The Inner Quaternary Ammonium Ion Receptor in Potassium Channels of the Node of Ranvier

CLAY M. ARMSTRONG and BERTIL HILLE

From the Department of Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14620 and the Department of Physiology and Biophysics, School of Medicine, University of Washington, Seattle, Washington 98195

ABSTRACT Quaternary ammonium ions were applied to the inside of single myelinated nerve fibers by diffusion from a cut end. The resulting block of potassium channels in the node of Ranvier was studied under voltage-clamp conditions. The results agree in almost all respects with similar studies by Armstrong of squid giant axons. With tetraethylammonium ion (TEA), pentyltriethylammonium ion (C_5), or nonyltriethylammonium ion (C_9) inside the node, potassium current during a depolarization begins to rise at the normal rate, reaches a peak, and then falls again. This unusual inactivation is more complete with C_9 than with TEA. Larger depolarizations give more block. Thus the block of potassium channels grows with time and voltage during a depolarization. The block reverses with repolarization, but for C_9 full reversal takes seconds at -75 mv. The reversal is faster in 120 mM KCl Ringer's and slower during a hyperpolarization to -125 mv. All of these effects contrast with the time and voltage-independent block of potassium channels seen with external quaternary ammonium ions on the node of Ranvier. External TEA, C_5, and C_9 block without inactivation. The external quaternary ammonium ion receptor appears to be distinct from the inner one. Apparently the inner quaternary ammonium ion receptor can be reached only when the activation gate for potassium channels is open. We suggest that the inner receptor lies within the channel and that the channel is a pore with its activation gate near the axoplasmic end.

INTRODUCTION

The tetraethylammonium ion (TEA) blocks potassium channels of the excitability mechanism in squid giant axons and in amphibian myelinated nerve fibers. However, there are significant differences in action depending on the type of nerve and the side of the membrane to which TEA is applied. For the squid axon, TEA must be in the axoplasm to block channels (Tasaki and Hagiwara, 1957; Armstrong and Binstock, 1965), but in myelinated

fibers TEA is effective from inside (Koppenhöfer and Vogel, 1969) or outside (Koppenhöfer, 1967; Hille, 1967 *a*) the membrane. The simplest case is with TEA outside a node of Ranvier of *Rana pipiens* where voltage-clamp experiments show that potassium currents at all voltages are reduced by a constant factor without any change in the kinetics of the currents (Hille, 1967 *a*). The result indicates that a fixed fraction of potassium channels is blocked throughout the depolarizing test pulse in the voltage clamp. The action of TEA on the outside of nodes of *Xenopus laevis* is similar except that there is also some slowing of the kinetics of the currents (Koppenhöfer, 1967).

TEA and other quaternary ammonium ions act in a different way when injected into the axoplasm of squid giant axons. First, the block is more complete for outward than for inward potassium currents, leading to so-called anomalous rectification (Armstrong and Binstock, 1965). Second, the blocking action does not develop until after potassium channels open. Hence, the degree of block is not constant throughout a depolarizing pulse, and the time-course of potassium currents is altered. With TEA the change in time-course looks almost like a simple quickening of the kinetics (Armstrong, 1966). With more hydrophobic quaternary ammonium ions, the shape of the potassium currents is more extensively changed: potassium currents rise after a depolarization but quickly inactivate down to a low level again (Armstrong, 1969, 1971). These observations are of general interest because they lead to the conclusion that the potassium channel is a pore with the normal activation gate at the inner end.

Recently Koppenhöfer and Vogel (1969) found that when the cut end of a myelinated nerve lies in a pool of TEA, the TEA diffuses into the axoplasm and up to the next node of Ranvier. Combining this novel technique with the voltage clamp, Koppenhöfer and Vogel observed that TEA inside the node reduces the amplitude and quickens the kinetics of potassium currents. Thus there are receptors for TEA on both sides of the nodal membrane which seem at least somewhat different. Our experiments confirm this difference and show that the TEA receptor on the inside of the node is similar to the receptor on the inside of the squid axon. Most importantly, the internal TEA receptor, as we shall call it for brevity, binds TEA and other more hydrophobic quaternary ammonium ions, but only after potassium channels open. A preliminary report of this work has appeared (Hille and Armstrong, 1971).

MATERIALS AND METHODS

The methods have been described in detail elsewhere (Hille, 1971). Single myelinated nerve fibers from the sciatic nerve of *Rana pipiens* were studied under voltage-clamp conditions by the method of Dodge and Frankenhaeuser (1958). All experiments were done at 5°C. Following Koppenhöfer and Vogel (1969), the 120 mM

KCl in one of the end pools of the nerve chamber usually contained an added quantity of a quaternary ammonium ion to be tested, and the internode in that pool was cut. Sometimes the cut was in the current-passing pool (pool E of Dodge and Frankenhaeuser, 1958). This had the advantage of giving fast results, because the cut could be close (500 μm) to the node under investigation, and the disadvantage of changing the calibration of the current record, because the cut reduces the resistance of the current path to the node. At other times, the cut was in the high impedance, recording pool (pool C) 1100 μm from the node, giving a slower onset of drug action with no change of the current calibration. The node under investigation was usually bathed in a Ringer's solution containing 115 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, and 1 mM tris(hydroxymethyl)aminomethane buffer, pH = 7.4. Where stated, quaternary ammonium ions or tetrodotoxin were added to the standard Ringer solution. Sometimes the node was bathed in a potassium Ringer solution containing 120 mM KCl, 2 mM $CaCl_2$, and 1 mM buffer. The following ammonium ions were tested: tetraethylammonium bromide (TEA), pentyltriethylammonium bromide (C_5), and nonyltriethylammonium bromide (C_9) all from Eastman Organic Chemicals, Rochester, N. Y.

All membrane potentials are given as inside minus outside and, accordingly, outward membrane current is called positive current. The current and voltage were photographed from the oscilloscope screen after correction of the currents for leakage and capacity. The correction was performed electronically by subtracting from the total membrane current the sum of a signal proportional to the membrane voltage and an exponentially decaying transient proportional to the steps of voltage. The leak subtractor was balanced to leave no membrane current change during a hyperpolarizing pulse, and achieved in a simpler way the same correction for leakage and capacity that is usually done by calculation after the experiment is over (Hille, 1967 *a*). In many cases only the steady leakage current was subtracted because it became evident that the capacity correction depends on the membrane conductance.

RESULTS

Internal TEA Acts Differently from External TEA

The left side of Fig. 1 shows a complete family of ionic currents recorded in the voltage clamp under normal conditions. With moderate depolarizations there are large, transient, inward sodium currents, and with stronger depolarizations there are large, maintained, outward potassium currents. The right side of Fig. 1 shows the same measurements repeated 12 min after one internode has been cut in 20 mM TEA. The cut is in pool C, 1100 μm from the node. Sodium currents are virtually unaffected by the internal TEA while potassium currents are reduced by 60% or more. The potassium currents I_K also reach their maximum value more quickly than before.

Changes in the kinetics of I_K are more easily seen in Fig. 2. Each test pulse depolarizes the node from the holding potential (−75 mv) to +75 mv and elicits a brief transient of outward sodium current followed by a

more maintained, outward potassium current. The four traces were taken at different times after the internode was cut in 20 mM TEA in the E pool, 650 μm from the node. The 1 min trace is still normal. In the subsequent traces, I_K rises at almost the same initial rate as in the 1 min trace but then

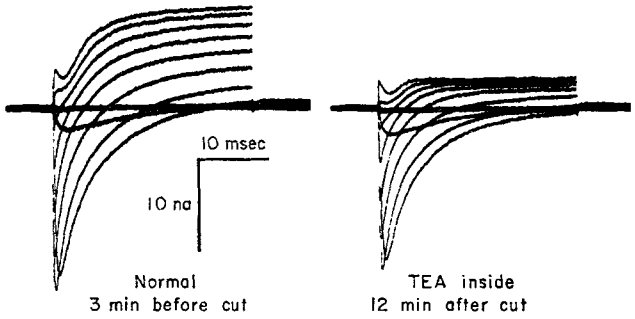

FIGURE 1. Block of I_K with internal TEA. A family of voltage-clamp currents for a normal node of Ranvier bathed in Ringer solution (left) and for the same node 12 min after cutting the internode in the C pool in 20 mM TEA (right) is shown. The 10 clamp voltages span the range -60 to $+75$ mv in 15-mv steps. Currents were corrected for leakage and capacity.

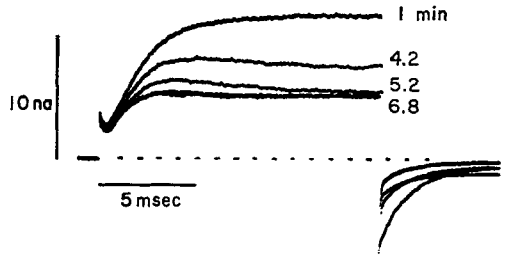

FIGURE 2. Development of the blocking effect of TEA after cutting in the internode. Superimposed voltage-clamp currents were all measured at $+75$ mv at different times after cutting the internode in the E pool in 20 mM TEA; corrected for leakage. The initial quick outward step of current is the sum of capacity and outward sodium currents (see Fig. 1 for comparison).

levels off earlier and actually falls again slightly. Note that although the earlier leveling off of I_K looks like a quickening of the kinetics of activation with TEA, I_K never rises faster than it does without TEA. As is shown later, the earlier flattening and secondary fall of I_K reflect a gradual blocking of channels which develops after they open. The secondary fall (inactivation) of I_K is less evident in the 6.8 min trace than in the two earlier ones. Indeed, in several experiments the secondary fall was absent or at least very small (see Fig. 1). When the node is repolarized at the end of the test pulse, there is a tail of inward current, which decays with a half-time of about 2 msec.

The initial amplitude of this tail is also progressively diminished as TEA diffuses into the node. The inward tail and the outward I_K are depressed to about the same degree.

The effect of internal TEA on the steady-state current-voltage relations was investigated using 50–100 msec depolarizing pulses to obtain maximal activation of potassium channels. Interference from sodium currents was eliminated by bathing the node in 100 nM tetrodotoxin. The internode was cut in 20 mM TEA in pool C. Steady-state K currents were depressed by internal TEA at all voltages, although not to the same degree. The depression was slightly but consistently stronger for the largest depolarizations. For example, after 15–18 min, I_K in two nodes was reduced to 43% and 37% of the control value at +25 mv and to 37% and 31% at +75 mv. After 38 min I_K in one node was reduced to 19% at +10 mv and to 15% at +75 mv.

Thus, we find three consistent but small differences between the actions of internal and external TEA at the node of Ranvier. TEA inside (*a*) causes I_K to flatten off sooner during a depolarization, (*b*) sometimes produces a secondary inactivation of I_K, and (*c*) depresses I_K more strongly at higher depolarizations. The same three effects are observed more clearly with TEA inside squid giant axons (Armstrong and Binstock, 1965; Armstrong, 1966). Fortunately the differences between the internal and external quaternary ammonium ion receptors are more convincingly demonstrated in the experiments with more hydrophobic ammonium ions to be described.

Internal C_9 Produces Strong Inactivation of I_K

ONSET OF INACTIVATION In squid axons a low axoplasmic concentration of C_9 (nonyltriethylammonium ion) produces a very marked inactivation of potassium currents, so that, during a maintained depolarization, I_K at first increases to an almost normal value, but then spontaneously decreases to a small fraction of its peak value. An example of the same behavior with C_9 inside a node of Ranvier is shown in Fig. 3. The traces show the current during a depolarization to +75 mv at various times after cutting the internode in 1 mM C_9. The current is normal 1 min after the cut, but at 8 min I_K begins to show a peak and subsequent decline, similar to the TEA effect but more pronounced. Despite the progressive decline of the late currents, the initial rate of rise of I_K remains almost the same in all the traces. The inward tails of current seen on return to −75 mv are also reduced by C_9. The steady-state block of I_K produced by cutting an internode in 1 mM C_9 is much stronger than the block produced by cutting in 20 mM TEA. Although the internal concentrations of C_9 and TEA are not known in these experiments, we feel safe in concluding that the internal quaternary ammonium ion receptor binds C_9 more strongly than TEA.

A complete family of voltage-clamp currents recorded from a C_9-contain-

ing node is given in Fig. 4. In the first part of the figure the internode has been cut but the C_9 effect has not yet developed and the traces have the usual configuration. In the second part, recorded 24 min later, the effect is well developed, and the changes seen in the previous figure can again be recognized. In addition, the experiment shows that C_9 depresses I_K much more at large depolarizations than at small depolarizations. Thus the blocking action of internal C_9 has a pronounced time dependence and voltage dependence. These properties, found to a much lesser degree with block by internal

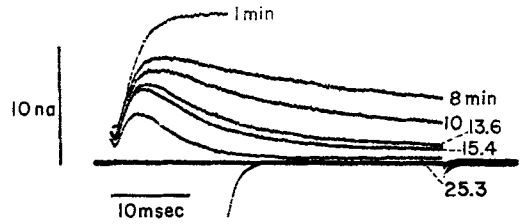

FIGURE 3. Development of the blocking effect of C_9 after cutting in the internode. Experimental details were as in Fig. 2 except that the E pool contained 1 mM C_9.

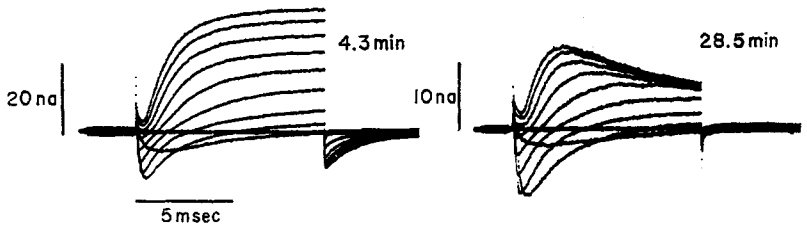

FIGURE 4. Voltage and time-dependent inactivation with internal C_9. Families of voltage-clamp currents are as in Fig. 1. Internode in the E pool was cut in 1 mM C_9; corrected for leakage.

TEA, serve to distinguish the internal and external actions of quaternary ammonium ions.

In one experiment the internode in the C pool was cut in 2 mM C_5. 30 min later I_K showed an early peak followed by inactivation to a steady level about 50% of the control value.

RECOVERY FROM INACTIVATION The block by C_9 is reversed by a long repolarization. The rate of recovery during the repolarization can be followed by depolarizing a second time, at various intervals after the end of the first pulse. The two pulses are shown diagrammatically in Fig. 5. Both pulses depolarize the node to +75 mv from the holding potential of −75 mv. Each frame in Fig. 5 superimposes the current during the first pulse (the larger, outward current of the pair) and the second pulse (the smaller current). The interval between the two pulses is given in milliseconds in the figure.

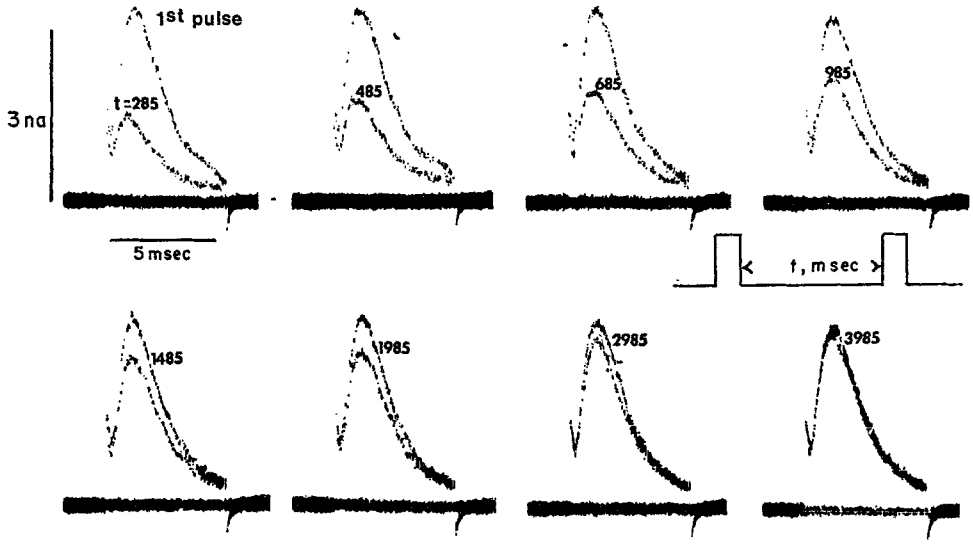

FIGURE 5. Time-course of recovery from inactivation with C_9. The voltage program consists of two 6-msec depolarizing pulses (+75 mv), as shown in the *inset*, separated by a variable time (t) given in milliseconds in each frame. The three superimposed traces are the baseline, the response to the first pulse (larger current), and the response to the second pulse (smaller current). Corrected for leakage; same node as Fig. 3.

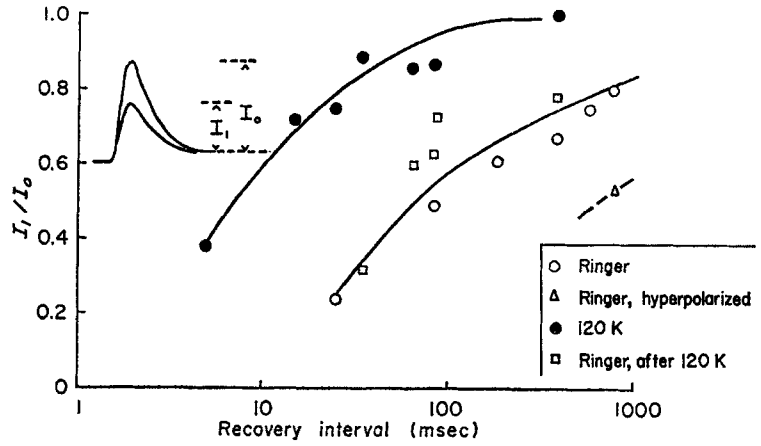

FIGURE 6. Time-course of recovery from inactivation with C_9. Amplitude of peak I_K in the second pulse is relative to the amplitude in the first pulse from an experiment like that of Fig. 5 (open circles). The other symbols are repetitions of the same kind of experiment but with a hyperpolarization to −125 mv between the two pulses or with isotonic K Ringer solution outside the node.

During the first pulse, I_K rises to a peak and then falls almost to zero as C_9 ions occlude potassium channels. The figure shows that 485 msec of repolarization is needed to obtain a 50% recovery from block. Full recovery requires roughly 4 sec.

The time-course of recovery for another node with C_9 inside is plotted in Fig. 6. The ordinate of the figure gives the ratio of the currents measured as in the inset of the figure, and the abscissa gives the interval between the two pulses. The open circles show the time-course of recovery in normal Ringer, with repolarization of the membrane to −75 mv. Raising the external K concentration to 120 mM speeds recovery at −75 mv considerably (filled circles), cutting the half-time of recovery from approximately 80 msec to about 8 msec. The squares in Fig. 6 show a redetermination of the recovery time-course in normal Ringer solution, after the determination in potassium Ringer. Recovery is somewhat faster than for the first determination, but still very much slower than in high potassium. Hyperpolarization of the membrane during the interval between the first and second pulses slows recovery tremendously, as the single triangle at 785 msec in Fig. 6 shows. For this one measurement the membrane was hyperpolarized to −125 mv. The considerable slowing of recovery by a hyperpolarization was seen and explored more thoroughly in another experiment, but unfortunately the original records were lost through a photographic error.

In accordance with the general pharmacological independence of sodium and potassium channels, sodium currents of normal size can be elicited throughout the period when potassium channels are still occluded by C_9.

External Quaternary Ammonium Ions Block Without Inactivation

External TEA reduces I_K with no secondary inactivation of the current and without appreciably changing the time-course of the current (Koppenhöfer, 1967; Hille, 1967 *a*). Fig. 7 shows that external C_5 also reduces I_K without changing its time-course. Other experiments show that external C_9 reduces I_K without giving the very pronounced secondary inactivation seen with internal C_9. Thus there is no kinetic evidence that the blocking action of external quaternary ammonium ions develops gradually following a de-

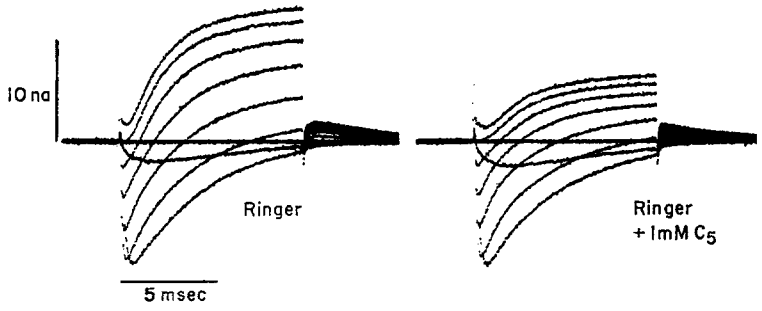

FIGURE 7. Block of potassium current with external C_5. A family of voltage-clamp currents in normal Ringer solution (left) and in a solution containing 1 mM C_5 (right) is shown. Potassium currents are reduced by 55% in C_5 and the rate of rise is reduced by about the same amount.

polarization as it seems to with internal quaternary ammonium ions. In addition, external C_5 and C_9 block less effectively than external TEA. With external application, 0.6 mM TEA blocks 80% of I_K (one determination; see also Hille, 1967 *a*), while 1 mM C_5 blocks 59% and 5 mM C_5 blocks 77% (each the average of two determinations on the same node). Finally, 0.1 mM C_9 has no detectable effect (three determinations) and 1 mM C_9 blocks 50% of I_K as well as 25% of I_{Na} (two determinations in one node). The effect of 1 mM C_9 was only partially reversible and was more like the damaging effect of a detergent than a simple interaction with the quaternary ammonium ion receptor.

DISCUSSION

Similarity to Squid Giant Axon

The results of our experiments with C_9 and TEA inside the node of Ranvier are extraordinarily like the results of analogous experiments on squid giant axons (Armstrong and Binstock, 1965; Armstrong, 1966, 1969, 1971). Four points of close similarity are as follows. (*a*) The blocking action is not present at the beginning of a depolarizing pulse and develops gradually after potassium channels open. (*b*) The block is strongest for large depolarizing pulses. (*c*) The block is stronger with C_9 than with TEA. (*d*) With C_9 the block reverses only slowly after the depolarization, at a rate which is slowed by hyperpolarization and speeded by elevating the external potassium concentration. These many similarities lead us to believe that the quaternary ammonium ion receptors inside the two kinds of axons are virtually identical.

On the basis of much more extensive experiments with the squid giant axon, Armstrong (1966, 1969, 1971) has developed the following model. Quaternary ammonium ions bind to a site within the potassium channel, directly in the path of the normal flow of potassium ions. The site has a hydrophobic component. Blocking ions can reach the site from the axoplasm but not from the external solution. Like potassium ions, the blocking ions are driven into the channel by diffusional and electrical forces and cannot enter a channel whose activation gate has not opened. Potassium ions entering the channel from the outside tend to push the blocking ions back into the axoplasm. The model accurately predicts the changes in I_K found with quaternary ammonium ions inside squid giant axon, and leads to the important physiological conclusions that the potassium channel is a pore rather than a carrier (Armstrong, 1971) and that the normal activation gate (n^4 gate) may be near the inner end of the pore.

The model also works well for the node of Ranvier. As in the squid work the following kinetic scheme is used for numerical calculations:

$$\text{closed channels} \underset{\text{kinetics}}{\overset{\text{Hodgkin-Huxley}}{\rightleftharpoons}} \text{open channels} \overset{k}{\underset{l}{\rightleftharpoons}} \text{blocked channels}$$

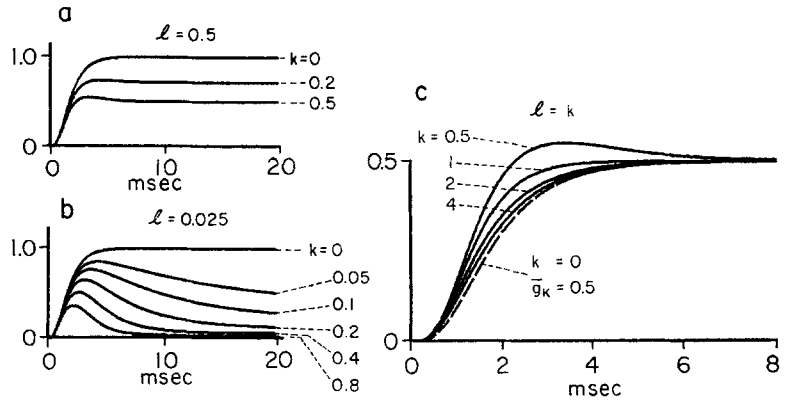

FIGURE 8. Potassium currents in the model. Time-courses of relative I_K calculated from the kinetic model for quaternary ammonium ion action are given in the text. In all of these curves the conventional Hodgkin-Huxley rate constants are given the values α_n = 1.0/msec and β_n = 0.0/msec and the blocking and unblocking rate constants l and k are given the values indicated in the figure (in units of milliseconds^{-1}).

where k is proportional to the axoplasmic concentration of quaternary ammonium ion. Fig. 8 *a* shows calculations of I_K which imitate the effects of internal TEA. The values of the rate coefficients k and l are given in milliseconds^{-1}. The top trace (k = 0) represents I_K in the absence of TEA. The other two traces, for two concentrations of internal TEA, show an earlier leveling off and small secondary inactivation of I_K similar to the observations of Fig. 2. Fig. 8 *b* shows the same kind of calculation for five concentrations of C_9 and a control (k = 0). The pronounced inactivation of I_K is similar to the observations of Fig. 3. As with the squid axon, the dissociation rate constant l is very much smaller for C_9 (0.025/msec) than for TEA (0.5/msec). This slower rate constant also fits with the stronger binding of C_9 and the slower recovery from inactivation with C_9. In agreement with the experiments, the calculations in Fig. 8 predict no slowing of the initial rate of rise of I_K with internal TEA or C_9. The model also predicts the observed speeding of recovery from inactivation with external K. The over-all qualitative agreement with the model emphasizes again the similarity between the frog node of Ranvier and the squid giant axon. We conclude that the potassium channel in the node of Ranvier is also a pore with the activation gate near the inner end. Furthermore, as our experiments with internal TEA agree in all respects with the experiments of Koppenhöfer and Vogel (1969), we can provide an explanation for their interesting findings.

Are the Internal and External Receptors in Fact a Single Receptor?

The four points of similarity between the internal TEA receptors in squid and frog are also four points of dissimilarity distinguishing the internal from external TEA receptor in frog. With external TEA the block does not vary

with time or with voltage, and more hydrophobic quaternary ammonium ions are less effective externally than TEA (Koppenhöfer, 1967; Hille, 1967 *a*, *b*). Thus, as Koppenhöfer and Vogel (1969) suggest, the internal and external TEA receptors seem different. Nevertheless the possibility exists of a single receptor site whose properties depend on the direction of approach of the blocking ion. Specifically, suppose that the blocking site were in the middle of a pore through the membrane, and that the inner end of the pore had a hydrophobic group which the outer end lacked. A C_9 ion entering the pore from inside would thus be stabilized in the blocking site by hydrophobic bonding from its hydrocarbon tail. A C_9 ion entering from the outside would not be stabilized this way. TEA could enter and leave the channel from both sides, pausing long enough at the blocking site to impede the passage of potassium ions. Although we do not endorse the concept of a single site, we also cannot refute it. Possibly flux measurements with radioactive TEA could help. With the squid giant axon, external TEA has no effect on I_K (Tasaki and Hagiwara, 1957; Armstrong and Binstock, 1965), so it is clear in that case that the blocking site cannot be reached from the outside. Vierhaus and Ulbricht (1971) have shown with fast solution changes that externally applied TEA reaches its blocking site on the node of Ranvier in less than 40 msec.

Location of the Activation Gate

The observations provide suggestive but far from conclusive evidence that the normal activation gate is at the inner end of the K channel. The most important evidence is that internal quaternary ammonium ions must await the opening of the activation gate before they can block, while external quaternary ammonium ions seem to have access to their blocking sites at all times. A simple interpretation is that the gate lies between the axoplasm and the inner quaternary ammonium ion receptor in the channel. In fact, however, the data do not show conclusively that external TEA can reach its blocking site at all times. The observation is that external TEA reduces I_K without changing its time-course. The simplest explanation is that a certain fraction of the channels is blocked at all times whether open or closed (Hille, 1967 *a*), but an alternative is that the binding and unbinding of TEA to open channels is so rapid that a steady state with regard to TEA blockage is established as fast as channels can open. In this case closed channels need not be blocked by TEA. Fig. 8 *c* shows that this alternative explanation could fit the observations. The dashed line marked $\bar{g}_K = 0.5$ represents I_K for half the channels blocked at all times. The full curves are predictions from the same kinetic model as was used earlier for internal TEA, setting l equal to k so that half the channels finally become blocked. As l and k are increased from a value appropriate for internal TEA (0.5/msec) to 4/msec,

the time-course of I_K approaches the dashed line. Thus only an order of magnitude increase of l and k changes the shape of I_K from the appearance with internal TEA to near that with external TEA, so that the choice between blocking of all channels or blocking only of open channels by external TEA is still open. As yet there is no evidence that external TEA does bind only to open channels, but if there were, the major argument for the inner location of the activation gate would be lost.

Another argument for the inner location of the gate comes from the effect of hyperpolarization. Hyperpolarization causes a change in the channel that results in slower recovery from inactivation. A simple interpretation is that the change is a closing of the activation gate which traps the quaternary ammonium ion in the channel. Again, this would mean that the gate lies between the axoplasm and inner quaternary ammonium ion receptor in the channel.

The Tail of Current, I_p

As described earlier a tail of inward current sometimes appears upon repolarization of a node from a large test pulse. The tail of current appears always in *Xenopus laevis*, sometimes in *Rana pipiens* from Mexico and the southwestern United States (Figs. 2–4), and never in *Rana pipiens* from Vermont (Fig. 1; and Dodge, 1963). Frankenhaeuser (1962, 1963) ascribed the tail to a secondary sodium current he called I_p which is activated by the test pulse with kinetics somewhat similar to those of I_K. The turning on of I_p was slower than for I_K, and the turnoff was faster. Because external TEA (Koppenhöfer, 1967) and internal TEA (Koppenhöfer and Vogel, 1969) and C_9 block this tail current, we believe that the current is produced by potassium channels. Some or all of the channels may face a restricted space where K^+ ions accumulate or are depleted during a long depolarization. Hence we ascribe Frankenhaeuser's I_p to a small change in electromotive force in potassium channels rather than to the activation of other channels. The tail of current I_p is not blocked by tetrodotoxin and is not reduced by replacing all the external sodium ion by tetramethylammonium ion (Hille unpublished).

This work was supported by United States Public Health Service Grants NS 08951 and NS 08174.

Received for publication 21 October 1971.

REFERENCES

ARMSTRONG, C. M. 1966. Time course of TEA^+-induced anomalous rectification in squid giant axons. *J. Gen. Physiol.* **50**:491.

ARMSTRONG, C. M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injection in squid axons. *J. Gen. Physiol.* **54**:533.

ARMSTRONG, C. M. 1971. Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. *J. Gen. Physiol.* **58**:413.

ARMSTRONG, C. M., and L. BINSTOCK. 1965. Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. Gen. Physiol.* **48**:859.

DODGE, F. A. 1963. A study of ionic permeability changes underlying excitation in myelinated nerve fibers of the frog. Ph.D. Thesis. The Rockefeller University, New York. (University Microfilms, Inc., Ann Arbor, Mich., No. 64-7333).

DODGE, F. A., and B. FRANKENHAEUSER. 1958. Membrane currents in isolated frog nerve fibre under voltage clamp conditions. *J. Physiol. (London).* **143**:76.

FRANKENHAEUSER, B. 1962. Instantaneous potassium currents in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol. (London).* **160**:46.

FRANKENHAEUSER, B. 1963. A quantitative description of potassium currents in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol. (London).* **169**:424.

HILLE, B. 1967 *a*. The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. Gen. Physiol.* **50**:1287.

HILLE, B. 1967 *b*. Quaternary ammonium ions that block the potassium channel of nerves. *Biophys. Soc. Annu. Meet. Abstr.* 19a.

HILLE, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* **58**:599.

HILLE, B., and C. M. ARMSTRONG. 1971. Similarity of the inner part of K channels in frog and squid nerve. *Biophys. Soc. Annu. Meet. Abstr.* 53a.

KOPPENHÖFER, E. 1967. Die Wirkung von Tetraäthylammoniumchlorid auf die Membranströme Ranvierscher Schnürringe von *Xenopus laevis*. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **293**:34.

KOPPENHÖFER, E., and W. VOGEL. 1969. Effects of tetrodotoxin and tetraethylammonium chloride on the inside of the nodal membrane of *Xenopus laevis*. *Pfluegers Arch.* **313**:361.

TASAKI, I., and S. HAGIWARA. 1957. Demonstration of two stable potential states in the squid giant axon under tetraethylammonium chloride. *J. Gen. Physiol.* **40**:859.

VIERHAUS, J., and W. ULBRICHT. 1971. Rate of action of tetraethylammonium ions on the duration of action potentials in single Ranvier nodes. *Pfluegers Arch.* **326**:88.