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IONIC CHANNELS IN NERVE MEMBRANES

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CONTENTS

1

IONIC CHANNELS IN NERVE MEMBRANES

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I. INTRODUCTION

Bernstein (1902, 1912) attributed the electrical potentials of excitable cells to a selective permeability of the cell membrane to ions. The membrane hypothesis has been so fruitful that it is now possible to predict most of the electrical responses of an axon from measurements of its permeability to ions. The permeability responds to any change in the membrane potential, and, in most axons, can vary from a nearly complete selectivity for potassium ions to a nearly complete selectivity for sodium ions. Although the mechanisms underlying the permeability changes remain obscure, some questions have already been answered.

This review focuses on two questions:

1. Do the several permeant ions share a common pathway through the membrane or are the pathways for different ions different?
2. Is the ionic permeability a diffuse property of broad areas of membrane or is it localized in rare specializations of the membrane?

These questions have received little attention until recently, yet the relevant literature is already vast. The discussion here is restricted to the three best understood axons, giant axons of squid and lobster and myelinated fibers from frogs, and concentrates on a few of the many experiments. Much interesting work on other axons, electric organs and muscles, supports the conclusions of this review, but cannot be discussed for lack of space.

This review concerns the nature of the ionic permeabilities. The reader may want to consult other reviews (Hodgkin, 1964; Noble, 1966; Cole, 1968) for a discussion of the relationship between permeability changes and the excitation of nerve cells.

II. THE HODGKIN-HUXLEY MODEL

This section describes electrical measurements of ionic permeability in axons and some of the models and theories now used to summarize and explain the results. Particular attention is given to experiments suggesting a difference between the ionic pathways.

A. *The Voltage Clamp*

The voltage clamp is an electrical technique for measuring movements of ions across the membranes of excitable cells. In ordinary use it can follow the time course of a movement of 10^6 ions that lasts 0.5 msec. The theory and execution of voltage clamping are authoritatively reviewed elsewhere (Hodgkin, Huxley and Katz, 1952; Dodge and Frankenhaeuser, 1958; Moore and Cole, 1963; Julian, Moore and Goldman, 1962a). The following is a simplified outline of the method.

In most neurophysiological work, current is applied as a stimulus and the ensuing changes in potential are measured. The voltage clamp reverses the process: the experimenter applies a voltage and measures the flow of current. In practice, the membrane potential is forced to follow prescribed step changes by a negative feedback amplifier that passes the necessary current across the membrane. Without the voltage clamp, the membrane current is partly a displacement current that charges the membrane capacity and partly an ionic current. However, in a voltage clamp with step changes of voltage, the membrane capacity is charged at a constant level for most of the time, and the observed membrane current is purely ionic. Hence the voltage clamp measures the movement of ions.

Two simplifying experimental observations enable the investigator to identify which ions carry the current. First, because each ion tends to move passively down its electrochemical potential gradient, basic thermodynamic arguments can be used to predict whether the net movement of an ion is inward or outward at a given potential. An ion contributes no membrane current when the membrane is clamped at the equilibrium potential for that ion. Second, because the permeability changes are relatively insensitive to the concentration of the major ions Na, K and Cl, the movements of one ion may be stopped by replacing that ion by an impermeant species with little change in the movements of other ions.

Hodgkin, Huxley and Katz (1952) found that the ionic current of squid giant axons develops in several stages following a step depolarization. In this review the membrane potential is defined as the inside potential minus the outside potential and is typically -65 mV in a resting squid axon. In the first milliseconds after a step to 0 mV transmembrane potential the ionic current, initially small and outward, grows to a large inward current only to reverse again and become large and outward. The permeability and the selectivity of the membrane change with time. By replacing the Na ions by impermeant ions and by measuring equilibrium potentials, Hodgkin and Huxley (1952a) separated the ionic currents into two major components, one carried by Na ions and the other by K ions, and one minor component, the "leakage" carried by unidentified ions.

From the viewpoint taken in this review, the most significant result of the experiment is the difference between the time courses of sodium current and potassium current. The sodium current increases to a peak soon after a step depolarization, but then declines to a negligible value after a few milliseconds. The potassium current rises more slowly to a steady value. Somehow during a

step depolarization the permeability of the membrane to Na ions is activated rapidly and then inactivated, whereas the permeability to potassium is activated with more delay and is not inactivated (Fig. 1).

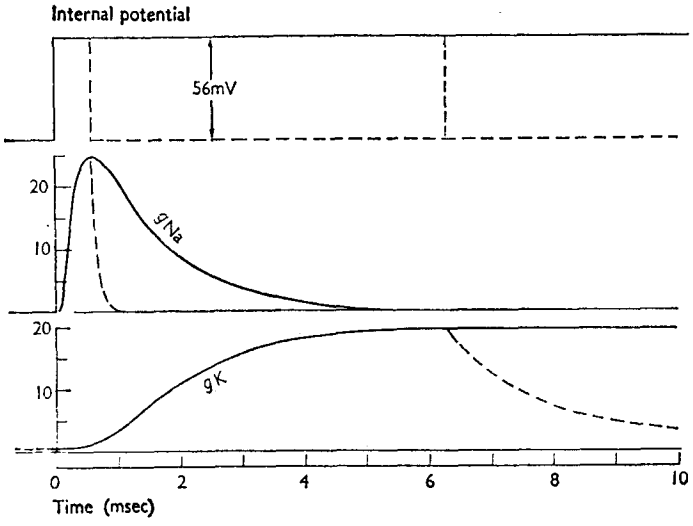

FIG. 1. Time course of Na and K conductance following voltage clamp steps from −65 mV to −9 mV in a squid giant axon. Continuous curves are for maintained depolarization. Broken curves are for a return to the resting potential. Conductance in μmho cm^{-2}. Temperature 8.5°C. (From Hodgkin, 1964, based on Hodgkin and Huxley, 1952a, b.)

B. *The Hodgkin–Huxley Equations*

Hodgkin and Huxley (1952b, c, d) incorporated their observations in a precise mathematical model. They showed that the relationship between current and voltage at any instant in time is linear, as it would be in an "ohmic" conductor. This meant that an expression like Ohm's law could be applied, to relate the ionic movements to the driving forces, rather than the more complicated theoretical expressions like the Goldman (1943) flux equation. For the sodium current I_{Na}

$$I_{Na} = g_{Na}(E - E_{Na})$$

where g_{Na} is called the sodium conductance, E is the membrane potential, and E_{Na} is the potential at which the net movement of Na ions stopped, the sodium equilibrium potential. The total ionic current I_i is the sum of I_{Na}, I_K, and I_L, where I_L stands for leakage current. The expression for total ionic current is then

$$\begin{aligned} I_i &= I_{Na} + I_K + I_L \\ &= g_{Na}(E - E_{Na}) + g_K(E - E_K) + g_L(E - E_L). \end{aligned}$$

A description of how the conductances g_{Na}, g_K and g_L depend on those parameters that could be varied experimentally completes the model. The value of

g_L does not change with time or voltage and is set equal to a constant g_L. Although the degree and rate of activation of g_{Na} and g_K increases with increasing depolarization, the conductances never exceed a limiting value. In the model, g_{Na} and g_K represent this maximum conductance of the permeability mechanisms. An increasing fraction of the maximum permeability is activated with increasing depolarization. The relationship is written

$$g_{Na} = m^3 h \bar{g}_{Na}$$
$$g_K = n^4 \bar{g}_K$$
$$g_L = \bar{g}_L$$

where m^3h and n^4 represent the fractions of the sodium and potassium permeabilities that are "turned on". The coefficients h, m and n are continuous functions of potential and time. They are dimensionless parameters that vary between

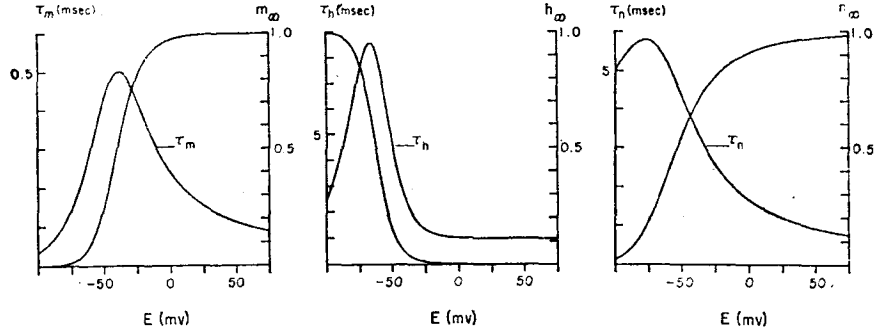

FIG. 2. The steady-state values and time constants for the parameters h, m and n in the Hodgkin–Huxley model. Note different time scales. The resting potential is $E = -65$ mV. (Kindly prepared by Dr. W. L. Hardy.)

zero and one with first order kinetics and rate constants that depend on the potential. The S-shaped rise of permeability observed after a depolarizing step (Fig. 1) is imitated by raising the parameters m and n to higher powers.

The kinetic changes of the parameters of the model are summarized by the three graphs in Fig. 2. At any membrane potential E, the values of h, m and n relax towards steady-state values h_∞, m_∞ and n_∞ along an exponential time course of time constant τ_h, τ_m and τ_n. At the resting potential m_∞ and n_∞ are small and h_∞ is large. Thus the sodium and potassium permeabilities, proportional to m^3h and n^4, are relatively small at rest. After a depolarization, m increases rapidly activating the sodium permeability, but h begins to decrease at the same time, and the sodium permeability eventually becomes inactivated. The potassium permeability is activated by the slow increase of n. In the model, the changes of h, m and n depend on the membrane potential but not on each other or on the flow of current. These equations successfully describe the propagated action potentials and the subthreshold responses of normal squid giant axons.

C. *The Meaning of the Equations*

A well-developed mathematical model of a phenomenon contains clues to the underlying physical processes. Hodgkin and Huxley realized that the model's success did not show that their choice was correct. The ideas were hypotheses awaiting further experimental tests. The ionic permeability is controlled by a structure responding to the electric field on the membrane. Hodgkin and Huxley envisioned charged or dipolar controlling "particles" within the membrane that redistribute themselves after a change in the membrane potential. The particles open the pathways to ionic movement by occupying certain sites. The large conductance increases after small changes in potential could be imitated by assigning several electronic charges to each particle. More than one particle is needed to activate each pathway to explain the S-shaped time course of the conductance increases. In the model, the controlling particles are represented by h, m and n, with m^3h being the probability of three m-particles and one h-particle occupying the sites for opening a pathway for Na ions, and with n^4 being the probability of four n-particles occupying the sites for opening a pathway for K ions. The empirical equations used to describe the rates of increase or decrease of h, m and n, as a function of potential are like Goldman's (1943) equation for the rate of movement of charged particles in an electric field.

The experiments suggest no interaction between the permeability changes to Na ions and to K ions. Although the activation of g_K overlaps with the inactivation of g_{Na}, there is no simple kinetic relation between the two processes and at each potential the degree of overlap differs. Hence, in the equations, all parameters of I_{Na} are kept separate from those of I_K as though two independent permeability mechanisms exist. The success of the equations is the first evidence that Na ions and K ions may not move through a common pathway.

D. *The Independence Principle*

The independence principle is a theory which assumes that the movement of an individual ion is independent of the movement of every other ion. The theory successfully predicts the observed dependence of I_{Na} on the Na concentration of the medium (Hodgkin and Huxley, 1952a). More critically, the independence principle also predicts the ratio of the influx to the efflux of Na ions at each membrane potential. This prediction has not been tested directly. However, the measured ratio of the total influx to total efflux of ^{24}Na summed over many action potentials seems to agree with the theory (Keynes, 1951; Hodgkin and Keynes, 1955a).

The independence of K movements was tested by a direct measurement of the fluxes of ^{42}K in an axon polarized by applied current (Hodgkin and Keynes, 1955b). Contrary to expectations, an increase of the external K concentration reduces the K efflux at a constant membrane potential. With an 80 mV change in potential, the flux ratio changes by a factor of 2400. The independence

principle predicts a factor of only 24. Effectively, a net outward movement of K prevents a tracer influx of K and vice versa. To explain the deviations from independence, Hodgkin and Keynes suggested that K ions might move in single file through narrow channels containing several ions in a row. Hence, in addition to differing in their selectivity, the pathways for Na and K movements differ structurally so that Na movements obey independence and K movements do not.

E. *Myelinated Nerves*

The Hodgkin–Huxley model has been reinterpreted and modified by many investigators. Some revisions arose because new methods gave new information. Others arose because the existing observations fit several types of equations and each equation could receive several physical interpretations. Recent reviews have summarized many of these developments (Hodgkin, 1964; Noble, 1966; Grundfest, 1961, 1966; Cole, 1968; Caldwell, 1968; Tasaki, 1968).

One extensive test of the form of the Hodgkin–Huxley model was its application to an entirely different type of nerve, myelinated fibers from frogs and toads (Dodge and Frankenhaeuser, 1958, 1959). Frankenhaeuser (1960a, 1963), working with *Xenopus laevis,* separated the ionic currents of the node of Ranvier into two major components, I_{Na} and I_K, and two minor components, I_L and Ip, where Ip was a small time-dependent current, possibly carried by sodium ions. In *Xenopus* the instantaneous sodium current and the instantaneous potassium current are nonlinear functions of the potential and can be more closely approximated by Goldman's (1943) flux equation than by Ohm's law. The time courses of the changes in sodium permeability P_{Na} and in potassium permeability P_K are very similar to those for the squid giant axon. They can be fitted by the kinetic model

$$P_{Na} = m^2 h \bar{P}_{Na}$$
$$P_K = n^2 k \bar{P}_K$$

where m^2h and n^2k represent the fraction of the maximum permeabilities $\bar{P}_{Na}$ and $\bar{P}_K$ that are "turned on". The parameters h, m, and n have the same significance as in the squid model. The parameter k gives an extremely slow (taking many seconds) inactivation of P_K with prolonged depolarization. A slow potassium inactivation exists in the squid giant axon as well (Ehrenstein and Gilbert, 1966). Koppenhöfer (1967) and Koppenhöfer and Schmidt (1968b) also working with *Xenopus* have found that n^4 fits the changes of P_K better than n^2.

Dodge (1961, 1963) analyzed the ionic currents of myelinated fibers from *Rana pipiens,* finding three components, I_{Na}, I_K and I_L. The changes are well approximated by

$$P_{Na} = m^3 h \bar{P}_{Na}$$
$$g_K = n^4 \bar{g}_K$$

For very long depolarizations (several seconds) a parameter for potassium inactivation is also needed (Hille, 1967b).

These experiments and voltage clamp experiments on other axons, especially lobster giant axons (Julian, Moore, and Goldman, 1962b), have defined some general properties of nerve fibers. In all nerves studied the currents can be separated into at least two components which are kinetically independent. The ionic permeabilities vary with time and voltage between negligibly small values and a limiting value which seems to represent a true maximum. The changes of the permeability are well described by the product of two independent factors, an activation factor and an inactivation factor.

On the other hand, the instantaneous current–voltage relationship and the exact form of the activation and inactivation factors seem specific to individual nerves. Frankenhaeuser (1960b) discussed the significance of the difference between the instantaneous current–voltage relationships of squid and toad nerves. The nonlinear relationship found in *Xenopus* nerves is expected for a symmetrical membrane bathed in asymmetrical solutions, whereas the linear relationship in squid axons can only be the consequence of an asymmetrical membrane. Hodgkin suggested a simple asymmetrical charge distribution. A suitable layer of positive fixed charge at the outer surface or of negative fixed charge at the inner surface of the membrane could make I_{Na} linear with voltage despite the asymmetry of the Na ion concentrations. A graded series of energy barriers with the highest outside and the lowest inside could have the same effect (Woodbury, 1969). The charge distribution or the sequence of energy barriers needed to make I_K linear with voltage is the converse of that for I_{Na} because the asymmetry of the K ion concentrations is in the opposite direction. Frankenhaeuser (1960b) concluded that the Na- and K-permeable sites of squid axons had to be separated from one another in squid axons to have such contrasting distributions of charge.

In the remainder of this review the difference between P_{Na} and g_{Na} is not of great importance, and for ease of discussion g_{Na} is applied to nodes of Ranvier as well as to giant axons.

F. *Modifications of the Kinetic Model*

Experiments with large hyperpolarizations preceding a depolarization reveal more delay in the rise of g_K in squid axons than the fourth power of the parameter n can match (Cole and Moore, 1960). The greater the conditioning hyperpolarization, the greater is the subsequent delay in the rise of g_K. The twenty-fifth power of n is needed to fit the observations following a hyperpolarization to -212 mV. The rise of g_K in myelinated nerve also cannot be described by n^4 after large conditioning hyperpolarizations (personal observation). The hypothesis that the power of n indicates how many particles traverse the membrane to activate the potassium permeability becomes less plausible as the number of particles required increases. Other apparently satisfactory kinetic models have been derived on the basis of the movement of an unlimited number of particles (FitzHugh, 1965) or even of single particles with interactions between sites (Tille, 1965). Whereas the rise of g_K is considerably delayed by large conditioning

hyperpolarizations, the time course of g_{Na} is not affected. Hence the degree of temporal overlap of g_{Na} and g_K varies greatly with the conditioning hyperpolarizations. The selective conditioning of K movements by large hyperpolarizations has been used to argue that the permeability pathways for Na and K are separated in the membrane (Narahashi and Moore, 1968).

Two physical models propose a coupling between the sodium and potassium permeability changes by assuming a common pathway for Na and K movements. Mullins's (1959, 1968) model supposes that ions pass through special pores in the membrane if the radius of a pore matches the radius of the ion and if certain other non-penetrating ions have not blocked the pore. After a depolarization the average radius of the pores shifts from the potassium radius to the sodium radius and back again. At the same time, non-penetrating ions leave the pores. Goldman's (1964) model supposes that ions bind to the negative charges of certain phospholipid molecules before moving through the membrane. The sites are normally occupied by Ca ions, but when the membrane is depolarized, the dipole of the site turns to make first a Na specific then a K specific site. It is unknown whether these models can match the experimental results because they do not have a complete predictive form. Both theories would probably predict a coupling between the activation and the inactivation of the sodium conductance. One mathematical model with this kind of coupling fits many experiments (Hoyt, 1963, 1968). Like the Hodgkin–Huxley model, Mullins's and Goldman's models propose that the electric field in the membrane acts on charged or dipolar controlling particles to activate the conductances.

In the models with convertible sites, one site can contribute to g_{Na} and to g_K but not simultaneously. Mullins (1968) has proposed that his model would have to be abandoned if under some conditions the sum of g_{Na} and g_K exceeded the maximum value $\bar{g}_{Na}$. As is shown below, the temporal overlap of g_{Na} and g_K can be increased considerably by pharmacological treatments, but in no case is Mullins's criterion known to be exceeded. However, the wide range in overlap from almost none to almost complete argues against any model with a kinetic dependence of the opening of a K site on the closing of a Na site.

G. *Ionic Specificity*

The Hodgkin–Huxley equations suggest that axons are inexcitable in the absence of Na ions, but experimentally this is not always the case. Certain cations, notably the Li ion, are good substitutes for the Na ion. In the voltage clamp, the permeability to Li parallels the permeability to Na in time course and amplitude (Moore, 1958; Cole and Moore, 1960; Chandler and Meves, 1965; Hille, 1968a). These observations can be incorporated into the model by replacing the term $m^3h\bar{g}_{Na}(E-E_{Na})$ by the product of m^3h and a new factor that includes the effects of Na ions, of Li ions, and of any other sodium substitutes present. The form of this new factor has not been investigated experimentally.

Many organic cations such as ammonium, guanidinium, hydrazinium, and hydroxylammonium ions are sodium substitutes (Lorente de Nó, Larramendi,

and Vidal, 1957; Tasaki, Singer and Watanabe, 1966). In the squid giant axon the permeability to NH_4 ions is the sum of two kinetic components, for NH_4 is simultaneously a sodium substitute and a potassium substitute (Lüttgau, 1961; Binstock and Lecar, 1967, 1969). One component has a time course m^3h and a permeability of 0.3 times P_{Na}. The other has a time course n^4 and a permeability of 0.3 times P_K.

Even the K ion is a sodium substitute in the sense that there is a component of g_K or P_K that follows the time course m^3h. However, the effective P_K is so much smaller than P_{Na} that this phenomenon is not normally important. Frankenhaeuser and Moore (1963) found the ratio $P_{Na}:P_K$ to be 1:1/20 during the initial increase of permeability in myelinated nerves. Using perfused squid giant axons Chandler and Meves (1965) found the following permeabilities of sodium substitutes relative to sodium: Li, 1.1; K, 1/20; Rb, 1/40; Cs, 1/61.

External Rb ion is a K substitute with a permeability almost equal to P_K (Pickard, Lettvin, Moore, Takata, Pooler, and Bernstein, 1964; Müller-Mohnssen and Balk, 1966). No ions other than Rb and NH_4 have been shown to be K substitutes in a voltage clamp experiment. Attempts to do so have failed because of deviations from the independence principle in the test solutions (Frankenhaeuser and Moore, 1963; Chandler and Meves, 1965).

These experiments show that there is not an absolute distinction between Na movements and K movements. The distinction lies rather between the kinetic components of permeability proportional to m^3h and to n^4. Chandler and Meves (1965) observed that the permeability proportional to m^3h has a selectivity $P_{Na}:P_K$ that is independent of the value of m or h. Theories that gradually convert a sodium pathway into a potassium pathway by a continuous change of the radius of a pore or of some other property of the system are inconsistent with their observation.

H. *Conclusion*

Voltage clamp experiments have identified several components of ionic movements. In the following discussion the pathways for the movements are called Na channels, K channels, and leakage channels. These terms tentatively denote the concept of pathway without requiring that different ions have different pathways or even that pathways be discrete, localized structures.

A prepulse to −50 mV inactivates the Na channels. A prepulse to −80 mV removes inactivation. A prepulse to −200 mV strongly delays the opening of K channels. In all cases the time course of the opening of Na channels bears no simple relation to the time course of the opening of K channels, and the responses are successfully predicted by assuming independent kinetics for different channels. The kinetic differences between Na and K channels would have been detected even if there were no difference in the ionic selectivities.

The ionic channels differ in their structure as well as in their kinetics. A Na channel is selective for Na and Li ions and is less permeable to many other ions including K. It may frequently be unoccupied (independence) and, in the squid

giant axon, may be asymmetrical with the highest energy barrier at its outer end. A K channel accepts K and Rb ions and is less permeable to other ions including Na. It may retain several K ions at one time, and may be asymmetrical with the highest energy barrier at its inner end.

In the simplest interpretation, Na and K channels are independent structures occupying different regions of the membrane. Any model with a gradual conversion of a channel from a condition of greater Na selectivity to a condition of greater K selectivity is untenable. However, the possibility that a Na channel opens and closes before being converted into a K channel which then opens cannot be rigorously excluded. The major difficulty with this type of coupled system is the apparent kinetic independence of g_{Na} and g_K and the major structural reorganization necessary in converting a Na channel into a K channel. As further evidence of independence and of structural difference accumulates, the coupled systems must be made increasingly complex to work.

The stimulating experiments of Tasaki's laboratory have uncovered many new phenomena relevant to ionic selectivity and the separation of channels. It will not be possible to analyze these phenomena until they are described in quantitative kinetic experiments with the voltage clamp, but Tasaki (1968) believes that his experiments require a common pathway for the simultaneous movements of all ions. As he suggests, absolutely selective channels are not likely. The alternative of several relatively selective channels, as described here, appears to fit all his qualitative experiments.

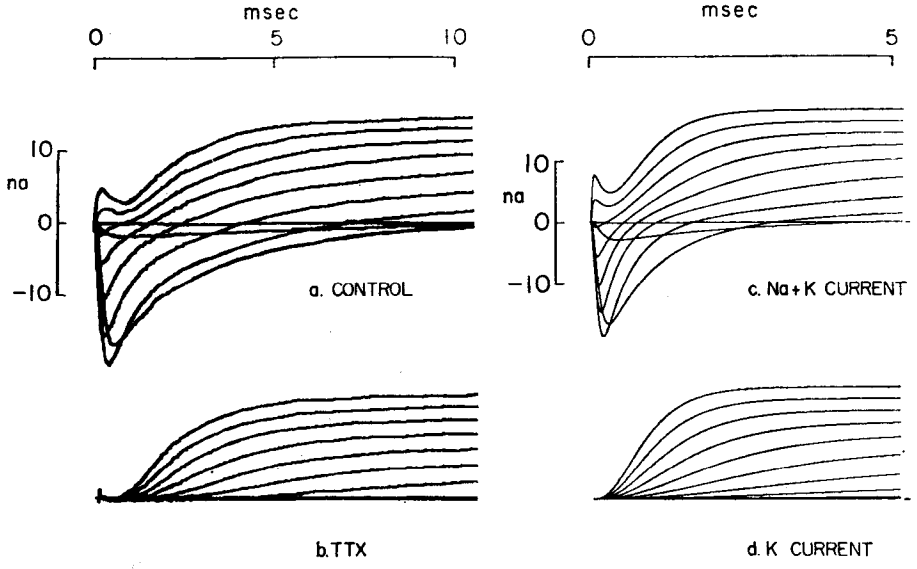

FIG. 3. Voltage clamp currents (minus leakage currents) in a node of Ranvier in (a) Ringer's solution and (b) 300 nM TTX (Hille, 1966) compared with (c) the sum of Na and K currents and (d) K current alone computed from a model node (Dodge, 1961). The currents correspond to potentials spanning the range from −60 mV to +75 mV in 15 mV steps. Outward current is positive. Temperature (a, b) 13°C; (c, d) 22°C.