

Permeability of the Sodium Channel in *Myxicola* to Organic Cations

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ABSTRACT The relative permeability of sodium channels to organic cations was determined in the *Myxicola* giant axon. Ionic currents under potential control were measured in seawater and in sodium-free solutions containing the organic cation. The measured reversal potential and the Goldman equation were used to obtain the relative permeabilities. The permeability sequence was found to be: sodium > hydroxylamine > hydrazine > ammonium > guanidine > formamidine > aminoguanidine \gg methylamine. Measurements were also made on sodium and several of the organic cations at different concentrations. The relative permeabilities of the ions were found to be independent of concentration. Qualitatively, the permeability sequence for the *Myxicola* giant axon was similar to that of the frog node of Ranvier.

INTRODUCTION

Hille (1968*a, b*, 1970, 1971, 1972, 1975*a*) proposed a model wherein a key ionized acid group within the pore of the channel controls the ionic selectivity of the sodium channel for excitable membranes. The model is based on measurements of sodium channel reversal potential with various permeable organic ions in the sodium-free bathing media. The notion of some ionic binding (Hille, 1975*b*) to a single key site may explain other features of movement through the sodium channel, such as ion competition for the site, the highly specific action of certain ionizable toxins such as tetrodotoxin (TTX) and saxitoxin (STX), and the pH dependence of the channel conductance.

Hille's (1971) experimental studies were performed on single frog nodes of Ranvier. The experimental studies reported here on the sodium channel currents and reversal potentials were made on the giant axon of the marine worm *Myxicola infundibulum*. From previous studies on the squid giant axon (Chandler and Meves, 1965, Binstock and Lecar, 1969, Moore et al., 1966) and on *Myxicola* giant axon (Ebert and Goldman, 1976) it was expected that the overall ion selectivity pattern would be qualitatively similar regardless of species. However, a detailed quantitative study would show whether there are variations in the nature of the sodium selective channel from phylum to phylum or whether there is a unique channel structure for excitable membranes such as might be expected if there were a unique sodium channel protein. Since frog and *Myxicola* blood differ by a factor of 4 in ionic strength, comparative measurements also

provide a test of whether the ionizable group can be shielded by increases in external ionic strength.

METHODS

Myxicola were obtained from the Maritime Research Associates, Deer Island, New Brunswick, Canada. Methods for preparing and voltage clamping the axon were as described by Binstock and Goldman (1969). Liquid junction potentials were corrected according to the values of Cole and Moore (1960) which were shown to be suitable for *Myxicola* axons (Binstock and Goldman, 1971).

Membrane currents were obtained from oscillographic recordings and Polaroid photographs or directly recorded on-line in digital format with a PDP-11/20 minicomputer and stored on magnetic tape for later analysis. Records were later displayed on a Tektronix 4010 (Tektronix, Inc., Beaverton, Ore.) and hard copies were made for additional study.

The peak current in the sodium channels is obtained by subtracting the leakage current, a single value measured several milliseconds after the initiation of the capacitive transient, from the measured peak current. The leakage current is assumed to be symmetrical and linear about the resting or holding potential. Therefore, the leakage can be determined from the hyperpolarizing pulses, where the sodium current is negligible.

The reversal potential (at zero current), E_r , was obtained from plots of peak current vs. voltage. The permeability was obtained from the change in the measured reversal potentials, $-\Delta E_r$, of the sodium channel, by using the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949) when switching from NaSW to a sodium substitute (S) seawater,

$$-\Delta E_r = E_r, S - E_r, Na = \frac{RT}{F} \ln \left(\frac{P_S}{P_{Na}} \right) \left(\frac{[S]_0}{[Na]_0} \right), \quad (1)$$

where the concentrations (activities) are contained in the brackets. The substituted ions are identified by the name of their free amine although the species of interest is actually the cation; Eq. (1) therefore applies to cations only. For example, the pH of hydrazine and hydroxylamine was measured and used to calculate cation concentration for Eq. (1). Any error in measuring $-\Delta E_r$ as a result of any errors in determining leakage currents is at most several millivolts.

The control solution, NaSW, was made up of: 430 mM NaCl, 10 mM KCl, 10 mM CaCl₂, 50 mM MgCl₂, 5 mM tris (hydroxymethyl) aminomethane (Tris), pH 7.4. In a number of cases NaSW was K-free. NaSW also had various concentrations of Na other than 430 mM. Concentrations were 322.5 mM, 215 mM, and 107.5 mM. Tris or tetramethylammonium (TMA) was added to bring the equivalent to 430 mM. These are designated $3/4$ NaSW, $1/2$ NaSW, and $1/4$ NaSW, followed by Tris or TMA. The test solutions, NH₄SW, etc., contain the same salts except that all of the NaCl was replaced by an osmotically equivalent quantity of the test salt. The following salts were tested: hydroxylamine · HCl; hydrazine · 2HCl mixed with hydrazine; NH₄Cl; guanidine · HCl; formamidine acetate; aminoguanidine · HNO₃; dimethylamine · HCl; methylamine · HCl; and acetamidine acetate. The purity of the salts used met or exceeded the American Chemical Society's standard for high purity. Some salts were rated highest purity at 97+ % or better. To reduce the concentration of a particular ion, either Tris or TMA was substituted. Na acetate SW was used as a control solution for testing formamidine.

RESULTS

Fig. 1 shows families of voltage-clamped currents for the *Myxicola* giant axon in sodium seawater (NaSW), hydroxylamine seawater (OHNH₃SW), and hydrazine

seawater ($\text{NH}_2\text{NH}_3\text{SW}$). The curves in NaSW are the normal Na currents through the Na channel plus leakage current. In hydroxylamine and hydrazine seawater (Fig. 1) the voltage-clamp currents are similar to the Na currents except that they are smaller; thus, the inward currents in Na-free bathing solutions must be carried by the inward movement of hydrazine and hydroxylamine ions. The permeability of hydroxylamine and hydrazine rises and falls with the same time course as that of P_{Na} . It appears that the sodium channels are quite permeable to hydroxylamine and hydrazine. Other organic cations are also permeable to a lesser extent.

Fig. 2 shows the reversal potentials obtained from current-voltage plots for the family of curves in Fig. 1. The average change in reversal potential, $-\Delta E_r$, for

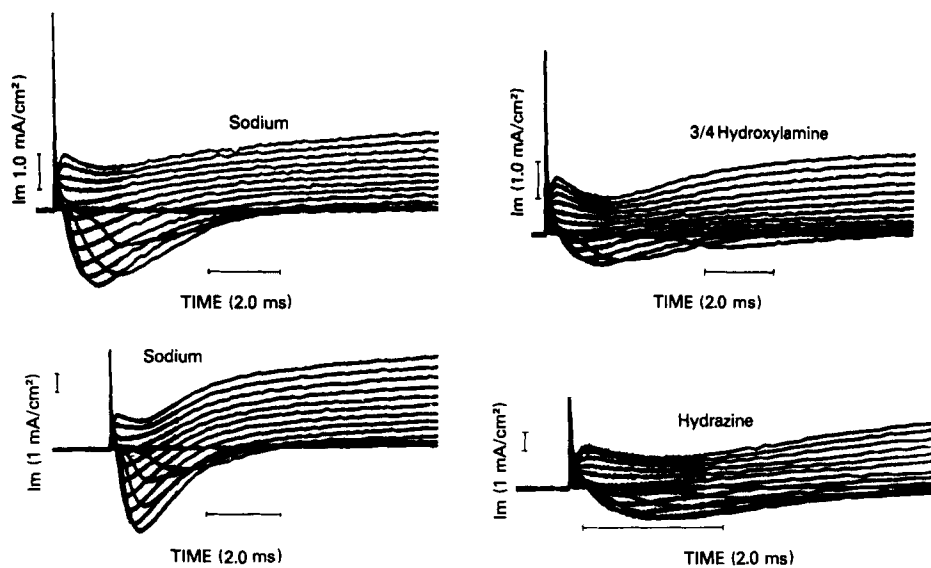


FIGURE 1. Families of voltage clamped currents in sodium seawater, hydroxylamine seawater, and hydrazine seawater. $3/4$ hydroxylamine- $1/4$ TMA were used instead of full hydroxylamine. Leakage is included.

hydrazine was 15.8 ± 0.5 mV. According to Eq. (1), this corresponds to a permeability ratio of 0.54 ± 0.01 . For hydroxylamine the average change in potential was 4.5 ± 1.5 mV, corresponding to a permeability ratio of 0.85 ± 0.05 (See Table I). The currents for hydroxylamine were considerably less than that predicted by the independence principle.

Hydroxylamine at full strength produced a deleterious effect on the axon. These so-called "pharmacological" effects made it difficult to obtain consistent currents. Therefore, $3/4$ hydroxylamine, $1/4$ TMA was used with full Na in NaSW as the reference. The change in reversal potential, $-\Delta E_r$, was 10 mV, corresponding to a permeability ratio of 0.67. Full and $1/2$ hydroxylamine were also used, with $1/2$ being referred to $1/2$ NaSW. The difference was made up with TMA. The change in reversal potential, $-\Delta E_r$, was 6 mV, corresponding to a

permeability ratio of 0.79 (See Table I). These differences observed between the node of Ranvier (Hille, 1971) and *Myxicola* axon are due to the time involved in doing the experiment. The longer the "drug" is bathing the axon the more difficult it is to get good results. It is therefore necessary to work as fast as possible and perhaps reduce the concentration of the ion under test.

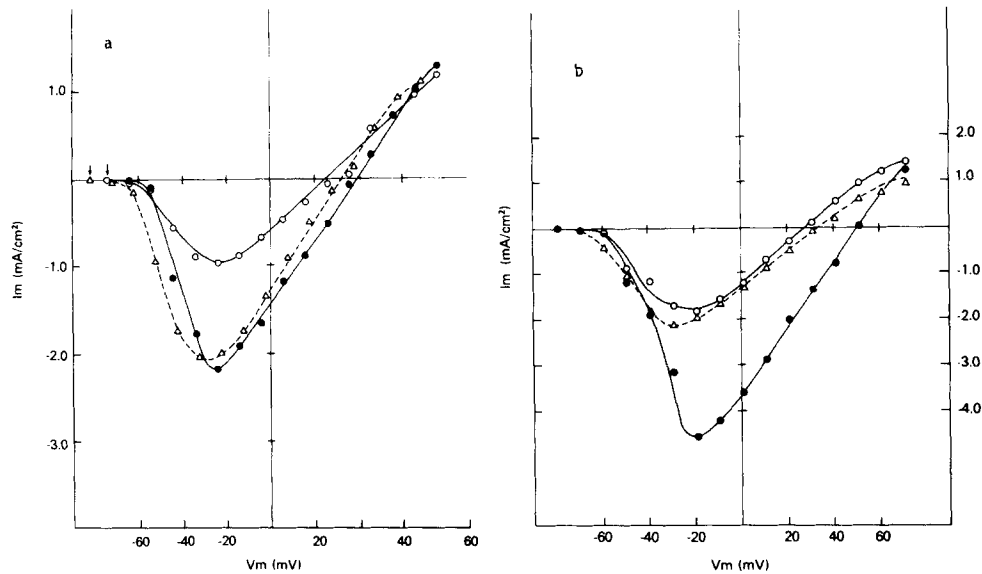


FIGURE 2. Leakage-corrected current-voltage plots for the family of curves in Fig. 1 showing the reversal potentials. (a) NaSW, closed circles; $3/4$ Hydroxylamine SW, $1/4$ TMA, open circles; NaSW, open triangles. (b) NaSW, closed circles; hydrazine SW, open circles; NaSW, open triangles.

TABLE I
PERMEABILITY RATIOS

S	P_S/P_{Na}	$-\Delta E_r$	Concentrations other than normal
Hydroxylamine (4)	0.85 ± 0.05	4 ± 0.5	
	0.67	10	Na; $3/4$ hydroxy, $1/4$ TMA
	0.79	6	$1/2$ Na, $1/2$ TMA; $1/2$ hydroxy, $1/2$ TMA
Hydrazine (3)	0.54 ± 0.01	15.3 ± 0.5	
	0.53	16	$1/2$ Na, $1/2$ TMA; $1/2$ hydrz, $1/2$ TMA
Ammonium (14)	0.20 ± 0.05	41.8 ± 6.2	
	0.20	40	Na; $1/2$ NH_4 , $1/2$ Tris
	0.22	38	Na; $1/2$ NH_4 , $1/2$ Tris
	0.28	32	$3/4$ Na, $1/4$ TMA; $3/4$ NH_4 , $1/4$ TMA
Guanidine (5)	0.17 ± 0.025	44.8 ± 4	
Formamidine (4)	$0.13 - 0.01$	51.5 ± 1.7	
Aminoguanidine (6)	0.13 ± 0.05	52.3 ± 9.5	

Number of measurements in parentheses.

There are other organic cations permeable in the sodium channel to a lesser extent. Fig. 3 shows families of voltage-clamped currents in NaSW, ammonium seawater (NH_4)SW, guanidine seawater ($\text{NH}_2\text{:C}(\text{NH}_2)_2$)SW, and formamidine seawater ($\text{NH}_2\text{:CHNH}_2$)SW. Fig. 4 shows the reversal potentials obtained from

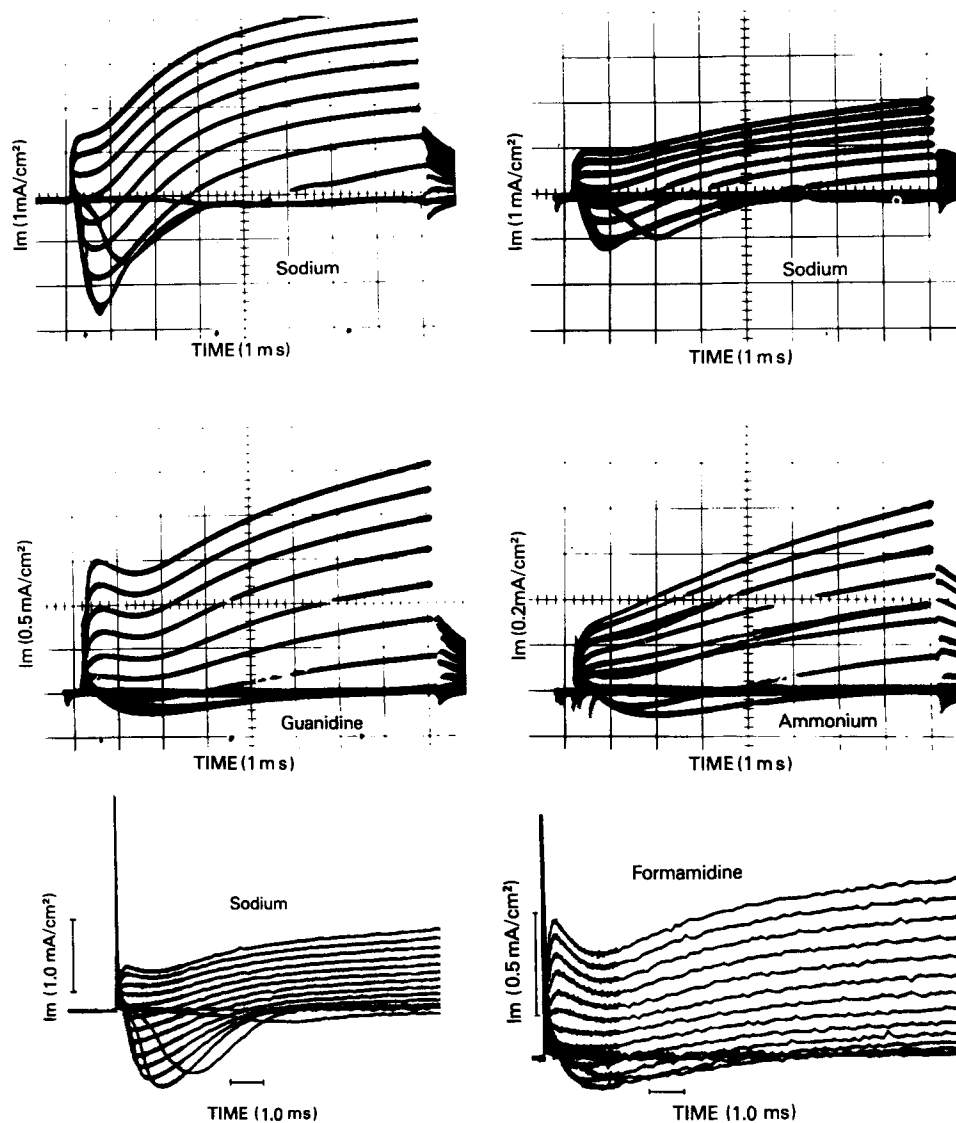


FIGURE 3. Families of voltage-clamped currents in sodium seawater, ammonium seawater, guanidine seawater, and formamidine seawater.

current-voltage plots for the family of curves. The changes in reversal potential are as follows: for NH_4 41.8 ± 6.2 mV, for guanidine 44.8 ± 4.0 mV, and for formamidine 51.5 ± 1.7 mV, corresponding to permeability ratios, respectively, of 0.20 ± 0.05 , 0.17 ± 0.025 , and 0.13 ± 0.01 . A summary of permeability ratios is given in Table I.

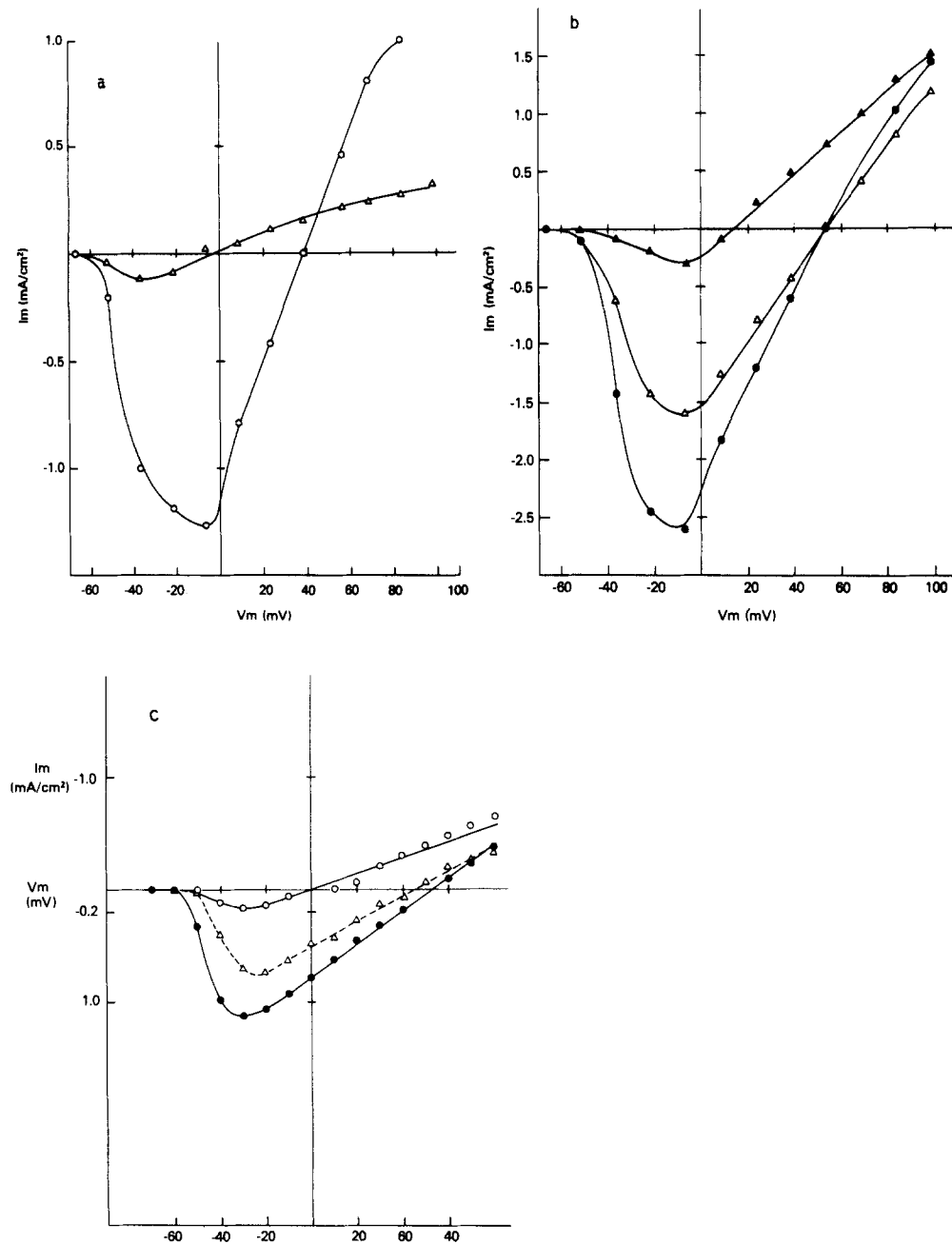


FIGURE 4. Current-voltage plots for the family of curves in Fig. 3 showing reversal potentials. (a) NaSW, open circles; ammonium SW, open triangles. (b) NaSW, closed circles; guanidine SW, closed triangles; NaSW, open triangles. (c) NaSW, closed circles; formamidine SW, open circles; NaSW, open triangles.

Fig. 5 shows current-voltage relationships for different concentrations of Na on a single axon. Fig. 6 shows a semilog plot of change in reversal potential with concentrations of several permeant ions including Na from Fig. 5. The different concentration for each ion fits the Nernst slope.

Some organic cations were tested at a reduced concentration. The Na in NaSW was also reduced in concentration by the same amount, the difference being made up by TMA. Fig. 7 (*upper*) shows families of currents of $1/2$ Na as the reference and $1/2$ hydrazine, and Fig. 7 (*lower*) shows similar currents for $3/4$ Na

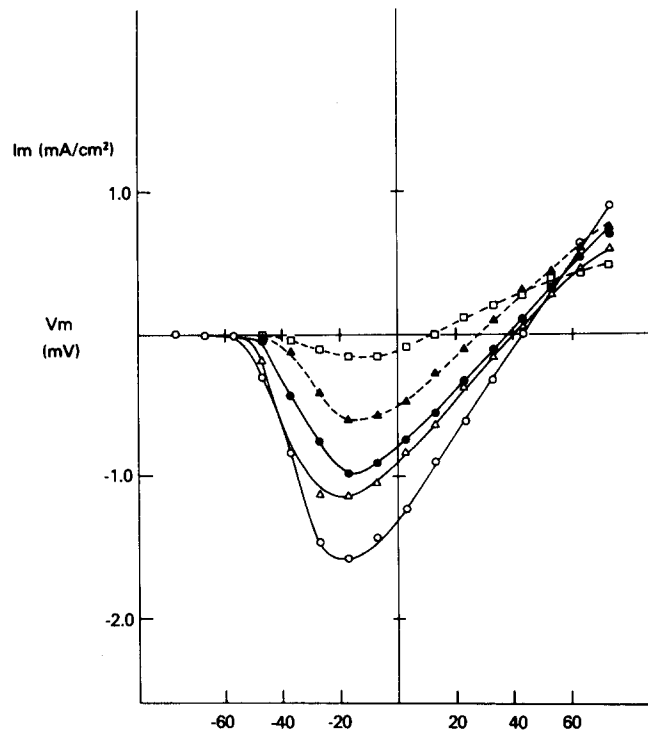


FIGURE 5. Current-voltage plots for different concentrations of sodium on a single axon. NaSW, open circles; $3/4$ NaSW, $1/4$ TMA, closed circles; $1/2$ NaSW, $1/2$ TMA, closed triangles; $1/4$ NaSW, $3/4$ TMA, open squares; NaSW, open triangles.

as the reference and $3/4$ NH_4 . Fig. 8 shows current-voltage relationship for the currents in Fig. 7 (*upper* and *lower*). The change in reversal potential obtained from Fig. 8 gave permeability ratios similar to that obtained for full concentration of the reference solution and the substituted ion. These are included in Table I. The change in reversal is included in Fig. 6.

Generally, the independence principle does not hold for most of the ions except possibly hydrazine and ammonium. The independence principle as formulated by Hodgkin and Huxley (1952) assumes that the movement of an ion is independent of the movement of every other ion. The currents of the other cations tested are much less than predicted by the permeability ratio. These

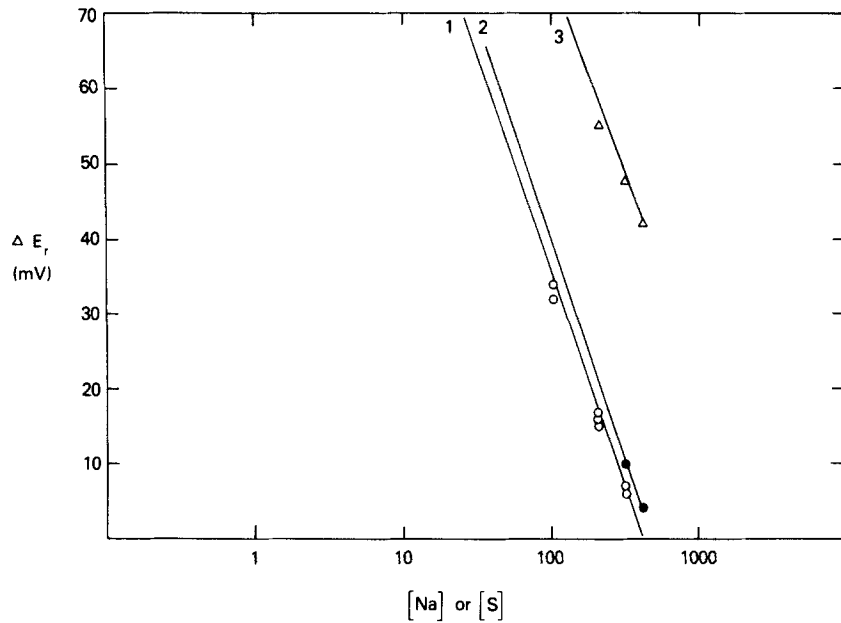


FIGURE 6. A semilog plot of change in reversal potential, $-\Delta E_r$, with concentration of several permeant ions. The concentration of Na or S is in millimoles. (1) Na, (2) hydroxylamine, (3) ammonium.

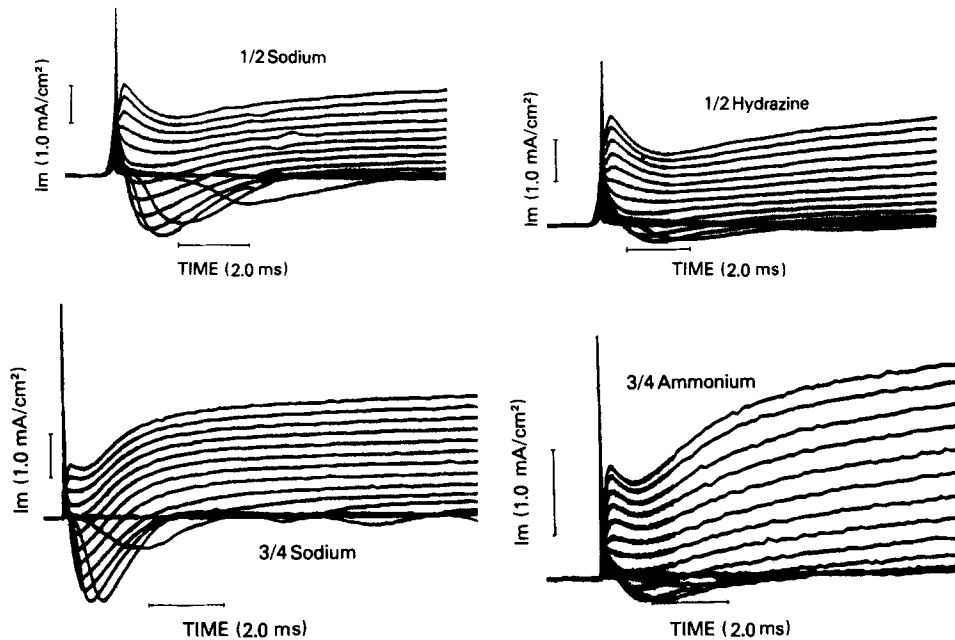


FIGURE 7. (Upper) Families of voltage-clamped currents for $\frac{1}{2}$ NaSW, $\frac{1}{2}$ TMA, and $\frac{1}{2}$ hydrazine SW, $\frac{1}{2}$ TMA. (Lower) Families of voltage-clamped currents for $\frac{3}{4}$ NaSW, $\frac{1}{4}$ TMA, and $\frac{3}{4}$ NH_4SW , $\frac{1}{4}$ TMA.

currents do not fit the predictions of the independence principle. All permeant organic cations tested are blocked by TTX.

Several methylated cations were tested. The permeability ratios of dimethylamine, methylamine, and acetamide as compared to sodium were less than 0.026. These cations gave no measurable inward currents. Also choline, TMA, and Tris, widely used sodium substitutes, gave no inward currents.

DISCUSSION

Experiments on permeability ratios in the sodium channel have been made on another phylum, namely, annelid, more specifically, *Myxicola infundibulum*, a

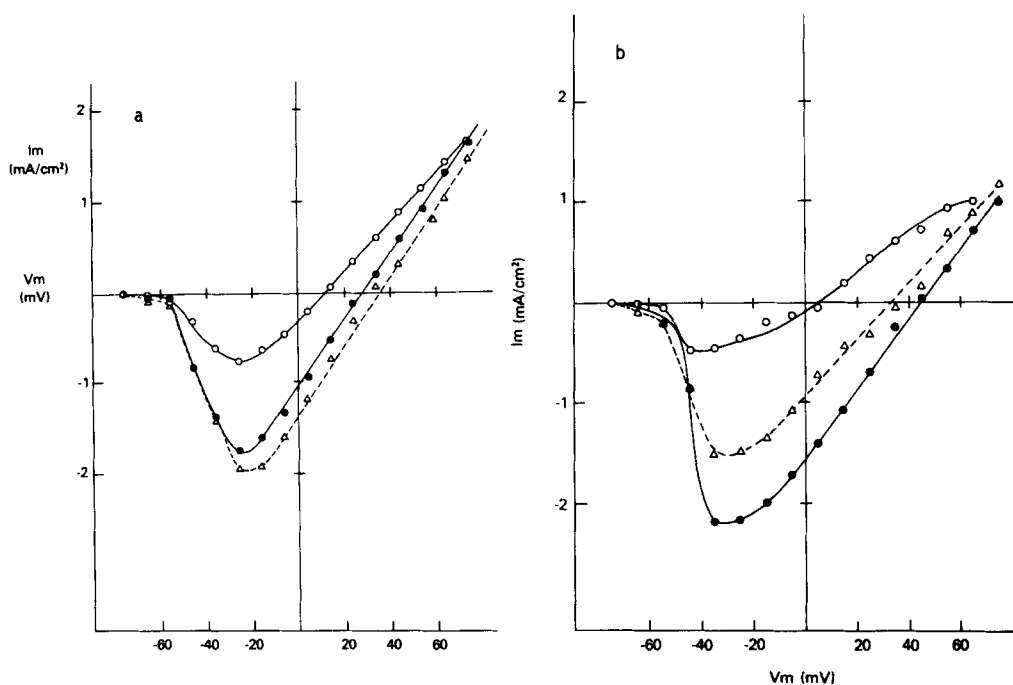


FIGURE 8. Current-voltage plots for the family of currents in Fig. 7a and b. (a) $\frac{1}{2}$ NaSW, $\frac{1}{2}$ TMA, open triangles; $\frac{1}{2}$ Hydrazine SW, $\frac{1}{2}$ TMA, open circles; NaSW, closed circles. (b) $\frac{3}{4}$ NaSW, $\frac{1}{4}$ TMA, closed circles; $\frac{3}{4}$ NH_4SW , $\frac{1}{4}$ TMA, open circles; $\frac{3}{4}$ NaSW, $\frac{1}{4}$ TMA, open triangles.

marine worm. Generally, the results obtained appear to be similar to that obtained for the node of Ranvier (Hille, 1971). The results are shown in Table II. The differences obtained are minor and may reflect the accuracies in the methods used and the methods themselves. One difference is that there is a permutation in the permeability sequence of *Myxicola* axon as compared to node of Ranvier (Hille, 1971) regarding guanidine and formamide. However, the difference in S:Na ratio is only 0.01.

Calculations of ionic permeability from voltage-clamp measurements can be made by: (1) comparing the amplitude of current or conductances of the test ion to Na; or (2) the reversal potential, E_r (at zero current) and the Goldman (1943)

and Hodgkin and Katz (1949) equation. In (1) the independence principle of Hodgkin and Huxley (1952) is used in the computation. The assumption is that the number of channels is the same in each solution and that there is no block or saturation of the open channel by either the control ion or the test ion. In (2) the reversal potential can be obtained in two different ways: (a) from peak current-voltage plots; and (b) from observation of the flat portion where the current is neither inward or outward. The I-V plots must assume a value of leakage to give a correct reading of the reversal potential when the current reads zero. In some cases this differs from the value obtained by observing the potential at which the current is zero. In both cases the Goldman (1943) and Hodgkin and Katz (1949) equation is used. See Methods. P_S/P_{Na} , the permeability ratio, is a measure of S to Na selectivity being referred to as the permeability ratio for S, with Na as the control or reference ion. The result does not depend upon the number of conducting channels. It is assumed that the selectivity is not changed by the

TABLE I I
COMPARISON OF PERMEABILITIES OF DIFFERENT
PREPARATIONS

S	P_S/P_{Na}			
	Myxicola	Node*	Squid	Frog muscle‡
Hydroxylamine	0.85	0.94		0.94
Hydrazine	0.54	0.59		0.31
Ammonium	0.20	0.16	0.27§	0.11
Guanidine	0.17	0.13	0.25	0.093
Formamidine	0.13	0.14		
Aminoguanidine	0.13	0.06		0.031

* Hille (1971).

‡ Campbell (1976).

§ Binstock and Lecar (1969).

|| Chandler and Meves; see Meves (1970).

different bathing medium and that it is not dependent on the voltage. The error in $-\Delta E_r$ is in the order of ± 5 mV, which corresponds to an error of $\pm 20\%$ in the permeability ratios. The equation used here to give a measure of selectivity seems to be insensitive to pharmacological alterations of conductance.

A detailed study was made with the ammonium ion on the squid giant axon (Binstock and Lecar, 1969), where the permeability ratio is 0.27. Also in the squid axon, for guanidine (Chandler and Meves, unpublished observations; see Meves, 1970), the permeability ratio is about 0.25. For *Myxicola* giant axon, the permeability ratio for ammonium is 0.20 and for guanidine 0.17. The values obtained for both species are higher than that obtained for the node of Ranvier (Hille, 1971). Also, aminoguanidine has a higher ratio. It can be suggested that the squid giant axons will have higher permeability ratios for the organic cations. *Myxicola* has the same environment as squid and this may be why some of the organic cation permeabilities are higher than in myelinated nerves.

Hydroxylamine, hydrazine, and methylamine cations are similar molecules. However, Hille (1971) found that in the frog node hydroxylamine and hydrazine are permeable in the sodium channel and methylamine is not. Methylamine is

very much smaller than formamidine, guanidine, and aminoguanidine, and yet the larger are permeable. This led Hille to the notion that the sodium channel is an oxygen-lined pore. One pair of oxygens is assumed to be an ionized carboxylic acid. Cations containing amino groups or hydroxyl groups pass through the sodium channel making hydrogen bonds to the oxygens. But methyl groups which are unable to form hydrogen bonds are too wide to go through. My *Myxicola* results confirm the striking observation that hydroxylamine and hydrazine are relatively permeable through the sodium channel, and methylamine is quite impermeable. This confirmation emphasizes the need for some model—such as Hille's—to explain why rather similar molecules have such different permeabilities.

Results obtained for reduced concentration of several organic cations are the same as that obtained for full concentration. It appears that the ionizable acid group is not shielded by increased ionic strength. This may be due to the location of the acid group being further inside the channel. It may also possibly be due to lack of room inside the channel, ions not being able to make a shield.

Table II shows a comparison of organic cation permeabilities in the sodium channel of several axon preparations and one muscle preparation. It is interesting to note that all of these permeabilities have a qualitatively similar pattern.

For *Myxicola*, then, the structure of the sodium channel seems to be quite similar to the sodium channels of other preparations, although they are distantly related. This suggests a universal selectivity filter, possibly part of a membrane protein.

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