The Selective Inhibition of Delayed Potassium Currents in Nerve by Tetraethylammonium Ion

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ABSTRACT The effect of tetraethylammonium ion (TEA) on the voltage clamp currents of nodes of Ranvier of frog myelinated nerve fibers is studied. The delayed K currents can be totally abolished by TEA without affecting the transient Na currents or the leakage current in any way. Both inward and outward currents disappear. In low TEA concentrations small K currents remain with normal time constants. The dose-response relationship suggests the formation of a complex between TEA and a receptor with a dissociation constant of 0.4 mM. Other symmetrical quaternary ammonium ions have very little effect. There is no competition between TEA and agents that affect the Na currents such as Xylocaine, tetrodotoxin, or Ca ions. The pharmacological data demonstrate that the Na, K, and leakage permeabilities are chemically independent, probably because their mechanisms occupy different sites on the nodal membrane. The data are gathered and analyzed by digital computer.

INTRODUCTION

The tetraethylammonium ion (TEA) is a nonbiological substance of unusual interest to neurophysiologists. Its many actions seem to center in two fundamentally different biological processes. As a small cation it can be confused with naturally occurring cations that serve as carriers of electric current through membranes. In this situation the TEA ion will either substitute as a satisfactory current carrier or inhibit the current-carrying mechanism. On the other hand as a quaternary ammonium ion TEA can be confused with natural chemical transmitter agents or their precursors. In this case the TEA ion will excite or block synaptic activity and interfere with the enzymes which transport, synthesize, and destroy transmitters or their precursors. Many references to these phenomena can be found in Raventós (1937), Schmidt (1965), and Grundfest (1961).

One of the actions of TEA is to prolong the action potentials of many nerve and muscle fibers (Loeb and Ewald, 1916; Cowan and Walter, 1937; Schmidt, 1965). In some cases the prolongation is known to be caused by an inhibition of the voltage-dependent potassium permeability. A nearly complete loss of the potassium conductance has been demonstrated for giant nerve cells in some ganglia of the mollusc, *Onchidium verruculatum*, bathed in 100 mM TEA (Hagiwara and Saito, 1959) and for nodes of Ranvier of *Xenopus laevis*, *Rana esculenta* (Schmidt and Stämpfli, 1966; Koppenhöfer and Weymann, 1965), and *Rana pipiens* (Hille, 1966 *a* and *b*) bathed in 5 mM TEA. The effect on nodes of Ranvier is maximal in 1 or 2 sec and is reversed in the same time by washing. The giant axon of the squid *Loligo pealii*, another mollusc, is insensitive to 100 mM TEA in the bathing medium, but a 40 mM internal concentration produces an extraordinary rectification. In this condition the potassium conductance is normal for inward currents and essentially nil for outward currents. When they described these phenomena Armstrong and Binstock (1965) suggested that internal TEA might be swept into the membrane with outward potassium currents, hence blocking, and flushed out again by inward currents.

It is the purpose of this paper to show that TEA is a specific inhibitor of the potassium conductance of the node of Ranvier. Its selectivity is as complete as that of the complementary agent, tetrodotoxin, a selective inhibitor of the sodium conductance (Narahashi, Moore, and Scott, 1964; Nakamura, Nakajima, and Grundfest, 1965 *a* and *b*; Takata, Moore, Kao, and Fuhrman, 1966; Hille, 1966 *b*). The pharmacological data suggest that the sodium, potassium, and leakage currents are carried by three entirely independent mechanisms. A preliminary report of some of this work has been given (Hille, 1966 *a*).

METHODS

The Nerve

A single large myelinated nerve fiber was dissected out of the sciatic nerve of *Rana pipiens* and one node of Ranvier was voltage-clamped according to the method developed by Dodge and Frankenhaeuser (1958). A rapid change of solution was achieved with an inlet and a vacuum outlet tube at opposite ends of the pool surrounding the node under investigation. The volume of the pool was about 0.15 cc. Routinely 1 or 2 cc of test solution were flushed through in 5 or 10 sec every 5 min. Voltage clamp measurements were begun after 3 min, and a new solution was applied when they were finished. In this way consecutively numbered experimental treatments were about 5 min apart. The nodes in the other pools were bathed in calcium-free isotonic KCl. The entire preparation including the salt bridges and calomel electrodes was in a brass block maintained at a constant low temperature by

circulating water from a regulated water bath. Low temperatures were preferred as they slowed the responses of the node, permitting better temporal resolution. The node was held clamped near the normal resting potential of −75 mv. A 40 msec prepulse at −120 mv eliminated any steady-state sodium inactivated before the test pulse.

The Ringer solution had the following composition (mM): NaCl 110, KCl 2.5, $CaCl_2$ 2.0, tris(hydroxymethyl)aminomethane buffer (pH 7.3)5. The 22 mM Ca Ringer was a normal Ringer made hypertonic by dissolving more $CaCl_2$. Other solutions of different composition were made by replacing some of the NaCl by KCl, tetramethylammonium bromide, tetraethylammonium chloride, tetrapropylammonium iodide, tetrabutylammonium iodide (all Eastman, gifts of Dr. R. Lorente de Nó), Xylocaine hydrochloride (gift of Astra Pharmaceutical Products, Inc., Worcester, Mass.), or tetrodotoxin (gift of Dr. T. Narahashi and Sankyo Company Ltd., Tokyo, Japan).

The Computer Analysis

The complete analysis of voltage clamp data requires lengthy mathematical calculations that can be performed by a digital computer if the data are stored in an appropriate form. The traditional photograph cannot be read by computers. Conventional AM or FM analogue tape recording lacks the high frequency response needed in the voltage clamp. On-line analog- to-digital conversion using fast digital logic and memory is the only practical technique. This method produces lists of numbers so long that they must be frequently transferred from the fast memory device to an indefinitely large storage medium such as digital magnetic tape.

In my experiments I use a Control Data Corporation 160 A digital computer on-line to control the sampling, storage, and transfer of voltage clamp data. Typically the responses to 20 different voltage clamp pulses are recorded in each experimental solution. The records in successive solutions are given consecutive numbers which have been used in the figures of this paper for identification. Each clamp test pulse of 25 msec is preceded by a 40 msec hyperpolarizing prepulse. Every 50 μsec throughout a 50 msec period starting just before the end of the prepulse the computer memory receives two 8-bit numbers, one the digital equivalent of the current and one of the voltage. These become a stored list of 2000 numbers. About 350 msec later exactly the same prepulse and test pulse are repeated and a second list of similar numbers is received. These two lists are averaged together to reduce the high frequency noise. The average voltages of the prepulse, of the test pulse, and of the following base line are calculated by averaging the appropriate voltage numbers. Similarly the current numbers starting 18 msec after the test pulse are averaged for the current base line. Aside from these four averages only the current points during the test pulse need to be saved. After the early transients of current in the first few milliseconds of the test pulse, the currents vary so slowly that they may be satisfactorily represented by one point every 500 μsec instead of every 50 μsec. This is accomplished by averaging each 10 consecutive current numbers after the first 2 msec. Averaging these points markedly improves the signal to noise ratio (see for example Fig. 5) as well as shortening the

data list. The computer takes less than 0.5 sec on-line to compute all the averages and to reduce the data from the 4000 points received to the 100 to be saved. In another small fraction of a second the 100 points are recorded on magnetic tape for later analysis, and the whole system is ready to receive a new pair of lists from a new test pulse.

Since the experiments are recorded in a form that can be read by a computer, their analysis is readily automated. I use a Fortran program and the same digital computer for the analysis. First the leakage current is calculated and subtracted (see next paragraph). Figs. 1, 3, and 5 include some time courses of the voltage clamp currents drawn directly by the computer on its digital plotter after subtracting the leakage current. Then the potassium steady-state current, I_{Kss}, and time constant, τ_n are determined by fitting the function:

$$I_K = I_{Kss}[1-\exp(-t/\tau_n)]^4$$

to the late currents. Dodge (1963) showed that fitting this function derived from the Hodgkin-Huxley (1952 *b*) equations can achieve a separation of currents equivalent to that achieved with the original sodium-substitution method (Hodgkin and Huxley 1952 *a*). Finally the sodium current is obtained by subtracting the potassium current function. Fig. 3 includes three time courses of the sodium currents obtained by subtraction of the leakage and potassium currents from experimental data. The currents are calibrated by assuming that the resting resistance of the normal node is 40 megohm (Tasaki, 1955).

The squid axon has an ohmic leak (Hodgkin and Huxley 1952 *b*). The leakage current is a rectangular step if the potential is displaced in a rectangular step. A freshly dissected frog node also generally exhibits an ohmic leak; however, after several hours in the isolated state or after a poor dissection, there can appear in addition to the ohmic leak an exponentially decaying current that resembles the capacitative surge except that it may fall with time constants as long as 0.5 msec. This current is probably the same as the capacitative artifact in the same preparation discussed by Dodge and Frankenhaeuser (1959). A similar phenomenon was seen by Takata, Pickard, Lettvin, and Moore (1966) using a sucrose gap on the lobster axon. The amplitude of the extra current is always proportional to the change of the membrane potential in a step displacement in either direction and is insensitive to drugs, changes in the ionic composition of the medium, or changes in frequency response of the voltage clamp. All these properties suggest that, unlike the ohmic leakage current, the extra current is a capacitative rather than an ionic current through the membrane. If the standard parallel 40 megohm resistance and 2 $\mu\mu$f capacitance of the nodal equivalent circuit are supplemented by a third parallel element containing a 20 megohm resistance and a 15 $\mu\mu$f capacitance in series, the observed currents of a typical case can be imitated. My interpretation is that the myelin on either side of the node gradually lifts away from the axon for several microns, thus exposing a new large area of axon membrane that contributes the extra capacitance. The series resistance would then be the resistance of the newly formed external gap between the myelin and the exposed membrane. Therefore, when necessary, I have used the procedure of

Takata, Pickard, Lettvin, and Moore (1966) who calculate the leakage current as the sum of an ohmic leak and an exponentially decaying extra leak.

RESULTS

TEA Reduces $\bar{g}_K$

The voltage-dependent potassium permeability is most conveniently studied in the absence of the transient sodium currents seen in normal nerves. Tetrodotoxin (TTX) achieves this condition (Hille, 1966 *b*). Fig. 1 shows the time

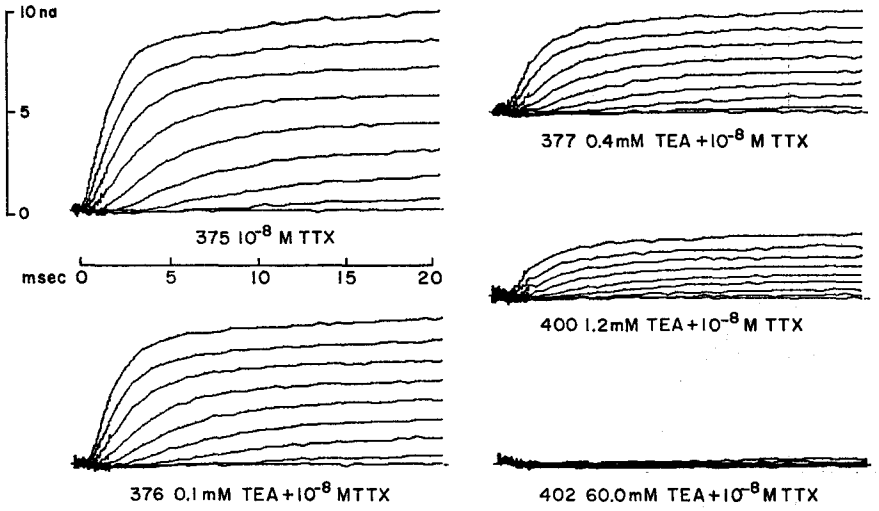

FIGURE 1. Potassium currents in TEA. The time courses, drawn by the computer, of the voltage clamp currents minus leakage current in five different solutions. Usually the responses to 19 depolarizing clamp voltages spaced at 7.5 mv intervals are recorded in each experiment. For clarity only nine curves have been drawn, spaced at 15 mv intervals from −60 to +60 mv. Before the node was treated with tetrodotoxin (TTX) the maximum inward sodium current was 15 na. In TTX no inward currents remain. Please note that in the numbering system used by the computer (octal), 400 is the immediate successor of 377 (T = 17°C).

course of the voltage clamp currents minus leakage current of a node in 10^{-8} M TTX. As the leakage current is removed mathematically and the sodium current pharmacologically, the tracings represent potassium current alone. The five families of curves at increasing concentrations of TEA show a progressive abolition of the outward potassium current. Fig. 2 illustrates the resolution of these curves into the time constant τ_n (left) and the steady-state current-voltage relation (right) and demonstrates that the primary effect of TEA is to reduce the amplitude of the potassium currents without affecting the time constants of the changes of the potassium conductance. Because the amplitude is reduced by the same proportion at all voltages, the

effect can be described as a reduction of the maximum potassium conductance, $\bar{g}_K$.

Similar results are obtained in the absence of TTX. Fig. 3 shows the time course of the voltage clamp current minus leakage current of a node in Ringer's. Both early sodium currents and late potassium currents are present. As TEA is added to the Ringer's solution (records 443 and 444 of Fig. 3) the potassium currents are reduced. The sodium and potassium currents can now be separated by the procedure described in the Methods. As in the previous experiment the steady-state current-voltage relations, given by the

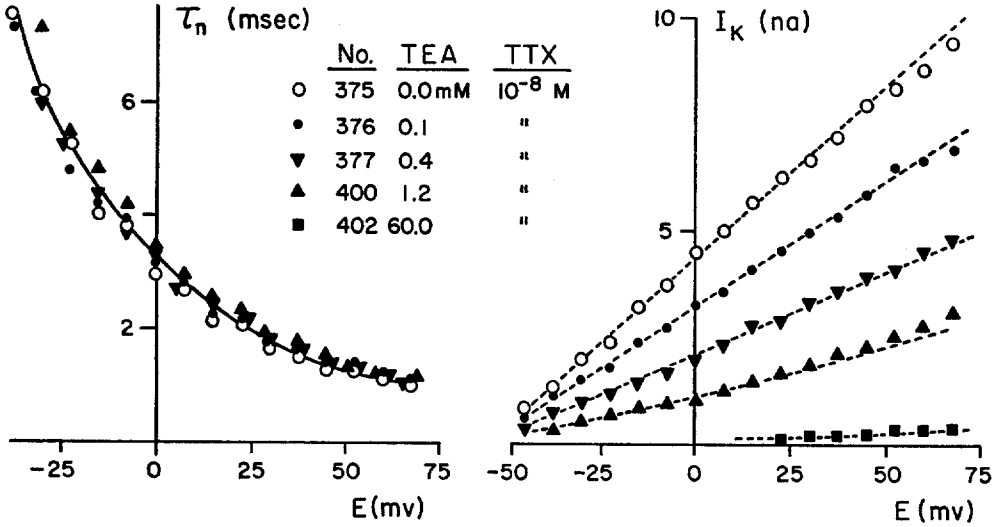

FIGURE 2. Analysis of potassium currents in TEA. The time constants τ_n (left) and steady-state current-voltage diagram (right) of the potassium currents of Fig. 1 (T = 17°C).

dotted lines of the right hand side of Fig. 4, show a uniform reduction of the potassium currents at all voltages while the time constants τ_n (not illustrated) are unchanged. Again the effect is a reduction of $\bar{g}_K$.

If TEA reduces $\bar{g}_K$ in a simple manner, inward as well as outward potassium currents should decrease. I have tested this in two ways. When a normal node is hyperpolarized after a long depolarization there is a brief "tail" of inward potassium current during the return of the potassium-carrying system from a high conductance state to a low conductance state. This tail is eliminated by TEA. Much larger inward potassium currents can be produced in isotonic KCl solutions. As Frankenhaeuser (1962) showed with *Xenopus* nodes held at about −75 mv in high KCl, small depolarizations give rise to delayed inward potassium currents and depolarizations beyond 0 mv give delayed outward potassium currents. On repolarization to −75

mv from any depolarization, there is a large tail of inward potassium current. *Rana* nodes exhibit all these potassium currents in 115 mM KCl and all of them are abolished by 12 mM TEA. Thus the action of TEA is adequately represented as a simple reduction of $\bar{g}_K$.

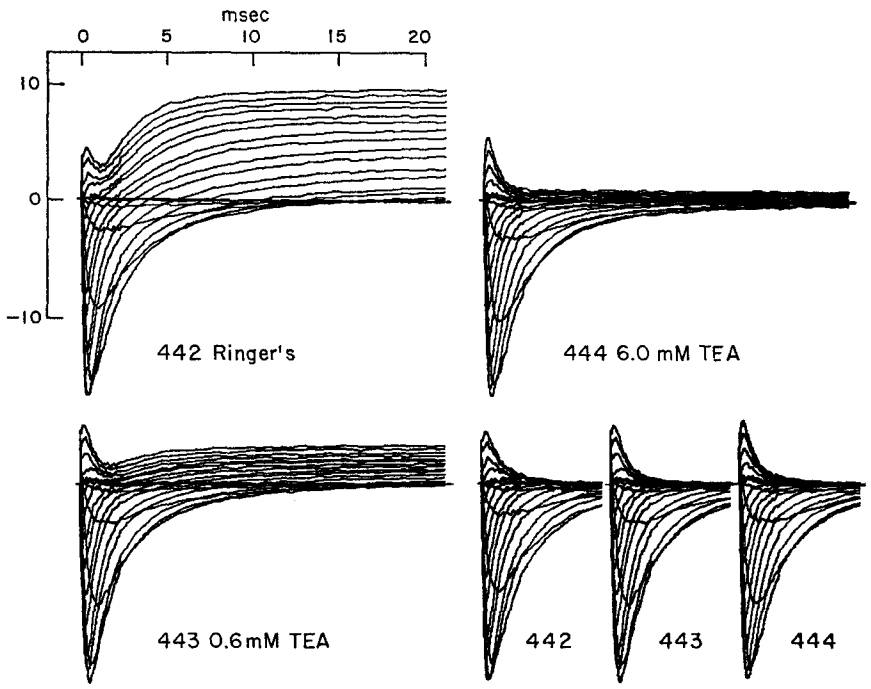

FIGURE 3. Sodium and potassium currents in TEA. The time courses, drawn by computer, of the voltage clamp currents of a node in three different solutions. The leakage current has been subtracted from the three longer records. The three shorter curves are the first few milliseconds of the previous records but with both the leakage and the potassium functions subtracted. Thus they are the experimental sodium currents. All 19 curves have been drawn, spaced at 7.5 mv intervals from −67.5 to +60 mv. The time scale is the same for all records (T = 11°C).

I_{Na} and I_L Are Insensitive to TEA

The experiment of Figs. 3 and 4 also illustrates the insensitivity of the sodium-carrying system to TEA. The three short records in the lower right hand side of Fig. 3 are from the same data as the three longer records in the figure but the potassium current function has been subtracted mathematically. Thus these are sodium currents. They seem indistinguishable. The sodium currents are analyzed in Fig. 4. On the right hand side the solid line indicates the points of the peak current-voltage relation. On the left hand side are the time constants, τ_m and τ_h, of those currents that are large enough to permit

measurement. Since the sodium currents turn on in a few hundred microseconds at 11°C and the measurements are made every 50 μsec, there is an uncertainty of 50 μsec in the determination of τ_m. The separation between the two lines drawn in the graph of τ_m represents the band of uncertainty. This experiment shows that within the uncertainty of the measurement there is no change of τ_m, of τ_h, or of the peak current-voltage relation attributable to TEA. This conclusion has been verified at concentrations of TEA up to 60 mM in normal nodes. At 60 mM TEA the voltage-dependence of the steady-state sodium inactivation was unchanged.

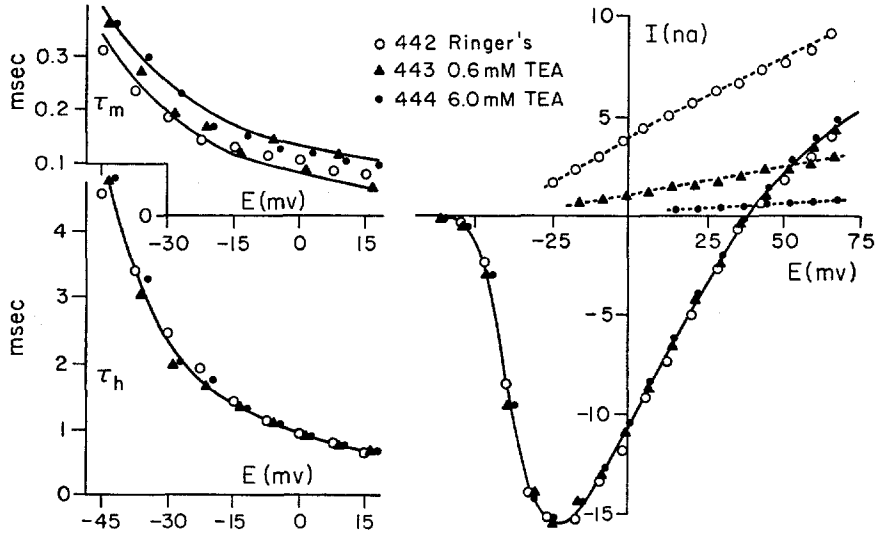

FIGURE 4. Analysis of sodium currents in TEA. The time constants τ_m and τ_h (left) and the current-voltage relations (right) of the voltage clamp currents of Fig. 3. The steady-state current-voltage relation is indicated by the dashed line and the peak current-voltage relation by the solid line. The two lines drawn in the graph of τ_m define the band of uncertainty of the measurement as described in the text (T = 11°C).

It seemed desirable to test the effect of isotonic TEA on the sodium current. In an isotonic TEA solution there is no sodium, and the sodium currents would be outward and small unless the axoplasm were loaded with sodium. The nodal axoplasm can be loaded with ions by temporarily breaking down the diffusion barrier by a strong electric shock. The resistance recovers, often completely, over a period of a few seconds after the shock. If the shock is given in Ringer's solution the sodium equilibrium potential is greatly reduced, outward sodium currents become large, and the potassium currents virtually disappear. After such treatment the potassium-carrying system may be damaged, so that it is unable to produce delayed inward currents in isotonic KCl, but the sodium system seems normal except for the

unusual equilibrium potential, attributable to the high internal sodium concentration.

Fig. 5 shows the time course of the voltage clamp currents minus leakage current (left) and the peak current-voltage diagram (right) of a node after several strong hyperpolarizing shocks. The curves represent sodium current alone because the damaged node has no potassium current. In normal Ringer's there are both inward and outward sodium currents with an equilibrium potential near 0 mv suggesting that the axoplasm contains nearly 110 mM sodium after loading. In 110 mM TEA there are almost no inward

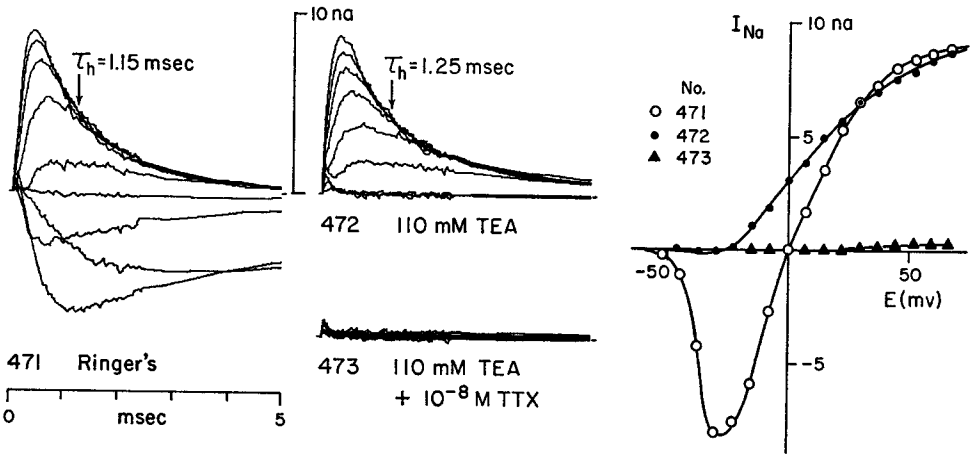

FIGURE 5. Sodium currents in TEA. The early time courses, drawn by the computer, of the voltage clamp currents minus leakage (left and center) and the peak current-voltage relation (right) of a node that has been loaded with sodium by the strong shock technique described in the text. The time courses are spaced at 15 mv intervals from −52.5 to +67.5 mv. The two arrows labeled τ_h indicate the time constant of the sodium inactivation process at +67.5 mv (T = 4°C).

currents, but there are outward currents above −25 mv. At large depolarization the outward currents are nearly identical in amplitude and in time course in Ringer's and in 110 mM TEA. The arrows on records 471 and 472 indicate the value of τ_h at +67.5 mv in Ringer's and TEA. They are essentially the same. Therefore nearly isotonic TEA does not change the sodium permeability system significantly. The third set of curves in Fig. 5 shows that TTX abolishes all the currents in isotonic TEA, proving that they are indeed sodium currents alone. A similar test of outward sodium current kinetics in 110 mM TMA shows that this ion also causes no change.

In the Introduction it is mentioned that quaternary ammonium ions might themselves carry current through the membrane. It is already known that

NH_4 ions carry early current about one-fourth as well as sodium ions in *Rana* nodes (Dodge, 1963). When care is taken to rinse away the external sodium from a normal node, there are no early currents in 110 mM TEA or in 110 mM TMA, and above −25 mv there are definite outward sodium currents. These observations show that these quaternary ions are at least 20 times poorer current carriers than sodium ions. A 110 mM solution of the quaternaries is equivalent to a solution with less than 6.5 mM sodium ions.

The leakage conductance does not change in 60 mM TEA. In the experiment illustrated in Figs. 1 and 2 the leakage conductances in the four concentrations of TEA shown are 97, 98, 100, and 100 relative to a control value of 100. The small variation is within the reproducibility of such a measurement. However, in 110 mM TEA three different nodes gave relative conductances of 60, 63, and 65, respectively.

TEA Has a Receptor

TEA probably acts by binding to a receptor on the extracellular side of the nodal membrane. The effects are rapid and reversible. Even after an hour in 10 mM TEA, the node recovers in seconds on being washed with Ringer's. Other symmetrical quaternary ammonium ions have little activity. Tetramethylammonium ion has no effect at 110 mM. Only 50% reduction of the potassium conductance occurs in 60 mM tetrapropylammonium ion or tetrabutylammonium ion. As an aside to the problem of receptor specificity, it is known that isotonic choline, a quaternary ammonium ion, reduces the potassium conductance changes of *Rana* and *Xenopus* nodes by 20 to 30% (Dodge and Frankenhaeuser, 1959; Dodge, 1963). This leads to the artifact of "crossing-over" or apparent reversal of the sign of the sodium currents when choline is used in the classical method of separation of currents by ionic substitution. By using a series of normal and hydroxylated quaternary ammonium ions I have been able to show that choline depresses the potassium conductance because it is a chemical relative of TEA.

While this information shows that the receptor is selective, it does not demonstrate what properties are important for binding. It should be possible to determine the number of molecules per receptor required to produce an effect by analyzing the dose-response relationship. Fig. 6 presents a summary of data from 11 nodes together with a theoretical curve assuming a receptor complex of one TEA ion per receptor with a dissociation constant of 4×10^{-4} M. The agreement is fair. If more than one TEA were required per receptor, the theoretical line would be steeper at the midpoint, so it seems reasonable to assume that only one TEA ion is required to produce an effect at a particular receptor. These data cannot be used to determine the number of receptors per node.

TEA Action Is Independent of Agents Modifying I_{Na}

Agents that affect the sodium current, such as TTX, Xylocaine, or calcium ions, do not interact pharmacologically with TEA. The current-voltage diagram in Fig. 7 shows that when TTX has completely abolished the sodium current (filled circles), the dose-response to TEA is the same as when the sodium current is unimpaired (open circles). Also abolishing the sodium current by 1.2 mM Xylocaine does not diminish the effectiveness of 6 mM TEA (Hille, 1966 *b*). Elevated calcium ion concentrations are known to raise the threshold to stimulation (Brink, 1954). In the voltage clamp the parameters of the sodium permeability system in high calcium are described approximately by saying that all rate constants are the same as before except that they are displaced by a constant voltage to more positive internal volt-

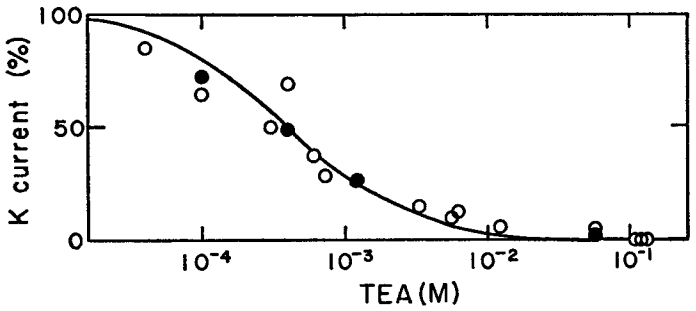

FIGURE 6. Dosage-response to TEA. The maximum potassium conductance, $\bar{g}_K$, at various concentrations of TEA relative to that in Ringer's solution. The filled circles are from the experiment in Figs. 1 and 2 in which the sodium currents had been eliminated by TTX. The solid line is the dose-response relationship of a hypothetical system in which one TEA ion binds reversibly to its receptor to produce a fraction of the inhibitory effect. The curve is identical to a simple adsorption isotherm or "rectangular hyperbola."

ages (Frankenhaeuser and Hodgkin, 1957; Blaustein and Goldman, 1966). The filled inverted triangles of Fig. 7 show the effects of 22 mM Ca, 11 times normal, on the current-voltage diagram of an otherwise normal node. In this solution τ_m, τ_h, and the peak sodium conductance relation were displaced approximately 22 mv in the direction of more positive internal potentials. At the same time the potassium currents were unchanged in amplitude and kinetics. This result is typical of several such experiments. A few measurements indicated that the steady-state sodium inactivation relation was also displaced almost as much as the peak sodium conductance relation. In a minority of cases there was a voltage shift of τ_n in the same direction as, but never as much as, the shift of the sodium constants. The upright filled triangles of Fig. 7 show that 6 mM TEA neither prevents 22 mM Ca from

displacing the peak sodium-voltage relation nor does the calcium prevent TEA from depressing the potassium conductance. I conclude from this evidence that the TEA receptor is not the receptor mediating the action of TTX, Xylocaine, or calcium.

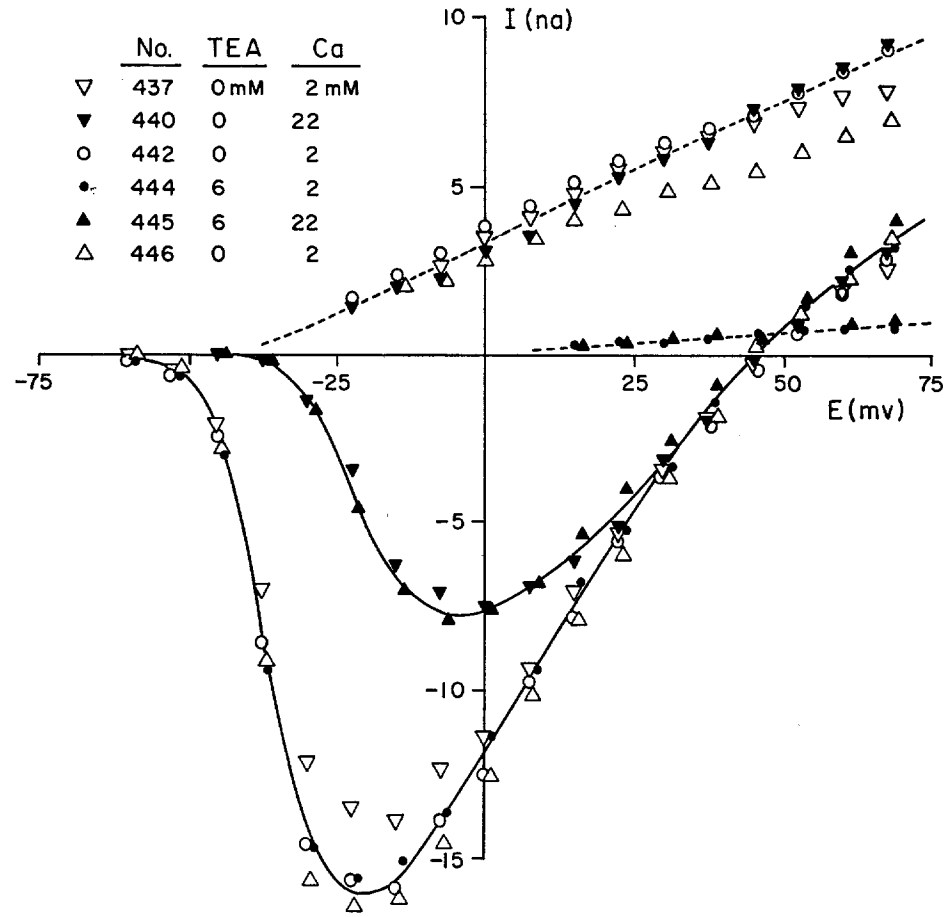

FIGURE 7. TEA and calcium. The peak sodium (solid lines) and steady-state potassium (dashed lines) current-voltage relations of a node in solutions containing TEA and calcium. The leakage currents have been subtracted. It can be seen that TEA and calcium exert their effects independently. The data are from the same node as the experiment in Figs. 3 and 4. Note that 440 is the immediate successor of 337 (T = 11°C).

DISCUSSION

The results presented indicate that there is only one effect of TEA on the excitability mechanisms of the frog node: the voltage-dependent potassium conductance is reduced. There have been reports that in addition to changing $\bar{g}_K$, TEA lengthens the time constant of potassium activation, τ_n, and the

time constant of sodium inactivation, τ_h (Schmidt and Stämpfli, 1966; Koppenhöfer, 1966). Because the turn off of the sodium current and the turn on of the potassium current overlap considerably in time, effects on $\bar{g}_K$ or on $\bar{g}_{Na}$ could easily be thought to be changes of τ_h or τ_n as well unless an accurate method of current separation is employed. Schmidt and Stämpfli used an indirect method involving current clamping. Koppenhöfer used a voltage clamp. The probability that *Rana pipiens* nodes differ from *Rana esculenta* and *Xenopus laevis* nodes in the sensitivity of these rate constants seems small. I hope that this point can be resolved. Although they did not study the question in detail, Armstrong and Binstock (1965) state that the sodium current parameters of the TEA-containing squid axon seem normal. It is clear from their work, however, that the effects of TEA on the potassium currents of the squid axon are different both in site of action and in the nature of the depression of the conductance from those seen in *Rana* fibers.

The actions of TTX and TEA on the frog node are complementary in that one inhibits the depolarizing mechanism and the other the repolarizing mechanism of the membrane. Each can reduce the maximum conductance of one ionic component to zero without affecting the time constants or the leakage system. Neither interferes with the action of the other. The complementarity is the same as that found between urethane and TEA in ganglion cells of *Onchidium* (Hagiwara and Saito, 1959). A second kind of complementarity exists between TTX and veratrine. One of the actions of veratrine on the frog node seems to be a suppression of the sodium inactivation mechanism, leading to maintained inward sodium currents during depolarizing voltage clamp pulses. These sodium currents are sensitive to the effects of Xylocaine (personal observation), TTX, and calcium (Ulbricht, 1966). There is indirect evidence that the potassium currents in veratrinized nerves are normal and fully sensitive to 5 mM TEA (Schmidt, 1965). Hence while the sodium currents of normal nerves are mostly inactivated by the time that large potassium currents appear, the veratrine-poisoned nerve exhibits the unusual property of simultaneously large sodium and potassium currents.

Hodgkin and Huxley (1952 *b*) demonstrated the *electrical* and the *mathematical* independence of three components of the ionic currents. Together all the pharmacological data on TTX, TEA, and veratrine demonstrate the *chemical* independence of the same three components. The selective destruction of the potassium system by large electric shock illustrated earlier is an additional manifestation of independence. These phenomena are easily interpreted if the current-carrying mechanisms for the sodium, potassium, and leakage currents are *spatially* separated from each other as well. If the ions actually pass through one of three qualitatively different specializations of the nerve membrane, the words "sodium channel," "potassium channel," and "leakage channel," now used in the literature, refer to these distin-

guishable specializations.[1] Presumably these channels are the receptors for TEA, TTX, or local anesthetics to which they bind in unknown fashion. Possibly the blockade is a simple obstruction and the suppression of inactivation is a simple propping open; however, there is no independent evidence to support or eliminate such molecular mechanisms in the frog node. Those potassium channels which have complexed with TEA will be closed while those uncomplexed will be fully normal and will account for the small potassium currents with normal kinetics seen in Fig. 1. A similar explanation could apply for the actions of TTX and veratrine on the sodium channel. In veratrine as in TTX the sodium current consists of a normal component and an altered component.

The above reasoning does not readily describe the action of high or low calcium concentrations. If it did the sodium currents should be resolvable into two components. One component would be the response of sodium channels lacking a bound calcium and the other the response of those with a bound calcium. One should be able by adding varying proportions of the components to match all the sodium currents at all concentrations of calcium. This cannot be done. Three explanations can be offered. There may be many calcium-binding sites on each channel; there may be only one site but the calcium complex forms and dissociates many times during 1 msec; or the channel may be affected by some property of the membrane that responds gradually to an increasing density of bound calcium.

The leakage channel accounts for most of the conductance of the resting frog nerve. When a node is placed in 5 mM TEA, the membrane depolarizes about 6 mv (Schmidt and Stämpfli, 1966), suggesting that the potassium channel plays a part in the determination of the resting potential as well. The sodium channel also contributes, especially at low calcium concentrations (see Schmidt, 1965). As pointed out by Finkelstein and Mauro (1963), the simultaneous contribution of three independent ionic systems to the resting potential ought to be described by a theory that takes explicit account of the individual current-voltage characteristics of each of the three systems. Physical theories that start with the assumption that there is only one kind of region through which all ions flow cannot satisfy this requirement. For this reason the Goldman (1943) equation, commonly used to calculate resting potentials and fluxes, must eventually be replaced by a more complete theory.

Note Added in Proof An elegant voltage clamp study of the effects of TEA on *Xenopus laevis* nodes has appeared (Koppenhöfer, E. 1967. *Arch. Ges. Physiol.* **293**:34)

[1] The names sodium and potassium applied to the channels indicate the principal ion flowing through the channel in normal conditions. There are many situations in which other ions contribute a significant fraction of the fluxes.

that is in substantial agreement with my results, except that τ_n is reported to be lengthened 70% by 0.3 mM TEA. The dissociation constants of the receptor complex have been reported for some TEA analogues (Hille, B. 1967. *Abstr.* 11th *Meet. Biophys. Soc.* 19).

I am glad to acknowledge long and fruitful discussions with Dr. Frederick A. Dodge, who conceived and constructed the prototype on-line voltage-clamp-to-computer system.
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