

Effects of Strychnine on the Potassium Conductance of the Frog Node of Ranvier

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ABSTRACT The nature of the block of potassium conductance by strychnine in frog node of Ranvier was investigated. The block is voltage-dependent and reaches a steady level with a relaxation time of 1 to several ms. Block is increased by depolarization or a reduction in $[K^+]_o$ as well as by increasing strychnine concentration. A quaternary derivative of strychnine produces a similar block only when applied intracellularly. In general and in detail, strychnine block resembles that produced by intracellular application of the substituted tetraethylammonium compounds extensively studied by C. M. Armstrong (1969. *J. Gen. Physiol.* **54**: 553-575. 1971. *J. Gen. Physiol.* **58**: 413-437). The kinetics, voltage dependence, and dependence on $[K^+]_o$ of strychnine block are of the same form. It is concluded that tertiary strychnine must cross the axon membrane and block from the axoplasmic side in the same fashion as these quaternary amines.

INTRODUCTION

Strychnine recently has been shown to affect both sodium and potassium currents in the squid axon membranes (Shapiro et al., 1974). Earlier experiments had shown that strychnine has some effects on the action potentials in toad myelinated fibers (Maruhashi et al., 1956). This present paper is intended to extend the study to the effects of strychnine on a voltage-clamped frog node of Ranvier. It is further designed to answer several questions raised by the previous work on squid axons. First is whether strychnine produces its voltage- and time-dependent block of potassium channels in a manner resembling that of certain quaternary ammonium ions (Armstrong, 1971). These ions act from the inside of the axon, moving into potassium channels after the channels are open for ion flow. The block is counteracted by the external potassium ions. These compounds act similarly on frog nodes of Ranvier (Armstrong and Hille, 1972).

Armstrong (1971) has estimated rate constants for the blocking reaction. The present paper reports similar experiments and analyses to compare the details of strychnine blockage with that produced by the quaternary ammonium (QA) ions.

MATERIALS AND METHODS

The methods for voltage clamping the frog (*Rana pipiens*) node of Ranvier are those of Hille (1971). This is a modification of the voltage clamp apparatus of Dodge and

Frankenhaeuser (1958) and involves the use of four fluid pools separated by three Vaseline gaps. Two high-bandwidth, high-gain feedback amplifiers control the voltage in two of the pools. Current is measured by the change in voltage in one of the end pools. The internode in that pool serves as a resistive lead and the conversion of measured pool voltage into current depends on measurement of the resistance of that lead.

Since the axon is cut in the two end pools, the internal composition at the start of the experiment can be varied by altering the contents of these pools. As a rule, the axon is cut in isotonic (120 mM) KCl. In a few experiments *N*-methylstrychnine (NMS) was added to one end pool after initial voltage clamp data were obtained. In these cases the axon in the current-passing pool was recut in a new solution containing NMS. Since the resistance of the current-passing pathway was changed by this procedure, recalibration was necessary. Several minutes are required for the NMS to diffuse the approximately 500 μm from the cut end to the region inside the node.

The standard Ringer's solution bathing the node contained 115 mM NaCl, 2 mM KCl, 2 mM CaCl_2 , and 2 mM Tris (hydroxymethyl) aminomethane buffer, pH 7.4. In high-KCl solutions, some of the sodium was replaced by potassium. The external calcium and buffer concentrations were maintained throughout. Experiments on potassium currents were performed with 200 nM tetrodotoxin (TTX) (Sigma Chemical, Corp., St. Louis, Mo.). Contamination with the sodium channel current was minimal. Leakage current, along with some capacity current, was subtracted electronically in the manner of Armstrong and Hille (1972). The measured leakage value was used to obtain the resistance of the axon lead from the current pool. All measurements were at 10°C.

Strychnine base and strychnine sulfate were obtained from ICN K & K Laboratories Inc., Plainview, N.Y. Strychnine contains a tertiary nitrogen with a pK_a of 7.45 in 120 mM KCl. We had previously postulated (Shapiro et al., 1974) that strychnine acts in the charged form on the inside of the membrane. Since strychnine is effective when applied externally we proposed that it crosses the membrane, probably largely or entirely in the uncharged form. In order to test this hypothesis I synthesized a simple quaternary derivative, *N*-methylstrychnine by dissolving the free base in a large excess of iodomethane (Eastman Kodak Corp., Organic Chemicals Div., Rochester, N.Y.). The reaction occurs at room temperature and yields a fine precipitate which was filtered and washed copiously with cold methanol. The precipitate was then dissolved in water and an aliquot was titrated. No group with a pK_a between pH 5 and pH 9 was found. The precision of the titration was such that unreacted strychnine must have been <0.1% of the product.

RESULTS

Strychnine Blocks Potassium Channels

The block of potassium channels by strychnine closely resembles that seen in squid axons. A typical family of currents following steps from -80 mV to more positive potentials is seen in Fig. 1. For depolarizations to positive membrane potentials, the currents have a characteristic shape, first rising to a peak with kinetics close to that of control currents and then declining with an exponential time course to a low plateau level. The heights of the peaks and plateaus as well as the exponential decay rates are all functions of step voltage.

In an earlier paper on squid giant axons (Shapiro et al., 1974) the parameter extracted from such records of I_K was the ratio I_K peak/ I_K control (steady state) as a function of E_m . However, if the dip in I_K is due to some first-order or pseudofirst-order block of the channels by strychnine, then the plateau level of I_K is a measure of the equilibrium value of the block, and it is the ratio of these

steady currents rather than the ratio of peak currents which is of theoretical interest. The peak I_K value is a function of the equilibrium level of the block as well as of the relative time constants of this block and of the potassium activation.

A plot of the steady-state currents before and after application of 1 mM strychnine is shown in Fig. 2 and a plot of the calculated potassium conductances for the same experiment is shown in Fig. 3. The block is voltage dependent. At this strychnine concentration there is little block of I_K at negative inside potentials. In contrast, with the highest depolarizations there is a 90% block of I_K . The shape of the I_K-E_m curve after strychnine is variable. The magnitude of a negative slope region (as in Fig. 2) depends on the axon. However, nearly all axons exhibit a negative slope conductance above 100 mV, and often above 40 mV (Fig. 2). The shape of the G_K-E_m curve after strychnine is more consistent. There is always a segment over which G_K decreases approximately exponentially with increasing depolarization.

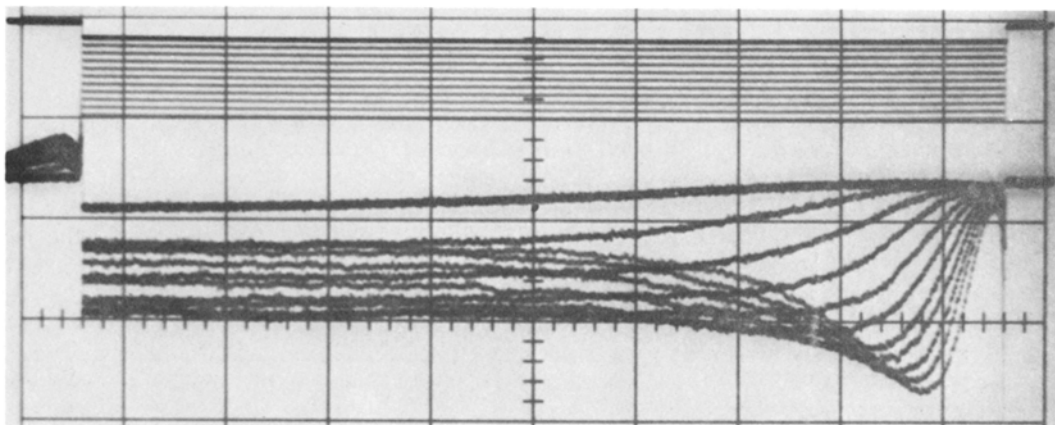


FIGURE 1. *Top traces*, Family of potassium currents in node treated with 1 mM strychnine. *Bottom traces*, Voltage records. Holding potential, -80 mV; steps to -48 , -32 , -16.0 , . . . 112 mV. Grid: 1.1 nA \times 2 ms.

We define Y_∞ as the fraction of unblocked channels in the steady state. Then the steady-state block with strychnine is $1 - Y_\infty$. The voltage dependence of block is graphed in Fig. 4.

In this and all other cases the observations are fit reasonably well by equations of the form:

$$Y_\infty = 1/1 + \exp[(E_m - E_A)/E_B], \quad (1)$$

where E_m , E_A , and E_B are in millivolts; E_A and E_B are constants which vary slightly from node to node; E_A is the value of the midpoint of the sigmoid curve; and E_B is inversely related to the steepness of the sigmoid. The continuous line in Fig. 4 is the solution of Eq. (1) with $E_A = 46$ mV and $E_B = 38$ mV.

The constants were calculated for seven axons bathed in 1 mM strychnine and the results are tabulated in Table I.

This equation can be derived from the Boltzmann equation where the propor-

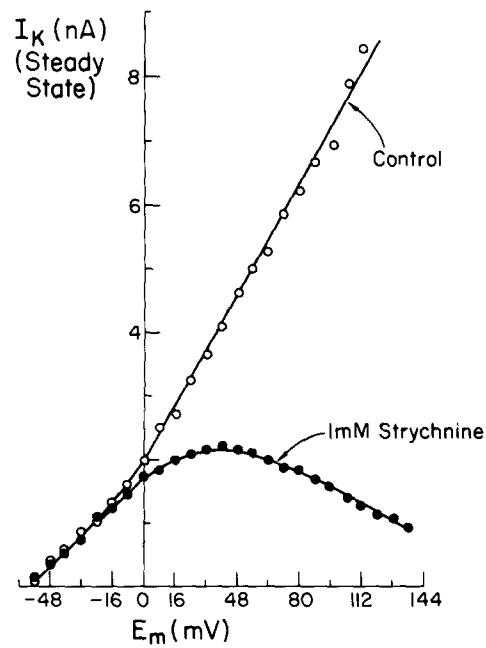


FIGURE 2. Potassium currents before and after strychnine. Open circles, controls; closed circles, 1 mM strychnine.

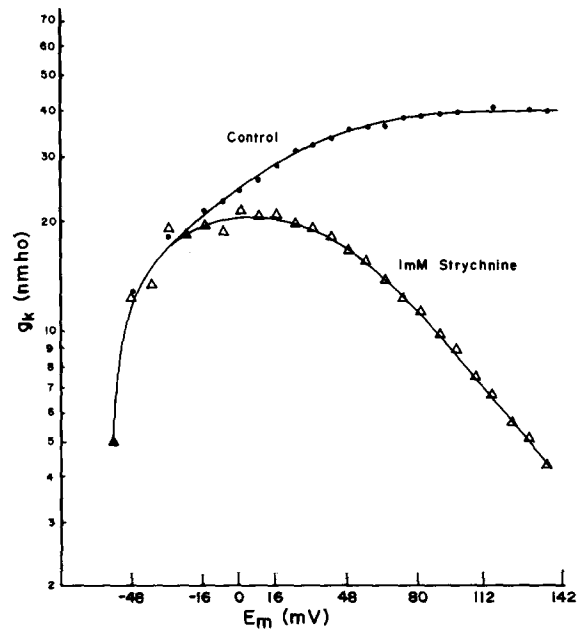


FIGURE 3. Conductances from same experiment as in Fig. 2. Calculated assuming $E_K = -80$ mV. Circles, control; triangles, 1 mM strychnine.

tion of molecules at a blocking site in the channel, $1 - Y_\infty$, is related to potential by the following equation:

$$1 - Y_\infty = 1/1 + \exp [(W + ze\delta E_m)/kT], \quad (2)$$

where W is the work required to move the strychnine from the axoplasm to the

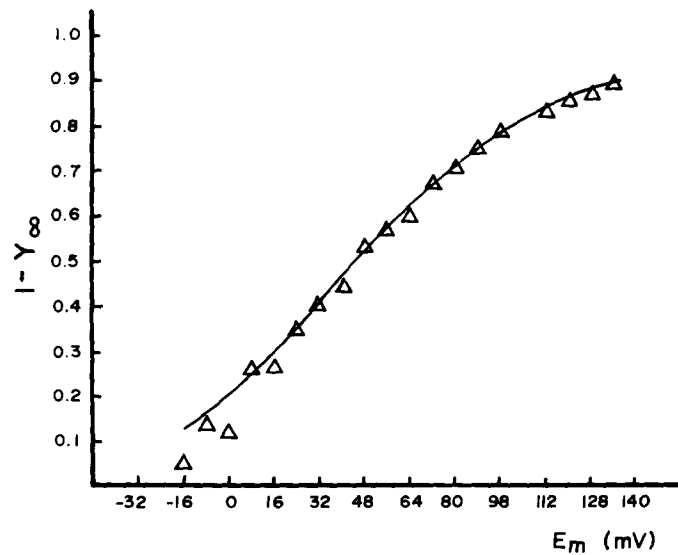


FIGURE 4. Potassium channel block ($1 - Y_\infty$) as a function of membrane potential. Same experiment as Figs. 2 and 3. Smooth curve calculated from Eq. (1) in text.

TABLE I
CONSTANTS CALCULATED FOR SEVEN AXONS BATHED IN
1 mM STRYCHNINE

	E_A	E_B
	mV	mV
	46	38
	58	40
	62	36
	40	41
	68	53
	65	33
	15	40
Mean (\pm) SD	50.6 \pm 18.7	40.1 \pm 6.3

blocking site when $E_m = 0$; z is the valence of strychnine; e is the electron charge; δ is the fraction of the membrane field through which strychnine must be moved to block the channel; k is Boltzmann's constant; and T is the absolute temperature. At 10°C, $kT/ze = 25$ mV.

Increasing the strychnine concentration decreases E_A . There is no obvious

systematic change in E_B . For lower strychnine concentrations Eq. (1) may cease to be valid since the block remains incomplete even at very positive voltages, i.e. the high voltage asymptote for $1 - Y_\infty$ is less than 1.

The exponential decay rates of the currents are themselves voltage dependent. Inspection of the current time courses in Fig. 1 shows that for small

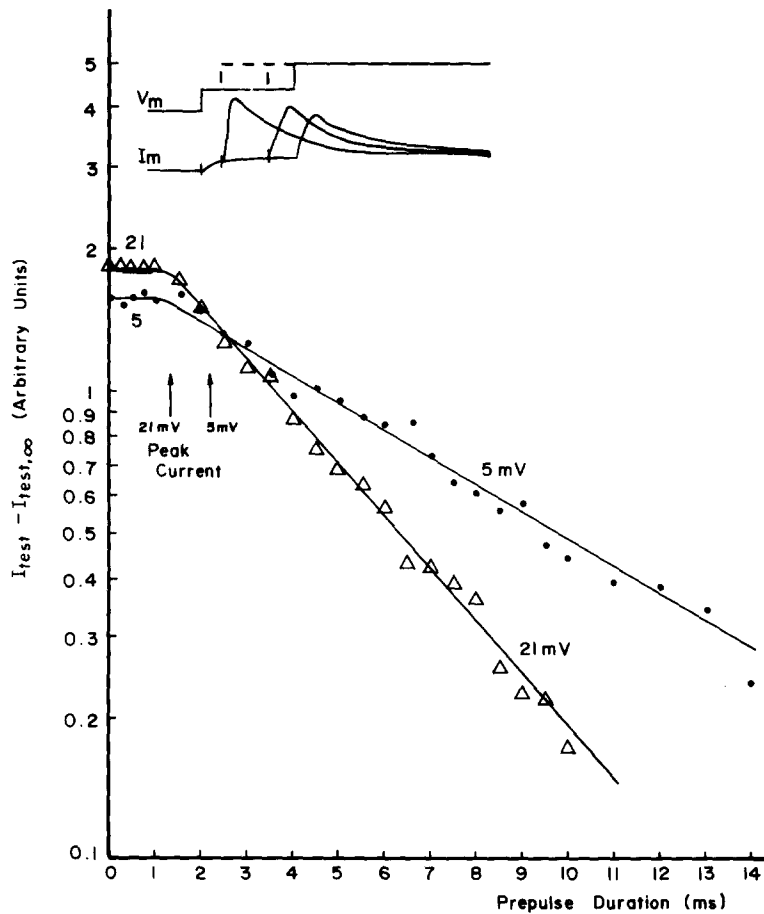


FIGURE 5. Potassium channel block as a function of pulse duration. I_{test} is value of peak test current. $I_{test,\infty}$ is I_{test} for very long conditioning pulses. Pulses sequence as in inset. I_m in inset is not data but for illustration only. Circles, 5 mV (E_m) prepulse; triangles, 21 mV (E_m) prepulse. Arrows indicate time of prepulse peaks.

depolarizations, to negative E_m , there is no hump in the current trace. The final current values are approached monotonically although they are significantly reduced from the controls. For example, equilibrium currents at -8 mV are reduced by 15%. Although the decay time constants vary with the axon, typical values from the currents plotted in Fig. 5 are: 48 mV, 2.7 ms; 72 mV, 1.5 ms; 88 mV, 1.1 ms; 200 mV, 1.1 ms. A saturation of the blocking rate at high voltages was observed in all cases. The limiting value in nine cases of nodes bathed in 1

mM strychnine ranged between 0.8 and 1.2 ms. The block is faster at higher strychnine concentrations, but no quantitative estimation of this effect was made.

The block does not begin at the onset of depolarization. In order to study the development of block more clearly, strychnine-treated nodes were depolarized in two steps. The first, smaller, conditioning step was of variable duration. The second, test step was to a potential at which the potassium activation was several times faster than the strychnine block, usually +80–90 mV. The equilibrium potassium current was unaffected by the conditioning pulse. In contrast, the peak test current varied with the duration of the conditioning pulse. After long conditioning pulses the peak test current approaches a minimum value ($I_{\text{test}, \infty}$). After very short conditioning pulses there was little reduction in peak test current. The value $I_{\text{test}} - I_{\text{test}, \infty}$, is plotted against prepulse duration for two prepulse potentials in Fig. 5. On a semilogarithmic plot the two functions closely approximate a straight line for prepulse durations over 3 ms, indicating that block develops exponentially. Conditioning pulses shorter than 1 ms do not reduce the height of the test pulse. The delay observed in the case of strychnine block corresponds roughly to the time to peak current in the conditioning pulse. The delay is less after larger depolarizing conditioning pulses.

An experiment similar to that in Fig. 5 was performed except that the amplitude rather than the duration of the conditioning pulse was varied. All conditioning pulses were 25 ms long, sufficient to attain a near-equilibrium block level. The ratio of peak test pulse to that seen with no conditioning pulse was calculated and subtracted from unity. The resulting value, representing the fraction of channels blocked during the conditioning pulse, is plotted against conditioning pulse voltage in Fig. 6. The function is sigmoid and strongly resembles that of Eq. (1). It is consistent with a model in which the prepulse determines the number of blocked channels. The same experiment also provides information on the equilibrium potassium conductances as a function of conditioning pulse voltage. Instead of measuring the peak test current, I took the current flowing immediately after the step to the test voltage. This function should resemble the conductance-potential curve calculated from the plateau levels of the partially blocked potassium currents. Such measurements describe a curve (Fig. 7) which is almost superimposable (except for a voltage shift) on the steady-state conductance-voltage curve in Fig. 3, obtained from a different node.

The strychnine block appears to be a genuine conductance decrease. The block developing at one voltage reduces the current seen in steps to other voltages, although any change in voltage will eventually alter the degree of block and, of course, the number of activated channels. The block develops with first-order kinetics with one major qualification. There is a 1 to several ms lag before the blocking reaction commences.

The Block Depends on $[K]_o$

So far, a dependence on voltage, time, and strychnine concentration has been described. Block also depends on the external concentration of potassium ion. Other ions such as sodium, lithium, and tetramethylammonium seem to be

without effect. A plot of I_K (plateau levels) against E_m for solutions with constant (either 2 mM or 10 mM) $[K]_o$ but with widely (0–117 mM) varying concentrations of sodium, lithium, or tetramethylammonium ions show all points lying along the same curve. The potassium channel block is insensitive to these ions. In

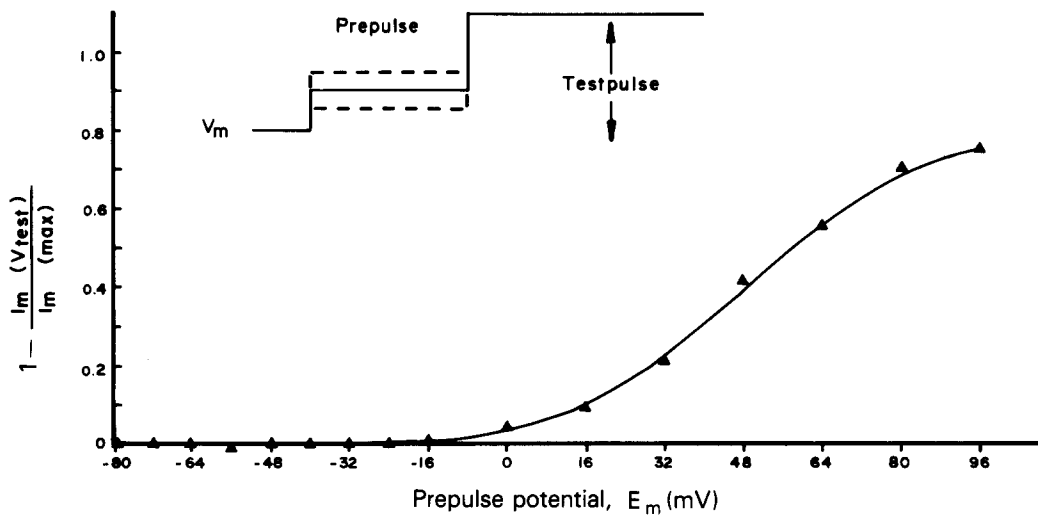


FIGURE 6. Peak amplitude of a test pulse after long conditioning pulses of variable amplitude. All values normalized. Pulse sequence as in *inset*.

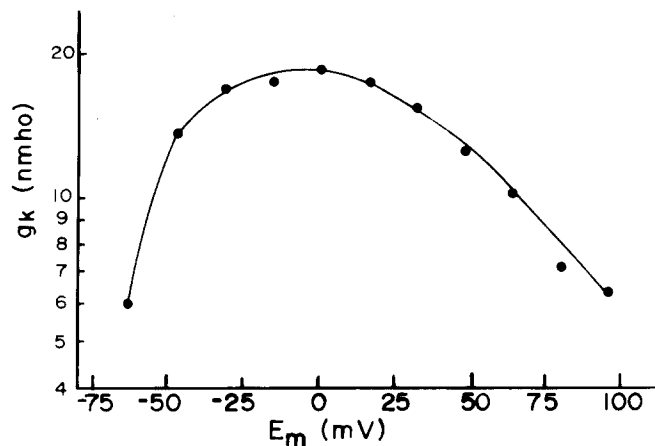


FIGURE 7. Potassium conductances after long conditioning pulse of variable amplitude in strychnine-treated node. G_K determined from "instantaneous" current upon repolarization to -112 mV.

contrast, block is decreased by elevating $[K]_o$. Frankenhaeuser (1962) showed in normal fibers that I_K varies with $[K]_o$ in a manner satisfactorily described by the "independence principle." I have confirmed this. Changes of $[K]_o$ from 10 to 60 to 120 mM in normal fibers produce no systematic change in current after

correction according to the equation. However, with nodes bathed in strychnine at different external potassium concentrations, the corrected currents were larger for greater $[K]_o$. One such experiment is shown in Fig. 8. The lower the external potassium the stronger the block of potassium channels. Qualitatively the effect is best shown at very positive membrane potentials. Under such conditions an increase in external potassium ions results in an increase in outward potassium current. This is of course a change in the direction exactly opposite to that predicted from the independence relation.

Potassium currents in high potassium and strychnine display another interesting feature which can be seen in Fig. 9. In normal fibers the tail currents after repolarization to -80 mV decline monotonically. But after strychnine they

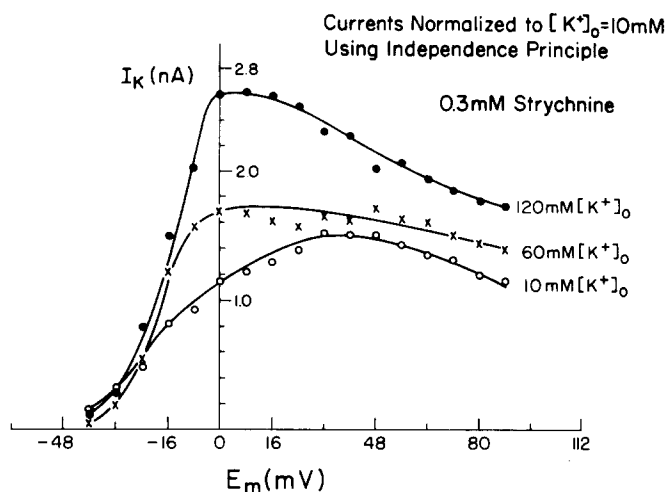


FIGURE 8. Potassium currents in 0.3 mM strychnine as a function of E_m for three values of $[K]_o$. All currents normalized to $[K]_o = 10$ mM by independence principle. Filled circles, 120 mM; crosses, 60 mM; open circles, 10 mM.

increase to a peak and then smoothly decline. The decline is also slower than that of the control.

N-Methylstrychnine Produces a Voltage-Dependent Block when Applied Internally

The charged quaternary derivative of strychnine should cross membranes less readily than the parent compound. If its action resembles that of strychnine it should provide an opportunity to determine whether strychnine acts from the inside or outside of the membrane. Current-voltage measurements were made on a normal node and then NMS was introduced into one end pool and the axon was recut. Fig. 10 shows a tracing of the potassium current 20 min after exposure to NMS. The current shows the peak and subsequent exponential decay to a plateau characteristic of external strychnine application. Since only $50 \mu\text{M}$ NMS was applied to the cut end of the fiber, the actual level at the inner surface of the nodal membrane was probably smaller. The voltage-dependence of the conductance block can be seen in Fig. 11. In this experiment the axon was actually cut

twice in two different NMS concentrations. The equilibrium current-voltage curve shows a clear negative slope region at high potential after introduction of NMS at concentrations below 1 mM. In contrast, NMS applied externally never produces a voltage-dependent block or a hump or peak of the potassium currents. If the external concentration is greater than 500 μM some relatively

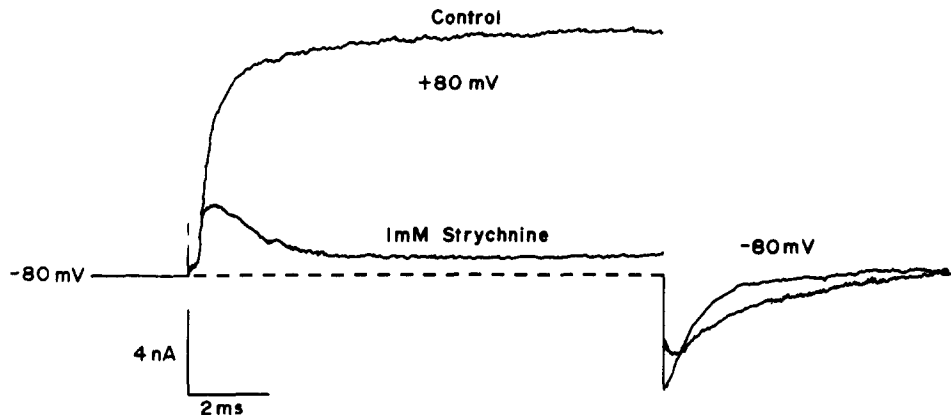


FIGURE 9. I_m before and after application of 1 mM strychnine. $[\text{K}]_o = 60 \text{ mM}$. Pulse to +80 mV. Dashed line is zero current.

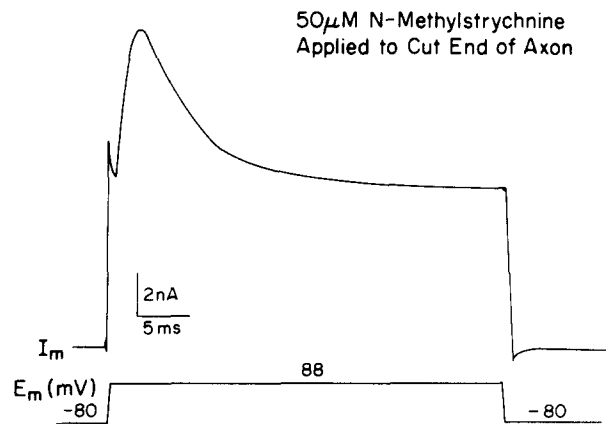


FIGURE 10. Potassium current 20 min after recutting axon in 50 μM NMS.

voltage-independent block is produced (Fig. 12). As in untreated nodes the potassium conductance in such NMS-treated nodes approaches a maximum at large membrane potentials.

Strychnine Can Washout Rapidly

Although there is evidence that strychnine may block from the inside of the axon, in most cases it can be removed rapidly upon washing. Such a washout is shown in Fig. 13. The node had been exposed to $5 \times 10^{-4} \text{ M}$ strychnine for 17

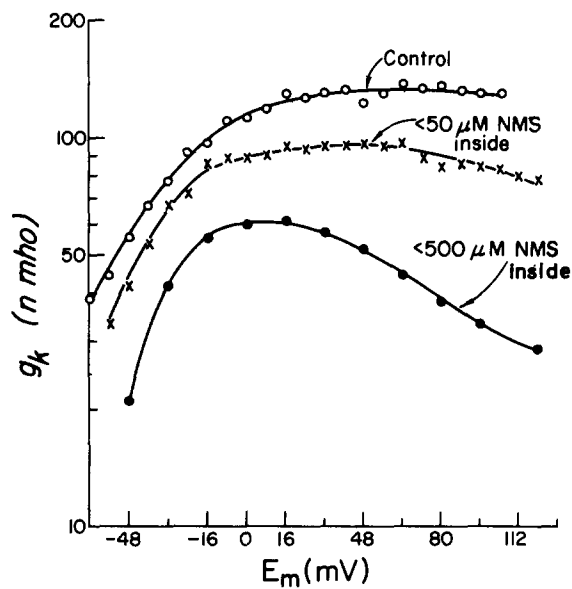


FIGURE 11. Potassium conductance as a function of E_m for an axon before cutting in NMS (open circles); 30 min after cutting in 50 μM NMS (crosses); and 15 min after recutting again in 500 μM NMS.

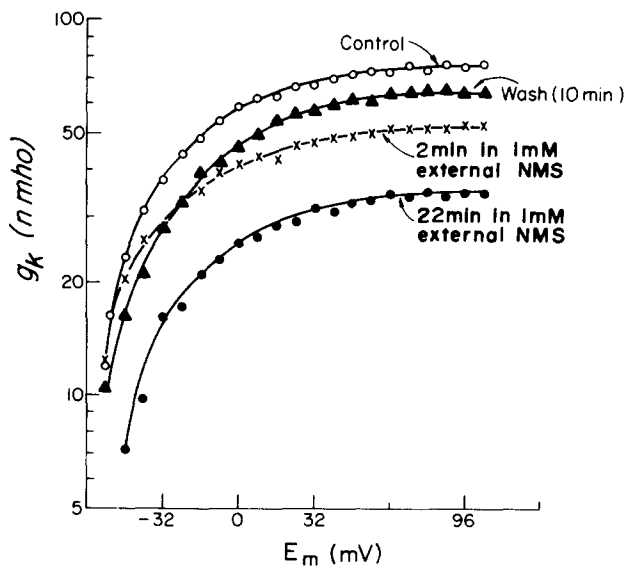


FIGURE 12. Potassium conductance as a function of membrane potential before (open circles), 2 min after (crosses), and 22 min after (filled circles) application of 1 mM NMS to bath. Filled triangles, 10 min after wash.

min. Then it was washed and the recovery monitored by measuring the equilibrium potassium current to a pulse to 48 mV every 10 s. The smooth curve is for a simple exponential removal with a time constant of 125 s. This is close to that for removal of TTX but several orders of magnitude slower than for the removal of tetraethylammonium ion (TEA).

DISCUSSION

Strychnine produces a voltage- and time-dependent block of both sodium and potassium channels. The quaternary derivative causes a similar block when applied internally. The block of potassium channel approaches an asymptotic value exponentially after an initial lag period. The exponential time course

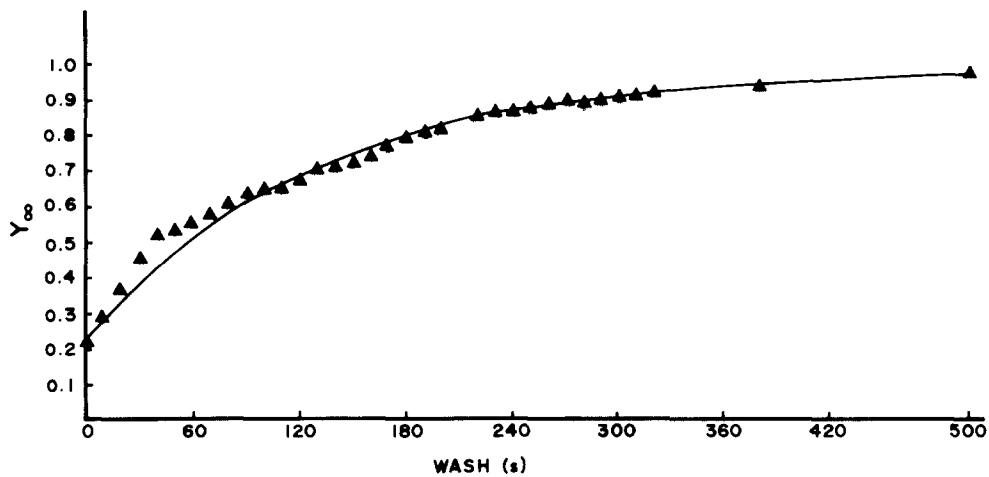
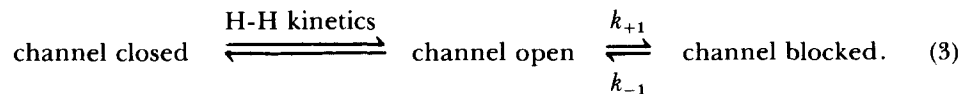


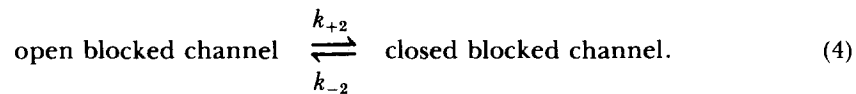
FIGURE 13. Rate of recovery from block produced by exposure to 0.5 mM strychnine for 17 min. Test pulse to 48 mV was repeated every 10 s. Smooth curve would be produced if the absolute rate of removal of strychnine declined exponentially with time. Time constant of decline is 125 s.

suggests a first-order or pseudofirst-order process. The lag time is consistent with a requirement that the channel be open before it can be blocked.

The simplest form of Armstrong's (1969) model for the block of potassium channels by internal QA ions is:



Armstrong concluded that only open channels could be blocked. Conversely, blocked channels had to be unblocked before they could close. In a later paper (Armstrong, 1971) Armstrong has proposed an additional reaction:



However, at -60 mV and standard $[K]_0$, k_{+2} is 0.01 ms^{-1} and this reaction is less significant than the blocking reaction. To a first approximation Eq. (3) gives a reasonable fit of the data. The blocking reaction is assumed to occur with the QA in great excess and thus is pseudofirst order, much like an enzyme-substrate reaction with an excess of substrate. The model predicts that as the concentration of QA is increased the rate of blocking should be increased and the peak and equilibrium currents decreased. The initial rate of rise of potassium currents should be unaffected.

The kinetic scheme in Eq. (3) also implies that if a sufficient number of channels is blocked, then upon repolarization there may be a transient increase in the tail current rather than a monotonic decrease. This would occur if the rate of channel unblocking exceeded the rate of closing and were predicated on the assumption that blocked channels must pass through an open state before closing. Another prediction is that the rate of decline in tail currents should be different in the presence of QA since the transition blocked-to-open may be rate limiting.

Nodes treated with strychnine resemble those treated with QA (Armstrong and Hille, 1972) in several basic features. In both cases two pulse experiments (Fig. 5; Armstrong, 1969) show that block begins after a delay and is part of the basis for the kinetic model which states that only open channels can be blocked. Also, in both cases potassium currents may reach a peak and then decline exponentially to a plateau level. For small depolarizing pulses or for low QA or strychnine concentrations the potassium current approaches its equilibrium state monotonically. Armstrong (1969) and Armstrong and Hille (1972) have modeled this behavior and demonstrated that the presence of a peak in the potassium current depends on the relative values of the normal H-H potassium rate constants, α_n and β_n and the QA blocking rate constants, k_{+1} and k_{-1} .

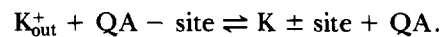
Armstrong (1971) found that an increase in QA concentration does increase the blocking rate and decrease the peak and equilibrium potassium currents. Strychnine has the same effect. Armstrong observed characteristic humps in the current tails after repolarization. These tail currents declined more slowly than those seen before QA perfusion. A similar change in the current tails is seen after exposure to strychnine.

The Block of Potassium Channels by QA or Strychnine is Voltage Dependent

The voltage dependence of the block is plotted in Fig. 4. The fit to the theory, Eq. (1), is reasonably good. The mean value of E_B from Table I is 40.1 mV. Since this is approximately $25 \text{ mV}/\delta$, δ , the fraction of the membrane field through which strychnine must be moved to block a channel, is about 0.62 . This lies between the values of δ calculated by Hille (1975 *a, b*), 0.20 for TEA, and 0.70 for methylammonium ion, for a simple single-site block by these compounds. Other models could account for the voltage dependence of the strychnine-induced block. In one model a charged particle in the membrane might have to move through the field to expose or position a strychnine binding site. In a third model a voltage-dependent reaction could compete or otherwise interfere with strychnine block. For example, if external potassium ions moved toward the

axoplasmic face of the membrane (presumably through ionic channels) to interfere with strychnine block we might observe a similar voltage dependence.

Armstrong (1971) found that recovery from block was accelerated by increasing the external potassium concentration. The kinetic model in Eq. (3) and (4) fits both the high and low $[K]_o$ cases. Increasing potassium increases k_{-1} but does not affect the other rate constants. Armstrong (1975) notes that this result indicates that potassium ions are displacing QA from the blocking site, not simply competing with them, but by a reaction of the form



However, the evidence which Armstrong (1971) presents demonstrates the increase in k_{-1} due to an increase in $[K]_o$ only for internally negative potentials, -60 mV and -100 mV, and his rate constants are therefore from recovery kinetics. The only other point tabulated is at $+90$ mV. In this case increasing $[K]_o$ had no effect on any calculated rate constants.

The experiments on the effect of $[K]_o$ on strychnine block did not include observations on the rate of recovery. Rather, the amount of block was compared at three different external potassium concentrations (Fig. 8). At potentials above -20 mV increasing $[K]_o$ increased Y_∞ . In the Armstrong kinetic equations this can result from a decrease in k_{+1} and/or an increase in k_{-1} . In order to calculate the rate constants, the rate of block as well as the extent of block must be measured. Because of experimental difficulties this was possible at only one potential in the experiment illustrated in Fig. 8. The time constant for the exponential approach to equilibrium block during a pulse to $+112$ mV was decreased from 1.1 to 0.9 ms when $[K]_o$ was increased from 10 mM to 110 mM. It is immediately clear, since this can be produced only by an increase in either or both unidirectional rate constants, that k_{-1} increases. Calculations of the rate constants, with no better than 10% precision gives the following values:

$$10 \text{ mM: } k_{+1} = 0.78 \text{ ms}^{-1}; k_{-1} = 0.09 \text{ ms}^{-1},$$

$$110 \text{ mM: } k_{+1} = 0.79 \text{ ms}^{-1}; k_{-1} = 0.28 \text{ ms}^{-1}.$$

This is consistent with the model which proposes that extracellular potassium ions displace strychnine from the blocking site, perhaps by electrostatic repulsion (Armstrong, 1975; Hille, 1975a). Strychnine may well differ from Armstrong's C_9 (nonyltriethylammonium ion) in that even the small inward potassium flux at $+112$ mV can interfere with its binding. Of course, at $+112$ mV in essentially isotonic $[K]_o$ the net potassium flux through open channels is outward. The displacement of strychnine from blocking sites by external potassium ions at that potential may indicate that the block is primarily affected by the unidirectional flux and is relatively insensitive to outward flux.

Of the four cations tested, only potassium interfered with block. This implies that either the inward flowing ion must pass the selectivity filter before getting sufficiently near the blocking site to displace strychnine, or the displacement reaction itself has a similar selectivity sequence.

The variable E_A in Eq. (1) should be a function of the dissociation constant of the blocking site-strychnine complex and the concentration of strychnine in the

axoplasm. An increase in strychnine concentration should move the curve to the left. This has been observed. An increase in the dissociation constant should move it to the right. If the concentration in the axoplasm is assumed to be the same as in the bath, then it is apparent that K_{diss} is a little above 1 mM because a positive E_m is required to produce 50% block. The K_{diss} of the blocking site can be calculated to be about 2.5 mM.

Since we know the equilibrium levels of block and the time constants of association at each of several voltages we can calculate the unidirectional rate constants for the proposed pseudofirst-order blocking reaction. Typical values for one node are shown in Table II.

We can utilize this information to make a rough and speculative estimate of the energy barriers presented to a strychnine molecule approaching or leaving a blocking site. I assume a single energy barrier for the blocking reaction and apply absolute rate theory as used for a single barrier by Woodhull (1973), and for a multibarrier reaction by Hille (1975*b*). The assumptions other than those in

TABLE II
UNIDIRECTIONAL RATE CONSTANTS FOR PROPOSED
PSEUDO FIRST-ORDER BLOCKING REACTION FOR ONE
NODE

E_m	k_{+1}	k_{-1}
mV	ms ⁻¹	ms ⁻¹
56	0.32	0.23
72	0.44	0.21
88	0.64	0.19
112	0.72	0.14
128	0.80	0.12

the rate theory itself are that k_{+1} and k_{-1} measured are those of the actual binding, the "transmission coefficient" of the reaction is 1.0, and the concentration of the strychnine in the axoplasm is the same as in the bath. We can then calculate two numbers at each voltage. U_{+1} is the voltage-independent barrier which axoplasmic strychnine must cross to reach the blocking site; U_{-1} is the voltage-independent barrier which strychnine at the blocking site must cross to reach the axoplasm. Since the rate constants are determined for $E_m \neq 0$, we must subtract the energy involved in movement of strychnine through the electric field. I assumed that the energy barrier is half-way down the field between the axoplasm and binding site. The energies U_{+1} and U_{-1} should be independent of the voltage if the correct δ is chosen. A value of about 0.5 gave a good fit, slightly better than the 0.6 calculated from the fit to the Boltzmann equation alone. The calculated values for U_{+1} and U_{-1} are 9.6 and -12.9 kcal/mol, respectively. The sum implies that the free energy of the strychnine-site complex is 3.3 kcal/mol less than that of unreacted strychnine in the cytoplasm (when $E_m = 0$)—a number consistent with a dissociation constant of about 2 mM.

The quaternary strychnine derivative NMS produces a voltage-dependent block only from the axoplasmic side of the node. Its action is so like that of strychnine that it is most probable that strychnine crosses the membrane in its

uncharged form and then acts at the axoplasmic surface probably in the cationic form. Fig. 14 compares the molecular structure of the most potent of the QA ions tested by Armstrong, phenylpropyl triethylammonium ion (ϕC_3) with NMS. The ϕC_3 molecule is almost superimposable on NMS except for the presence of a methyl group on NMS and an ethyl on ϕC_3 . ϕC_3 would be superimposable on *N*-ethylstrychnine.

NMS applied to the cut end of the axon has effects equivalent to strychnine applied at about 10 times the concentration in the bath. Either the concentration of intracellular strychnine is much lower than in the bath, or NMS is more potent or somehow concentrated as it travels down the core of the axon. If one ignores the last possibility this means that NMS and possibly strychnine, too, have significantly different k_{diss} , k_{+1} , and U_{+1} than those calculated above. k_{diss} and U_{+1} would be smaller, k_{+1} larger.

We have seen that strychnine block of potassium channels resembles that by QA ions and that, indeed, the molecular structure of the most potent QA, ϕC_3 , is nearly superimposable on that of strychnine. Armstrong has estimated the

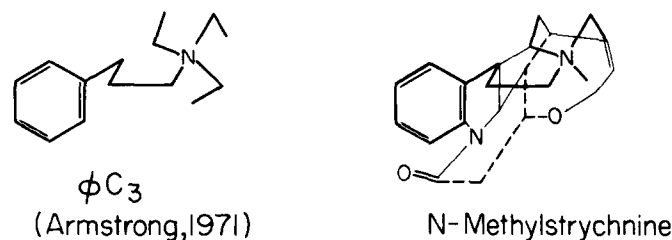


FIGURE 14. Structures of two potent potassium channel blockers, ϕC_3 and NMS. Common structure is drawn in more heavily in NMS.

value of the rate constants for several QA's. Typical values are, at 90 mV, $k_{+1} = 0.63 \text{ ms}^{-1}$, $k_{-1} = 0.03 \text{ ms}^{-1}$ for nonyltriethylammonium ion (C_9), and $k_{-1} = 0.60 \text{ ms}^{-1}$ for pentyltriethylammonium (C_5) ion. The general rule is that as the chain length of the R group of R-triethylammonium increases, k_{-1} decreases but k_{+1} is changed little if at all. Dissociation constants (measured at +50 mV) change with chain length and range: for straight chains, from 2 mM for C_1 to 5 μM for C_9 . ϕC_3 binds even more tightly than C_9 . By assuming that potassium and QA ions enter potassium channels with equal ease Armstrong has calculated the single channel conductance for potassium ions. The figure, about 3 pmho, is in reasonable agreement with other more direct measurements.

The estimates of the rate of strychnine block are subject to errors, the greatest of which is the estimation of axoplasmic strychnine concentration. Yet the agreement between the strychnine and QA constants is striking. The forward reaction rate is evidently similar for all the QA ions. The rate of removal of strychnine is between that of C_5 and C_9 . As for C_9 , external potassium ions only affect k_{-1} .

The parallels are so extensive that I conclude that strychnine must be blocking the channels by the same mechanism. Although a tertiary amine, strychnine must be crossing the membrane and, in its charged form, mimicking QA ions.

The actual mechanism of QA blockage is not known and the strychnine data may add a little to our efforts in trying to solve the problem. The strychnine data ordinarily are well fit (Fig. 4) by Eq. (1) and the assumption of a single site. However, there are two consistent deviations from prediction. First, for >1 mM strychnine the block at negative potentials is less than expected from fitting Eq. (1) to the data at potentials greater than 35 mV. This slight systematic deviation probably could be better fit by a two-site model (see Hille, 1975*a*), but this has not been done.

Second, the block at low strychnine levels is incomplete even at large depolarizations and appears to approach some asymptote. This might be explained by postulating a specific set of rules for movement of both potassium and strychnine into, along, and out of the channel. Hille (1975*a*) has discussed some of the consequences of different rules on the voltage-dependence of block and concludes that with appropriate adjustments a wide variety of behavior could be explained.

Third, strychnine is anomalous in the large value of δ . Other compounds with large values of δ are small ions, such as methylammonium. As the ionic radii increase the value of δ tends to decrease. For TEA δ is 0.2. It is hard to imagine a molecule as large as strychnine actually penetrating through 0.6 of the dielectric of the membrane as it traverses a potassium channel unless the axoplasmic half of the potassium channel is much larger than the dimensions of a potassium ion. However, the voltage dependence of the blocking reaction might be due to another mechanism entirely.

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