Ionic Selectivity of the Sodium Channel of Frog Skeletal Muscle

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ABSTRACT The ionic selectivity of the Na channel to a variety of metal and organic cations is studied in frog semitendinosus muscle. Na channel currents are measured under voltage clamp conditions in fibers bathed in solutions with all Na^+ replaced by a test ion. Permeability ratios are calculated from measured reversal potentials using the Goldman-Hodgkin-Katz equation. The permeability sequence was $Na^+ \approx Li^+ \approx$ hydroxylammonium > hydrazinium > ammonium > guanidinium > K^+ > aminoguanidinium in the ratios 1:0.96:0.94:0.31:0.11:0.093:0.048:0.031. No inward currents were observed for Ca^{++}, methylammonium, methylguanidinium, tetraethylammonium, and tetramethylammonium. The results are consistent with the Hille model of the Na channel selectivity filter of the node of Ranvier and suggest that the selectivity filter of the two channels is the same.

INTRODUCTION

The ionic selectivity of axon sodium channels has been well described (Chandler and Meves, 1965; Hille, 1971, 1972, 1975 *b, c;* Meves and Vogel, 1973). This paper extends the study of Na channel permeabilities to skeletal muscle using the muscle voltage clamp method described in the first of this series of papers. In the past such experiments have been difficult to perform on muscle for the lack of a voltage clamp technique sufficiently fast to provide good resolution of Na channel currents that also permits the many necessary solution changes without disturbing the preparation.

In nerve the Na channel is permeable to many small cations (Chandler and Meves, 1965; Tasaki et al., 1966; Hille, 1971, 1972). In particular, Hille was able to characterize the ionic selectivity at the frog node of Ranvier by systematically testing the permeability to an exhaustive list of small organic and metal cations. He proposed that a 3.1 × 5.1-Å oxygen-lined pore would explain the exclusion of all impermeant monovalent cations tested on simple geometric and chemical grounds (Hille, 1971, 1972, 1975 *c*). The observed selectivity sequence of permeant cations and deviations from independence have been successfully described by a four-barrier Eyring rate theory model of the sodium channel that includes a negatively charged site at this selectivity filter (Hille, 1975 *b, c*). As is shown here Na channel permeabilities observed for frog muscle are nearly identical to those observed for frog node and are thus also consistent with this model. This work has appeared in preliminary form (Campbell, 1974, 1975).

METHODS

Voltage Clamp Preparation

The voltage clamp method is that described in the previous paper (Hille and Campbell, 1976) except experiments were performed before all the improvements described there had been made. Specifically, compensation for the attenuating effects of the impedance Z_{ED} were not available. The abbreviations and nomenclature in Hille and Campbell (1976) are used throughout.

A piece of a single muscle fiber is removed from the dorsal head of a frog semitendinosus muscle and placed across partitions separating the four pools of the recording chamber. Pool A, containing the area of membrane under voltage clamp control, is filled either with normal Ringer or Ringer with all the sodium replaced by a test ion. The other three pools contain 120 mM CsF. Cutting the fiber ends in CsF eliminates mechanical activity and contributes to the electrical fidelity of the voltage clamp (see the preceding paper). Of importance to this study, cutting in CsF also raises the Na channel reversal potential, presumably by replacing some of the Na^+ and K^+ ions inside the fiber with impermeant Cs^+ ions. The importance of a high reversal potential for measuring the permeability to relatively impermeant ions will be discussed later.

Two feedback amplifiers are used. One is required for potentiometric recording of the membrane potential which appears as $-E_M$ in pool A. The other amplifier supplies the end of the fiber in pool E with the membrane current required to hold this potential to the voltage clamp command potential. Membrane current is not measured explicitly, but is assumed to be proportional to the voltage in the current-injecting pool (V_E). The reservations in this assumption are discussed in the previous paper and in a later section on errors. All records were corrected electronically for leakage and capacity currents. The frequency response of the amplifiers used in these experiments is lower than shown in the first paper but still adequate for rapid voltage clamp control. Compensation for capacity and leakage currents permits all other current to be measured starting about 80 μs after the voltage step.

Recording and Analysis

Ten to twenty current traces, in response to voltage clamp steps spaced about 7.5 mV apart, are recorded on a storage oscilloscope and then on film. Peak Na channel currents (measured as V_E) are graphed to determine the reversal potential. Reversal potentials from bracketing runs in normal Ringer (E_{Na}) are averaged and subtracted from the reversal potential measured in the test solution (E_S). The resulting change in reversal potential is used to calculate the desired permeability ratio from the Goldman-Hodgkin-Katz voltage equation (Goldman, 1943; Hodgkin and Katz, 1949). The change in reversal potential between the two solutions is given by (Hille, 1971):

$$E_S - E_{Na} = 2.303\ (RT/F)\ \log_{10}\ (P_S[S]/P_{Na}[Na]),$$

where $2.303\ RT/F$ is 55.2 mV at 5°C, [S] is the activity of test ion in test Ringer, [Na] is the activity of Na^+ in the control Ringer, and P_S/P_{Na} is the ratio of channel permeabilities to the test and Na^+ ions. This way of determining P_S/P_{Na} is used because unlike methods based on flux or current relations it does not assume independence and does not depend on the number of channels open (Hille, 1971, 1975*c*). It does assume that the selectivity of the channel is independent of membrane voltage and the solution bathing the fiber. These assumptions may not be true. For some models of the Na channel selectivity filter even the Goldman-Hodgkin-Katz voltage equation does not hold strictly true and permeability ratios may depend on other experimental conditions (Hille, 1975*b*). Nevertheless,

the above method seems to be the most useful way to determine selectivity of a channel for comparison with other results as long as these reservations are kept in mind.

Activities

For 0.1 mol/kg solutions at 25°C the activity coefficients are: LiCl, 0.790; NaCl, 0.778; KCl, 0.770 (Robinson and Stokes, 1965). For 0.07 M $CaCl_2$, the activity coefficient is 0.55. The Guggenheim convention (Butler, 1968; Shatkay, 1968) defines the single ion activity coefficient for Ca^{++} as the square of the activity coefficient of the salt, in this case 0.30. For the metal cations the activity coefficients are in the ratio 1.02:1.00:0.99:0.39 for Li^+, Na^+, K^+, and Ca^{++}. These ratios are used with the cation concentrations to compute the activities required by the above equation. Since activity data are not available for the organic cations tested, their activity coefficients were assumed equal to that of 0.1 mol/kg NaCl for the calculation of permeability ratios.

Solutions

The CsF solution used in the end pools contains 120 mM CsF with 1 mM imidazole or tris(hydroxymethyl)aminomethane buffer, pH 7.4. The composition of the test solutions is given in Table I. Test solutions generally contain 110 mM NaCl or an osmotically equivalent concentration of a Na substitute, 2 mM $CaCl_2$, and 4 mM tris(hydroxymethyl)aminomethane buffer, pH 7.4. For simplicity the solutions are named "Na Ringer," "Li Ringer," and so on according to the test ion.

Hydrazine and hydroxylamine have pK_a's below 8 and the pH of these solutions is adjusted to 5.97 and 5.79 to obtain a higher concentration of the ionized species. The hydrazinium and hydroxylammonium Ringer also have slightly different concentrations of other ions as a result of dilution that occurred when adjusting them to their pH. The osmolarity of the Na Ringer is 203 mosM, hydroxylammonium Ringer 154 mosM. All others tested had osmolarities within 5% of the Na Ringer value.

TABLE I

COMPOSITION OF TEST SOLUTIONS

Ringer	Anion	$[S^+]$	Comment
Na	Cl^-	110	
1/8 Na	Cl^-, Br^-	13.8	Osmolarity maintained with 96.2 mM TMA Br.
Li	Cl^-	110	
K	Cl^-	110	4 mM CsCl added for some experiments.
Ca	Cl^-	89.5	
Hydroxylammonium	Cl^-	52.9	pH = 5.79; $[S^+]$ calculated from pH and $[S]_{total}$; $[Ca^{++}] = 1.6$.
Hydrazinium	Cl^-	68.9	pH = 5.97; $[S^+]$ calculated from pH and $[S]_{total}$; $[Ca^{++}] = 1.3$.
Ammonium	Cl^-	110	
Guanidinium	Cl^-	110	
Aminoguanidinium	NO_3^-	110	
Methylammonium	Cl^-	110	
Methylguanidinium	$SO_4^=$	73.3	
Tetramethylammonium	Br^-	110	
Tetraethylammonium	Br^-	110	

Except as noted, all solutions also contain 2 mM $CaCl_2$, 4 mM tris(hydroxymethyl)-aminomethane buffer and have a pH between 7.3 and 7.4.

Errors

The previous paper describes several current-dependent voltage errors. At maximum sodium currents these errors may total 20 mV, but since the net membrane current at the Na channel reversal potential is roughly $^1/_{10}$ the peak value an error of only about 2 mV is expected. In addition, the method described below for measuring attenuation artifact includes this error, therefore the attenuation correction simultaneously reduces current-dependent errors in reversal potential measurement.

Another error might be expected to arise from contributions of Na channels in the membrane of the transverse tubular system which presumably is under poor voltage clamp control. The voltage clamp calculations illustrated in Fig. 12 of the first paper show that at least for the model chosen there is a negligible effect of tubular sodium conductance on the reversal potential.

The current signal in these experiments was not corrected for the capacitative component of the current-injecting pathway Z_{ED} as described in the first paper. Because this error is also proportional to the membrane current it should not greatly affect the measurement of reversal potential. In fact, all of the dynamic errors taken together apparently have little effect on measured reversal potential changes. For instance, when the A pool solution is changed from normal Na Ringer to Ringer with $^7/_8$ of the NaCl replaced by the impermeant salt tetramethylammonium (TMA) bromide, the average change in reversal potential measured in 16 experiments is −48.1 mV at 5°C while the theoretical change predicted by the Nernst equation is −49.8 mV. This is the method used to determine the amount of "attenuation artifact," a voltage error found when this voltage clamp method is used to study the node of Ranvier (Dodge and Frankenhaeuser, 1958; 1959; Hille, 1971). In that preparation the attenuation may be 10–20%. For the muscle preparation the attenuation averaged 3.5%, and the average reversal potential changes used to calculate permeability ratios were corrected by this amount. This 1.7-mV deviation from the Nernst prediction, if artifactual, implies that the combination of all errors has only a very small effect on the reversal potential change measured between these two solutions.

The reversal potential changes are corrected for the differences in junction potential that the control and test Ringer solutions make with the agar bridge in pool A. These potentials are measured with respect to a Beckman 38402 ceramic junction saturated KCl reference electrode (Beckman Instruments, Inc., Fullerton, Calif.). The measured junction potentials are given in Table II. For most solutions the correction is less than 2 mV.

RESULTS

The observed changes in reversal potential are listed in Table II. For permeant ions the average reversal potential changes are corrected for attenuation and junction potentials and then used to calculate permeability ratios. Reversal potential changes are expressed as mean ± SEM. In solutions showing no inward currents, no reversal potential can be measured. For these impermeant ions the potential that first gives a measurable transient outward current is taken as the upper limit of the reversal potential and used to calculate a limit to the permeability ratio. In the case of impermeant ions the lowest limit on permeability is reported rather than an average.

Lithium

Sodium channels of muscle are about as permeable to lithium as they are to sodium. In Fig. 1, the voltage clamp series in Li Ringer is nearly identical to the

TABLE II

REVERSAL POTENTIAL CHANGES AND PERMEABILITY RATIOS IN SODIUM CHANNEL OF MUSCLE

Fiber	1/8 Na	Li	K	Ca	Hydroxyl-ammonium	Hydra-zinium	NH_4	Guani-dinium	Amino-guanidinium	Methyl-ammonium	Methyl-guanidinium	TMA	TEA
1								34		>109			
2		1.5											
3		1.5											
4		−1.5	77					69		>95			
5		0.0						48					
6		0.0	72					52		>100			
7		−1.5	57					57					
8	48							71				>104	
9	48			>66				63		>104		>101	
10	49			>77				57		>104		>112	
11	48			>54				49		>97		>103	
12	42					31		45		>109		>109	
13	46				22	38	51	60				>92	
14	49		81*		15	37		57					
15	46					40		57					
16	37		72*				49	48					
17	43		75*	>61		34		51					
18	49		78*	>68	22	41	54	55					
19	49			>75	18	38	55	55					
20	48								84		>103		>109
21	48								77		>98		>92
22	48								81		>115		>109
23	49								80		>89		>94
MEAN	46.7	0.0	73.1	>77	19.3	37.0	52.3	54.6	80.5	>109	>115	>112	>109
SEM	0.84	0.55	2.96		1.7	1.3	1.4	2.2	1.4				
Junction potential	+1.4	+0.5	−2.4	+4.2	−0.8	+1.0	−1.8	+0.5	−0.2	−0.9	−4.5	+0.9	+2.3
After corrections		0.5	73.2	>84.2	19.1‡	39.3‡	52.3	57.0	83.1	>111.9	>114.4	>116.9	>115.2
P_S/P_{Na}		0.96	0.048	<0.093	0.94	0.31	0.11	0.093	0.031	<0.009	<0.013	<0.008	<0.008

* The K Ringer in these experiments also contained 4 mM CsCl as explained in the text.

‡ Corrected also for concentration of ionized species.

For impermeant ions the largest limit on reversal potential change was used to compute the limit for the permeability ratio. Potentials are in millivolts.

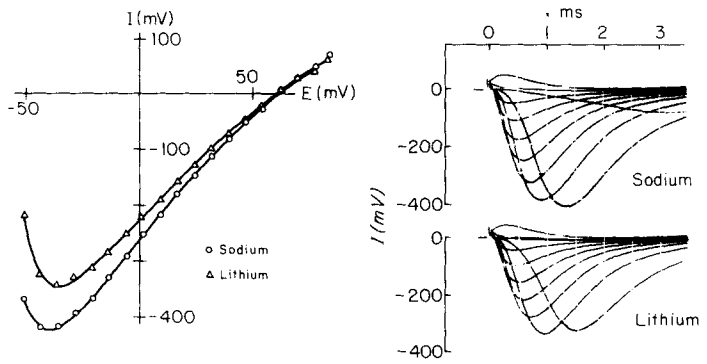

FIGURE 1. Lithium currents in the muscle Na channel. (Left) Peak current-voltage relations in Na and Li Ringer. The smooth curves were drawn by hand. Although the reversal potential in the two solutions is the same, the maximum currents in Li Ringer are about 20% less than in Na Ringer. (Right) Voltage clamp series from the same experiment showing currents for voltage steps spaced 15 mV apart. Fiber ends were cut in 120 mM CsF. Fiber 5, 5°C.

control series in Na Ringer. The corresponding current-voltage relation shows almost no change in reversal potential upon substituting the Li Ringer. In six experiments Li Ringer lowered the reversal potential 0.5 ± 0.6 mV, for a permeability ratio P_{Li}/P_{Na} of 0.96. Although the muscle Na channel is about as permeable to Li^+ ions as it is to Na^+ ions, the current-voltage relation of Fig. 1 shows that the currents in Li Ringer are 16–20% less than in Na Ringer. Evidently Li^+ ions block Na channels in addition to passing through them. In other words Li^+ ions violate the independence principle (Hodgkin and Huxley, 1952; Hille, 1975 *c*) in the muscle Na channel. As is shown below, some other permeant ions show similar deviations from independence.

Potassium

Potassium is the only other metal ion tested found to be measurably permeant in the Na channel. It is also one of the most difficult to test. After a minute or two in K Ringer with Cl^- as the anion, the muscle swells and the membrane often develops large irreversible leaks destroying the preparation. In later experiments preparations bathed in isotonic K_2SO_4 survived well. In addition, the high external concentration of potassium increases the potassium permeability of other channels giving rise to large rectifying background currents that must be subtracted to determine currents in Na channels. For this reason four experiments were performed in K Ringer with 4 mM CsCl added to help keep these extra potassium conductances blocked. In Table II the reversal potential changes from these experiments are noted with an asterisk (*). In seven experiments the reversal potential in K Ringer was 73.2 ± 3.0 mV lower than in Na Ringer, corresponding to a permeability ratio P_K/P_{Na} of 0.048. Sodium is about 21 times more permeant than potassium in the Na channel of muscle.

Hydroxylammonium and Hydrazinium

Two of the most permeant sodium substitutes tested are hydroxylammonium and hydrazinium. Voltage clamp series and current voltage relations from a fiber bathed in these solutions are shown in Fig. 2. In four experiments, changing the bathing medium to hydroxylammonium Ringer with 52.9 mM hydroxylammonium ions gave an average reversal potential change of -19.1 ± 1.7 mV. After correcting for concentration the permeability ratio is 0.94. Although the sodium channel is almost as permeable to hydroxylammonium as it is to sodium, the currents in hydroxylammonium Ringer are only half the size expected from the same concentration of Na^+ ions. The currents are expected to be 22% smaller just from the low pH (5.79) of the solution (Hille, 1968; Woodhull, 1973; Campbell and Hille, 1976). In addition the hydroxylammonium ions may also block channels directly. The hydroxylammonium Ringer was toxic to the fiber and measurements in this solution had to be completed within a minute to avoid irreversible damage. In seven experiments hydrazinium Ringer lowered the reversal potential by 38.0 ± 1.3 mV. After compensating for the concentration hydrazinium ions (68.9 mM) the permeability ratio is 0.31. Currents in hydrazinium are about 35% less than predicted by independence, while at pH 5.97, the blockage expected from H^+ ions is only 16%. Fig. 2 also shows a positive shift in the depolarization required to open Na channels in both hydrazinium and hydroxylammonium Ringer. This calcium-like effect is due in part to the low pH of these solutions, although guanidinium Ringer shows a similar but smaller effect at pH 7.4.

Other Permeant Organic Cations

Ammonium, guanidinium, and aminoguanidinium are also permeant to the muscle Na channel. Ammonium Ringer reduced the reversal potential by 52.3 ± 1.4 mV for a permeability ratio of 0.11. Fig. 3 shows a series of voltage clamp currents for a fiber in guanidinium Ringer. The middle trace is near the reversal

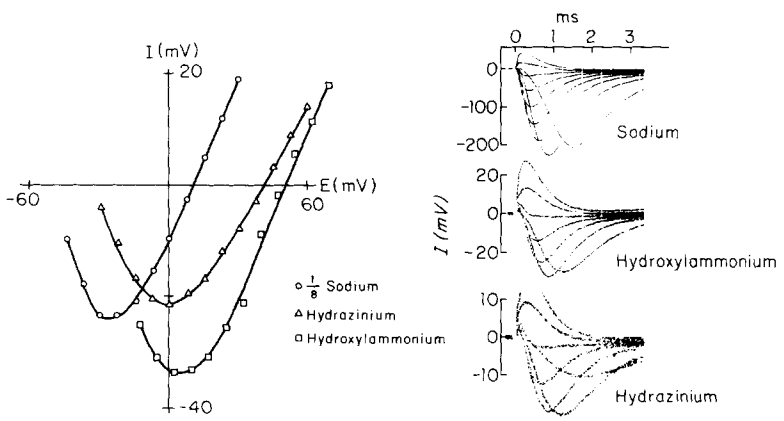

FIGURE 2. Hydroxylammonium and hydrazinium currents in the muscle Na channel. (Left) Peak current-voltage relations. (Right) Voltage clamp series from the same experiment. Fiber ends were cut in CsF. Fiber 13, 5°C.

potential and shows a curvature in the base line characteristically produced in this solution and also in K and ammonium Ringer. The leakage subtraction procedure in these solutions seems to be in error maybe as the result of these ions unblocking some other membrane conductance. In 17 experiments guanidinium Ringer lowered the reversal potential an average 57 ± 2.2 mV for a permeability ratio of 0.093. Aminoguanidinium, the least permeant ion tested that still gives measurable inward currents, lowered the reversal potential by 83.1 ± 1.4 mV in four experiments, corresponding to a permeability ratio of 0.031.

Methylated Cations

The voltage clamp series of Fig. 3 shows no inward currents for a fiber in methylammonium Ringer. Without an inward current a reversal potential for methylammonium ion cannot be determined, however the first potential giving an outward current does place a lower limit on the reversal potential. Out of seven experiments, the largest limit of reversal potential change observed for methylammonium was −111.9 mV, corresponding to a permeability ratio of less than 0.009. The limit cannot be extended much beyond this value because sodium currents do not turn on below depolarizations to about −50 mV and E_{Na}'s of fibers cut in CsF are not seen above about +80 mV. The maximum change that could possibly be measured is thus about 130 mV, corresponding to a permeability ratio of 0.0044. Since many of the impermeant ions shift the current voltage relation of Na channels, requiring even greater depolarization to open them, the practical limit is closer to 115 mV. In these experiments, the lowest permeabilities observed were those of TMA and tetraethylammonium (TEA) Ringer. For both of these solutions the permeability ratio was less than 0.008. Fig. 3 also shows a voltage clamp series for a fiber in TMA. It is

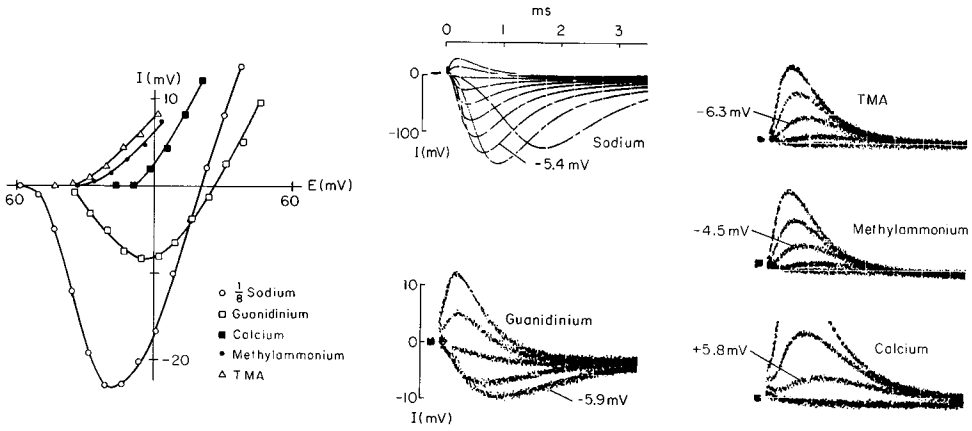

FIGURE 3. Currents from a fiber bathed in Na, guanidinium, tetramethylammonium, methylammonium, and Ca Ringer. (Left) Peak current-voltage relations. (Right) Voltage clamp series from the same experiment. For reference the value of the voltage step is shown for one trace of each series. Voltage steps were spaced 15 mV apart. Fiber ends were cut in CsF. Fiber 11, 5°C.

indistinguishable from the currents recorded when the test solution is TEA. Neither solution supports inward currents in sodium channels.

DISCUSSION

Previous Studies on Muscle

There have been no previous studies of muscle Na channel selectivity, but muscle action potentials have been observed in sodium-free solution. Keynes and Swan (1959) measured skeletal muscle action potentials in Li Ringer and found them nearly identical to action potentials in Na Ringer. They concluded that the permeability of the excited muscle membrane to lithium was about the same as the permeability to sodium. This is consistent with the P_{Li}/P_{Na} of 0.96 reported here. Hydrazinium, although less permeant than sodium or lithium, will also support action potentials in sodium-free solution. Muscle fibers in a variety of different concentrations of hydrazinium solution gave action potentials 27 mV smaller than when in Na solution at the same concentration (Koketsu and Nishi, 1966). This is also quite consistent with the hydrazinium permeability ratio of 0.31 reported here. Comparison with selectivity data from nerve appears later in this discussion.

Calcium

The question of calcium permeability of muscle membrane has special interest because of the role calcium plays in the activation of contraction. It has been suggested that release of calcium from the sarcoplasmic reticulum is triggered by calcium (Endo et al., 1970; Ford and Podolsky, 1970) presumably carried inside the cell as a result of membrane electrical activity. In vertebrate heart and crustacean muscle, surface membrane currents carry a significant proportion of the calcium required for activation (Fatt and Ginsborg, 1958; Beeler and Reuter, 1970). The Na channels of squid axons perfused with CsF pass calcium ions (Meves and Vogel, 1973). In my study, no inward currents were seen in Ca Ringer. This measurement is made more difficult by the threshold-raising effect of high calcium solutions (Frankenhaeuser and Hodgkin, 1957; Hille, 1968; Campbell and Hille, 1976). Thus, potentials which might have given measurable inward currents through an open Na channel might have been insufficient to open channels against the "hyperpolarizing" effect of the solution. Also, although reversal potentials give a P_{Ca}/P_{Na} of about 0.1 for the squid axon Na channel (Meves and Vogel, 1973), the actual currents are quite small (Baker et al., 1971; Meves and Vogel, 1973) as if Ca^{++} ions block Na channels as they pass through (Woodhull, 1973; Campbell and Hille, 1976). Calcium currents of the size seen by Meves and Vogel would have been near the limit of resolution of the apparatus used in these experiments. The limit of 0.093 for P_{Ca}/P_{Na} reported here certainly does not eliminate the possibility of some calcium entering the fiber through the Na channel. No search was made for calcium currents through other channels.

Potassium

Selectivity against potassium is important for the function of sodium channels. With a permeability ratio P_K/P_{Na} of 0.048, the muscle Na channel is more

selective against potassium than that of the nerves studied (see Hille, 1972, for a discussion of potassium permeability of nerve Na channels). The experiments in K Ringer are difficult, and more error might be expected in the results, but it is doubtful that the error in reversal potential would be as great as the 14 mV required to bring the permeability ratio up to the 0.086 measured for the node of Ranvier. The most likely source of errors is an incomplete exchange of solutions during the haste required for a successful K Ringer experiment, or from an increased series resistance error caused by the greater leakage in K Ringer. However, both of these errors would tend to make the permeability ratio even lower. Since the muscle Na channel is also about twice as selective as nerve against aminoguanidinium, the only permeant ion tested less permeant than potassium, there may indeed be a slight difference in the selectivity filters of the two channels.

Comparison to Node, Hille Pore Model

The sodium substitutes tested in this study are representative cations from the larger list studied by Hille on the node of Ranvier. A comparison of the permeability ratios in muscle and node is given in Table III. Taking the value of 0.96 for the lithium permeability ratio as insignificantly different from the 0.94 ratio for hydroxylammonium, the sequences are identical. In both tissues the permeabilities of lithium and hydroxylammonium are about the same as sodium, and in both tissues aminoguanidinium is the largest ion giving inward currents. As in the node, no methylated cations are permeant. Methylammonium is nearly identical in size and shape to both hydroxylammonium and hydrazinium, but methylammonium is impermeant while hydroxylammonium and hydrazinium permeate easily. Indeed, aminoguanidinium and guanidinium are much larger than methylammonium yet they are permeant. The pore selects very strongly against methyl groups, and this ability to discriminate is an

TABLE III

NA CHANNEL PERMEABILITIES OF FROG NODE AND FROG SKELETAL MUSCLE

Ion	P_{ion}/P_{Na}	
	Muscle	Node
Lithium	0.96	0.93
Potassium	0.048	0.086
Hydroxylammonium	0.94	0.94
Hydrazinium	0.31	0.59
Ammonium	0.11	0.16
Guanidinium	0.093	0.13
Aminoguanidinium	0.031	0.06
Calcium	<0.093	<0.11
Methylguanidinium	<0.013	<0.010
Methylammonium	<0.009	<0.007
Tetraethylammonium	<0.008	<0.008
Tetramethylammonium	<0.008	<0.005

Node permeability ratios are from Hille (1971, 1972).

important feature of the selectivity filter model proposed by Hille (1971, 1972, 1975 *c*). To explain the exclusion of the impermeant organic cations he proposed a 3.1 × 5.1-Å oxygen-lined pore as a model of the selectivity filter. Those ions that cannot fit through this size of pore are not permeant. The formation of hydrogen bonds between hydroxyl or amino groups and the oxygens lining the pore allows the hydrogen bond donors (−OH or −NH of the ion) to approach the acceptors (−O of the pore) about 0.8–0.9 Å closer than if hydrogen bonds were not formed. Thus cations containing methyl groups, which cannot form hydrogen bonds, appear larger to the selectivity filter than the apparently similar amino- or hydroxyl-substituted cations. This model successfully predicts the exclusion of methylated organic cations, and, in Hille's experiments, the exclusion of several nonmethylated cations that are too large for the pore in spite of hydrogen bonding.

Although this simple steric model explains some important properties of the Na channel selectivity filter, it is insufficient to account for the ionic selectivity sequence or deviations from independence. Hille (1975 *b*) has further refined the model using Eyring rate theory with four energy barriers along the channel. One barrier comprises the selectivity filter. Its height includes the energy required to dehydrate the ion sufficiently for passage through the narrow filter and the energy gained by approaching the negative charge of an ionized carboxylic acid thought to be part of the filter structure (Hille, 1971, 1972, 1975 *a,b,c*). The observed selectivity sequence is the same as Eisenman (1962) affinity sequence X suggesting that the negative charge of the selectivity filter is a high field strength site. In Hille's model deviations from independence result from the depth of an energy well between two of the barriers.

This expanded model predicts much of the behavior of sodium channels in the node. Not predicted are shifts in channel gating caused by test ions. Gating and selectivity appear to be separate functions, affected differently by test conditions.

Minor differences exist in the observations from frog node and frog skeletal muscle. The muscle permeability to hydrazinium, and aminoguanidinium, and K^+ ions is about half that of nerve, and unlike nerve the muscle currents in ammonium Ringer are less than predicted by the independence principle. Perhaps the differences are artifactual, or maybe they result from small differences in channel shape caused by small differences in the surrounding membrane environment. Nevertheless the selectivity sequence of the two channels is not significantly different and the Hille pore model applies well to both. Apparently the underlying structures of the two channels are the same. This is not surprising since the sodium channels of axons from very distantly related species are also quite similar. The final paper of this series presents additional evidence of similarities between the sodium channels of frog node and muscle.

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