The Permeability of the Sodium Channel in *Myxicola* to the Alkali Cations

G. A. EBERT and L. GOLDMAN

From the Department of Physiology, School of Medicine, University of Maryland, Baltimore, Maryland 21201. Dr. Ebert's present address is the Department of Physiology and Biophysics, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129.

ABSTRACT Relative permeabilities to the alkali cations were determined, from the reversal potential (V_{Rev}), for the Na channel of internally perfused voltage-clamped *Myxicola* giant axons. P_{Li}/P_{Na} and P_K/P_{Na} are 0.94 and 0.076, respectively. Rb and Cs are not measurably permeant. V_{Rev} vs. the internal Na activity was well described by the constant field equation over a 300-fold range of internal Na concentrations. In agreement with findings on squid axons, the P_K/P_{Na} was found to increase when the K content of the internal perfusate was reduced (equivalent per equivalent substitution with TMA). Internal Rb and Cs also decreased the P_K/P_{Na}. The order of effectiveness of internal K, Rb, and Cs in increasing the Na selectivity of the Na channel was

$$Cs > Rb \geq K.$$

External Li increases the P_K/P_{Na} but this may be due to the formation of LiF internally. It may be that substances do not have to traverse the channel in order to affect the selectivity filter. Evidence is presented which suggests that the selectivity of the Na channel may be higher for Na in intact as compared to perfused giant axons. It was concluded that the channel selectivity properties do not reflect only some fixed structural features of the channel, but that the selectivity filter has a labile organization.

INTRODUCTION

It is not at all clear that one can uniquely determine the nature of the Na translocation process from the sequence of permeabilities of the Na channel to the alkali cations (see Hille, 1972; and Mullins, 1975 for alternative interpretations of the same data). However, whatever the detailed nature of the translocation process is, the permeability sequence ought to reflect its properties. And, studies under internal perfusion would seem to provide the preferred method for making such observations (Chandler and Meves, 1965).

We present here observations on the relative permeability of the Na channel to the alkali cations in internally perfused *Myxicola* axons in order to characterize its selectivity properties and for comparison with the selectivity properties of other preparations. We have also been able to confirm the results of Cahalan and Begenisich (1975) and the earlier results of Chandler and Meves (1965) who found that the Na channel selectivity properties are affected by the composition of the bathing media, rather than reflecting some invariant feature of the Na

channel structure. In addition, evidence is presented which suggests that the Na to K permeability ratio in giant axons is greater in intact than in internally perfused preparations.

METHODS

Myxicola were obtained from Maritime Biological Laboratories, Deer Island, New Brunswick, Canada. Methods for preparing and voltage clamping the axons were as in Binstock and Goldman (1969). Internal perfusion was achieved as described in detail by Ebert and Goldman (1975). In brief, a single cannula (OD approximately 200 μm) is steered down the length of axon to be perfused and slowly withdrawn. During the first 3 min of perfusion the internal medium contained 1 mg/ml papain (Calbiochem, San Diego, Calif.) and thereafter was enzyme free.

Permeabilities were determined from the measured reversal potential (V_{Rev}) of the Na channel, using the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949),

$$V_{Rev} = \frac{RT}{F} \ln \frac{P_{Na}a_{Nao} + P_x a_{xo}}{P_{Na}a_{Nai} + P_x a_{xi}} \quad (1)$$

where P_{Na} and P_x are the permeabilities to Na and some other cation, a_{Nao}, a_{xo} are the external and a_{Nai}, a_{xi} the internal activities of the two ions, and R, T, and F have their usual significance. As described in the Results section, for some experiments either a_{Nai}, a_{xo} or both were zero and for some experiments more than two cations were present. Activities were computed using activity coefficients from Robinson and Stokes (1959). In experiments in which the effects of different internal concentrations of some ion were determined, test solutions were prepared by equivalent per equivalent substitution of one fluoride salt for another. These substitutions were assumed to be without effect on the activity coefficient of the ion under investigation. Potentials are reported as absolute membrane potential (inside minus outside) and have been corrected for liquid junction potentials measured for each experiment as described by Ebert and Goldman (1975). V_{Rev} was determined as that potential at which outward current could just be detected from the form of the current record (Goldman and Binstock, 1969). The advantages of this method are that it is not necessary to know the magnitude of the leak current and that the leak current is not required to hold stable over some period of time. In practice the membrane potential is stepped to some potential where there is net inward current or at least to well below reversal. The potential is increased in 1 mV steps until a change in the current wave form (i.e. a flattening of the early part of the record) is detected. This is taken as V_{Rev}. Always by 1–3 mV of further depolarization one sees a clear hump of outward current which continues to grow in amplitude as the potential is increased. A series of current records much like those in perfused axons showing reversal in an intact *Myxicola* axon are given in Fig. 3 of Goldman and Binstock (1969). Note that V_{Rev} is theoretically independent of surface charge if anions are impermeant.

The reliability of this reversal method was checked by Goldman and Binstock. In five axons V_{Rev} was determined as described above and also by noting the intersection of the leak-corrected peak transient current-voltage curve with the voltage axis. Leak corrections were made by repeating the measurements in ASW containing 10^{-6} M tetrodotoxin (TTX) and subtracting the two sets of values. The mean difference between the V_{Rev} values determined with the two methods was only 3 mV. The reversal method therefore seems to be both precise and accurate.

A difference between perfused and intact *Myxicola* axons is that the leak current is considerably greater in perfused preparations. This produces an error in the determina-

tion of V_{Rev} due to the series resistance. Compensated feedback (Hodgkin, Huxley, and Katz, 1952) was not used in these experiments, as the ringing produced by compensation made the detection of V_{Rev} very difficult. Rather, all reported V_{Rev}'s have been corrected for this series resistance error by subtracting a potential equal to the product of the measured leak current at V_{Rev} and the series resistance (R_s). R_s was measured for each axon from the jump in potential produced by brief constant current pulses and corrected for the rise time of the current pulse as described by Binstock et al. (1975). Mean R_s was 8.8 Ω cm^2 as compared to 13 Ω cm^2 for intact *Myxicola* (Goldman and Schauf, 1972). This difference is consistent with the idea that about half the R_s in intact axons originates from the thickness of axoplasm between the internal recording electrode and the membrane, and that *Myxicola* axoplasm has about 2.5 times the resistivity of the perfusion medium (Carpenter et al., 1975; Gilbert, 1975*a*). The corrections were generally 3 to 10 mV.

Artificial seawater (ASW) was K-free and had the following composition (mM): 440 Na, 10 Ca, 50 Mg, 560 Cl, 5 Tris, pH 7.9 ± 0.1. K-ASW had 440 mM KCl, and Li-ASW 440 mM LiCl in place of the NaCl. In some solutions (see Results) 10^{-6} M TTX (Sigma Chemical Co., St. Louis, Mo.) was added. Normal internal medium contained 500 mM KF, 1 mM Hepes, pH 7.9 ± 0.1, and 0.87 mM phenol red (Fisher Scientific Co., Fair Lawn, N. J.). For the experiments reported in Fig. 4, normal internal medium also contained 1 mM NaF. Other internal media contained varying amounts of NaF, RbF, CsF, or tetramethylammonium fluoride (TMA, Eastman Kodak Co., Rochester, N. Y.) substituted equivalent per equivalent for KF. All internal media were adjusted to be isosmotic with ASW with sucrose and checked by osmometer (osmette A, Precision Systems Inc., Sudbury, Mass.). Thus both the osmotic and the ionic strength of all perfusates were held nearly constant. Constancy of the ionic strength is particularly important for avoiding errors due to a change in the R_s. Perfusates are identified as 500 K, 200 K-300 Rb, etc. Temperature was 2° ± 1°C.

RESULTS

Voltage clamp experiments on internally perfused *Myxicola* axons have not been previously reported. In Fig. 1 a series of original current records from an axon bathed in ASW and perfused with 500 K have been reproduced. In this figure the holding potential equals the resting potential (−62 mV), but for all other experiments the test step in potential was preceded by a 25 msec conditioning pulse to −110 mV to remove short term inactivation (Goldman and Schauf, 1972). In addition, at least 5 s were allowed between test pulses to remove most of the slow inactivation (Goldman and Schauf, 1972; Rudy, 1975). In Fig. 2 the peak transient (Na) and steady-state delayed (K) current-voltage relations for another axon in ASW and 500 K are shown. Both the current records and the current voltage relations are much like those seen in intact *Myxicola* axons (Binstock and Goldman, 1969).

Effect of K_i

In squid, Chandler and Meves (1965) found that reducing the internal K concentration, $[K]_i$, from 300 mM to 24 mM increased the P_K/P_{Na} from 0.087 to 0.127. A further reduction to 12 K-12 Na, increased it to 0.194. They attributed this to the reduced ionic strength of these perfusates. However, Cahalan and Begenisich (1975) have shown that the effect is due to the reduced a_{K_i} and not to ionic strength, membrane potential, or to a P_{Cl} (Cahalan and Begenisich, per-

sonal communication), and found a P_K/P_{Na} of 0.38 in 60 K perfusates. This effect may also be seen in *Myxicola*.

Fig. 3 shows the Na:K permeability ratio of the Na channel (plotted as P_{Na}/P_K) as a function of a_{K_i} of the perfusate. Reduced K perfusates contained an equivalent amount of TMA. The external medium was always ASW. Each circle represents a single determination on an individual axon. The triangles are the means of replicate determinations on an individual axon, and the square (bars indicate standard error) the mean of 10 determinations on six axons perfused with 500 K.

Low a_{K_i} increases P_K/P_{Na}. With an a_{K_i} of 340 mM, mean P_K/P_{Na} in this series was 0.080 (V_{Rev} = 56.4 mV). At an a_{K_i} of 68 mM, mean P_K/P_{Na} was 0.143 (V_{Rev} =

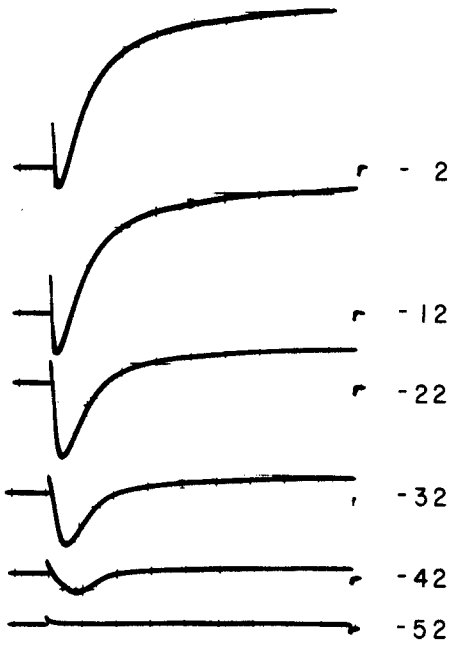

FIGURE 1. Voltage-clamped current records from an internally perfused *Myxicola* giant axon. The numbers at the right indicate the potentials stepped to in millivolts. Internal perfusate:500 K. External medium: ASW. Holding potential −62 mV. Scale 0.6 ma/cm², 2 ms.

81.4 mV) or a 1.8-fold increase. Over this range P_{Na}/P_K changed nearly linearly with a_{K_i}.

One axon was perfused with 500 TMA in an attempt to detect outward TMA current through the Na channel. No TMA current was seen. When the internal medium was switched to 500 K on this same axon, substantial outward K current could be recorded, and the action potential was 90 mV. These results indicate that the effect is actually attributable to the change in a_{K_i} and not to some permeability of the Na channel to TMA. These results also suggest that in *Myxicola* as in squid permeability ratios are sensitive to the experimental conditions rather than characterizing some fixed structural element of the channel. The permeability ratios reported below must be evaluated in the light of this finding.

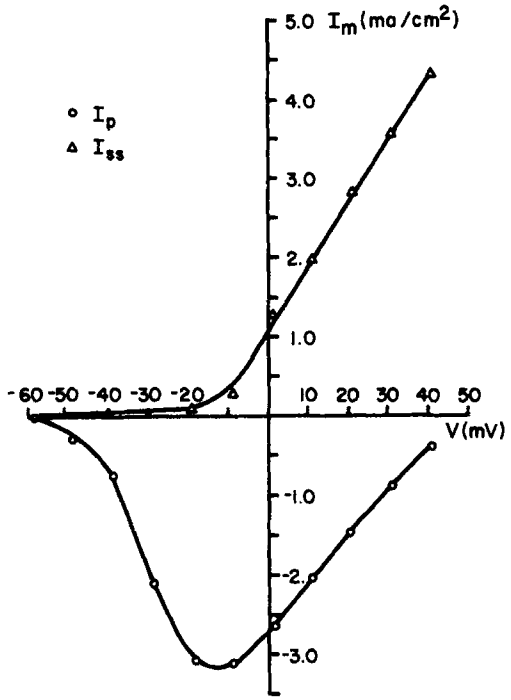

FIGURE 2. Peak transient (circles) and steady-state delayed (triangles) current-voltage relations in an internally perfused *Myxicola* axon. Each test step was preceded by a 25 ms conditioning step to −110 mV. Internal and external media: 500 K and ASW, respectively. Uncorrected for leak current.

Effect of Na_i

Fig. 4 shows V_{Rev} as a function of a_{Na_i}. The external medium was always ASW, and a_{Na_i} was increased by equivalent per equivalent substitution of NaF for KF. V_{Rev} at 0.68 mM a_{Na_i} is 56.8 ± 0.9 mV (mean of six determinations in six axons). All other points indicate an individual determination. The solid line was drawn according to Eq. 1, with $P_K/P_{Na} = 0.076$, and is clearly a good description of the data.

The good fit of Eq. 1 to these data does not contradict the results of the previous section, as for small changes in a_{Na_i} (and hence a_{K_i}) P_K/P_{Na} changes very little and for large a_{Na_i} values V_{Rev} is not very sensitive to P_K/P_{Na}.

Although the discrepancy between experimental and predicted values is small, it is always in the same direction. Chandler and Meves (1965) reported a similar result in squid. This discrepancy is in the opposite direction to be accounted for by the a_{K_i} effect on the P_K/P_{Na}.

Effect of Li-ASW

Table I gives the values for V_{Rev} and P_{Li}/P_{Na} for axons perfused with 500 K. P_{Li}/P_{Na} is computed from

$$\Delta V_{Rev} = \frac{RT}{F} \ln \frac{a_{Na_o}}{(P_{Li}/P_{Na})a_{Li_o}}, \quad (2)$$

where ΔV_{Rev} is the change in V_{Rev} when the external medium is changed between ASW and Li-ASW. $(P_{Li}/P_{Na})_1$ is the permeability ratio when the fiber was exposed first to ASW and only later to Li-ASW. $(P_{Li}/P_{Na})_2$ is the ratio when the axon had been exposed to Li-ASW before the determination in ASW.

The mean $(P_{Li}/P_{Na})_1$ is 0.77. This value is somewhat smaller than those reported in squid (Chandler and Meves, 1965), node (Hille, 1972), and frog muscle (Campbell, 1976) which are all near unity. The issue seems to be that exposure to Li-ASW increases the relative K permeability of the Na channel, as V_{Rev} in ASW tends to be lower in axons that have first been exposed to Li-ASW. Correspondingly, in each of the five experiments in which P_{Li}/P_{Na} was determined by measuring V_{Rev} in ASW after exposure to Li-ASW, P_{Li}/P_{Na} was near unity (mean 0.94).

The origin of the Li effect is not clear, but it may be related to the fact that

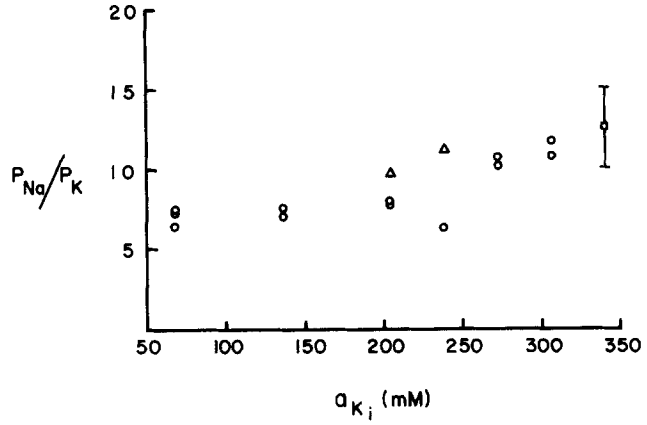

FIGURE 3. The Na to K permeability ratio (P_{Na}/P_K) as a function of the internal K activity (a_{K_i}) in *Myxicola*. Circles represent a single determination on an individual axon. The triangles indicate means of replicate determinations on a single axon, and the square and brackets indicate the mean plus standard error of 10 determinations in 500 K perfusate.

these axons all contain 500 mM KF. During the passage of inward Li current, therefore, the local concentration of LiF at the channel mouth could exceed its solubility, which is not very high. At any rate, the initial Li exposure does alter the channel in some way. P_{Li}/P_{Na} can be determined, therefore, only if this confounding Li effect is eliminated, and may be taken as 0.94.

Effect of Rb_i and Cs_i

Four axons were perfused with 500 mM RbF. In none of these could outward current be detected through the Na channel. For three of the axons the ASW was replaced with Tris-ASW to increase the driving force on Rb. Test steps to an absolute potential of nearly 160 mV still produced no detectable outward current. All these axons were capable of supporting action potentials when returned to ASW, and in the two cases where it was looked for, subsequent perfusion with 500 K produced a vigorous outward current through the Na channel. In one case where the external medium was ASW, no outward Rb

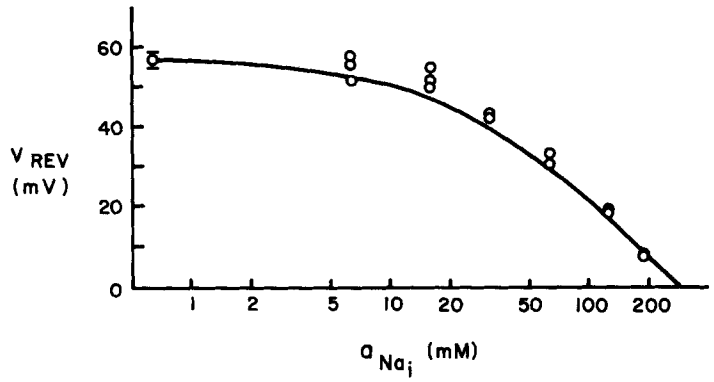

FIGURE 4. Reversal potential of the Na channel (V_{Rev}) as a function of the internal Na activity (a_{Na_i}) in *Myxicola*. The circle plus brackets at the left indicates the mean plus standard error of six axons perfused in 500 K − 1 Na. The solid line has been drawn according to Eq. 1 with $P_K/P_{Na} = 0.076$.

TABLE I

THE EFFECT OF Li-ASW ON V_{Rev}

Axon	External solution	V_{Rev}	$(P_{Li}/P_{Na})_1$	$(P_{Li}/P_{Na})_2$
		mV		
75M134	ASW	59		
	Li-ASW	51	0.65	
75M135	ASW	55.5		
	Li-ASW	50	0.74	
75M136	ASW	56		
	Li-ASW	55	0.88	1.02
	ASW	52.5		
75M137	ASW	58.5		
	Li-ASW	41	0.45	
75M138	ASW	57		
	Li-ASW	57	0.93	0.96
	ASW	56		
75M140	ASW	54.5		
	Li-ASW	56	0.97	
75M141	Li-ASW	52.5		0.92
	Li-ASW	51		0.87
	ASW	52.5		
75M144	Li-ASW	56		0.93
	ASW	55.5		
Mean			0.77	0.94

P_{Li}/P_{Na} was computed from Eq. 2. The internal medium was 500 KF and the temperature 2°C.

current was seen at an absolute potential of 119 mV, which means that $P_{Rb}/P_{Na} < 0.005$. We conclude that Rb ions are not measurably permeant in the Na channels of *Myxicola*.

Similar results were obtained on perfusion with 500 mM CsF. In the single axon tested no outward current through the Na channel was detected in Tris-ASW with test pulses to an absolute potential of 164 mV. On subsequent return to ASW and 500 K perfusate strong outward K current through the Na channel

TABLE II

THE EFFECT OF Rb_i AND Cs_i ON P_K/P_{Na}

Axon	Internal perfusate	V_{Rev}	$(P_K/P_{Na})_K$	$(P_K/P_{Na})_{Rb}$	$(P_K/P_{Na})_{Cs}$
		mV			
75M154	300 K				
	200 TMA	61.5	0.110		
	300 K				
	200 Rb	70		0.076	
75M156	300 K				
	200 TMA	59.5	0.120		
	300 K				
	200 Rb	75.5		0.061	
75M164	300 K				
	200 TMA	67	0.086		
	300 K				
	200 Cs	97.5			0.024
	300 K				
	200 TMA	70	0.077		
75M169	300 K				
	200 TMA	63.5	0.101		
	300 K				
	200 Rb	69.5		0.078	
	300 K				
	200 TMA	62	0.108		
	300 K				
	200 Cs	81			0.048
	300 K				
	200 TMA	60	0.117		
Mean			0.103	0.072	0.036

External solution was ASW. Permeability ratios were computed from Eq. 1 under the assumption that neither Rb, Cs, nor TMA was permeant. Temperature 2°C.

was recorded, and the action potential had an amplitude of 120 mV. Cs is also not measurably permeant in the Na channels of *Myxicola*.

The effects of perfusion with Rb and Cs are shown in Table II. In these experiments, axons, in ASW, were first perfused with 300 K-200 TMA solutions and the V_{Rev} was determined. The internal solution was then switched to 300 K-200 Rb or 300 K-200 Cs and the V_{Rev} was again determined. As a_{K_i} is constant the effects of Rb or Cs on P_K/P_{Na} may be computed under the assumption that both

these ions are negligibly permeant. The opposite kind of assumption, that both Rb and Cs are permeant and have no effect on P_K/P_{Na} produced negative permeability ratios and so is clearly not correct.

P_K/P_{Na} values in 300 K-200 TMA (subscripted K in Table II) agreed well with those determined in the previous series of TMA experiments (Fig. 3). The mean P_K/P_{Na} in 300 K-200 TMA of 0.103 fell to 0.072 in 300 K-200 Rb (i.e. slightly less than that expected in 500 K) and to 0.036 in 300 K-200 Cs. Hence internal K, Rb, and Cs all increase the Na selectivity of the Na channel.

Effect of K-ASW

Three intact axons were exposed to K-ASW to look for inward K current through the Na channel. Current through the Na channel was taken as the difference between the current in K-ASW and that in K-ASW + 10^{-6} M TTX. In these experiments 15 s were allowed between test pulses to permit nearly full recovery from slow inactivation. The results from one experiment are shown in Fig. 5. Identical results were obtained in the other two axons.

For large depolarizations, substantial outward current is seen. This must be

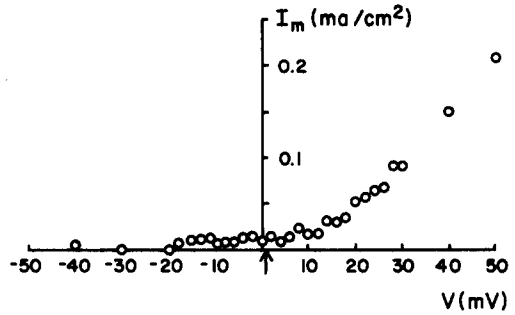

FIGURE 5. Na channel current-voltage relation for an intact *Myxicola* axon bathed in 440 K-ASW. The arrow indicates the V_{Rev} expected in this axon from that measured in ASW, and with a P_K/P_{Na} of 0.076.

largely due to the internal Na. However, for smaller depolarizations, the current-voltage relation asymptotically approaches the zero current axis; i.e., there is no detectable inward K current through the Na channel. The arrow indicates where V_{Rev} should have been given the measured V_{Rev} of this axon in ASW and assuming a P_K/P_{Na} of 0.076. The possible significance of these results is discussed below.

DISCUSSION

A striking difference between the Na channels in *Myxicola* and squid is that in *Myxicola* neither Rb nor Cs is measurably permeant, while in squid Chandler and Meves (1965) reported a P_{Rb}/P_{Na} of 0.025 and a P_{Cs}/P_{Na} of 0.016. Hille (1972) also found that Rb and Cs were not measurably permeant in the node, using a leak correction. The *Myxicola* results were obtained without a leak correction and confirm Hille's results on another preparation with a different methodology. Moore et al. (1966) also found that Cs was not measurably permeant in intact squid axons. The significance of such differences lies in that models for the ion translocation process ought to be able to accommodate them.

TABLE III

RELATIVE PERMEABILITIES TO THE ALKALI CATIONS OF THE Na CHANNEL IN VARIOUS PREPARATIONS

Preparation	Internal solution	P_{Li}/P_{Na}	P_K/P_{Na}	P_{Rb}/P_{Na}	P_{Cs}/P_{Na}	Reference
Loligo axon	500 K	–	0.11	–	–	Binstock and Lecar (1969)
	530 K	–	0.069	–	–	Cahalan and Begenisich (1975)
	450 K	–	0.10	–	–	Adelman (1971)
	300 K	1.14	0.083	0.025	0.016	Chandler and Meves (1965)
	Intact	1.0	0.083*	0.083	‡	Moore et al. (1966)
Dosidicus axon	550 K	–	0.04	–	–	Rojas and Atwater (1967)
	550 K	–	0.10	–	–	Atwater et al. (1969)
	275 K	–	0.091	–	–	Rojas and Taylor (1975)
Rana axon	Cut preparation	0.93	0.086	‡	‡	Hille (1972)
Rana muscle	Cut preparation	0.96	0.048	–	–	Campbell (1976)
Myxicola axon	500 K	0.94	0.076	‡	‡	This paper

* Neither the current records nor the current-voltage curve indicate measured inward K current.
‡ Not measurably permeant.

Some collected values for permeability ratios of the alkali cations for the Na channel in various preparations are given in Table III. Only permeability values obtained from V_{Rev} and with relatively high K perfusates are included. Several studies in which permeability ratios were obtained from relative current magnitudes (Frankenhaeuser and Moore, 1963; Rojas and Keynes, 1975) have been omitted.

Some significant features emerge from the table. P_{Li}/P_{Na} is remarkably constant. In a variety of preparations and under a variety of conditions, P_{Li}/P_{Na} always remains near unity. P_K/P_{Na}, however, displays a somewhat broader range of values, spreading over more than a factor of two. Very likely there are factors other than the a_{K_i}, not yet accounted for, which regulate the P_K/P_{Na}.

In both squid and *Myxicola* reducing $[K]_i$ produces a clear increase in P_K/P_{Na}. For *Myxicola* axons, internal K, Rb, and Cs all seem to increase the selectivity of the Na channel for Na, in the order of effectiveness

$$Cs > Rb \geq K.$$

External Li seems to have the opposite effect, although this may be due to the formation of LiF internally. Both Cs and Rb (and presumably LiF) are not measurably permeant in the Na channel in *Myxicola*. It may be, then, that ions do not have to traverse the channel in order to modify its selectivity properties. Note that the structure regulating selectivity discriminates very little between Rb and K, while their permeabilities are very different.

Whatever the basis for the effect, these results indicate that ion selectivities do not reflect only some fixed aspects of the channel structure. Rather, the selectivity filter seems to have a labile organization. This had already been proposed by Rojas and Atwater (1967). Further experiments along this line may be useful in elucidating the chemical structure of the channel. In this regard, Cahalan and Begenisich, in squid, (personal communication) have already found a number of

internal cations which affect the Na channel selectivity properties, while Chandler and Meves (1965), in a limited number of experiments, showed that neither replacement of internal Cl with SO_4 nor reduced internal pH had any effect on P_K/P_{Na}.

In the view of Hille (1972), the ability of the Na channel to select between the different alkali cations arises entirely from a high field strength site in the channel, of the type of the Eisenman (1962) sequence X or XI, to which Na ions in some way temporarily bind in their passage through the channel. In this case substances which affect the permeability ratios must act to alter the properties of this site. An alternative view is that of Mullins (1959, 1975) who assumed that there was no formal charge in the channel, but that polarizable oxygen groups lining the channel substituted for the coordination shell provided by the waters of hydration in the aqueous phase. The selectivity ratios in this view arise from a distribution of channel sizes. In this case it would be the distribution function which is affected by, e.g. internal K. In this respect it would be of interest to examine the effects of various internal and external media on the selectivity of the K channel. In the view of Hille (1973), the K channel lacks the high field strength site of the Na channel, and one might expect internal and external cations to affect the selectivity properties of the K channel in quite a different way than that in which they affect the Na channel. Effects on, e.g. P_{NH_4}/P_K in the two channels (Binstock and Lecar, 1969) might be interesting to study in this respect.

There is now sufficient information available to compare some of the selectivity properties of intact and perfused *Myxicola* axons. Goldman and Binstock (1969), on intact *Myxicola,* made an extensive study of V_{Rev} of the Na channel as a function of $[Na]_o$. The reversal method (see Methods) was used. Liquid junction potentials were corrected according to the values of Cole and Moore (1960), which were shown to be suitable for *Myxicola* (Binstock and Goldman, 1971). The V_{Rev} vs. log $[Na]_o$ relation displayed a nearly perfect Nernst slope down to one-fourth of the $[Na]_o$ in ASW. V_{Rev} in ASW was 71 mV (mean of 17 axons). However such data do not allow one to determine a P_K/P_{Na} if the $[Na]_i$ and $[K]_i$ are not known.

Recently, Gilbert (1975*a*) has reported values for $[Na]_i$ and $[K]_i$ in *Myxicola* giant axons of 14.9 and 321 mmol/liter fiber H_2O, respectively. These values were determined from flame analysis of extruded axoplasm, which may be obtained in large quantities from *Myxicola*. The $[K]_i$ value would seem to be highly reliable, as it may be used to accurately predict the measured V_{Rev} values for the K channel in intact *Myxicola* over a nearly 10-fold range of $[K]_o$ (Binstock and Goldman, 1971). The $[Na]_i$, however, can only be an upper limit due to the possibility of contamination from the body fluids.

Using Gilbert's $[Na]_i$ and $[K]_i$ values and our measured P_K/P_{Na} on perfused axons of 0.076, a V_{Rev} of 56.7 mV is computed from Eq. 1, while the experimental value for intact axons is 71 mV. This discrepancy is much too great to attribute to experimental error in the determination of V_{Rev}. An obvious possibility is that some of the internal Na in *Myxicola* is sequestered or not in free solution in the axoplasm. However, Gilbert (1975*a*) has concluded that at least 90% of the measured Na in *Myxicola* axoplasm is freely exchangeable, and that

the activity coefficient for Na in axoplasm is very near to that in seawater. Nor is this discrepancy due to a simple error in the estimation of $[Na]_i$. V_{Rev} computed as above but assuming $[Na]_i$ is zero produces a V_{Rev} of 68 mV, and to reconcile these values on this basis one would need to assume that intact axons had no internal Na. With Gilbert's internal cation values and the experimental value of 71 mV, a P_K/P_{Na} in intact axons of about 0.02 or a selectivity for Na over K of about 50:1 is obtained.

A similar result is obtained for squid axons. For *Loligo forbesi* Keynes and Lewis (1951) reported $[Na]_i$ and $[K]_i$ values of 53 and 371 mmol/liter fiber H_2O. Very similar values were reported for *L. pealei* by Steinbach and Spiegelman (1943). These seem to be the lowest estimates of $[Na]_i$ available (see Gilbert, 1975*b*, Table II, for a survey of these values). Taking Chandler and Meves P_K/P_{Na} value from perfused axons we compute a V_{Rev} of about 40 mV, while Moore and Adelman (1961) found about 50 mV in their careful experimental study of V_{Rev}. The measured V_{Rev} indicates a selectivity of Na over K of about 138:1 in intact axons. Other published estimates of Na and K contents in squid (Gilbert, 1975*b*, Table II) lead only to higher computed selectivities for Na over K in intact axons. To attribute these discrepancies in P_K/P_{Na} to overestimates of $[Na]_i$ would require that in the most favorable case 60% of the measured Na_i or about 30 mmol must be somehow dispensed with. This seems rather unlikely, especially as the axons on which the electrical measurements were made ought not to have especially low $[Na]_i$ values.

A more likely explanation would seem to be that the P_K/P_{Na} is substantially lower in intact as compared to perfused giant axons. Possibly, just as perfusion removes something from the axon needed to maintain active transport (Baker et al., 1968) it also removes something needed to maintain a high Na selectivity of the Na channel. Indeed inward K current through the Na channel could not be detected in intact *Myxicola* axons (Fig. 5), nor did Moore et al. (1966) present any direct evidence of inward K current in intact squid axons.

It might be argued that the high $[K]_o$ in these intact *Myxicola* experiments has produced a large fall in the P_K/P_{Na} over that in ASW. With either explanation the results of Fig. 5 provide further evidence of the lability of the selectivity filter in the Na channel.

We thank Drs. G. Ehrenstein and L. J. Mullins for critical reading of the manuscript.
This work was supported by National Institutes of Health research grant #NS 07734-07 to L. Goldman.

Received for publication 11 March 1976.

REFERENCES

ADELMAN, W. J., JR. 1971. Electrical studies of internally perfused squid axon. *In* Biophysics and Physiology of Excitable Membranes. W. J. Adelman, Jr., editor. Reinhold Publishing Corporation, New York. 274–319.

ATWATER, I., F. BEZANILLA, and E. ROJAS. 1969. Sodium influxes in internally perfused squid giant axons during voltage clamp. *J. Physiol. (Lond.).* **201**:657–664.

BAKER, P. F., R. F. FOSTER, D. S. GILBERT, and T. I. SHAW. 1968. Sodium transport and perfused axons. *Biochim. Biophys. Acta.* **163**:560–562.

BINSTOCK, L., W. J. ADELMAN, JR., J. P. SENFT, and H. LECAR. 1975. Determination of

the resistance in series with the membranes of giant axons. *J. Membr. Biol.* **21**:25–47.

BINSTOCK, L., and L. GOLDMAN. 1969. Current and voltage-clamped studies on *Myxicola* giant axons; effect of tetrodotoxin. *J. Gen. Physiol.* **54**:730–740.

BINSTOCK, L., and L. GOLDMAN. 1971. Rectification in instantaneous potassium current-voltage relations in *Myxicola* giant axons. *J. Physiol. (Lond.).* **217**:517–531.

BINSTOCK, L., and H. LECAR. 1969. Ammonium ion currents in the squid giant axon. *J. Gen. Physiol.* **53**:342–361.

CAHALAN, M., and T. BEGENISICH. 1975. Internal K^+ alters sodium channel selectivity. *Biophys. J.* **15**:261a.

CAMPBELL, D. T. 1976. Ionic selectivity of the sodium channel of frog skeletal muscle. *J. Gen. Physiol.* **67**:295–308.

CARPENTER, D. O., M. M. HOVEY, and A. F. BAK. 1975. Resistivity of axoplasm. II. Internal resistivity of giant axons of squid and *Myxicola*. *J. Gen. Physiol.* **66**:139–148.

CHANDLER, W. K., and H. MEVES. 1965. Voltage clamp experiments on internally perfused giant axons. *J. Physiol. (Lond.).* **180**:788–820.

COLE, K. S., and J. W. MOORE. 1960. Liquid junction and membrane potentials of the squid giant axon. *J. Gen. Physiol.* **43**:971–980.

EBERT, G. A., and L. GOLDMAN. 1975. Internal perfusion of the *Myxicola* giant axon. *Biophys. J.* **15**:495–499.

EISENMAN, G. 1962. Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2**(2, pt. 2):259–323.

FRANKENHAEUSER, B., and L. E. MOORE. 1963. The specificity of the initial current in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol. (Lond.)* **169**:438–444.

GILBERT, D. S. 1975*a*. Axoplasm architecture and physical properties as seen in the *Myxicola* giant axon. *J. Physiol. (Lond.).* **253**:257–302.

GILBERT, D. S. 1975*b*. Axoplasm chemical composition in *Myxicola* and solvability properties of its structural proteins. *J. Physiol. (Lond.).* **253**:303–319.

GOLDMAN, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37–60.

GOLDMAN, L., and L. BINSTOCK. 1969. Current separations in *Myxicola* giant axons. *J. Gen. Physiol.* **54**:741–754.

GOLDMAN, L., and C. L. SCHAUF. 1972. Inactivation of the sodium current in *Myxicola* giant axons; evidence for coupling to the activation process. *J. Gen. Physiol.* **59**:659–675.

HILLE, B. 1972. The permeability of the sodium channel to metal cations in myelinated nerve. *J. Gen. Physiol.* **59**:637–658.

HILLE, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *J. Gen. Physiol.* **61**:669–686.

HODGKIN, A. L., A. F. HUXLEY, and B. KATZ. 1952. Measurement of current voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol. (Lond.).* **116**:424–448.

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

KEYNES, R. D., and P. R. LEWIS. 1951. The sodium and potassium content of cephalopod nerve fibres. *J. Physiol. (Lond.).* **114**:151–182.

MOORE, J. W., and W. J. ADELMAN, JR. 1961. Electronic measurement of the intracellular concentration and net flux of sodium in the squid axon. *J. Gen. Physiol.* **45**:77–92.

MOORE, J. W., N. ANDERSON, M. BLAUSTEIN, M. TAKATA, J. Y. LETTVIN, W. F. PICKARD, T. BERNSTEIN, and J. POOLER. 1966. Alkali cation selectivity of squid axon membrane. *Ann. N.Y. Acad. Sci.* **137**:818–829.

MULLINS, L. J. 1959. An analysis of conductance changes in squid axon. *J. Gen. Physiol.* **42**:1013–1035.

MULLINS, L. J. 1975. Ion selectivity of carriers and channels. *Biophys. J.* **15**:921–931.

ROBINSON, R. A., and R. H. STOKES. 1959. Electrolyte Solutions. Butterworth & Co., Ltd. (Publishers), London.

ROJAS, E., and I. ATWATER. 1967. Effect of tetrodotoxin on the early outward currents in perfused giant axons. *Proc. Natl. Acad. Sci. U.S.A.* **57**:1350–1355.

ROJAS, E., and R. D. KEYNES. 1975. On the relation between displacement currents and activation of the sodium conductance in the squid giant axon. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **270**:459–482.

ROJAS, E., and R. E. TAYLOR. 1975. Simultaneous measurements of magnesium, calcium, and sodium influxes in perfused squid giant axons under membrane potential control. *J. Physiol. (Lond.).* **252**:1–28.

RUDY, B. 1975. Slow recovery of the inactivation of sodium conductance in *Myxicola* giant axons. *J. Physiol. (Lond.).* **249**:22p–24p.

STEINBACH, H. B., and S. SPIEGELMAN. 1943. The sodium and potassium balance in squid nerve axoplasm. *J. Cell. Comp. Physiol.* **22**:187–196.