differences in LA concentrations in solution. Another interpretation is that LAs in the bulk membrane do not directly equilibrate with the binding site, but that a hydrophobic component of the total energy for binding the LA to its site accounts for the strong correlation of blocking potency with hydrophobicity. Accordingly, the dissociation rates of LA from the site should be proportionately slower for the more potent compounds.

Chernoff examined these possibilities using a series of lidocaine homologues with potencies and hydrophobicities ranging over two orders of magnitude.¶¶ Despite the large range of potencies for phasic and tonic inhibition, the rates of binding to phasically activated channels differed minimally. This was supported with the hypothesis that the membrane was concentrating "free" LA near the phasic LA binding site. Dissociation rates from the phasically activated channel also differed by a small amount, threefold, which is too small to be consistent with the hypothesis that hydrophobic binding to the active site accounts for the observed potency difference.

Dissociation of LA from the closed Na^+ channel depends on LA charge, hydrophobicity, and size. As an example, the dissociation rates, partition coefficients, molecular weights, and net charges for homologues of lidocaine, including permanently charged and permanently neutral compounds, are listed in table 2. Under conditions where the amine LAs are maximally protonated (acid ex-

¶¶ Chernoff DM: Kinetics of local anesthetic binding to sodium channels: Role of pK$_a$. Ph.D. Dissertation, Mass. Inst. Technology, 1988.

TABLE 2. Dissociation of Lidocaine Homologues from Closed Na^+ Channels

Drug	z*	m.w.	P^+	P^0	$\lambda_r(s^{-1})$†	pH_0	Ref
QX222	+1	221	N.M.	U.P.	0.23	8.0	156
QX314	+1	263	0.034	U.P.	0.06	8.0	156
Lidocaine	+1	234	0.06	300	0.18	6.2	128
	0		U.P.	—	4–5	9–10	
5-HHX	0	235	U.P.	9	1–2	6–9.4	128
GX‡	+1	178	N.M.	U.P.	0.22	6.2	128
	0		U.P.	3	1.6	8.5	
Bupivacaine	+1	288	1.5	U.P.	0.05–0.01	6.2	128
	0		U.P.	2600	1.7	9.5	

From reference 155 and Chernoff DM: Kinetics of local anesthetic binding to sodium channels: Role of pK_a. Ph.D. Dissertation, Mass. Inst. Technology, 1988. Used with permission.

U.P. = undefined parameter.

N.M. = parameter not measured.

* Charge on the molecule (z) determined for aqueous solution at pH_0 two units above (z = 0) or below (z = 1) the measured pK_a.

† Rate of reversal Na^+ channel phasic block at the resting potential.

‡ GX: glycine xylidide, the N-desethylation product of lidocaine.

ternal *p*H), the smallest drug, the primary amine GX, leaves the site as rapidly as the diethylamine, lidocaine, and its trimethyl quaternary derivative, QX222, although the triethyl lidocaine derivative, QX314, and the larger bupivacaine leave three to four times more slowly. Under conditions that maximize the neutral species of the amine LAs (external *p*H 9), the leaving rate is accelerated tenfold for GX and more than thirtyfold for lidocaine and bupivacaine over the rates of their respective protonated species. All are comparable to the dissociation rate for permanently neutral 5-HHX, although the partition coefficients among these compounds differ up to one thousandfold. Thus, for escape of charged LAs from closed Na^+ channels, size is a determining factor, the rate being lower for drugs above a molecular weight of 250 and higher for those below, whereas for escape of the neutral compounds, molecular size has no influence.

Courtney has measured dissociation rates from closed channels of a more widely varying sample of LAs to test the role of LA size.[127] He found that smaller LAs dissociate more rapidly than larger ones and that moderate hydrophobicity aids departure of LAs from the binding site, although extreme hydrophobicity (for example, etidocaine and bupivacaine) lengthens the duration of LA occupancy more than would be predicted from less hydrophobic drugs. Courtney attributed much of this rate dependence to molecular size and proposed that LA molecules must depart from closed channels through a long, narrow passage (roughly 3.6 Å in radius) where the LA molecules literally scraped the walls.

Cooling increases LA inhibition of neuronal impulses. Lidocaine potency increases about tenfold when the temperature drops from room temperature to near 10° C, and phasic impulse blockade is also potentiated.*** The solution pK_a for lidocaine also rises slightly upon cooling, approximately halving the fraction in the neutral form in the bathing solution (from 9–4% at *p*H 7.2), while marginally increasing the protonated form in solution.[129] However, bulk uptake of protonated LA by bilayer membranes rises with cooling; the partition coefficient for protonated tetracaine increases from 3.1×10^3 at 25° C to 6.3×10^3 at 4° C, whereas that of the neutral species changes by less than 20%.††† The overall effect is to increase the protonated LA concentration within the membrane but not in the solution. This underscores the importance of the protonated species for channel blockade. However, potencies of uncharged LAs are also enhanced by cooling,[130,131] indicating that protonation alone does not account for this increased potency. At low temperatures (4° C) benzocaine develops a pronounced phasic action virtually absent at room temperature.[130] Stronger LA binding or slower diffusion of "free" drug away from the channel may explain the potentiation of phasic block by cooling.

ANTAGONISM OF LAS BY LIPOPHILIC ACTIVATORS OF THE NA^+ CHANNEL

Sodium channel gating is modified by a diverse group of compounds termed activators, which include alkaloids extracted from plants and amphibians, peptide toxins from scorpions and coelenterates, and certain synthetic insecticides, all of which increase the likelihood that a Na^+ channel will open and/or remain open.[99] These drugs are often used to resolve Na^+ channel gating transitions in by eliminating certain channel conformations. They thereby enable the identification of state-specific actions of other agents, showing which channel conformations are involved in the selective binding or blocking process.

As previously noted, LA inhibition of Na^+ currents is antagonized by the lipophilic activators veratridine[71] (VTD) and batrachotoxin (BTX),[41,58,70,132] and LAs reciprocally inhibit these activators.[41,133,134] VTD and BTX probably bind to the same site on Na^+ channels;[135] they bind more rapidly to open than to closed channels and thus show phasic responses themselves, converting channels to a drug-modified form more rapidly if the membrane is depolarized repetitively during their initial application.[70,132,136–139] In axons, the effects of BTX are virtually irreversible whereas those of VTD can be reversed by washing away the drug.[70,140]

*** Strichartz G, Bokesch P, and Zimmermann M: unpublished observations on frog and cat.

††† Sanchez V, Ferrante F, Cibotti N, Strichartz G: unpublished observation.

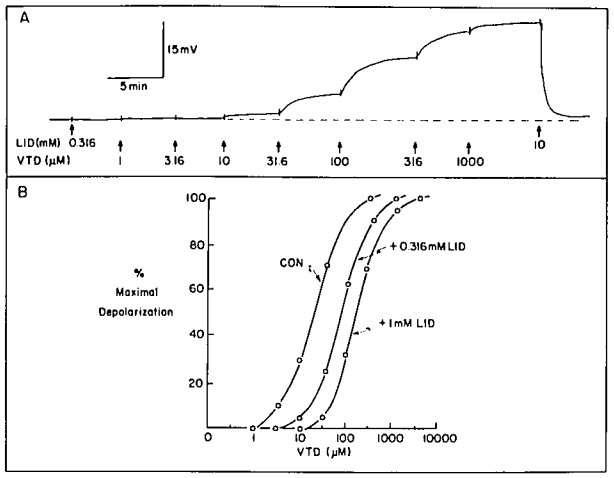


FIG. 10. The inhibition by lidocaine of veratridine's (VTD) actions on the resting potential. (*A*) Depolarization of the compound resting potential (average of resting potential in all fibers in the frog sciatic nerve) by incrementally increasing concentrations of VTD, in a nerve exposed continuously to 0.316 mM lidocaine (LID). The eventual elevation of LID to 10 mM almost completely reverses VTD's effect. (*B*) A series of dose-response curves demonstrates the competitive antagonism of veratridine (VTD) by LID. The steady-state depolarization produced by VTD on the frog sciatic nerve is plotted against the VTD concentration for control nerves in LA-free solution (CON), and for nerves incubated in LID at the two concentrations noted. Parallel shifts in the normalized dose-response curves are described by competitive inhibition of VTD by LID with a calculated inhibitory dissociation constant of 93 μM; *p*H 7.2; T = 20–22° C. (Reprinted from reference 70, with permission.)

Experiments in which both LAs and VTD or BTX are applied to Na^+ channels have produced apparently contradictory results. Pre-exposure of axons to LA slows the rate of BTX binding and the appearance of BTX-modified channels, but if an axon is first exposed to BTX, the pharmacologic effects and, by implication, the binding of BTX, cannot be reversed by subsequent LA addition.[70,140] By comparison, the effects of VTD can be both slowed and reversed by LA. Steady-state membrane depolarizations produced by VTD are depressed by LAs in a competitive manner. From such experimental data, inhibitory LA binding constants can be determined that agree closely with values from the tonic reduction of Na^+ currents (fig. 10). The stereopotency ratios for LAs for these two actions also agree.‡‡‡ These findings suggest that both the competition between LA and the highly lipophilic activators and the inhibition of Na^+ channel gating by LA correspond to one common LA binding site.

Competitive inhibition between two agents does not of itself require that they bind at the same site. Postma and Catterall measured the kinetics underlying the inhibition of BTX binding to Na^+ channels in cultured cells by a series of LAs.[68] Local anesthetics appeared to decrease the affinity of BTX for the Na^+ channel through an allosteric mechanism because they affected the rates of both binding and dissociation of BTX. These authors interpreted this result to mean that LAs and BTX must bind to allosterically coupled sites.[68,69] However, for highly lipid-soluble drugs like BTX, removal from the aqueous medium does not result in an immediate fall in membrane concentration. Maintained levels of BTX in the membrane will permit continued binding reactions, and as a result, the apparent off-rate will not be the true dissociation rate and, furthermore, will be modified by agents that prevent rebinding without a direct effect on dissociation *per se*. Under such conditions, what appears kinetically as an allosteric effect may be a direct, steric competitive inhibition.

SINGLE OR MULTIPLE SITES FOR LA ACTION?

The presence of multiple binding sites for LAs is supported by some findings but is inconsistent with others. Both the reduction of Na^+ currents and the displacement of channel activators occur with a dependence on LA concentration that indicates that only one LA molecule need bind for the inhibitory reaction. This demonstrates the lack of positively or negatively cooperative LA binding, but does not address directly the possibility of multiple, independent sites. Ulbricht and his colleagues have tested the kinetics and steady-state levels of Na^+ current inhibition in axons bathed by solutions containing two different LAs.[100,141] Using tertiary amine and neutral LAs, they found responses consistent with a single site that could bind either one or the other drug. In contrast, Mrose and Ritchie demonstrated that mixtures of neutral and tertiary amine LAs had summed effects in inhibiting compound action potentials that were inconsistent with their binding at one and the same site.[142] These results must be qualified due to the "contamination" of Mrose and Ritchie's assay by LA inhibition of K^+ channels, an effect that is known to compromise a LA's impulse blocking potency[28] and that could mask real differences in LA binding to Na^+ channels. A different experimental approach measured the ability of permanently charged or neutral LAs to inhibit Na^+ flux through BTX-activated channels in cultured neurons and concluded that separate sites for the two types of LAs were necessary to explain the exclusively competitive inhibition.[143]

The previously noted antagonism of activator binding by LA also occurs with a variety of antiarrhythmic and anticonvulsant agents[69,144,145] and even some organic inhibitors of calcium channels.[146] Separate inhibitory binding sites for neutral and permanently charged drugs un-

‡‡‡ LeeSon S, Strichartz G: unpublished observations.

derlie this competitive inhibition as judged by kinetic and equilibrium binding studies with batrachotoxin.[68,143] The stereopotency and apparent affinity of LAs at these "competitive" sites is similar to that for inhibition of activator-free channels. In addition, a rapid and reversible noncompetitive inhibition by LAs of BTX-activated Na^+ channels is observed in natural and reconstituted membranes.[67,147] This fast inhibition requires much higher concentrations of LA than are sufficient to displace BTX slowly or to inhibit activator-free channels, and it has the opposite stereopotency. There is thus evidence for three LA binding sites in BTX-activated channels, two of which competitively displace BTX slowly but with high affinity, and a third that accounts for a rapid, noncompetitive inhibition of the channel. All aspects of Na^+ channel physiology are, however, modified by BTX, and the observed inhibitions by LAs may correspond to binding at sites that are either absent or unimportant in BTX-free channels.

LAs are also competitive inhibitors of two other activators, one of which (veratridine) binds loosely and reversibly to the classical activator site,[41] the other of which (brevetoxin) binds at a separate site, synergistically and mutually potentiating the actions of veratridine.[148] Local anesthetics rapidly and competitively antagonize depolarization induced by either brevetoxin or veratridine and act with the same equilibrium inhibitory dissociation constants and stereopotency as for the inhibition of action potentials and Na^+ current. Because LAs antagonize the actions of brevetoxins and veratridine so similarly, it is likely that the LA binding site is coupled allosterically to one of these activators sites. The actions of brevetoxins are independent of depolarization and show no use dependence,[148] whereas those of veratridine and of BTX are strongly use dependent, developing much more rapidly in repetitively depolarized membranes than in membranes at rest.[70,97] This phenomenon is the corollary of phasic inhibition by LAs and suggests an intriguing possibility that a single site exists for the activators BTX and VTD and for LAs, one type of ligand binding leading to channels that have a greater tendency to activate, the other resulting in diminished activation.

On balance, the experimental evidence favors a single binding site on the Na^+ channel for charged and tertiary amine LAs. This site controls the capability of the channel to activate, inhibiting formation of the open state when bound by LA and also affecting the binding of activator drugs at another (and perhaps the same) site. A second site for neutral LAs may explain the channel blocking actions of alcohols, general anesthetics, and other nontraditional agents.

Channel Conformations and LA Actions

Having described a LA binding site based on the features of phasic and tonic inhibition, we now return to examine the nature of the phasically activated state. Which of the several states of the channel that are promoted by membrane depolarization—intermediate closed, open, or inactivated (figs. 3 and 6)—participate in the LA binding reactions that underly phasic inhibition?

The apparent enhancement of channel inactivation by LA, together with the antagonism of LA action by the activator drugs,[149] which also suppress channel inactivation, led to the concept that selective LA binding to inactivated Na^+ channels was the basis of both tonic and phasic block.[17,30,33,34] Further evidence supporting this hypothesis came from experiments on squid axons where the inactivation process had been abolished by digestion of the membrane from the axoplasmic surface by Pronase, a mixture of proteolytic enzymes.[150] Pronase-treated channels that activate normally but do not inactivate are resistant to phasic block by most LAs,[110,151,152] contrasting with their behavior before Pronase treatment and supporting the proposed role of the inactivated state in phasic inhibition.

Recent experiments qualify the exclusive role of inactivation, however. First, phasic inhibition by LAs is preserved[38-40] when the oxidant chloramine-T (CT) rather than Pronase is used to remove inactivation[153] or when less-extensive proteolysis occurs.[154] From this we conclude that: 1) activation alone is sufficient to support phasic block, and therefore 2) Pronase-induced resistance to phasic block probably results from the modification of a LA binding site. This site is proteinaceous and located at the inner surface of the channel where it is accessible to large, superficially acting enzymes.

Second, the participation of different states of the channel has been examined in studies using repetitive depolarizations of different duration, ranging from pulses too brief to open any channels to those six times longer than the time required for complete inactivation[48,***] (fig. 11; note the logarithmic abscissa). The extent of steady-state phasic inhibition varies with pulse duration in a drug-specific manner, increasing little for GX, more for 5-HHX, and most for lidocaine and bupivacaine. Since the interval between pulses remained constant in these experiments, the level of phasic inhibition reflects the degree of LA binding that occurred during the activating pulse, that for GX is almost maximal at 0.2 ms, well before most of the Na^+ channels have reached the open state and before any inactivation can be measured. The other LAs produce progressively greater phasic block with longer pulses, indicating continued binding to inactivated as well as open states of the channel.

The kinetic basis of this duration-related increase in phasic inhibition for lidocaine is shown in figure 10 B (note the linear abscissa). The rate constant for phasic binding during the depolarization (k_{+1}) increases rapidly over times that include maximal channel opening, then

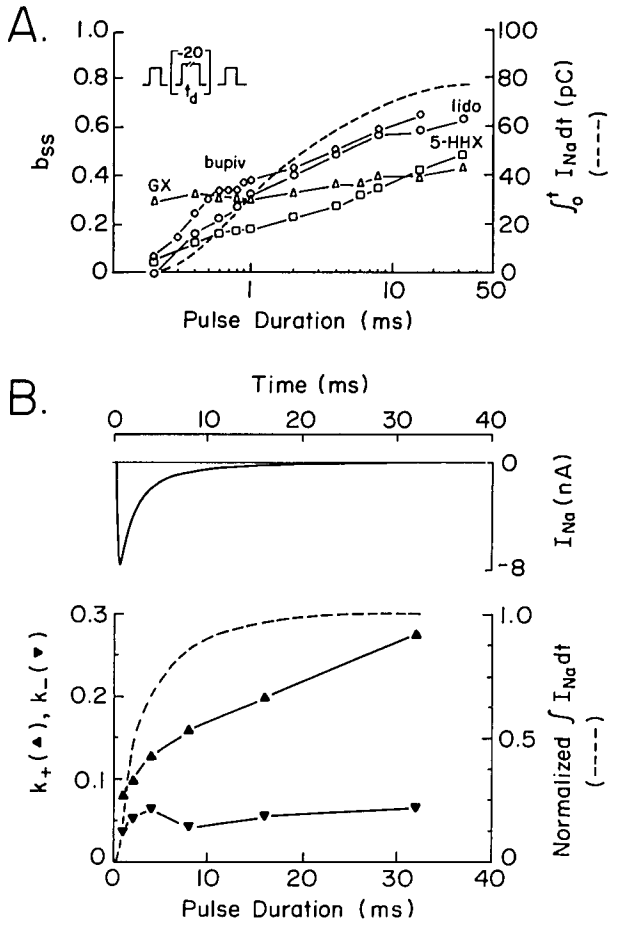


FIG. 11. (*A*) The dependence of steady-state phasic block on the duration of the "conditioning" pulse (note the logarithmic time scale). The dashed line shows the integral of Na^+ current, a measure of the openness of the channels, over the pulse duration range (0.2–32 ms). The inset shows the pattern of applied pulses; b_{ss} is the ratio of current measured by test pulse applied after the conditioning pulses (in brackets) to the test current that precedes conditioning. Almost full phasic block develops from the shortest pulses for some LA (GX = glycine xylidide) whereas others (*e.g.*, Lido = lidocaine) are more sensitive to pulse duration. (Reprinted from reference 48, used with permission.) (*B*) The rates for lidocaine binding (k_+) and dissociation (k_-) as a function of depolarizing pulse duration (note the linear time scale). The dissociation rate is independent of pulse duration, but the binding rate increases strongly with pulse duration, even for long pulse durations where channel inactivation is complete. Toad myelinated nerve. T = 13° C; pH_o = 7.3.

more slowly for periods that are dominated by the inactivated state; while the phasic dissociation rate during depolarization (k_{-1}) is essentially independent of pulse width. The rate of LA unbinding from closed channels is also independent of the length of the depolarization and of its amplitude; likewise it is unaffected by chloramine-T-induced inhibition of inactivation. Local anesthetics dissociate from closed Na^+ channels at the same rate regardless of how the phasic block was induced or whether the channel was inactivated or not.

Third, reversal of phasic block by some LAs depends on the membrane potential between depolarizing pulses in ways that are inconsistent with LA trapping by the inactivated state. Recovery from inhibition by quaternary LAs is slower when the membrane is held at more negative potentials between pulses and is accelerated by brief, small depolarizations.[29,155] Bound charged LAs thus dissociate more rapidly from the activated than the closed channel (activation becomes decreasingly probable at more negative potentials but is favored by brief depolarization), and at the same slower rate from resting or inactivated channels (because channel inactivation is less probable as the membrane potential is made more negative).[156] The holding membrane potential imposed before and between conditioning depolarization exerts a different influence in modulating inhibition by tertiary amine LAs. Prolonged hyperpolarization reduces but cannot reverse completely the tonic inhibition.[30,33] As such, negative potentials also maximize the channel population in the resting state and thus remove the population of inactivated channels (fig. 3); the inhibition remaining at large hyperpolarization can be taken as a measure of the LA affinity for the resting state.[42] Furthermore, tertiary amine LAs show more rapid recovery from phasic inhibition at more negative potentials, perhaps because the neutral species can escape more easily from channels in the resting rather than the inactivated conformation. By extension of these rate differences to the equilibrium binding at constant membrane potentials, we would argue that LAs have a higher affinity for activated rather than resting channels and that driving channel states by voltage to a "least-activated" conformation thus accelerates dissociation.

During one long depolarization, noninactivating Na^+ channels remain open long enough to reach equilibrium levels of LA binding. This binding and its proportional inhibition, like the steady-state phasic inhibition of inactivating channels, increases with increasing membrane depolarization.[39,48] However, unlike the traditional phasic effects, the voltage-dependence of LA inhibition at equilibrium approaches that of channel activation.[40,154] The probability of LA binding parallels that of channel activation. Depolarization thus appears to accomplish an inhibition by LAs largely through the gating of Na^+ channels. Any direct contribution of membrane potential to the LA binding reaction *per se* must be quite weak.

The role of slow inactivation of the Na^+ channel in LA actions has been well established for antiarrhythmic agents inhibiting cardiac Na^+ currents by Khodorov *et al.*[51,157,158] Long depolarizations, which characterize cardiac action potentials, induce the transition of Na^+ channels to slow inactivated states that recover to an activatable form in tens to hundreds of milliseconds, depending on the resting

membrane potential. Antiarrhythmics like lidocaine have a high affinity for the slow inactivated states that explains their therapeutic actions at concentrations (10^{-5} M) where their effects on Na^+ channels at rest are immeasurably small.[49] The overall contribution of slow inactivation to LA actions in nerve is less obvious, for depolarizations are much briefer and LA concentrations are much higher (10^{-3} M), both conditions that favor resting or activated channel block.[17,42,144]

The emerging picture of the LA binding site is one of little structural specificity, accomodating many types of amphiphilic§§§ bases, with some broad restrictions in size but only a weak stereospecificity.[144] The site is altered by gating of the channel between activated and closed states; activation may make the site more accessible and increase its affinity for LAs. Once bound, however, the LA greatly restricts the conformational changes producing channel activation. Extracellular protons can somehow reach bound LAs at the closed conformation site, but not at the activated site, and hydrophobic interactions contribute more to LA binding to the closed than to the activated state. The dependence of LA kinetics on LA size and hydrophobicity suggest that binding to activated channels occurs primarily from an apolar phase of the membrane and that dissociation from closed channels involves passing through a narrow crevice or pore. Hydrophobicity thus appears to determine the rate at which a LA reaches its binding site, whereas drug charge (protonation) appear to determine whether it will stay there.

Mechanisms of Spinal and Epidural Anesthesia

Despite advances in the study of LA-Na^+ channel interactions, a number of clinically important questions remain unanswered. For example, although blockade of Na^+ channels is clearly paramount for peripheral nerve block, the mechanism of LA action in epidural and spinal anesthesia may be more complex. Studies to date have merely indicated the presence or absence of signal transmission through the spinal cord or have studied the time-dependent kinetics of "wash-in" and "wash-out" of LAs in spinal cord regions, spinal roots, or in spinal fluid.[159–161]

Many membrane-associated proteins other than Na^+ channels are affected by LAs. These include adenylate cyclase,[162,163] guanylate cyclase,[164] calmodulin-sensitive proteins,[165] and the ion-pumping enzymes Na^+/K^+-ATPase[166,167] and Ca^{2+}/Mg^{2+}-ATPase.[168] In addition, the action of phospholipase A_2, important in prostaglandin and prostacylin generation, and of phospholipase C, essential for inositol trisphosphate-mediated activation of protein kinase C, are both inhibited by LAs.[169,170] The effects of LAs on membrane-associated enzymes and second-messenger systems operating in the cytoplasm are extensive.

Synaptic transmission in the spinal cord also may be inhibited directly by LA through the modification of postsynaptic receptors as well as the blockade of presynaptic calcium channels that must function to stimulate the release of transmitters. The prototypical postsynaptic receptor, the nicotinic acetylcholine receptor (nAChR) that mediates chemical transmission at the neuromuscular junction, is inhibited by LAs. Concentrations of LA equal to those required to block nerve impulses reduce chemically gated postjunctional currents of the nAChR.[171–173] The binding site is accessible to LAs in the external solution, and the molecular mechanism appears to involve channel "block" at a site separate from the agonist (ACh) binding site to which it is allosterically coupled.[174] Inasmuch as the features of the nAChR-activated channel may be general attributes of chemically gated ion conductances, other synapses also may be susceptible to LAs.

Presynaptic sites for LA inhibition within the spinal cord also exist. Recent patch-clamp studies of cultured dorsal root ganglion neurons indicate the presence of several types of Ca^{2+} channels.[175] Inhibition of these channels reduces the amount of neurotransmitter released during depolarizations.[176] Calcium channels also are inhibited by local anesthetics.[177,178] Binding of dihydropyridines (high-affinity calcium-channel antagonists) to Ca^{2+} channels is antagonized by LA with the same potency order and stereospecificity for their inhibition of Na^+ channels.[179] The Ca^{2+}-dependent release of norepinephrine triggered by depolarization of synaptosomes, which is insensitive to TTX (10^{-6} M), is totally blocked by 1 mM tetracaine¶¶¶ that is not so different from the tetracaine concentration in spinal fluid attained during spinal anesthesia (ca. 180 μM), strongly implicating LAs as direct inhibitors of neuronal transmitter release.

The broad, nonselective action of LAs are also apparent in the heart. Experiments on single isolated myocardial cells show that lidocaine and tetracaine, in addition to inhibiting the voltage-dependent Na^+ currents, also reduce the amplitudes of K^+ and Ca^{2+} currents.[180,181] This lack of pharmacologic specificity is reciprocal: agents that primarily block Ca^{2+} channels, such as dihydropyridines and verapamil, at higher concentration also inhibit Na^+ channels.[146,182]

It seems likely, therefore, that LAs will bind to sites other than Na^+ channels during spinal and epidural anesthesia and that binding to these other sites may contribute to anesthesia. To be sure, the early event in epidural anesthesia may be a blockade of impulses in spinal roots

§§§ Amphiphilic compounds are soluble in both aqueous and hydrocarbon solvents.

¶¶¶ Ohizumi Y: Personal communication.

(for anatomical reasons),[183] but the full effect of LA may well involve other sites as the block develops in time and the drug diffuses into the cord.[184] The assumption that spinal and epidural anesthesia result exclusively from Na^+ channel inhibition along axons remains unproven. Therefore, an encompassing view of LA actions within the spinal cord should include their effects on a variety of ion channels and other membrane-related activities, such as second-messenger systems, effects that may contribute to spinal and epidural anesthesia as well as to the toxic side effects of LAs on brain and heart.

Summary

Impulse block by LA occurs through the inhibition of voltage-gated Na^+ channels. Both protonated and neutral LAs can inhibit Na^+ channels though interference with the conformational changes that underly the activation process (the sequence of events that occurs as channels progress from the closed resting state to the open conducting state). The occlusion of open channels contributes little to the overall inhibition.

Local anesthetic inhibition of Na^+ currents increases with repetitive depolarizations in a process called phasic block. Phasic block represents increased LA binding, either because more channels become accessible during depolarization or because the channel conformations favored by depolarization bind LA with higher affinity. The details of phasic block are dependent on LA chemistry: certain LAs bind and dissociate quite rapidly, others act more slowly; some LAs interact effectively with closed states that occur intermediately between resting and open states, others favor the open channel, and still others have a higher affinity for inactivated states. Channel activation accelerates LA binding, and LAs may bind more tightly to activated and inactivated than to resting channels. In this regard, both the modulated receptor and the guarded receptor hypotheses are valid. In binding to activated and inactivated channels, LAs prevent the conformational changes of activation and antagonize the binding of activator agents that poise channels in activated, open states. These reciprocal actions are one aspect of the concerted conformational rearrangements that occur throughout Na^+ channels during gating.

The LA binding site may exist in the channel's pore, at the membrane-protein interface, or within the protein subunits of the channel. Judging from its susceptibility to intracellular proteases and its accessibility to LAs with limited membrane permeability (*i.e.*, quaternary LAs in the cytoplasm), the site lies nearer to the cytoplasmic than the external surface of the membrane. Nevertheless, protons in the external medium influence the dissociation of LA from the closed channel.

Binding of LAs at the inhibitory site is weak and loose. If one accounts for the membrane-concentrating effects of LA hydrophobicity that are expressed as membrane: buffer partition coefficients equal to $10^2 - 10^4$, then the apparent LA affinities are low. The equilibrium dissociation constants calculated on the basis of free drug in the membrane are 1–10 mM, with a correspondingly weak binding to the inhibitory LA site. The stereospecificity of LA action is also relatively nonselective, suggesting a loose fit between ligand and binding site. We speculate that a LA molecule acts by fitting into an amphipathic

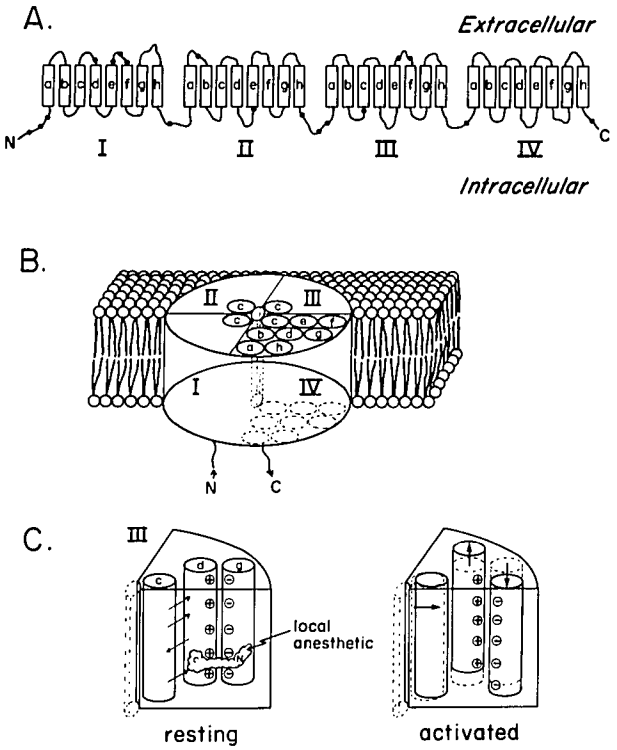


FIG. 12. A speculative model for the molecular mechanism of LA action. (*A*) The primary sequence of the large subunit of the Na^+ channel has four repeating domains (I–IV), each containing six to eight sequences of amino acids that probably form α-helical structures spanning the nerve membrane, denoted by the rectangles lettered a–h. (*B*) Montal *et al.* have postulated that these helices pack together in approximately fourfold symmetry with the polar edges of the four "c" helices forming the lining of the ion pore, projecting through the center of the complex. For simplicity, only the extracellular (*top*) and intracellular (*bottom, dashed elipses*) edges of the helices are shown in *B*. (Adapted from reference 185, used with permission.) (*C*) A stripped-down view of quadrant III shows the postulated gating mechanism. Helix d is coupled to the pore-forming helix c and contains a series of basic amino acids that form a strip of positive charge. These charges are stabilized in the low dielectric milieu of the membrane interior by a strip of negative charges counterposed to them on helix g. Membrane depolarization activates the channel by pulling the g helix in, pushing the d helix out, and thereby moving the c helix to open the channel pore. We speculate that a local anesthetic binds at a site near or on the gating helices, as drawn on the resting conformation, and prevents these conformational changes.

pocket located on or between membrane-spanning regions of the Na^+ channel (fig. 12). In proposed models for the tertiary and quaternary structure of the Na^+ channel, each of four homologous regions (I–IV) of one large polypeptide contain six to eight membrane-spanning α helices (a–h). Several of these helices are composed of acidic or basic amino acid side chains that are aligned along one edge of the helix, forming ion pairs with each other (*e.g.*, segments d and g, fig. 12 C).[185] Each ion pair constitutes an electric dipole, and when the membrane is depolarized these dipoles shift locally, the electrically coupled helices slide in or out of the membrane (accounting for gating current), and the pore-forming regions of the channel (designated as the helices marked c) change conformation, permitting ion passage, thus opening the channel. We propose that LAs bind at the dipole-containing helices such as to inhibit their rearrangement in response to membrane depolarization. The site's availability to LA depends on the channel's conformation and, reciprocally, LA binding limits the Na^+ channel to certain conformations. Smaller LAs enter and leave the site relatively quickly, larger ones enter and leave more slowly and may not be able to reach the deeper recesses. Intermediate-sized LAs enter relatively rapidly, can reach deeply, and leave at rates that are dependent on the drug's overall shape and structural flexibility. This model is speculative, but it provides an alternative to the widely promulgated "open-channel blocking" diagrams and, we believe, explains the experimental results equally well.

Research on LA mechanisms will likely extend in two directions in the near future. In one, biochemical investigations will define a binding site or sites for LAs on Na^+ channels while further biophysical studies will compare the pharmacology of LAs acting upon different types of Na^+ channels and will resolve the molecular details of the inhibitory mechanism. In the other direction, the full range of activities affected by LAs will be explored, and possible cellular and molecular mechanisms for spinal and epidural anesthesia and for toxic LA effects upon CNS, heart, and elsewhere will be defined. Both approaches should improve the understanding, practice, and safety of local anesthesia.

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