Biophys. Struct. Mechanism 1, 1—16 (1974)

Ionic Channels through the Axon Membrane* (A Review)

Werner Ulbricht**

Physiologisches Institut der Universität Kiel

Received January 31, 1974

Abstract. Ionic channels are discrete sites at which the passive movement of ions takes place during nervous excitation. Three types of channels are distinguished. 1. Leakage channels that are permanently open to various cations. 2. Na channels that open promptly on depolarization but slowly close again (inactivate) on sustained depolarization and that are predominantly permeable to Na^+ ions. 3. K channels that on depolarization open after some delay but stay open and that are mainly passed by K^+ ions. The selectivity sequence of the Na channels of the squid axon (or frog nerve) is as follows: $Na^+ \approx Li^+ > (Tl^+) > NH^+_4 \gg K^+ > Rb^+$, Cs^+; that of K channels is: $(Tl^+) > K^+ > Rb^+ > NH^+_4 \gg Na^+$, Cs^+. Na channels are selectively blocked by tetrodotoxin (TTX) or saxitoxin (STX), K channels by tetraethylammonium ions (TEA). Either channel type is reversibly blocked when one drug molecule binds to one site per channel, the equilibrium dissociation constant of these reactions being about 3×10^{-9} MTTX (or STX) and 4×10^{-4} M TEA, respectively. Because of their specificity and high affinity, TTX and STX are used to "titrate" the Na channels whose density appears to be of the order of $100/\mu m^2$. The "gates" of the channels operate as a function of potential and time but independent of the permeating ion species. Drugs (e.g. veratridine) and enzymes (e.g. pronase, applied intraaxonally) cause profound changes in the gating function of the Na channels without influencing their selectivity. This points to separate structures for gating and ion discrimination. The latter is thought to be, in part, brought about by a "selectivity filter" of which detailed structural ideas exist. Recent experiments suggest that the gates of the Na channels are controlled by charged particles moving within the membrane under the influence of the electrical field.

Key words: Selectivity — Tetrodotoxin — TEA — Channel Density — Gating Function.

The nervous impulse consists of a brief change in membrane polarization, the action potential, that travels down the nerve fibre. In most nerves the action potential is brought about by a transient flow of Na^+ ions into the fibre that is followed by an outflow of K^+ ions whereby the ions move passively through the membrane, i. e. down their respective electrochemical gradients. It has long been suggested that this movement of ions takes place at discrete membrane sites which have been termed "channels" although details of their structure have as yet to be worked out. The present review describes recent experiments that may help in this formidable task.

Behaviour of Channels

As a rule several types of channels are distinguished. Their average behaviour can best be defined with the aid of the classical current pattern that is observed when the nerve membrane is forced, in a socalled "voltage clamp" experiment, to undergo a sudden depolarization, say by 56 mV as in Fig. 1. This figure stems from

* Lecture presented at the Annual Meeting of Deutsche Gesellschaft für Biophysik, Jülich, September 1973.

** Supported by the Deutsche Forschungsgemeinschaft.

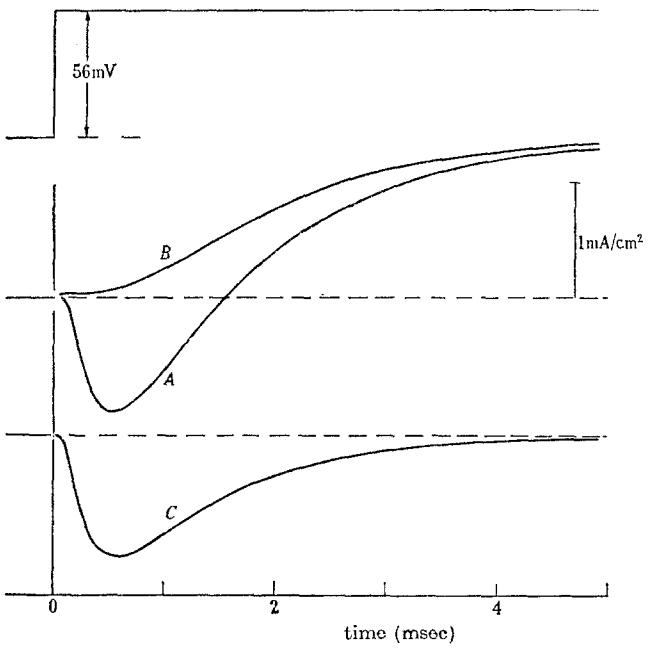

Fig. 1 A—C. Separation of membrane current into components carried by Na and K. A depolarization by 56 mV was applied at $t = 0$ and in artificial seawater (400 mM Na) curve *A* was obtained. *B*, in Na-poor (40 mM) seawater with no net movement of sodium for this potential so that *B* constitutes the K current (plus leak). *C*, Na current as difference between *A* and *B*. 8.5 °C. From Hodgkin (1957); by permission of author and the Royal Society

an experiment on a squid giant axon and curve A shows the total membrane current when the fibre is in normal seawater. The current is at first inward (downward) but it soon becomes outward and tends towards a steady level. When the external Na is reduced to such a concentration that the driving force acting on these ions vanishes during the depolarizing impulses, the monotonic current curve B is found. Since we can get rid of the Na component by this procedure, B represents the K component containing a small constant leakage current whose carrier is undefined. By subtraction, then, the transient Na curve C is obtained. Curve C describes the average behaviour of one type of channels that open promptly when the membrane is depolarized but slowly close again by "inactivation" on sustained depolarization. We shall call these channels "Na channels" since they normally and mainly let pass Na^+ ions through the membrane. By analogy we whall postulate from curve B that there are K channels that on depolarization open after some delay but stay open and that are normally and predominantly used by K^+ ions. K as well as Na channels promptly close when the membrane potential is brought back to its resting level. It appears worth mentioning here that terms as "prompt opening", "slow closing" etc. describe the average kinetic behaviour of the channels as derived from the macroscopic phenomenon of the ionic current. The real individual channel may well exist in only two states: fully opened or completely closed.

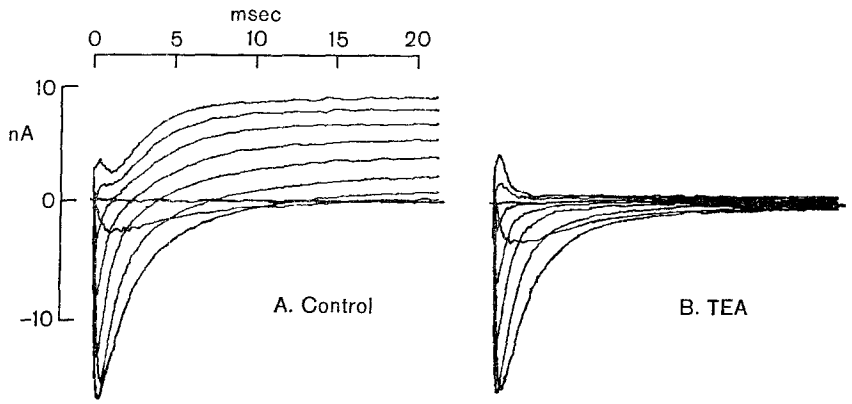

Fig. 2 A and B. Voltage clamp currents, in nA per nodal area (corrected for leakage currents) of a frog nerve fibre, in Ringer solution (A) and 6 mM TEA (B). Underlying depolarizing pulses in steps of 15 mV; 11 °C. From Hille (1970, based on his paper of (1967); by permission of author and Pergamon Press

Finally there are channels of a third type through which the leakage current passes. They are permanently open and do not appear to discriminate well between monovalent cations (Hille, 1973).

Fig. 1 also demonstrates that during a depolarizing impulse the Na current is already being inactivated when the K current rises. This observation has led to the idea of a single type of channel that after opening first favours the passage of Na^+ ions but within 1 msec changes its selectivity towards K^+ ions (Mullins, 1959). This hypothesis has been questioned since each of the two current components (Na and K) can be separately blocked by drugs (Mullins, 1968; Narahashi and Moore, 1968), however only recent experiments of a different kind appear to have definitely refuted the one-channel hypothesis (Armstrong *et al.*, 1973).

Specific Blocking Agents

The inhibitor of the K current component is the tetraethylammonium ion (TEA). In squid axons TEA blocks only when applied internally (Tasaki and Hagiwara, 1957; Armstrong and Binstock, 1965) while in nodes of Ranvier of frog nerve fibres both external (Koppenhöfer, 1967; Hille, 1967) and internal application (Koppenhöfer and Vogel, 1969) are effective. Fig. 2 illustrates the effect of external TEA on the latter preparation. The family of ionic currents on the left was obtained in normal Ringer solution with depolarizing impulses of increasing amplitude. The currents on the right were observed with the same procedure after 6 mM TEA had been added to the Ringer solution. This caused the outward delayed currents to vanish completely while the early inward currents remained unaffected. With lower drug concentrations it can be shown that TEA reduces only the amplitude of the K current without interfering with its kinetics and an obvious explanation would be that the drug simply reduces the number of operational K channels. The quantitative dependence of this inhibition on TEA concentration suggests that a K channel is completely blocked when one TEA ion

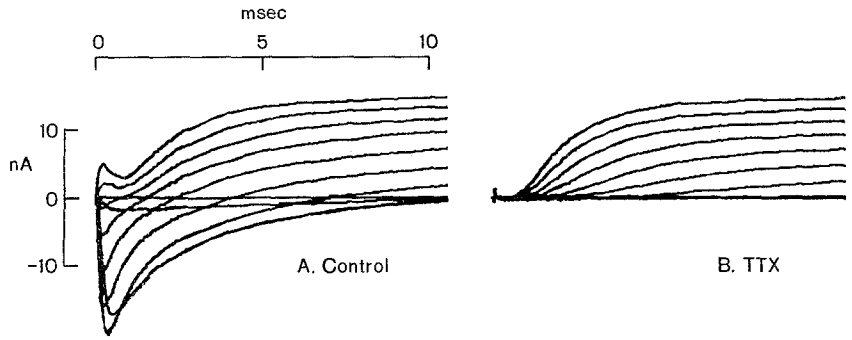

Fig. 3 A and B. Voltage clamp currents, in nA per nodal area (corrected for leakage currents) of a frog nerve fibre in Ringer solution (A) and 300 nM TTX (B). Underlying impulses depolarized, in 15 mV steps, the membrane between internal potentials of − 60 and + 75 mV; 13 °C. From Hille (1970, based on his paper of 1966); by permission of author and Pergamon Press

binds to one receptor per channel. This fully reversible reaction is very fast (Vierhaus and Ulbricht, 1971b) and its equilibrium dissociation constant, K, is 0.4 mM (Hille, 1967). Substitution of only one ethyl group of TEA by a methyl group renders the molecule rather ineffective so that K increases to 15 mM (see Hille, 1970). Peculiar effects of other TEA derivatives, when applied to the inside of the nerve fibre, will be described later (p. 11).

The specific inhibitors of the Na channel are tetrodotoxin (TTX) and saxitoxin (STX). Their structure is complicated and although the toxins have similar molecular weights of a little over 300 they are not chemically related; details can be found in the reviews of Kao (1966) and of Evans (1972). TTX is found in puffer fish and some kind of salamander. STX is produced by dinoflagellates on which certain shellfish feed that may considerably concentrate the toxin which, therefore, is also known as "paralytic shellfish poison". From either toxin as little as about 3 nM (3×10^{-9} M) suffices to block, at equilibrium, 50% of the Na channels (Hille, 1968; Cuervo and Adelman, 1970). Hence in 300 nM TTX no trace of the Na current is observable as shown in the right part of Fig. 3 which by comparison with the control also demonstrates that TTX (like STX) does not influence the delayed current through the K channel.

Because of the highly specific effect of such low toxin concentrations the underlying pharmacological reaction deserves our special interest. In particular it is hoped that we shall eventually understand the structure of the toxin receptor as an important part of the Na channel. Hille (1968) postulated from equilibrium experiments that a Na channel is promptly and completely blocked when one toxin molecule binds to a specific channel site. It is conceivable that the toxin literally plugs the external mouth of the channel. Interestingly, the inner mouth does not seem to bear a similarly complementary structure since even very high intra-axonal concentrations of TTX (Narahashi *et al.*, 1966; Koppenhöfer and Vogel, 1969) or STX (Narahashi, 1971) remain without any blocking effect.

The rate of TTX action has been studied in squid axons (Cuervo and Adelman, 1970) but in this preparation a cell layer impedes the diffusional access to the

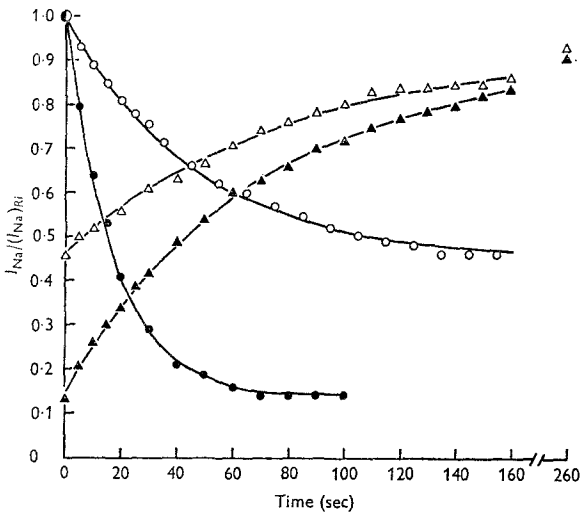

Fig. 4. Development of and recovery from TTX-induced inhibition of Na current, I_{Na}. Ordinate, I_{Na} relative to its value before the application of 3.1 nM TTX (open symbols) or 15.5 nM TTX (filled symbols). Abscissa, time after change of solution. Circles and triangles refer to measurements during onset and offset, respectively. Node of Ranvier at room temperature. From Schwarz *et al.* (1973); by permission of The Journal of Physiology

membrane. In nodes of Ranvier the experimental situation is much more favourable (Vierhaus and Ulbricht, 1971a) and in recent experiments (Schwarz *et al.*, 1973) the rates of the reaction between TTX and its receptor, R, at the nodal membrane have fully confirmed Hille's scheme that can be written

$$\mathrm{TTX} + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{TTX}.R\,,$$

where TTX.R is the drug-receptor complex and k_1 and k_2 are the rate constants of association and dissociation, respectively. Since the toxin only reduces the amplitude of the Na current without changing its time course or the general dependence of the Na permeability on membrane potential, the onset of TTX action can simply be studied with periodic depolarizing impulses of constant amplitude. Fig. 4 illustrates such an experiment in which successively two TTX concentrations, 3.1 nM (open circles) and 15.5 nM (closed circles), have been applied. Obviously, increasing the TTX concentration accelerates the block and it does so quantitatively in accordance with the reaction scheme which also requires the offset rate on washout to be independent of concentration. The curves through the triangles show that this is indeed observed. Although the time constants involved in Fig. 4 are remarkably large they appear to be governed solely by the reaction and it can be shown unequivocally that the toxin reaches the membrane without disproportionate delay. At 20 °C the mean $k_1 = 3.3 \times 10^6\ \mathrm{M^{-1}\,sec^{-1}}$ and the mean $k_2 = 1.4 \times 10^{-2}\ \mathrm{sec^{-1}}$. Temperature experiments yield Arrhenius activation energies of 13.7 and 20.5 kcal/mole for k_1 and k_2, respectively. This and interesting results of the interaction between H^+ and TTX at the Na channels (Wagner and Ulbricht, 1973, 1974) offer clues to the reaction and the receptor.

Counting Channels

Since TTX and STX show such a high affinity for the channel receptor, attempts have been made to determine the number of Na channels by some kind of toxin titration (Moore *et al.*, 1967; Keynes *et al.*, 1971). In principle, nerve preparations of known membrane surface are successively equilibrated in a small volume of toxin solution whose concentration is gradually diminished by binding to the Na channels. The loss in toxin content is determined as loss in blocking potency i.e. by an bio-assay. If the extracellular space is measured separately and allowed for and under the assumption that no unspecific binding takes place, an upper limit of the receptor (= channel) density is obtained. It is between 36 and 75 sites/μm^2 for thin unmyelinated nerve fibres of lobster, crayfish and rabbit, i.e. preparations that yield a large membrane area per weight. Similar experiments on myelinated fibres in which less than 0.1% of the surface belongs to the excitable membrane show that unspecific binding is indeed relatively low (Keynes *et al.*, 1971). This was recently confirmed when ^{3}H-labelled TTX and STX became available for binding studies in nerves (Colquhoun *et al.*, 1972; Henderson *et al.*, 1973a, b) and nerve membrane homogenates (Benzer and Raftery, 1972). These measurements revealed a component of Langmuir binding whose equilibrium constant is comparable to that postulated in pharmacological experiments. Also, the measurements seem to confirm the astonishingly low density of Na channels of the order of 100/μm^2, an order at which one can arrive from independent experimental evidence (see p. 14) or by theoretical arguments (Hille, 1970). It should be mentioned here that Henderson and Wang (1972) and Benzer and Raftery (1973) have succeeded in solubilizing a membrane component that very specifically binds TTX as tested with ^{3}H-labelled toxin. This component has a molecular weight of the order of 500,000 and may be part of the Na channel. Hence its further purification and chemical characterization is eagerly awaited. Unfortunately, the K channels cannot be "titrated" similarly since the equilibrium dissociation constant of TEA binding is much too large. Armstrong (1966, 1969), from an intricate interpretation of the effects of intraaxonal application of quaternary ammonium ions, estimates the K channel density of the squid membrane to be about 70/μm^2.

Selectivity of Channels

As mentioned before the Na channel owes its name to the ion species that normally and predominantly passes through it. But already at the turn of the century Overton (1902) observed that a frog muscle fully maintained its function in a solution in which Na^+ ions had been replaced by Li^+ ions. Obviously the Na channel does not greatly distinguish between the two ion species and for the squid axon this was assessed in a quantitative way by Chandler and Meves (1965) when they studied the channel's selectivity. Before their experiments are described the following digression may be fitting.

In Fig. 1 it was demonstrated that the early peak of the total ionic current was nearly all Na current, I_{Na}. Hence if the peak currents are plotted as a function of the membrane potential, E, at which they are observed in the voltage clamp, the typical $I_{Na}(E)$ relation of Fig. 5 is obtained. This curve through the triangles illustrates how I_{Na} increases on depolarization as more and more Na channels are

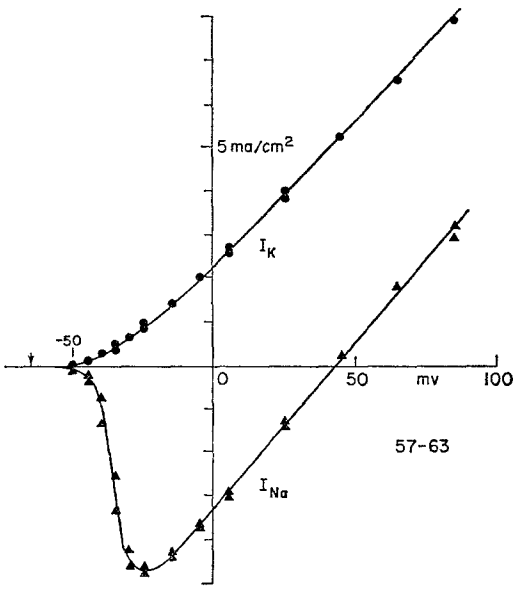

Fig. 5. Current-voltage curve of squid axon membrane. Ordinate, current density in mA/cm², inward current plotted downward. Abscissa, membrane potential in mV (axon interior relative to outside), depolarization to the right. Arrow marks holding potential between pulses (− 65 mV). Filled triangles give peak = sodium currents, I_{Na}, filled circles stationary outward = potassium currents, I_K. From Cole and Moore (1960); by permission of authors and Journal of General Physiology

open simultaneously. However, the current decreases again on further depolarization since the driving force acting on the Na^+ ions diminishes as the potential approaches the equilibrium potential for these ions. At this potential, I_{Na} vanishes and it reverses its sign beyond.

In general terms the reversal potential can be derived from the constant field equation (Goldman, 1943; Hodgkin and Katz, 1949)

$$E = \frac{RT}{F} ln \frac{P_K(K^+)_o + P_{Na}(Na^+)_o + P_{Cl}(Cl^-)_i}{P_K(K^+)_i + P_{Na}(Na^+)_i + P_{Cl}(Cl^-)_o} \tag{1}$$

which relates the membrane potential, E, at zero current to the activities, (), of the ions on both sides of the membrane (subscript o for outside, i for inside) and the respective permeability coefficients, P. The symbols R, T and F have their usual meaning. If $P_{Na} \gg P_K$, P_{Cl} as during the peak of the early current, Eq. (1) simplifies to the Nernst equation for the Na ion distribution

$$E_{Na} = \frac{RT}{F} ln \frac{(Na^+)_o}{(Na^+)_i} \tag{2}$$

which indeed gives a good description of the reversal potential in the normal solution as in Fig. 5.

Chandler and Meves (1965), however, have done their experiments on squid axons whose axoplasm had been squeezed out and substituted by a K-containing but Na-free solution. When this perfused axon was in its normal external medium,

i.e. Na-rich seawater, the observed peak currents were rather similar to those plotted in Fig. 5. In particular, during a very strong depolarizing pulse the early current was outward, but in contrast to Fig. 5 this current could not be due to a Na efflux. Chandler and Meves have shown that this current is carried by K^+ ions passing, though less readily, through the Na channel. Therefore, this K efflux has to be distinguished strictly from that underlying the delayed current through the K channel. In the pertinent experimental situation where there are no Na^+ ions on the inside of the membrane, E_{Na} is no longer defined by Eq. (2). Rather, the reversal potential, E_e, has to be derived from the Goldman-Hodgkin-Katz Eq. (1) for the present case with $(Na^+)_i = (K^+)_o = 0$ and $P_{Cl} = 0$, (the latter being suggested by independent evidence):

$$E_e = \frac{RT}{F} ln \frac{P_{Na}(Na^+)_o}{P_K(K^+)_i} . \tag{3}$$

When the ion activities are known the determined E_e yields the permeability ratio P_K/P_{Na} which is henceforth taken as a measure of the selectivity of the Na channel, the pathway of the early current. This ratio is 1/12 in squid axons; other monovalent cations (with the exception of Li^+) are even less permeant.

In myelinated nerve fibres intraaxonal perfusion is not feasible. To study the selectivity of the Na channel one has to measure successively the reversal potential, $E_{e,\,Na}$, in normal saline and then $E_{e,\,X}$ in a solution in which the Na^+ ions are substituted by other monovalent cations X^+ whose activity is $(X^+)_o$:

$$\Delta E_e = E_{e,X} - E_{e,\,Na} = \frac{RT}{F} ln \frac{P_X(X^+)_o}{P_{Na}(Na^+)_o} . \tag{4}$$

The difference in reversal potential, ΔE_e, again yields the relative permeability, P_X/P_{Na}, of the Na channel to X^+ ions. In Table 1 a few of the permeability ratios determined by Hille (1971, 1972) on nodes of Ranvier are compared to those found in the squid experiments. The sequence of relative permeabilities is similar in the two preparations and in both Rb^+ and Cs^+ ions are very poor Na substitutes. Another remarkable point which was not tested in the squid axon is that although hydroxylamine readily passes through the channel, methylhydroxylamine does practically not. Hille (1971) observed with several permeant organic cations that introducing a methyl group rendered them impermeable although they were not larger than other permeant cations bearing hydroxyl or amino groups. This puzzling finding led Hille to postulate a selectivity filter through which a penetrating ion has to pass. As illustrated by Fig. 6A the filter is thought to be lined by 8 oxygens that are grouped around a pore opening of 3.1×5.1 Å. The salient point is that the oxygens may serve as donors of hydrogen bonds that are temporarily formed to $-NH_2$ or $-OH$ groups, as acceptors, of the permeating cations. Since hydrogen bonding allows the acceptor to approach the donor more closely than the van-der-Waals contact, cations whose van-der-Waals thickness is larger than the pore cross-section may permeate if hydrogen bonds can be formed. This is illustrated in Fig. 6B for the case of hydroxylamine by the two overlapping hydrogen-oxygen contacts. A methylated cation of comparable van-der-Waals size would be excluded since methyl groups cannot form hydrogen bonds. Fig. 6C depicts a Na^+ ion and a water molecule entering the filter where the water makes a hydrogen bond. Actually 3 to 4 water molecules accompany each Na^+ ion as a partial

Table 1. Relative permeability, P_X/P_{Na}, of the Na channel to cations X^+

X^+	P_X/P_{Na}	
	frog nodal membrane[a]	squid giant axon[b]
Sodium	1.0	1.0
Hydroxylamine	0.94	—
Lithium	0.93	1.1
Ammonium	0.16	0.27
Potassium	0.086	0.083
N-Methylhydroxylamine	<0.056	—
Caesium	<0.013	0.016
Rubidium	<0.012	0.025

[a] Hille (1971, 1972); his method permits the estimation of only upper limits for the last three cation species.

[b] Chandler and Meves (1965) who did not test hydroxylamine and N-methylhydroxylamine; value for ammonium from Binstock and Lecar (1969).

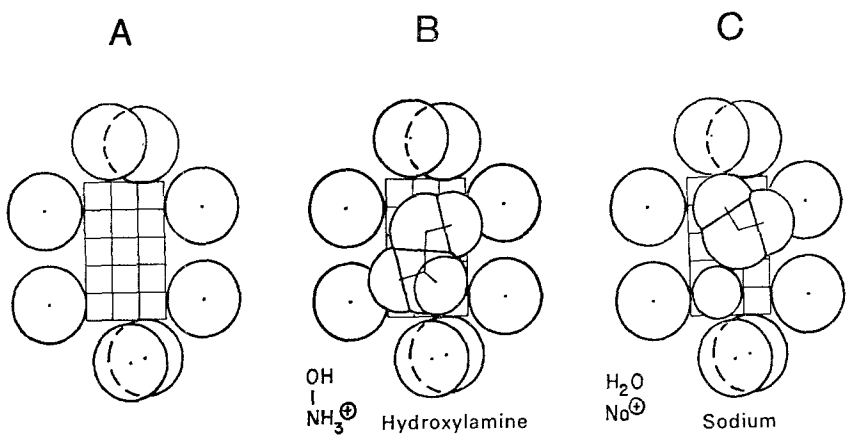

Fig. 6A—C. Hypothetical selectivity filter of the Na channel. *A*, cross-section of pore with 3 × 5 Å grid lined by 8 oxygens (from adjacent groups) 6 of which are approximately in one plane. The lower pair of overlapping oxygens assumedly belongs to an ionized carboxylic acid (pK_a of 5.2) whose protonation blocks the channel. This would explain the TTX-like action of low pH. *B*, hydroxylamine passing through the filter; O-H contacts overlap where hydrogen bonds are made. *C*, a sodium ion and a water molecule passing, the latter, too, forming a hydrogen bond. From Hille (1971); by permission of author and The Journal of General Physiology

hydration shell; they are outside the plane shown in Fig. 6C. In free solution the cation is exclusively surrounded by the oxygens of the water dipoles whereas in the pore the oxygens of its lining assume part of the solvation function.

A pure sieving theory of permeability, even if it involves a sophisticated filter like the one just described, cannot readily explain the permeability sequence of the cations, in particular as size does not play the only role: hydroxylamine is as permeable as the much smaller Li^+ ion (see Table 1). Therefore Hille (1972) had to incorporate into his hypothesis Eisenman's (1962) electrostatic model for cation selectivity of glass electrodes which had already been favoured by Chandler and Meves (1965). For details of this theory and its adaptation to organic cations the reader is referred to the papers of Eisenman and of Hille. Finally, it should be mentioned that TTX and STX render the Na channel impermeable for any cation species (see review of Hille, 1970), hence, as emphasized before, these toxins specifically block the channel rather than interact with Na^+ ions.

The selectivity of the K channel has also been tested in squid axons (Moore *et al.*, 1966; Binstock and Lecar, 1969; Hagiwara *et al.*, 1972) and nodes of Ranvier (Lüttgau, 1961; Hille, 1973). In spite of differences in the methods and in some results there is agreement that the K channel is about as permeable to Rb^+ ions as it is to K^+ ions, that NH^+ ions do pass but less readily and that Cs^+ and Na^+ ions are practically impermeable. Strangely, the permeability of Tl^+ ions is about twice as high as that of K^+ ions. The organic cations that fit through the Na channel are excluded from the K channel for which Hille (1973) has also postulated a selectivity filter. In the nodal membrane it appears to be lined by 5 oxygens around a lumen whose diameter is 3.0 to 3.5 Å. This would force a K^+ ion on penetration to abandon all but 2 water molecules of its hydration shell. A similar selectivity barrier has been deduced from squid experiments (Bezanilla and Armstrong, 1972).

Activation and Inactivation

The preceding paragraphs have illustrated how studies of ionic discrimination may give valuable clues to the structure of one channel constituent, the selectivity filter. The other, equally important property of the channels, their ability to open and close on a change of membrane potential is often visualized to reside in a structure that is called "gate" for convenience. Is the gate identical with the filter? This idea is tempting since the filter constitutes a strategically located constriction where a small change could easily block the channel. At present we cannot give an unequivocal answer to this question but for the Na channel there exist findings in favour of separate structures for gating and discrimination. The evidence is again obtained with the help of a drug as demonstrated by the voltage clamp records of Fig. 7. The membrane current is that of a Ranvier node treated with the alkaloid veratridine. On depolarization an inward current is observed (continuous trace) that is obviously carried by Na^+ ions since it disappears in a Na-free solution (interrupted trace). The important point, however, is revealed by the time scale that is in sec rather than in msec as in the previous record from an unpoisoned axon (Fig. 1). A detailed study has shown that the alkaloid increases the time constant of opening and closing of the Na channels by a factor of about 10^4 and that it eliminates the inactivation (on sustained depolarization) while selectivity and susceptibility to TTX block are unimpaired (Ulbricht, 1969a). Clearly, these dissociated effects strongly suggest that gate and filter are separate structures.

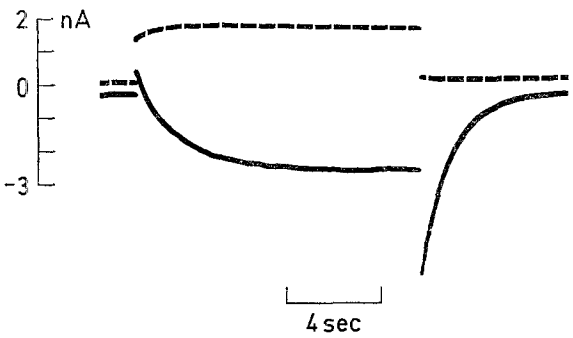

Fig. 7. Time course of membrane current of a Ranvier node treated with veratridine (15 μM); voltage clamp experiment at 21.7 °C. Current calibration in nA per nodal area, inward current negative. Continuous trace observed with normal Na concentration, interrupted trace in a Na-free solution (choline substituting for sodium); in either case a 12 sec depolarization by 30 mV was applied. Note that inward current during the pulse and inward current tail following the pulse are seen only in the presence of Na. From Ulbricht (1969b)

Fig. 7 also demonstrates that inactivation does not necessarily follow on opening of the Na channels. Several drugs including certain insecticides and venoms inhibit or slow the inactivation (see e.g. the reviews of Hille, 1970 and Narahashi, 1971). A particular interesting example is given in Fig. 8 which depicts Na currents—K currents being suppressed by TEA—of a squid axon following internal treatment with pronase, a mixture of proteolytic enzymes (Armstrong *et al.*, 1973). After 12 min of this treatment the inactivation is completely abolished and the current record C superficially resembles that of the veratrinized membrane but in contrast to Fig. 7 the activation of P_{Na} has hardly changed at all. Obviously, normal inactivation is linked to a protein structure, the "inactivation gate", that can be destroyed apart from the "activation gate" by the enzyme. Since pronase does show these effects only when applied internally the "inactivation gate" must be located at or near the inside of the axon membrane.

Not only can inactivation be removed by drugs but it can also be pharmacologically produced in K channels where it does not normally exist, at least not on a msec time scale. Fig. 9A presents K currents (I_{Na} was suppressed by TTX) of a squid axon into which a TEA derivative, nonyltriethylammonium (C_9) ions has been injected. Comparison with the control in Fig. 9B shows that the outward currents rise as normal but subsequently decrease to a low residual level i.e. they inactivate. Armstrong (1969, 1971) has studied this phenomenon extensively and arrives at the following, much simplified explanation. The activation gate of the K channel is located near its inner mouth. When the gate opens on depolarization it may admit, with hydrated K^+ ions, a C_9 ion that fits (like TEA^+) into the relatively wide initial segment of the channel. There it binds to a receptor and thus blocks the efflux of K^+ ions. C_9 cannot move through the channel since the adjacent segment is too narrow and may have the features of (or be) the selectivity filter. On repolarization the gate of a blocked channel closes slowly if at all so that the C_9 ion may eventually diffuse back into the axoplasm. External application of C_9

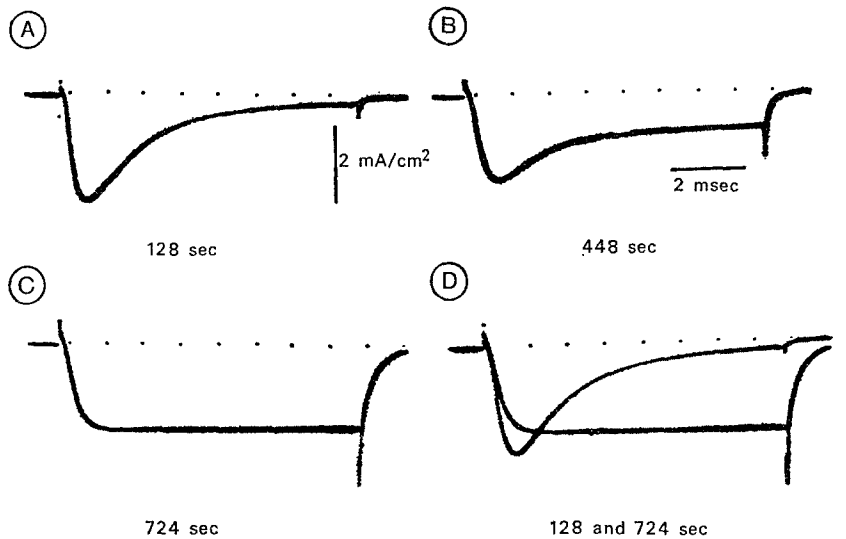

Fig. 8A—D. Effect of internally applied pronase on inward currents of squid axon membrane. Axon was in artificial seawater and filled with a K-rich solution containing, besides 1 mg pronase/ml, 15 mM TEA to block the K channels. Voltage clamp records obtained at 8 °C during depolarizing pulses to $E = 0$ mV preceded by conditioning pulses of − 100 mV. *A*, 128 sec after pronase perfusion when I_{Na} inactivation was still normal. *B*, after 448 sec, inactivation remained intact in only part of the Na channels. *C*, after 724 sec inactivation was completely abolished. *D*, superimposed traces from *A* and *C* to show that activation was hardly affected. From Armstrong *et al.* (1973); by permission of authors and The Journal of General Physiology

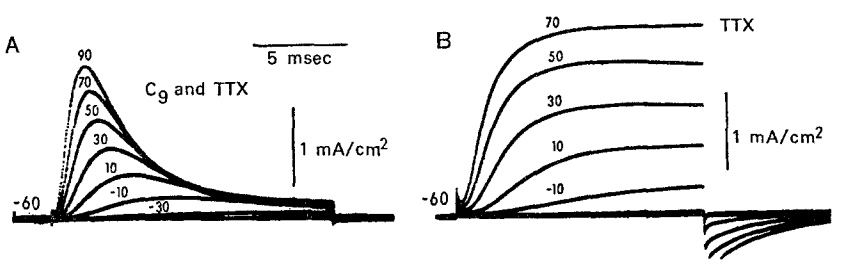

Fig. 9A and B. Squid membrane currents in seawater containing 100 nM TTX. *A*, from an axon into which nonyltriethylammonium (C_9) had been injected; final axonal concentration 0.11 mM. *B*, from an uninjected axon; 9 °C. From Armstrong (1971); by permission of author and The Journal of General Physiology

(or TEA, see p. 3) has no effect in squid axons but in the node of Ranvier it blocks like TEA i.e. irrespective of time and potential (Fig. 2). Nevertheless, when applied internally by diffusion from the cut ends of the fibre (a method introduced by Koppenhöfer and Vogel, 1969) C_9 leads to K current inactivation as in the squid axon (Armstrong and Hille, 1972).

Gating Current

An obvious question arises: could the drug-induced inactivation serve as a model for the natural inactivation of the Na channel? No conclusive evidence has been produced so far suggesting that Na inactivation is the result of a reversible channel blockade by particles that stem from the phases adjacent to the membrane. However, it has repeatedly been suggested that on depolarization charged particles move *within* the membrane thereby opening channels—or closing them as in the case of inactivation. This idea was proposed by Hodgkin and Huxley (1952) as a possible physical basis to their equations describing the membrane current. The sigmoid onset of I_{Na} (see Fig. 1) e.g. could be accounted for if one assumes that Na^+ ions can pass through the membrane only if three charged particles have moved to a certain region of the membrane under the influence of the electrical field. If the permeability were controlled by particles bearing a single charge one would expect from Boltzmann's principle an e-fold change in permeability per RT/F i.e. 25 mV at room temperature. Since in squid axons P_{Na} increases this much for a depolarization by only about 4 mV, it would seem that six single charges or three double charges etc. must be moved simultaneously. Clearly, similar models can be developed involving dipoles. At any rate, movement of charge should, in principle, become detectable as current. Hodgkin and Huxley (1952) already assumed this gating current (as it is nowadays often called) to be very small and hence completely masked by the ionic current. This becomes clear if one realizes that only six charges are supposedly moved to open a Na channel through which many thousand Na^+ ions pass during an action potential. Hence detection of a gating current appears promising only after suppressing most of the ionic current. Chandler and Meves (1965) achieved this by perfusing a squid axon with a RbCl solution and placing it in Na-free choline seawater since Rb^+ (Table 1) and choline ions hardly penetrate at all through the Na channel. These authors were not able to detect any gating current and concluded from the resolution of their method an upper limit of 100 Na channels/μm^2 i.e. the same order of magnitude as deduced from the TTX titration experiments.

Recently, the search for the gating current in the squid axon membrane has been resumed by several investigators using electronic averaging devices to increase the sensitivity of their current measurements (Armstrong and Bezanilla, 1973; Keynes and Rojas, 1973; Meves *et al.*, 1974). An example is given in Fig. 10 which stems from Armstrong and Bezanilla who worked on perfused axons. The lower trace depicts a portion of the usual I_{Na} observed in seawater when the axon was filled with a K-rich solution to imitate the axoplasm; the current was obtained by a depolarizing pulse from $E = -70$ mV to 0 mV. Later the perfusate was changed to a CsF solution and the axon was transferred to Na-free and K-free Tris seawater so that, again, only impermeable cations were present. Now the axon was alternately pulsed from -70 to 0 mV and from -70 to -140 mV i.e. each time either de- or hyperpolarized by 70 mV to eliminate by the averaging process all symmetrical components like the leakage current. The upper trace in Fig. 10 shows (at a 200 times increased sensitivity) that after averaging 2×2000 pulses a small transient outward current remains; it reaches its peak when, normally, I_{Na} sets in. The authors varied the stimulus program to study this current which they interpret as follows. At rest most of the positively charged particles are located

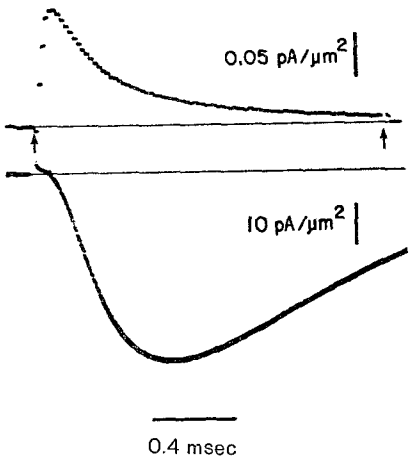

Fig. 10. Sodium current (lower trace) and presumed gating current (upper trace). For the lower trace the axon was in artificial seawater, internally perfused with 275 mM-KF + 400 mM sucrose and depolarized by 70 mV. For the upper trace the axon was in K-free Tris seawater and perfused with 550 mM-CsF. The trace depicts the averaged current as obtained by alternately de- und hyperpolarizing the membrane by 70 mV. The pulse duration is marked by the two arrows; 3.5 °C and a holding potential of −70 mV for both traces. From Armstrong and Bezanilla (1973); by permission of authors and Nature

near the inner edge of the membrane where they close most of the channels. On *hyper*polarization the remaining stray particles move into the closing position thereby instantaneously causing an inward current that is soon exhausted. On *de*polarization the particles move from their resting position towards the outer edge of the membrane thereby opening the channels. The concomitant outward current too sets in immediately but decreases more slowly; it vanishes when the particles have reached their final position corresponding to the pulse potential. What one sees in the upper part of Fig. 10, then, is the difference between the two current components. A comparable contribution by "inactivating" particles moving in opposite directions is not expected since inactivation is too slow.

Asymmetrical currents as the one illustrated by Fig. 10 have been observed by all investigators also in TTX-treated axons. This seems to support the idea that gate and TTX receptor belong to separate structures. In other respects the findings of the three groups of investigators and their interpretations differ. For example, Armstrong and Bezanilla (1973) calculate from their current records with sufficiently large impulses a density of Na channels of about $50/\mu m^2$ while the experiments of Keynes and Rojas (1973) yield eight times as much. Meves *et al.* (1974) state, more cautiously, that their asymmetry currents *could* be caused by the intramembrane movement of dipoles that control the Na permeability. However the conflicting results and interpretations may eventually be reconciled, these experiments deserve our special attention since the gating mechanism undoubtedly poses the central question in the physiology of excitable membranes.

I should like to thank Drs. H.-H. Wagner and M. Mályusz for reading the manuscript.

References

Armstrong, C. M.: Time course of TEA^+-induced anomalous rectification in squid giant axons. J. gen. Physiol. **50**, 491—503 (1966)

Armstrong, C. M.: Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injected in squid axon. J. gen. Physiol. **54**, 553—575 (1969)

Armstrong, C. M.: Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. J. gen. Physiol. **58**, 413—437 (1971)

Armstrong, C. M., Bezanilla, F.: Currents related to the movement of the gating particles of the sodium channels. Nature (Lond.) **242**, 459—461 (1973)

Armstrong, C. M., Bezanilla, F., Rojas, E.: Destruction of sodium conductance inactivation in squid axons perfused with pronase. J. gen. Physiol. **62**, 375—391 (1973)

Armstrong, C. M., Binstock, L.: Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. J. gen. Physiol. **48**, 859—872 (1965)

Armstrong, C. M., Hille, B.: The inner quaternary ammonium ion receptor in potassium channels of the node of Ranvier. J. gen. Physiol. **59**, 388—400 (1972)

Benzer, T. I., Raftery, M. A.: Partial characterization of a tetrodotoxin-binding component from nerve membrane. Proc. nat. Acad. Sci. (Wash.) **69**, 3634—3637 (1972)

Benzer, T. I., Raftery, M. A.: Solubilization and partial characterization of the tetrodotoxin binding component from nerve axons. Biochem. biophys. Res. Commun. **51**, 939—944 (1973)

Bezanilla, F., Armstrong, C. M.: Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. J. gen. Physiol. **60**, 588—608 (1972)

Binstock, L., Lecar, H.: Ammonium ion currents in the squid giant axon. J. gen. Physiol. **53**, 342—361 (1969)

Chandler, W. K., Meves, H.: Voltage clamp experiments on internally perfused giant axons. J. Physiol. (Lond.) **180**, 788—820 (1965)

Cole, K. S., Moore, J. W.: Ionic current measurements in the squid giant axon membrane. J. gen. Physiol. **44**, 123—167 (1960)

Colquhoun, D., Henderson, R., Ritchie, J. M.: The binding of labelled tetrodotoxin to non-myelinated nerve fibres. J. Physiol. (Lond.) **227**, 95—126 (1972)

Cuervo, L. A., Adelman, W. J.: Equilibrium and kinetic properties of the interaction between tetrodotoxin and the excitable membrane of the squid giant axon. J. gen. Physiol. **55**, 309 to 335 (1970)

Eisenmann, G.: Cation selective glass electrodes and their mode of operation. Biophys. J. **2**, Suppl. 259—323 (1962)

Evans, M. H.: Tetrodotoxin, saxitoxin and related substances: their application in neurobiology. Int. Rev. Neurobiol. **15**, 83—166 (1972)

Goldman, D. E.: Potential, impedance, and rectification in membranes. J. gen. Physiol. **27**, 37—60 (1943)

Hagiwara, S., Eaton, D. C., Stuart, A. E., Rosenthal, N. P.: Cation selectivity of the resting membrane of squid axon. J. Membrane Biol. **9**, 373—384 (1972)

Henderson, R., Ritchie, J. M., Strichartz, G. R.: The binding of labelled saxitoxin to mammalian non-myelinated nerve fibres. J. Physiol. (Lond.) **232**, 53—54P (1973a)

Henderson, R., Ritchie, J. M., Strichartz, G. R.: The binding of labelled saxitoxin to the sodium channels in nerve membranes. J. Physiol. (Lond.) **235**, 783—804 (1973b)

Henderson, R., Wang, J. H.: Solubilization of a specific tetrodotoxin-binding component from garfish olfactory nerve membrane. Biochemistry **11**, 4565—4569 (1972)

Hille, B.: The common mode of action of three agents that decrease the transient change in sodium permeability in nerves. Nature (Lond.) **210**, 1220—1222 (1966)

Hille, B.: The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. J. gen. Physiol. **50**, 1287—1302 (1967)

Hille, B.: Pharmacological modifications of the sodium channels of frog nerve. J. gen. Physiol. **51**, 199—219 (1968)

Hille, B.: Ionic channels in nerve membranes. Progr. Biophys. molec. Biol. **21**, 1—32 (1970)

Hille, B.: The permeability of the sodium channel to organic cations in myelinated nerve. J. gen. Physiol. **58**, 599—619 (1971)

Hille, B.: The permeability of the sodium channel to metal cations in myelinated nerve. J. gen. Physiol. **59**, 637—658 (1972)

Hille, B.: Potassium channels in myelinated nerve: selective permeability to small cations. J. gen. Physiol. **61**, 669—686 (1973)

Hodgkin, A. L.: Ionic movements and electrical activity in giant nerve fibres. Proc. roy. Soc. B **148**, 1—37 (1957)

Hodgkin, A. L., Huxley, A. F.: A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (Lond.) **117**, 500—544 (1952).

Hodgkin, A. L., Katz, B.: The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. (Lond.) **108**, 37—77 (1949)

Kao, C. Y.: Tetrodotoxin, saxitoxin and their significance in the study of excitation phenomena. Pharmacol. Rev. **18**, 997—1049 (1966)

Keynes, R. D., Ritchie, J. M., Rojas, E.: The binding of tetrodotoxin to nerve membranes. J. Physiol. (Lond.) **213**, 235—254 (1971)

Keynes, R. D., Rojas, E.: Characteristics of the sodium gating current in the squid giant axon. J. Physiol. (Lond.) **233**, 28—30P (1973)

Koppenhöfer, E.: Die Wirkung von Tetraäthylammoniumchlorid auf die Membranströme Ranvierscher Schnürringe von *Xenopus laevis*. Pflügers Arch. ges. Physiol. **293**, 34—55 (1967)

Koppenhöfer, E., Vogel, W.: Wirkung von Tetrodotoxin und Tetraäthylammoniumchlorid an der Innenseite der Schnürringsmembran von *Xenopus laevis*. Pflügers Arch. **313**, 361—380 (1969)

Lüttgau, H. C.: Weitere Untersuchungen über den passiven Ionentransport durch die erregbare Membran des Ranvierknotens. Pflügers Arch. ges. Physiol. **273**, 302—310 (1961)

Meves, H., Shaw, T. I., Vogel, W.: Asymmetry currents in squid giant axons. Pflügers Arch. **347**, R 33 (1974)

Moore, J. W., Anderson, N., Blaustein, M., Takata, M., Lettvin, J. Y., Pickard, W. F., Bernstein, T., Pooler, J.: Alkali cation selectivity of squid axon membrane. Ann. N.Y. Acad. Sci. **137**, 818—829 (1966)

Moore, J. W., Narahashi, T., Shaw, T. I.: An upper limit to the number of sodium channels in nerve membrane? J. Physiol. (Lond.) **188**, 99—105 (1967)

Mullins, L. J.: An analysis of conductance changes in squid axon. J. gen. Physiol. **42**, 1013—1035 (1959)

Mullins, L. J.: A single channel or a dual channel mechanism for nerve excitation. J. gen. Physiol. **52**, 550—553 (1968)

Narahashi, T.: Neurophysiological basis of drug action: Ionic mechanism, site of action and active form in nerve fibers. In: Adelman, W. J. (Ed.), Biophysics and physiology of excitable membranes, p. 439. New York: Van Nostrand-Reinhold 1971

Narahashi, T., Anderson, N. C., Moore, J. W.: Tetrodotoxin does not block from inside the nerve membrane. Science **153**, 765—767 (1966)

Narahashi, T., Moore, J. W.: A single or dual channel in nerve membranes? J. gen. Physiol. **52**, 553—555 (1968)

Overton, E.: Beiträge zur allgemeinen Muskel- und Nervenphysiologie. II. Mittheilung. Über die Unentbehrlichkeit von Natrium (oder Lithium-) Ionen für den Contractionsact des Muskels. Pflügers Arch. ges. Physiol. **92**, 346—386 (1902)

Schwarz, J. R., Ulbricht, W., Wagner, H.-H.: The rate of action of tetrodotoxin on myelinated nerve fibres of *Xenopus laevis* and *Rana esculenta*. J. Physiol. (Lond.) **233**, 167—194 (1973)

Tasaki, I., Hagiwara, S.: Demonstration of two stable potential states in the squid giant axon under tetraethylammonium chloride. J. gen. Physiol. **40**, 859—885 (1957)

Ulbricht, W.: The effect of veratridine on excitable membranes of nerve and muscle. Ergebn. Physiol. **61**, 18—71 (1969a)

Ulbricht, W.: Effect of temperature on the slowly changing sodium permeability of veratrinized nodes of Ranvier. Pflügers Arch. **311**, 73—95 (1969b)

Vierhaus, J., Ulbricht, W.: Effect of a sudden change in sodium concentration on repetitively evoked action potentials of single nodes of Ranvier. Pflügers Arch. **326**, 76—87 (1971a)

Vierhaus, J., Ulbricht, W.: Rate of action of tetraethylammonium ions on the duration of action potentials of single nodes of Ranvier. Pflügers Arch. **326**, 88—100 (1971b)

Wagner, H.-H., Ulbricht, W.: Interaction of tetrodotoxin and H ions at the nodal membrane. Pflügers Arch. **339**, R 70 (1973)

Wagner, H.-H., Ulbricht, W.: Do TTX and H^+ compete for the same site of the sodium channel? Pflügers Arch. **347**, R 34 (1974)

Prof. Dr. W. Ulbricht
Physiologisches Institut der Universität Kiel
D-2300 Kiel
Olshausenstraße 40—60
Federal Republic of Germany