The Inhibition of Sodium Currents in Myelinated Nerve by Quaternary Derivatives of Lidocaine

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Abstract The inhibition of sodium currents by quaternary derivatives of lidocaine was studied in single myelinated nerve fibers. Membrane currents were diminished little by external quaternary lidocaine (QX). QX present in the axoplasm (<0.5 mM) inhibited sodium currents by more than 90%. Inhibition occurred as the sum of a constant, tonic phase and a variable, voltage-sensitive phase. The voltage-sensitive inhibition was favored by the application of membrane potential patterns which produce large depolarizations when sodium channels are open. Voltage-sensitive inhibition could be reversed by small depolarizations which opened sodium channels. One explanation of this observation is that QX molecules enter open sodium channels from the axoplasmic side and bind within the channels. The voltage dependence of the inhibition by QX suggests that the drug binds at a site which is about halfway down the electrical gradient from inside to outside of the sodium channel.

Introduction

The electrical activity of excitable cells results from conductance changes of the cell membranes. These macroscopically observable conductance changes have been attributed to the opening and closing of microscopic channels which are the pathways for ionic current through the cell membranes (see Hille, 1970; Armstrong, 1971). The time-course and voltage dependence of conductance changes have been known since Hodgkin and Huxley (1952) first analyzed ionic currents of the squid giant axon using the voltage-clamp technique. Yet the nature of individual ionic channels remains obscure.

One approach to characterizing ionic channels is to study the variety of molecules which block ionic currents. The ability of small molecules to selectively inhibit sodium or potassium currents is one piece of evidence supporting the existence of separate sodium and potassium channels (Hille, 1967; Narahashi et al., 1966; Armstrong, 1966).
This paper reports the inhibition of sodium currents in myelinated nerves by quaternary derivatives of the local anesthetic lidocaine. The results suggest a model in which quaternary lidocaine (QX) binds within open sodium channels preventing the passage of sodium ions. When the results of QX inhibition are combined with measurements of the permeability of various cations through sodium channels (Hille, 1971, 1972) a three-dimensional structure for the sodium channel emerges. The dimensions include a minimum width for the axoplasmic entrance of open channels (6 × 8 Å), a maximum width for the selectivity structure (3 × 5 Å) and a linear arrangement of ion gating structure followed by QX binding site and then selectivity filter in passing through the sodium channel from the axoplasm to the outside.

**METHODS**

*Nerve Preparation*

Single myelinated fibers were studied under voltage-clamp conditions after the method of Dodge and Frankenhaeuser (1958). Fibers averaging about 15 μm diameter were dissected from the sciatic nerve of the frog,*Rana pipiens*. The chamber in which the fiber was mounted and the voltage-clamp instrumentation were like the ones described by Hille (1971).

The nerve fibers were clamped at 6°-7°C, to a holding potential of -75 mV. At the beginning of each experiment the internodes bordering the node to be clamped lay in an isotonic (0.12 M) KCl solution. One internode was cut in this KCl solution at least 30 min before any experimental measurements were made. Drugs were diffused down the axon by first replacing the KCl solution surrounding the uncut internode by a KCl solution containing the drug. The internode was then cut. There was no immediate difference between membrane currents measured before and after cutting the second internode.

*Recording and Calibration*

Membrane currents and potentials were displayed on an oscilloscope and recorded on 35 mm film. Capacitative and leak components of membrane currents were subtracted electrically (see Armstrong and Hille, 1972). The voltage "attenuation artifact" noted by Dodge and Frankenhaeuser (1958, 1959) was determined by decreasing the sodium concentration in the bathing medium and measuring the change of the reversal potential of sodium currents. Using the Nernst value for the theoretical change in the sodium reversal potential, the recorded membrane potentials were calculated to be 85% of the actual membrane potentials. Membrane potentials reported here have been corrected for this attenuation by multiplying the measured values by 1.18.

Computer solutions were derived on a Raytheon PB 440 digital computer (Raytheon Computer Operation, Santa Ana, Calif.).
Assay of Voltage Conditioning

The inhibition by QX drugs described in the Results section can be modulated by imposing certain voltage patterns across the axon membrane. This process is termed "voltage conditioning." The following terms apply to the formation and measurement of voltage conditioning:

- \( E_c \) = conditioning pulse; depolarizing pulses, usually of 5 ms duration applied at 1 s\(^{-1} \), which increase inhibition produced by QX.
- \( E_t \) = test pulse; depolarizing pulse used to measure size of sodium currents.
- \( I_t \) = test currents; currents flowing during \( E_t \); peak \( I_t \) is the value used to assay sodium conductance, hence to infer QX inhibition and voltage conditioning.
- \( E_{rev} \) = reversing pulse; small depolarizing pulse, used to remove voltage-conditioned inhibition rapidly.
- \( E_{pp} \) = prepulse; usually hyperpolarizing pulse, 50 ms in duration, which immediately precedes conditioning pulses \( (E_{ppc}) \), test pulses \( (E_{ppr}) \), and reversing pulse \( (E_{pps}) \).

The peak current values after conditioning have been divided by \( I_{-75} \), the value of peak current after the membrane was held at \(-75 \text{ mV}\) for several minutes. The size of the control currents, \( I_{-75} \), diminishes slowly and continuously during the experiment. This decrease probably results from both the normal degeneration of the nodal preparation and from an increasing concentration of axoplasmic QX due to diffusion. To account for the continuous reduction of currents, measurements of the control current during a "normalizing" pulse precede each voltage conditioning measurement.

Solutions

Ringer solutions bathing the node contained 115 mM NaCl, 2 mM CaCl\(_2\), 2.5 mM KCl, and 2 mM morpholinopropanesulfonic acid (MOPS), pH = 7.0. In most
experiments the bathing solution also contained 6 mM tetaethylammonium bromide to inhibit K⁺ currents. The 1/4 Na-Ringer and 1/2 Na-Ringer solutions contained 100.6 mM and 57.5 mM tetrarmethylammonium bromide, respectively, replacing an equimolar amount of NaCl. Tetralkalammonium salts were purchased from Eastman Organic Chemicals Div., Eastman, Kodak Co., Rochester, N. Y., MOPS from Calbiochem, La Jolla, Calif. The quaternary lidocaine salts, QX-222 Cl and QX-314 Br (Fig. 1), were generously donated by Dr. Bertil Takman of the Astra Pharmaceutical Company, Worcester, Mass.

RESULTS

Inhibition of Sodium Currents by Internal Quaternary Lidocaine Derivatives

Sodium currents are studied most clearly when the potassium currents through the node are absent. In the experiments reported here more than 90% of the potassium current was removed pharmacologically by 6 mM tetaethylammonium ion present in the Ringer solutions bathing the node (Hille, 1967). The remaining currents are due almost entirely to ions passing through sodium channels and will be referred to as sodium currents (Hille, 1972).

The currents follow the usual time-course of sodium currents (Hodgkin and Huxley, 1952a; Dodge and Frankenhaeuser, 1958), growing to a peak value shortly after the beginning of a depolarizing pulse and diminishing gradually during the remainder of the depolarization (Fig. 2a). Currents are inward for small depolarizations (-45 mV), outward for the largest depolarizations (+75 mV).

Figure 2. Families of voltage-clamp currents for a node bathed in Ringer (a), in Ringer containing 0.5 mM QX-314 (b), and in Ringer, 20 min after cutting the internode in 0.12 M KCl containing 0.5 mM QX-314 (c). The time-courses of the ionic current in sodium channels at 10 different voltages starting at -75 mV and spaced at 15-mV intervals are superimposed. Inward currents are negative, outward positive. One internode was cut in 0.12 M KCl 30 min before measuring the first family of currents (trace a). In trace c the steady state of inhibition has not yet been reached.
Fig. 2 shows three families of voltage clamp currents: (a) With the node bathed in normal Ringer solution, (b) in Ringer solution containing 5 mM quaternary lidocaine, and (c) bathed in normal Ringer solution with quaternary lidocaine present inside the node. In three different nerves the amplitude of the currents was reduced by 7.3 ± 2.3% when 5 mM QX-314, the triethyl lidocaine derivative, was outside the node. In contrast, QX-314 inside the node at lower concentrations strongly inhibits sodium currents. After the internode of the nerve fiber is cut in an isotonic KCl solution containing 0.5 mM QX-314, sodium currents diminished by approximately 90%. Because the currents at all membrane potentials are diminished while the sodium reversal potential remains the same, QX compounds probably inhibit by lowering the conductance of sodium channels in the node. Inhibition is observed within 30 min after cutting the fiber 0.5 mm from the clamped node. Apparently QX inhibits sodium currents after diffusing down the internode to the region within the node (see Koppenhöfer and Vogel, 1969), yet it has little effect from outside the node. This large inhibition remains through the rest of the experiment and will be referred to as the “tonic phase” of inhibition. The QX concentration in the nodal axoplasm is less than that at the end pool since subsequent cutting of the other end of the nerve fiber in the same concentration of QX results in an even greater tonic inhibition.

Enhancement of QX Inhibition after Depolarization Pulses

A striking feature of inhibition of QX compounds is an increase in inhibition after depolarizing pulses. Fig. 3 shows three traces of the time-course of sodium currents during a test pulse depolarization ($E_t$) to $-45$ mV with QX-314 inside the node. The currents are already reduced to $0.11 \pm 0.03$ of the currents without QX. The peak inward current is largest if the membrane is held at $-75$ mV for several minutes before the test pulse. When instead, the test pulse is preceded by a train of five depolarizing pulses to $+75$ mV of 5 ms duration and spaced 1 s apart, the test currents become smaller. They are smaller still when the test pulse is preceded by 20 pulses to $+75$ mV. This reversible depression, called “voltage-sensitive inhibition,” is the primary subject of this paper.

Control experiments on two nerve fibers cut in KCl containing no QX show normal sodium currents with none of the voltage-sensitive diminution associated with internal QX drugs. In the absence of these drugs the application of conditioning pulses is without effect on sodium currents.

In the terminology used “test currents” ($I_t$) are an assay of the extent of inhibition. These test currents flow during a voltage clamp to a standard “test voltage,” denoted $E_t$. Other voltage pulses applied before the test pulse can increase the inhibition due to the QX drug. These pulses are called
“conditioning pulses” and have potentials denoted by $E_c$. Conditioning pulses are usually of 5 ms duration. They are applied at least 1 s before the test pulse and should not be confused with the hyperpolarizing pulse which directly precedes the test pulse and which is used to remove normal sodium inactivation. These pulses, which immediately precede test pulses or conditioning pulses with no other voltage intervening, are called “prepulses” and have values denoted by $E_{ppt}$ or $E_{pps}$, respectively. The use of these terms is illustrated in Fig. 4.

Fig. 3. Inhibition by quaternary lidocaine increased by a sequence of depolarizations. The time-course of sodium currents with QX-314 present inside the nerve. All currents were measured during a depolarizing “test” pulse to $-45$ mV ($E_t = -45$). The largest peak current occurred after the membrane potential was held at $-75$ mV for several minutes before the test pulse (trace 0). Peak currents during the test pulse were diminished (trace 5) when their measurement was preceded by five depolarizations to $+75$ mV ($E_t = +75$) lasting 5 ms each and spaced 1 s apart. Preceding the test pulse by 20 of these depolarizing pulses ($E_t = +75$) further diminished the measured peak currents (trace 20). See Results section for experimental details.

Fig. 4 shows the effect of conditioning pulses on voltage-sensitive, drug-induced inhibition. The top of Fig. 4 presents the pattern of voltage pulses applied to the nerve membrane. In this experiment a number, $n$, of conditioning pulses of voltage $E_c$ are applied to the nerve before the test voltage. The conditioning pulses are spaced 1 s apart; the last conditioning pulse begins 1 s before the test pulse. Both the conditioning pulses and the test pulse are directly preceded by a fixed 50 ms long prepulse. Inhibition is enhanced by more positive conditioning pulses as well as by an increased number of conditioning pulses until some steady-state level of inhibition is reached. The extent of steady-state inhibition depends on the value of $E_c$. The same phenomenon is observed with QX-222 (trimethyl lidocaine) present internally, but was not investigated systematically.
The opening and closing of sodium channels is much faster than the reversal of voltage-conditioned inhibition. The average channel opens in 1 ms and closes in 10 ms while the reversal of block usually takes more than 20 s (Fig. 8 a). During the first test pulse after voltage conditioning the reversal of inhibition is insignificant. Hence, the currents measured during that test pulse are a valid measure of the steady state of voltage-sensitive inhibition.

**Figure 4.** Development of voltage-sensitive phase of sodium current inhibition. Top: pattern of voltages applied to the nerve. A number, n, of conditioning pulses (shown to the left of the arrow) is applied to the nerve before currents are measured during the standard test pulse of -45 mV (shown to the right of the arrow). The conditioning pulse voltage, E_c, is changed; conditioning prepulse, E_prep, is fixed. All pulses spaced 1 s apart. 

Bottom: peak inward current, I_t, during test pulse to -45 mV is reduced by an increasing number of conditioning pulses until steady state of inhibition is reached. Points are an average of three measurements of one axon.

Fig. 5 shows a semi-log plot of peak currents during steady-state inhibition versus the conditioning voltage, E_c. The increase in inhibition is small for conditioning voltages below 0 mV, but above 0 mV the inhibition increases dramatically. The solid lines are drawn from a model derived in the Discussion. The data of Fig. 5 refer to the voltage-sensitive phase of inhibition and do not include information about the tonic phase of inhibition. In these experiments the tonic inhibition was 91 ± 5%. The currents have been normalized to account for the slow degeneration of nodal currents (see Methods).
The problem is to determine which processes contribute to the voltage enhancement of inhibition. Depolarizations of the membrane normally produce: (a) changes of the electric field strength within every part of the nodal membrane; (b) opening of ionic channels in response to the changes of electric field; and (c) ionic currents, driven by both diffusion and voltage gradients, flowing through the channels. Voltage-sensitive inhibition could result from interactions of the drug with the nonconducting regions of the membrane or with the ionic channels in the membrane. Voltage sensitivity of a drug-channel interaction could arise from a direct effect of the electric field on the QX-channel interaction, or from the indirect effect of voltage-de-

![Figure 5. Steady state of voltage-conditioned inhibition as a function of conditioning voltage. The ordinate is the normalized size of test currents measured after conditioning to the steady state of inhibition, \( I_t(E_c) \). Conditioning was performed as in Fig. 4, top. Data points are the average of three measurements on each of four nerves. The vertical bars are the mean deviations from the mean for each experimental point. Solid lines are solutions of an equation derived in the Discussion.]()

pendent ionic currents whose charge carriers might interfere with QX binding. The following results resolve some of these possibilities.

**Voltage-Sensitive Inhibition Requires Open Sodium Channels**

Voltage enhancement of QX inhibition occurs under conditions which normally open sodium channels. Reviewing briefly, openness of sodium channels is given by the Hodgkin-Huxley (1952 b) formalism:

\[
"\text{openness}" = \frac{g_{Na}}{g_{Na}} = m^3h.
\]

For all sodium channels of the nodal membrane to be open both \( m^3 \) and \( h \) must be equal to unity. To simplify the discussion I will refer to \( m^3 \) and \( h \) "gates." These gates are open when \( m^3 \) and \( h \) have unity value and closed when they have zero value. In other words, if either \( m^3 \) or \( h \) gates are closed,
the channels cannot conduct ions. The $m^3$ and $h$ gates are functions of time and membrane potential and are easily changed in voltage-clamp experiments.

**The significance of $h$** Voltage enhancement of QX inhibition can be modified by conditions which control the value of $h$. Fig. 6a shows the results of an experiment in which the voltage, $E_{prep}$, of the prepulse preceding the $+75$ mV conditioning pulses was varied systematically. The normal result of changing the prepulse voltage is to poise $h$ gates at different degrees of openness before the depolarizing pulse. This also occurs in the presence of internal QX compounds, although $h$-gate kinetics are modified by the drug. (In two experiments, cutting the internode in 0.5 mM QX-314 shifted the curve for steady-state inactivation, $h_\infty$, less than $\pm 5$ mV along the voltage axis. But the time constants, $\tau_h$, were increased nearly twofold for some voltages when QX was present internally. Still, the prepulse duration of 50 ms is sufficiently long for the $h_\infty$ values to be reached. The slowing of the $h$-gate kinetics is not dealt with in this paper.)

When hyperpolarizing prepulses are used, voltage-enhanced inhibition develops rapidly, and with more positive prepulses it develops slowly (Fig. 6a). In either case the final steady-state inhibition is about the same. The rate of appearance of inhibition as a function of prepulse voltage is shown in Fig. 6b. The points are from experiments like the one of Fig. 6a and lie close to the graph of $h_\infty$ as a function of prepulse voltage, the solid curve through open circles which was determined by the method of Hodgkin and Huxley (1952b). Inhibition develops more slowly when $h$ is small than when $h$ is near unity. Evidently $h$ gates must be open for voltage-enhanced inhibition to occur.

The dependence of voltage-enhanced inhibition on $h$ explains another experimental finding. Different patterns of conditioning pulses resulting in the same total period of membrane depolarization have different effects. In particular, a train of short pulses which depolarizes the membrane for a time equal to the duration of one long depolarizing pulse is more effective than the long pulse in enhancing QX-related inhibition. Five pulses of 5 ms length decrease currents by 33% compared to an 11% decrease from one 25 ms pulse. Each of the short pulses results in a transient opening of sodium channels. The long pulse opens them once, but because of eventual inactivation as $h$ decreases, the channels remain closed for most of the depolarization. When the channel is closed because the $h$ gate is closed, less inhibition develops.

**The significance of $m$** There is evidence that the tonic phase of inhibition by QX develops more rapidly when the $m$ gates of sodium channels are open. This dependence on $m$ can be demonstrated once in each experi-
Figure 6 a. Development of voltage-sensitive inhibition depends on conditioning prepulse voltage, $E_{ppc}$. Top: pattern of applied voltages: same as in Fig. 4, but $E_c$ fixed (+75 mV) and $E_{ppc}$ changed. Bottom: the peak current (normalized) during the test pulse ($E = -45$ mV) is reduced more slowly as conditioning prepulse voltage becomes more positive. $I(-75)$ is the size of peak currents measured after membrane potential was held at -75 mV before the application of conditioning pulses (see Methods).

Figure 6 b. Inhibition by depolarizing pulses preceded by 50 ms pulse at $E_{ppc}$. The initial rate of appearance of the voltage-sensitive phase of inhibition as a function of conditioning prepulse voltage ($E_{ppc}$). Data from two measurements on each of three separate nerves during experiments like the one shown in Fig. 6 a. The solid line drawn through the open circles is an average of separate measurements of $h$ with QX present for each experiment; the midpoint potential differed by less than ±5 mV. The fastest initial rate is arbitrarily assigned the value of 100.

The node is clamped to -75 mV for 45 min after cutting the internode in a QX solution. Although the drug has had sufficient time to diffuse into the nodal axoplasm (see Fig. 2), currents flowing during small depolarizations (-30 mV) are reduced from controls by only 10%. In order to rapidly approach the tonic level of inhibition (ca. 90%) the membrane requires several depolarizing pulses to 0 mV. Apparently the inhibition appears slowly if $m^3$ gates of sodium channels are not open even if the $h$ gates are
completely open. Depolarizations to 0 mV open almost all the channels and the inhibition rapidly reaches the tonic level from which the reversible, voltage-sensitive phase is studied.

Once the tonic phase of inhibition from axoplasmic QX has been reached, it cannot be reversed to the previous, small level of inhibition. The onset of the voltage-sensitive phase of inhibition may depend on the openness of $m^3$ gates, but this is difficult to determine. The depolarizing pulses which are necessary to open $m^3$ gates also enhance the extent of inhibition and it is not possible to discriminate between the individual contributions from these two processes using only the steady-state results.

Reversing Voltage-Enhanced Inhibition

The enhancement of QX-related inhibition by depolarizing voltages is a reversible phenomenon. Reversal occurs very slowly when the membrane potential is held at -75 mV. Reversal of inhibition is much faster if small depolarizing pulses of 5 ms duration are applied to the node. For example, Fig. 7 shows the reversal of voltage-conditioned inhibition by “reversing pulses” ($E_{rev}$) of $-45$ mV applied to the membrane at different frequencies. Under such conditions the inhibition decreases with an exponential time-course. The lines drawn through the data points in Fig. 7 obey the equation

$$I_{test,r} = I_{test,oo} (1 - e^{-k\tau}),$$

where $I_{test,r}$ is the value of peak current during $E_{rev}$ measured at a time, $\tau$, after the first reversing pulse was applied, $I_{test,oo}$ is the peak current after the application of 60 reversing pulses. $k$ is a first-order rate constant with values $0.31 \pm 0.08$ s$^{-1}$, $0.12 \pm 0.02$ s$^{-1}$, and $0.063 \pm 0.007$ s$^{-1}$ for reversing pulses applied at 4 s$^{-1}$, 2 s$^{-1}$, and 1 s$^{-1}$, respectively. The rate of the reversal reaction is proportional to how frequently the reversing pulses are applied. Since small depolarizations increase the $m^3$ parameter, evidently the $m^3$ gates of sodium channels must be open before voltage-sensitive drug inhibition can be reversed.

Reversal rates also depend on the voltage of prepulses preceding the reversing pulses. Hyperpolarizing prepulses accelerate the reversal, whereas depolarizing prepulses slow it (Fig. 8a). The dependence of reversal rate on prepulse voltage appears very similar to the voltage dependence of $h$ (Fig. 8b, solid curve). Values of $h$ near unity correspond to a fast reversal of inhibition, values near zero correspond to a slow reversal. Apparently the reversal of voltage-enhanced inhibition cannot occur unless both the $m^3$ gates and the $h$ gates of sodium channels are open.

Changing the current in sodium channels has little effect on voltage conditioning. In two experiments normal Ringer solution (containing 115 mM Na$^+$) was replaced by Ringer in which an impermeant ion, tetramethyl-
ammonium ion, was substituted for half the sodium ions. The kinetics and the steady-state level of voltage-sensitive inhibition are the same in half-sodium Ringer and in normal Ringer. The steady-state inhibition from +75 mV conditioning pulses in normal Ringer is $I_{r(+75)}/I_{r(-75)} = 0.40 \pm 0.05$, in half-sodium Ringer $I_{r(+75)}/I_{r(-75)} = 0.38 \pm 0.07$. Over this range of external sodium concentrations there is little dependence of voltage conditioning on the ion influx through the sodium channel.

**Potassium Currents Are Also Affected by QX-314**

Two experiments were done without external tetraethylammonium ion, so that potassium currents could be measured. The compound QX-314,
when present internally, produces a delayed inhibition of potassium currents in frog myelinated nerves. Potassium currents appear normal at the beginning of large depolarizing pulses but become smaller as the pulse endures. The same phenomenon has been observed when derivatives of tetraethyl-

![Diagram](image)

**Figure 8 a.** Reversal of voltage-sensitive inhibition as a function of reversing prepulse voltage ($E_{ppr}$). After inhibition produced by 20 pulses at $+75$ mV, reversing pulses were applied at a frequency of 1 s⁻¹. Top: reversing pulse voltage pattern. The reversing pulse potential was fixed at $-45$ mV while the reversing prepulse potential ($E_{ppr}$) was set at the values noted in the figure. Test currents were always measured during a test pulse to $-45$ mV preceded by a prepulse to $-120$ mV. See legend of Fig. 7 for an explanation of the ordinate.

**Figure 8 b.** Reversal of inhibition by small depolarizing pulses preceded by 50 ms pulse at $E_{ppr}$. Data points are from two measurements on each of three axons. Rates are expressed in arbitrary units based on a value of 100 for the fastest rate. $h$ curve (solid line) determined as in Fig. 6 b.

ammonium ion are present inside squid axons (Armstrong, 1966, 1971) and in frog myelinated nerves (Armstrong and Hille, 1972). This is not surprising since QX-314 is a tetraethylammonium ion derivative, and it is independent evidence that the drug does reach the axoplasm inside the node.

**DISCUSSION**

**A Model for Voltage-Sensitive QX Inhibition**

The voltage-sensitive phase of QX inhibition exhibits three features: (a) Inhibition increases when the nodal membrane is subjected to a train of de-
polarizing pulses. (b) The \( h \) gate and probably the \( m^3 \) gate of sodium channels must be open for the inhibition to increase. (c) The inhibition can be reversed by conditions which open both \( h \) and \( m^3 \) gates at small membrane depolarizations. All these results suggest a direct interaction between QX molecules and sodium channels.

One simple model which takes all these features into account is that QX binds to, and dissociates from open \((C_o)\) sodium channels. QX cannot bind to or dissociate from closed channels \((C_c)\), but complexed channels \((C \cdot QX)\) can open and close in the normal manner. The channels which have QX bound to them cannot conduct ions. A general scheme to describe this model is:

\[
\begin{align*}
&\text{(open)} C_o \xrightarrow{QX} C_o \cdot QX \quad \text{(open, blocked)} \\
&\text{(closed)} C_c \quad C_c \cdot QX \quad \text{(closed, blocked)}.
\end{align*}
\]

The horizontal step represents the binding reaction to open channels. The vertical steps represent opening and closing of blocked and unblocked channels.

According to the model, during each conditioning pulse sodium channels open and the binding reaction proceeds until the channels close. After a sufficient number of pulses the binding reaction reaches equilibrium. The equilibrium state of the binding reaction corresponds to the steady state of voltage-sensitive inhibition.

Any quantitative model of QX inhibition must include a description of how the steady state of inhibition depends on conditioning voltage. There are two simple means by which voltage can displace the equilibrium of the binding reaction: (a) the reaction itself is directly influenced by voltage, or (b) the steps which provide and remove substrates for the reaction are voltage dependent, producing an indirect effect on the degree of inhibition. Each of these possibilities will be considered.

First, suppose that the actual QX-binding reaction is influenced by the membrane potential. This means that some component of the free energy of binding depends on the voltage difference between the phase where the drug is free and where it is bound. The derivation of equations describing voltage-sensitive binding appears in the Appendix. There it is demonstrated that both tonic and voltage-sensitive inhibition can arise from the sequence of reactions:

\[
C_o + QX \xleftarrow{K_1} C_o \cdot QX^1 \xrightarrow{K_2(\Delta E)} C_o \cdot QX^2,
\]

where \( K_1 \) is a voltage-independent and \( K_2(\Delta E) \) a voltage-dependent equi-
librium constant for two forms of the complex $C_0\cdot QX$. Normalized steady-state inhibition produced by conditioning voltage $E_1$ is then given by Equation 5:

$$\frac{I_1(E_1)}{I_1(E_0)} = \frac{1 + [QX][K_1 + K_1 K_2 \exp (\delta E_2/24)]}{1 + [QX][K_1 + K_1 K_2 \exp (\delta E_0/24)]}. \quad (5)$$

$E_2$ is the normalizing conditioning potential (see Methods), usually $-75$ mV, $[QX]$ is the drug concentration at the first binding site, and $\delta$ is the fraction of the membrane potential which affects the binding reaction, a dimensionless parameter with value between zero and one. The term on the left-hand side of the equation is the experimentally measured quantity, the normalized extent of inhibition. According to Equations 5 and 6 (see Appendix), tonic inhibition can be accounted for by assigning relatively large values to the first association constant, $K_1$. If the axoplasmic QX concentration is the concentration of QX at the cut internode, 0.5 mM, then $K_1 = 20 \text{ mM}^{-1}$ and $K_2 = 0.25$ results in 93% tonic inhibition. The mean value from five experiments is $91 \pm 5\%$.

The second step in the sequence of binding reactions satisfactorily describes the voltage-sensitive phase of inhibition. Graphs of Equation 5 are plotted as solid curves in Fig. 5. The constants $K_1$ and $K_2$ were assigned the values which produced correct solutions for tonic inhibition in the presence of 0.5 mM QX-314, 20 mM$^{-1}$ and 0.25, respectively. Values of $\delta$ from 0.5 to 0.7 generate an envelope of curves which encloses the data points. Solutions of Equation 5 which have values of $K_2$ as small as 0.1 and $\delta$ values as large as 0.8 also enclose the experimental results and give a steeper slope at more depolarized membrane potentials. The calculated slopes at $E_s = +60$ mV for $\delta = 0.5$, 0.6, and 0.7 are an $e$-fold change in current per 88, 64, and 47 mV, respectively. The mean slope from four experiments at $+60$ mV conditioning voltage is best fit by a value of $\delta = 0.55 \pm 0.11$.

The assumptions from which Equation 5 was derived place the binding reactions in a sequential order. An alternate scheme for the reaction is that the binding steps are independent processes which occur in parallel. The expression which can be derived from the parallel formulation is similar to that for the series formulation, as long as the voltage-independent affinity constant is much larger than the voltage-dependent constant. While it is not possible to distinguish between the two reaction schemes in these experiments, the physical interpretations of the data corresponding to the two models are similar.

Now consider the second possible mechanism for voltage-dependent inhibition. This mechanism assumes that the binding reaction itself is voltage independent, but the steps which provide and remove reactive channels produce an indirect voltage sensitivity of QX-inhibition. These steps, which
precede and follow the QX binding reaction, are the opening and closing of free and complexed sodium channels, symbolized by the vertical arrows in the general scheme for QX binding shown before. The gating of normal sodium channels is voltage dependent. If the voltage gating of the channels blocked by QX differs significantly from the gating of unblocked channels, then the vertical steps of the general scheme will have different equilibria at different voltages. In such a situation the QX complexing reaction will lie further towards completion when a series of depolarizing conditioning pulses opens blocked channels (C·QX) less than unblocked channels (C). The complexing reaction is then indirectly responsive to voltage, and is most responsive to those voltages for which the gating of complexed channels differs most from the gating of uncomplexed channels.

In contrast, the scheme which postulates a direct effect of voltage on the binding reaction assumes the voltage gating of blocked channels is the same as that of unblocked channels. The evidence does not permit the elimination of either mechanism. However, the indirect effect of voltage on the QX-inhibition seems the less probable mechanism for the following reason. Since the observed inhibition is most sensitive to conditioning voltages above +30 mV, the indirect mechanism requires that this be the voltage range in which gating of blocked channels differs most from that of unblocked channels. Gating differences can arise from $h$ gates or $m^3$ gates. The data of Fig. 8, summarizing results of the unblocking of complexed channels, imply that the $h$ gate of blocked channels has a voltage dependence very similar to that of unblocked channels. With regard to $m^3$ gates complexed channels become unblocked by reversing pulses which depolarize the membrane to $-45$ mV, so $m^3$ gates of QX-complexed channels are presumably open at this voltage. The $m^3$ gates of unblocked channels also have a strong voltage dependence at $-45$ mV, but change little above 0 mV. Furthermore, in other situations where gating functions are shifted by changes in external conditions, such as increases in external $H^+$ or $Ca^{2+}$, the $m^3$ function is always shifted in the same direction as the $h$ function (see Frankenhaeuser and Hodgkin, 1957; Hille, 1968). And if QX blocking shifts the $h$ function at all, it shifts it towards more negative membrane potentials (compare Figs. 6 b and 8 b). Hence, it seems unlikely that the $m^3$ gating of blocked channels differs from that of unblocked channels between $+30$ and $+75$ mV. For these reasons the indirect mechanism for voltage sensitivity seems a less probable factor; the direct effect of voltage on the stability of the QX-channel bond appears as the more probable explanation.

Interpretations of the Model

Throughout this paper the passive sodium conductance mechanism has been referred to as the sodium channel. Recent studies suggest that both sodium (Hille, 1970, 1972) and potassium (Armstrong, 1971) conductance mech-
anisms in nerve are pores through the axon membrane small enough to allow the discriminate passage of the appropriate ions. In this discussion emphasis is placed on the interpretation of the QX binding data which supports and extends the detailed existence of such ionic channels.

One interpretation of the sequential model is that there are two binding sites for QX molecules in the sodium channel. The first binding site has a much higher affinity for QX than does the second site. This first site is located near the axoplasmic opening of the channel and lies outside the region of the channel which can support an electric potential gradient. At this site the affinity for QX is unaffected by changes in the membrane potential. Still, the binding of QX to these high affinity sites, which accounts for the tonic phase of inhibition, has some requirement that the m³ gate of channels be open since it occurs much more rapidly when the membrane is depolarized transiently from $-75 \text{ mV}$. The second binding site has a much lower affinity for QX and is located well within the channel.

A different interpretation is that the QX receptor lies within the axon membrane phase yet not within the sodium channel. Presumably, QX molecules would bind to part of the sodium channel itself or possibly to the voltage sensor of the channel gate. The voltage dependence of the kinetics of QX binding would be attributed to an alteration of the binding site, such as a conformational change, with voltage changes. The dependence of steady-state inhibition on membrane potential could be explained exactly as it was for the in-channel binding. Although the greater inhibition by internally present QX is most parsimonisously explained by a model which limits the approach of external QX because of channel dimensions, the other, extra-channel model, cannot be discounted from presently available data.

**Asymmetry of Sodium Channels**

Quaternary lidocaine derivatives block sodium currents primarily from the axoplasmic side of the nodal membrane, in agreement with their action on squid axon (Frazier et al., 1970). This observation is analogous to the differential action of tetrodotoxin, which inhibits sodium currents from the outside of the squid axon but has no activity when present in the axoplasmic space (Narahashi et al., 1966). Apparently the internal and external openings of sodium channels are not very similar.

Two properties of sodium channels are the ability to open and close in response to different membrane potentials and the ability to determine which ions can pass through open channels. These separate functions, gating and ion selectivity, are most simply attributed to separate structures arranged in series in the channel. A size for the selectivity filter has been proposed from studies of permeant cations; it is $3 \times 5 \text{ Å}$ (Hille, 1971, 1972). Neither triethyl (QX-314) nor trimethyl (QX-222) lidocaine molecules can extend farther than $1.5 \text{ Å}$ into a filter with these dimensions. If part of the inhibition for
internal QX arises from drug molecules located within the sodium channel, the selectivity filter must be further from the axoplasmic side of the sodium channel than the QX binding site. The observation that \( h \) gates must be open for voltage-sensitive binding of internal QX compounds suggests that the \( h \) gate is between the axoplasmic surface and the binding site. The \( m^2 \) gate is probably located in the same region since it must be open for the reversal of voltage-sensitive binding. The overall order from inside to outside is then: gate, QX binding site, and selectivity filter.

The results of Rojas and Armstrong (1971) also suggest the location of the \( h \) gate adjacent to the axoplasmic membrane surface. They showed that normal inactivation of sodium channels in squid could be removed by perfusion of the axoplasmic space with the proteolytic enzyme pronase, whereas pronase in the bathing medium had no effect. Furthermore, quaternary lidocaine inside the node increases values of \( \tau_h \) at all membrane potentials, while causing only small shifts in the voltage dependence of the sodium conductance factors, \( m^2 \) and \( h \). QX molecules seem to act directly on the inactivating mechanism of unblocked sodium channels while affecting the voltage-sensing function only nominally.

**Two Other Voltage-Dependent Inhibitions**

Other investigators have postulated voltage-dependent binding reactions in ionic channels. Armstrong (1966, 1971) noted in voltage-clamp experiments on squid axons that inhibition of outward potassium currents by internal tetraethylammonium ions increased as the axon was depolarized. He proposed a voltage-dependent reversible reaction of tetraethylammonium ions with open potassium channels although he did not derive an explicit analytical expression for the inhibition.

Woodhull (1972) has proposed a voltage-dependent model to explain her observations on the inhibition of sodium currents by \( H^+ \) and \( Ca^{2+} \). Both \( H^+ \) and \( Ca^{2+} \) ions exhibit voltage-sensitive blocking activity when present at sufficient concentrations outside the node of Ranvier. Woodhull's model is very similar to the one developed here except that there is no requirement for open channels. Blocking is accounted for by the binding of ions at a negative (acidic) site which lies 20-30% of the way through the sodium channel from the outside. This location for an anionic site is within the range of possible locations for the QX binding site.

**Appendix**

**Derivation of Equations for Voltage-Sensitive Binding Reactions**

To explain the voltage-sensitive behavior of QX inhibition a model for equilibrium binding of drug to membrane site has been postulated. According to this model the
voltage-sensitive inhibition of sodium currents in the steady state depends on the equilibrium between QX-complexed and uncomplexed sodium channels (see Discussion). In discussing this equilibrium it is easiest to consider the binding reaction alone:

\[ C_o + QX \xrightarrow{\Delta E} C_o\cdot QX \]

where \( C_o \) refers to open sodium channels. When a term in \( \Delta E \), the potential difference affecting the reaction, is added to the free energy the equilibrium constant for the reaction becomes voltage dependent:

\[ K_a = \frac{[C_o\cdot QX]}{[C_o][QX]} = K_o \exp \left(-\frac{\Delta G(E)}{RT}\right), \tag{1} \]

where \( K_a \) is the observed association constant, \( K_o \) is the association constant when there is no membrane potential, \([C_o\cdot QX]\) is the number of open channels bound by drug, \([C_o]\) the number of open, unbound channels, and \([QX]\) the drug concentration at the binding site. \( \Delta G(E) \) is the voltage-dependent part of the free energy, \( R \) is the gas constant, and \( T \) the temperature (° Kelvin).

Since the model assumes that both unblocked and blocked channels open and close normally, \([C_o] = m^h[C] \) and \([C_o\cdot QX] = m^h[C\cdot QX] \), where \([C] \) and \([C\cdot QX] \) are the number of unblocked and blocked channels, respectively. Hence, Equation 1 can be written

\[ K_a = \frac{[C\cdot QX]}{[C][QX]} \cdot \tag{3} \]

The simplest form of \( \Delta G(E) \) is one which is linear with \( \Delta E \). Physically this could correspond to the coulombic influence of the membrane potential on some charged reactant or product species. Since the quaternary lidocaine is a monovalent cation and a reactant, I assume that the voltage-dependent free energy term is also proportional to the charge on this ion and has the form:

\[ -\Delta G(E) = \frac{ZF \delta E_o}{RT}, \tag{2} \]

where \( Z \) is the charge on the reactant, in this case equal to +1, \( F \) is Faraday's constant, \( \delta \) is the fraction of the applied membrane potential \( (E_o) \) which affects the reaction, \( E_o \) is equal to the conditioning voltage which induces the steady-state inhibition. At the experimental temperature (6°C) \( RT/F \) is equal to approximately 24.0 mV.

An expression for the number of channels not blocked by QX at any conditioning voltage \([C] \) can be derived from the previous assumptions plus the condition that the sum of blocked and unblocked channels always equals \([C]_{\text{total}} \):

\[ [C] = \frac{[C]_{\text{total}}}{1 + K_a[QX] \exp \left(\delta E_o/24\right)}. \tag{3} \]
Currents measured after voltage conditioning are proportional to the number of unblocked channels. The ratio of peak sodium currents flowing at the same test potential \(E_t\) through a membrane which has been conditioned at two different conditioning potentials, \(E_1\) and \(E_2\), is given by

\[
\frac{I_t(E_1)}{I_t(E_2)} = \frac{1 + K_s QX \exp (\delta E_2/24)}{1 + K_s QX \exp (\delta E_1/24)}.
\]

(4)

With appropriate values for \(\delta\) and \(K_s\), Equation 4 satisfactorily describes the voltage-sensitive phase of inhibition alone. However, it does not account for tonic inhibition. When the parameters \(\delta\) and \(K_s\) are adjusted to account for the tonic phase, the voltage dependence of the function becomes quantitatively incorrect. To account for both the tonic and voltage-sensitive phases one more reaction must be postulated in the model. This reaction is a voltage-independent binding of QX to the channels. One way to allow for this modification is with the reaction scheme:

\[
\begin{align*}
C_0 + QX & \xrightarrow{K_1} C_0 \cdot QX^1 \xrightarrow{K_2(\Delta E)} C_0 \cdot QX^2,
\end{align*}
\]

where \(K_1\) is a voltage-independent, \(K_2(\Delta E)\) a voltage-dependent binding constant. By applying the same assumptions used in deriving Equation 4 the expression for the ratio of peak currents after conditioning at two different potentials becomes:

\[
\frac{I_t(E_1)}{I_t(E_2)} = \frac{1 + [QX][K_1^2 + K_1 K_2 \exp (\delta E_2/24)]}{1 + [QX][K_1^2 + K_1 K_2 \exp (\delta E_1/24)]}.
\]

(5)

The extent of the tonic phase of inhibition can be calculated from Equation 5. Tonic inhibition is given by the fraction of all the sodium channels which are blocked in the steady state at \(-75\) mV membrane potential:

\[
\frac{[C \cdot QX_1] + [C \cdot QX_2]}{[C_{total}]} = \frac{[QX][K_1^2 + K_1 K_2 \exp (-758/24)]}{1 + [QX][K_1^2 + K_1 K_2 \exp (-758/24)]},
\]

(6)

where \([QX]\) is the drug concentration at the first binding site.

I wish to acknowledge support and encouragement from Dr. Bertil Hille.

This work is supported by U.S. Public Health Service Grants NS 05082, NS 08174, and FR 00374.

Received for publication 14 July 1972.

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