Sodium Channel Selectivity

Dependence on Internal Permeant Ion Concentration

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ABSTRACT The selectivity of sodium channels in squid axon membranes was investigated with widely varying concentrations of internal ions. The selectivity ratio, $P_{Na}/P_K$, determined from reversal potentials decreases from 12.8 to 5.7 to 3.5 as the concentration of internal potassium is reduced from 530 to 180 to 50 mM, respectively. The internal KF perfusion medium can be diluted by tetramethylammonium (TMA), Tris, or sucrose solutions with the same decrease in $P_{Na}/P_K$. The changes in the selectivity ratio depend upon internal permeant ion concentration rather than ionic strength, membrane potential, or chloride permeability. Lowering the internal concentration of cesium, rubidium, guanidinium, or ammonium also reduces $P_{Na}/P_{ion}$. The selectivity sequence of the sodium channel is: Na > guanidinium > ammonium > K > Rb > Cs.

INTRODUCTION

One approach toward a molecular understanding of transport through sodium channel is to consider its selectivity. Hille (1971, 1972, 1975a) has postulated that a 3 × 5-Å selectivity filter could govern which ions are permeant and has given perhaps the most detailed molecular interpretation for the selectivity of the channel. However, no theory has successfully accounted for the degree to which sodium is preferred over potassium by a sodium channel. It has appeared that the selectivity of sodium channels might be a fixed property of the filter region, being unaltered by a variety of pharmacological treatments (Hille, 1968; Narahashi, 1974), by changes in sodium inactivation (Armstrong et al., 1973), or by axon deterioration (Chandler and Meves, 1965). However, Chandler and Meves (1965) also observed that diluting the internal perfusion medium of a squid axon with isotonic sucrose appeared to reduce the selectivity of the channel. They suggested four possible causes for this result: (a) a change in ionic strength, (b) a change in internal potassium activity, (c) a small chloride permeability, or (d) an
effect of membrane potential upon selectivity. Our results show that, of these possibilities, the internal potassium concentration has the strongest effect upon the ratio of potassium permeability to sodium permeability. Furthermore, changing the internal activity of any of the five permeant ions tested leads to similar alterations of permeability ratios. A preliminary report of these results has been presented (Cahalan and Begenisich, 1975). The results indicate that selectivity is a variable property of the channel responding to changes in the ionic composition of the internal medium.

METHODS
Axon segments averaging 420 µm in diameter from the squid, Loligo pealii, were internally perfused and voltage clamped using the methods described in Begenisich and Lynch (1974). The membrane potential, V, was measured using an internal 0.56 M KCl pipette and an external 3 M KCl electrode. Membrane potentials have been corrected for the measured liquid junction potential at the 0.56 M KCl/internal solution interface. Reversal potentials were obtained in two experiments with a 3 M KCl internal pipette and were the same as the (corrected) values using the 0.56 M KCl electrode. This is an indication of the accuracy of the liquid junction potential corrections. The average resting potential of 65 axons bathed in K-free artificial seawater and perfused with 275 mM KF and 400 mM sucrose was -68.4 mV (range -57.3 to -75.3).

Electronic compensation for series resistance was employed throughout; 2-3 Ωcm² were compensated for internal solutions of normal ionic strength, while 4-7 Ωcm² were compensated for solutions of lower ionic strength. Series resistance errors are expected to make little difference in determining the sodium reversal potential (V_{rev}), since net ionic current at V_{rev} is small, consisting only of leakage current. In four experiments varying the series resistance compensation from 2 to 7 Ωcm² caused no detectable change in V_{rev}.

Solutions
We designed solutions to distinguish between possible effects of ionic strength versus potassium activity on the selectivity of sodium channels. For several different potassium activity levels we added varying amounts of the impermeant cation, tetramethylammonium (TMA), to raise the ionic strength. TMA was recrystallized from ethanol to eliminate contamination by permeant ions. Table I shows the solutions used for this series of experiments. Recrystallized tetraethylammonium (TEA) bromide was added to block most of the delayed potassium conductance which could interfere with the sodium reversal potential measurement. Single ion activity coefficients (Kielland, 1937) were used to determine the concentration of KF needed to keep the potassium activity approximately constant as the ionic strength was increased by TMA. The buffer for all internal solutions was 1 mM HEPES (N-2-hydroxyethyl-piperazine-N’-2-ethanesulfonic acid), and the pH was 7.2-7.4 at 5°C. Enough sucrose was added to make the internal solution isosmotic within 3% with the artificial seawater, which was composed of 440 mM NaCl, 10 mM CaCl₂, 50 mM MgCl₂, and 1 mM Tris buffer at pH 7.4. Additional Tris CI was used as a substitute for sodium in the low sodium artificial seawater.

Solutions containing internal test cations other than potassium are given in Table II. The rubidium and guanidinium solutions were maintained at constant ionic strength by the addition of TMA, while ionic strength was allowed to vary for the cesium and ammonium solutions. All solutions contained at least 50 mM fluoride ion to enhance the survival of the axon. TEA was added to solutions containing rubidium or ammonium ions to block their passage through potassium channels. Stock solutions of sucrose, KF, and
TMACI were tested by flame photometry for contamination by sodium ions. These measurements were kindly made by Dr. Donald Geduldig. The solutions used contained less than 0.5 mM Na representing a maximal error of about 3% in the calculated permeability ratio, $P_K/P_{Na}$.

**TABLE I**

| KF concn (activity) | TMACI concn | TEA Br concn | Sucrose concn | Total ionic strength |
|---------------------|--------------|--------------|---------------|---------------------|
| mM                  | mM           | mM           | mM            | mM                  |
| 530 (355)           | -            | 20           | -             | 550                 |
| 297 (199)           | 250          | 20           | -             | 550                 |
| 188 (127)           | 346          | 15           | -             | 550                 |
| 97 (65)             | 443          | 10           | -             | 550                 |
| 63 (43)             | 492          | 5            | -             | 550                 |
| 275 (199)           | -            | 15           | 365           | 290                 |
| 175 (127)           | 100          | 15           | 375           | 290                 |
| 90 (65)             | 190          | 10           | 375           | 290                 |
| 59 (43)             | 226          | 5            | 400           | 290                 |
| 80 (65)             | -            | 10           | 610           | 90                  |
| 50 (43)             | -            | 5            | 700           | 55                  |

**TABLE II**

| Test ion | Test ion concn | TMACI concn | TMAF concn | TEA Br concn | Sucrose concn | Total ionic strength |
|----------|----------------|--------------|-------------|--------------|---------------|----------------------|
|          | mM             | mM           | mM          | mM           | mM            | mM                   |
| Rb Cl    | 225            | -            | 50          | 15           | 400           | 290                  |
| 59       | 175            | 50           | 5           | 400          | 290           |
| CsF      | 550            | -            | -           | -            | -             | 550                  |
| 275      | -              | -            | -           | 400          | 275           |
| NH₄F     | 275            | -            | -           | -            | 10            | 380                  |
| 50       | -              | -            | 5           | 710          | 55            |
| Guanidinium Cl | 225     | -            | 50          | -            | 400           | 275                  |
| TMA      | 225            | 225          | 50          | -            | 400           | 275                  |

*Analysis*

The membrane potential was normally held between $-65$ and $-75$ mV. Depolarizing test pulses were preceded by a 60-ms hyperpolarization of 50 mV to remove sodium inactivation. Peak sodium currents were measured for each test pulse. Usually a linear leakage correction was applied from the leakage current measured during a 60-mV hyperpolarizing pulse, but in some experiments tetrodotoxin was added and the voltage clamp series repeated to provide a more accurate subtraction of the leakage current and any residual
potassium current not blocked by the internal TEA ions. The reversal potentials agreed
within 2 mV for either leakage subtraction method. Reversal potentials, $V_{rev}$, were
determined by interpolation of leak-corrected sodium current to the zero current axis of
the current-voltage relation. This method agrees well in practice with the method of
simply observing the characteristic change or turnover of the current trace as the
potential is increased in small increments from just below to just above the reversal
potential.

External sodium and internal potassium or the ions of Table II were the only measura-
ble permeant ions present excepting calcium present at 10 mM concentration and having
a $P_{Na}/P_{Ca}$ of perhaps 100, (Baker et al., 1971). Consequently we have ignored the small
degree of calcium permeability, and permeability ratios $P_{K}/P_{Na}$ or $P_{ion}/P_{Na}$ were calculated
from the reversal potentials by

$$V_{rev} = \frac{RT}{F} \ln \left( \frac{P_{Na}[Na]_{o}}{P_{ion}[ion]_{i}} \right)$$

(Goldman, 1943; Hodgkin and Katz, 1949), where $R$, $T$, and $F$ have their conventional
meanings with $RT/F = 24.0$ mV at $5^\circ C$. Single-ion activities (Kielland, 1937) rather than
concentrations were used in the permeability ratio computations. The calculations were
repeated using salt activity coefficients with no significant changes in the results.

**RESULTS**

**Dilution of the Internal Medium Increases $P_{K}/P_{Na}$**

Fig. 1 A shows sample voltage clamp records of sodium currents before and after
dilution of the internal KF medium by isotonic sucrose. The KF concentration in
this case was reduced from 275 to 50 mM as the ionic strength went from 290 to
55 mM. Tetraethylammonium ions were present to block the delayed turn-on of
potassium conductance. For the higher concentration of KF (Fig. 1 A, top)
currents are just inward for a depolarization to $+60$ mV and outward for the
next higher clamp step to $+70$ mV. When the internal solution was diluted with
additional sucrose, the reversal potential increased to between 70 and 80 mV. Fig. 1 B shows the relationship between peak sodium currents and the potential
for these two voltage clamp series. The reversal potential increased from 61 to 75
mV with dilution. The predicted increase in reversal potential $\Delta V_{rev}$, assuming
no change in the permeability ratio $P_{K}/P_{Na}$, can be computed from:

$$\Delta V_{rev} = \frac{RT}{F} \ln \left( \frac{[K]_{i}}{[K]_{i}'} \right).$$

Substituting $[K]_{i} = 199$ mM and $[K]_{i}' = 43$ mM gives an increase of 36 mV for the
predicted change in reversal potential rather than the measured 14-mV increase.
This discrepancy can be resolved if the permeability of potassium relative to
sodium increases with dilution. Then, from Eq. 1, $P_{K}/P_{Na}$ must increase from
0.12 to 0.31 when the potassium concentration is decreased from 275 to 50 mM.
In seven axons $P_{K}/P_{Na}$ increased to $0.276 \pm 0.03$ (mean $\pm$ SEM) when the internal
potassium was diluted to 50 mM by isotonic sucrose. These results are in
agreement with the findings of Chandler and Meves (1965) for dilutions of the
internal perfusion medium. From our results $P_{K}/P_{Na}$ decreased from $0.124 \pm 0.003$ (mean $\pm$ SEM, $n = 37$) to $0.078 \pm 0.004$ ($n = 9$) on replacing 275 mM KF
and sucrose by a solution containing 530 mM KF. The range of $P_K/P_{Na}$ with a variety of internal solutions is indicated in Table III.

**Chloride and Tetramethylammonium Are Not Measurably Permeant**

One alternative explanation to a change in the selectivity ratio for the experiments just described would be a small permeability to chloride ions. If chloride were permeant the reversal potential would be given by:

$$V_{rev} = \frac{RT}{F} \ln \left\{ \frac{P_{Na}[Na]o}{P_{K}[K]o + P_{Cl}[Cl]o} \right\}.$$  \hspace{1cm} (3)

Then, in order to duplicate the 14-mV increase in reversal potential observed in Fig. 1B, there would have to have been a $P_{Na}/P_{Cl}$ ratio of 31.5, while all permeability ratios remained constant in both solutions. Several experimental observations rule out the possibility of a chloride permeability of this magnitude. In one experiment the external chloride ions were replaced by larger isethionate ions with no measured change in the reversal potential. If $P_{Na}/P_{Cl} = 150$ and if isethionate ions are impermeant, replacing the external chloride by isethionate would lead to a just-detectable 2-mV change in the reversal potential. Hence $P_{Na}/P_{Cl}$ must be greater than 150 from this experiment.

Another experiment indicates that chloride must be even less permeant than this. All of the internal potassium ions were replaced by TMA ions, while the external solution contained 440 mM Tris Cl and no sodium. Currents were...
measured in response to depolarizing pulses as shown in Fig. 2A. The small transient current just after the voltage step may be charge movement in association with the gating process of sodium channels (Armstrong and Bezanilla, 1974; Keynes and Rojas, 1974). Then the same TMA solution with 1 mM sodium was introduced inside the axon. Adding 1 mM internal Na caused an outward bump of current for voltage pulses to +80 mV and above as shown in Fig. 2B. Since there were 275 TMA ions, 440 Tris ions, and 440 Cl ions for every sodium ion, the combined permeability of TMA, Tris, and Cl relative to sodium must have been less than 1/500 because current from 1 mM sodium was clearly visible for a depolarization to +80 mV, whereas no current in Na channels was recognized in the absence of internal sodium. In any event, a chloride permeability could not

TABLE III

| K activity | Ionic strength | V_m | P_K/P_Na | No. of axons |
|------------|---------------|-----|----------|-------------|
| mM | mM | mV±SEM | | |
| 355 | 550 | 57.3±1.2 | 0.078 | 9 |
| 199 | 550 | 58.7±0.9 | 0.132 | 6 |
| 199 | 290 | 60.1±1.0 | 0.124 | 37 |
| 127 | 550 | 62.0±1.1 | 0.180 | 3 |
| 127 | 290 | 63.4±1.0 | 0.170 | 3 |
| 65 | 550 | 73.3±5.4 | 0.217 | 2 |
| 65 | 290 | 70.1±5.8 | 0.249 | 3 |
| 43 | 550 | 76.2±2.8 | 0.293 | 8 |
| 43 | 290 | 76.9±2.0 | 0.285 | 8 |
| 43 | 55 | 77.6±2.7 | 0.276 | 7 |

FIGURE 2. (A) Currents with no known permeant ions. With Tris seawater outside and 225 TMACl + 50 TMAF inside only leakage and possibly gating currents are observed. Depolarizations are separated by 20 mV from −60 to +120 mV. (B) I_L, I_Gate, + I_Na with 1 mM sodium inside. Depolarizing pulses range from +40 to +120 mV in 20-mV increments. Note the difference in current scales for parts A and B.
account for the small change in reversal potential when the internal solution was
diluted with sucrose. This experiment also shows that TMA ions are acceptably
impermeant to serve as a means of increasing ionic strength without adding
permeant ions.

**Potassium Concentration not Ionic Strength Affects** $P_K/P_{Na}$

Eighteen experiments were done to determine whether dilution of the internal
KF solution reduces sodium channel selectivity by a decrease in potassium
concentration or by a decrease in ionic strength. Using solutions in Table I
potassium activity was varied at constant ionic strength, or ionic strength was
varied at constant potassium activity. Ionic strength was varied independently by
changing the concentration of impermeant TMA ions. In some experiments
Tris was used to vary the ionic strength with the same basic conclusions as for
internal TMA. Tris, however, also appeared to change the rectification of
sodium channels by blocking outward current more than inward current.

Fig. 3 A illustrates voltage clamp sodium currents for an experiment in which
potassium activity was varied from 355 to 43 mM while ionic strength was held
constant at 550 mM. In the top trace with a potassium activity of 355 mM sodium
currents turn over from inward to outward for a depolarization to between 50
and 60 mV. When the internal potassium activity was lowered to 199 mM the
reversal potential increased to between 55 and 65 mV and increased further to
between 70 and 80 mV for a potassium activity of 43 mM. Fig. 3 B shows the
current-voltage relations for this experiment. As in the dilution experiments
described previously $P_K/P_{Na}$ calculated from Eq. 1 varied from about 0.08 for
high K activity to 0.3 for low K activity even though ionic strength was held
constant in this case. The reversal potential for this axon in 275 KF and sucrose
(ionic strength, $\mu_s = 290$ mM) was the same value (62.5 mV) before and after the
solution changes at higher ionic strength, providing a good check on the stability
and reversibility of the measurements.

Fig. 4 illustrates an experiment in which two different potassium activity levels
and two different ionic strengths were directly compared. Changing the ionic
strength by itself had little effect on the measured reversal potentials. The
permeability ratios are shown again to depend on potassium activity and not on
ionic strength.

Table III summarizes the results of all experiments in which potassium activity
and ionic strength were varied independently inside the axon. There is little
effect of ionic strength on selectivity, but $P_K/P_{Na}$ increases as the potassium
activity is reduced.

**Other Permeant Ions Exhibit Concentration-Dependent Permeability**

Alterations in sodium channel permeability ratios with changes in the internal
concentration are not limited to the potassium ion, as revealed in experiments
with internal permeant ions other than potassium. Reducing the concentration
of cesium, rubidium, ammonium, or guanidinium inside the axon causes the
selectivity ratio $P_{ion}/P_{Na}$ to increase as shown in Table IV. For instance, guanidin-
ium at an internal concentration of 50 mM is as permeant as sodium, while at 225
Figure 3. (A) Sodium currents with varying internal K at constant ionic strength. TMACI was added to keep the ionic strength at 550 mM. Voltage clamp steps are separated by 10 mV, with the largest depolarizing pulse indicated to the left of each current family. (B) Current-voltage relations with constant ionic strength and varying internal potassium. Reversal potentials and permeability ratios $P_K/P_{Na}$ are shown in the table for each potassium activity.

Figure 4. Current-voltage relations for two different potassium activities and two ionic strengths in the same axon.

mM guanidinium is about one-half as permeant. The sequence of ion selectivity remains the same at high or low concentrations: Na > guanidinium > NH₄ > K > Rb > Cs. The order of guanidinium and ammonium in this sequence is reversed compared to the node of Ranvier (Hille, 1972).
$P_{K}/P_{Na}$ Is Unaffected by External Na or Membrane Potential

In seven experiments we measured the change in reversal potential produced by diluting the external Na to 1/4 normal. In five experiments the internal solution was 275 KF and sucrose, while in two other experiments the internal solution was 59 KF and 225 TMACI and sucrose. Tris was used as the external sodium substitute. The fourfold reduction in external sodium shifted the reversal potential by $-30 \pm 1.1$ mV (mean ± SEM). Since the expected change in reversal potential is $-32.6$ mV assuming no change in $P_{K}/P_{Na}$, lowering the sodium concentration or changing potential by 30 mV had little effect on the permeability ratio.

**DISCUSSION**

$P_{K}/P_{Na}$ as a Variable

A cornerstone of the ionic hypothesis is the finding that changes in the external sodium concentration produce changes in the sodium equilibrium potential in accord with the Nernst equation for a sodium electrode (Hodgkin and Huxley, 1952). In analogous experiments we find that changing the permeant ion concentration on the inside of the membrane changes the reversal potential less than would be expected from the Goldman-Hodgkin-Katz equation. If the selectivity ratio $P_{Na}/P_{ion}$ remains constant as the concentration of the ion is varied on the inside of the axon, there should be a shift in the reversal potential of $2.3 \frac{RT}{F}$, or 55 mV at 5°C, for a 10-fold change in concentration. However, we find that the shift is substantially less than 55 mV per 10-fold concentration change for any of the five permeant ions tested on the inside.

Fig. 5 A illustrates this effect for changes in the internal potassium activity at two ionic strengths. Reversal potentials do not change as much as predicted for a potassium electrode, when the internal potassium activity is varied. The line in Fig. 5 A represents a slope of 55 mV per 10-fold K activity change. The variable selectivity of the sodium channel, as expressed by $P_{Na}/P_{K}$ (regardless of ionic

| Ion         | $a_{ion}$ (mM) | $V_{rev}$ (mV) | $P_{ion}/P_{Na}$ | No. of axons |
|-------------|----------------|----------------|------------------|--------------|
| Cs          | 350            | 99.6±1.2       | 0.017            | 3            |
|             | 275            | 99.5±2.7       | 0.029            | 3            |
| Rb          | 162            | 88.7           | 0.045            | 4            |
|             | 43             | 94.3±5.2       | 0.196            | 3            |
| NH₄         | 199            | 39.4±0.7       | 0.301            | 4            |
|             | 43             | 59.7±4.6       | 0.592            | 3            |
| Guanidinium | 163            | 33.0±0.3       | 0.482            | 2            |
|             | 43             | 51.5±3.0       | 0.99             | 2            |

TABLE IV

REVERSAL POTENTIALS AND $P_{ion}/P_{Na}$ FOR OTHER IONS
strength), is plotted against the internal potassium activity in Fig. 5 B. The linear regression line provides the following empirical description of our results:

\[ \frac{P_{Na}}{P_K} = 0.029[K]_i + 2.21. \]  

(4)

For high levels of internal potassium we find good agreement with previous

![Figure 5.](image)

**Figure 5.** (A) Reversal potentials at different internal potassium activities. The reversal potentials, \( V_{rev} \), and SEM limits are plotted against the logarithm of the potassium activity ([K]_i), for two different ionic strengths (\( \mu = 550 \text{ mM}, \) and \( \mu = 290 \text{ mM} \)). The solid line has a 55 mV/decade (Nernstian) slope. (B) \( \frac{P_{Na}}{P_K} \) at different internal potassium activities. Filled circles are averages of all data, regardless of ionic strength. The solid line is the linear regression line for the data.

measure of the selectivity ratio, \( \frac{P_{Na}}{P_K} \) of about 12 (Chandler and Meves, 1965; Moore et al., 1966; Binstock and Lecar, 1969; Atwater et al., 1969; Hille, 1972), whereas for much lower potassium levels the selectivity ratio is reduced to about 3.5. Concentration-dependent permeability ratios have been found for \( \frac{P_{Na}}{P_K} \)
Concentration Dependence of Sodium Channel Selectivity

There are several control experiments which point to the activity of the internal permeant ion as the important variable for selectivity. First, as a check on the completeness of the internal solution change, we varied the concentration of sodium in the internal perfusion medium with no other permeant ions present. With sodium as the only permeant ion the reversal potential should be determined by $V_{\text{rev}} = \frac{RT}{F \cdot \ln([\text{Na}]_e/\text{[Na]}_i)}$. The measured reversal potentials agreed within 3 mV of the expected values, indicating that there was a complete exchange of the internal solution. Other consequences of this result are discussed below.

Second, a small amount of chloride or fluoride permeability cannot be responsible for the apparent change in the selectivity ratio. Changing the anion from chloride to isethionate on the outside did not alter the reversal potential. Furthermore, the presence of 1 mM Na in the internal solution (Fig. 2) was easily detectable when only Tris, TMA, Cl, and F were present in the internal and external solutions. Thus, the combined permeability of these four ions must be less than about 1/500 that of sodium. This very low degree of chloride permeability would not contribute significantly to the measured reversal potential.

Finally, as described in Results, neither ionic strength, external Na concentration, or membrane potential produced changes in permeability ratios. Therefore, the major part of the $P_{\text{Na}}/P_{\text{ion}}$ change can be attributed to changes in internal ion activity.

The concentration dependence for selectivity may be asymmetrical with respect to the membrane. Changes in the external sodium concentration did not alter the selectivity ratio, in contrast to the effect of the internal permeant ion concentration. It would be of interest in this connection to determine if external potassium or other ions in the absence of external sodium, have effects on the selectivity ratio.

Models for the Selectivity Change

The following sections consider several models for the observed behavior of the sodium reversal potential as the internal permeant ion concentration is varied. We have described our results in terms of changes in a permeability ratio calculated from the Goldman (1943), Hodgkin and Katz (1949) voltage equation, and will consider several models within this framework that might account for the data. One should bear in mind, however, that other formulations of the concept of permeability are possible depending upon the transport model for sodium through the membrane. For example, Heckmann (1972) has shown that for pores in which there is single-filing of ions past several sites in the channel, permeability can be concentration dependent and have asymmetrical properties. Likewise, Läuger (1975) and Hille (1975a,b) have shown that for one-ion pore

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1 Dr. L. Goldman and Dr. G. Ebert have recently performed similar experiments with changes of the internal potassium concentration in perfused *Myxicola* axon and find similar changes in the sodium channel selectivity ratio. We thank Drs. Goldman and Ebert for this communication.
models featuring saturable binding to sites in the channel, permeability is concentration dependent, though permeability ratios may remain constant for varying degrees of saturation. The failure of our observations to be described by a constant $P_K/P_{Na}$ may mean that there are structural changes in the membrane when the internal potassium is varied that alter the molecular properties of the filter region, or it may be that our concept of permeability needs to be modified because of nonindependent ion movement in ionic channels.

A restricted space with low $[K]$ or high $[Na]$ One possible explanation for our results would be that the concentration of potassium at the inner surface of the membrane is lower than in bulk solution. One might imagine a special reservoir forming part of the inner mouth of the channel, access to which might involve binding or some process to limit the concentration. This type of mechanism could lead to reversal potentials which shift less than expected when the potassium concentration in the perfusion medium is altered. Such a space, however, would have to have rather special properties, since, as described above, changes in internal sodium shift the reversal potential in accord with the Nernst equation.

Another possible explanation involving a restricted volume near the inner mouth of the pore is accumulation of sodium ions. However, again the accumulation mechanism would have to have very unusual properties, as different amounts of accumulation for different internal ions would have to be postulated. Also, we find good agreement between the reversal potentials for instantaneous sodium currents and peak sodium currents. Finally, as described above, our experiments with changing the internal sodium concentration are in accord with the Nernst equation without postulating internal sodium accumulation. For these reasons it seems unlikely that a simple mechanism involving internal sodium accumulation can explain our results.

Barrier models We have investigated the possibility that an Eyring rate model for transport through the channel (Woodbury, 1971; Läuger, 1973; Hille, 1975b) might afford one approach to explain concentration-dependent permeability ratios. Hille has pointed out several features of the four-barrier, one-ion pore with regard to selectivity, including the fact that the model has selectivity ratios that depend on membrane potential. The lack of effect of membrane potential in the experiments with external sodium concentration changes puts one restriction on the types of energy profile we can consider. With this restriction it is necessary to conclude that no single set of energy barriers and wells, one for sodium and one for potassium, can account for concentration-dependent permeability ratios. Within the model it would be necessary to alter the height of barriers arbitrarily either by raising sodium barriers or by lowering potassium barriers when the internal potassium concentration is reduced. Thus the approach does not lend itself toward a clarification of the basic mechanism underlying concentration-dependent permeability ratios.

A site-controlling selectivity Another mechanism for explaining our results might be a model in which a site at the inner membrane surface or a site within the membrane accessible from the inside, controls the conformation of
the sodium channel filter region. If the sites were completely occupied by potassium, \( P_{Na}/P_K \) would be some maximum value. The actual selectivity ratio for an ensemble of channels at a given potassium activity would be determined by the fractional occupancy of the site by potassium. The higher the internal potassium concentration, the greater the occupancy of the site, and hence, the larger the value of \( P_{Na}/P_K \).

It is perhaps not unreasonable to expect some rearrangement of the local molecular environment of the filter region in response to the presence of an ion in the channel. The site model just developed might reflect just this sort of interaction. Let us suppose that potassium ions interact with the filter region of the channel as they permeate and leave the filter in an altered conformation with a relatively high \( P_{Na}/P_K \). After the departure of a potassium ion the filter then relaxes back to its original state with a lower \( P_{Na}/P_K \). One need only suppose that this relaxation time of the molecules comprising the filter region is longer than the interval between entry of potassium ions, perhaps on the order of a microsecond. Then, on the average, the selectivity ratio would reflect the "memory" of the filter having been altered by a potassium ion through a redistribution of channels between the altered and original (unoccupied) states.

Further experiments are needed to test these ideas more completely. A complete series of selectivity ratios at several concentrations (instead of only two in the present study) of another permeant ion such as guanidinium, ammonium, or rubidium, would be one approach. Experiments need to be done to determine if permeability ratios can be altered by ions on the outside of the membrane. Experiments testing the ability of one ion to alter another's selectivity ratio could also be done.

Conclusions

Our basic experimental finding is that reversal potentials do not shift in accord with the Goldman (1943), Hodgkin and Katz (1949) voltage equation (see Fig. 5 A), when the internal potassium concentration is varied. Two fundamentally different possibilities exist to explain this finding. First, there may be a structural change in macromolecules comprising a selectivity filter of the sodium channel resulting in a less selective filter when internal potassium is lowered. Second, there is no structural or chemical change within the membrane, but our conception of permeabilities must be modified due to limitations in the Goldman, Hodgkin, and Katz equation, possibly because of difficulties in the assumption of independent ion movements. Future experiments in this area must be accompanied by the development of new ways to consider permeability in systems violating the assumptions that are implicit in the constant field equation.

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REFERENCES

ARMSTRONG, C. M., and F. BEZANILLA. 1974. Charge movement associated with the opening and closing of the activation gates of the Na channels. J. Gen. Physiol. 63:535-552.

ARMSTRONG, C. M., F. BEZANILLA, and E. ROJAS. 1973. Destruction of sodium inactivation of squid axons perfused with Pronase. J. Gen. Physiol. 62:375-391.

ATWATER, J., 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., A. L. HODGKIN, and E. RIGG. 1971. Depolarization and calcium entry in squid giant axons. J. Physiol. (Lond.). 218:709-755.

BEGENISICH, T., and C. LYNCH. 1974. Effects of internal divalent cations on voltage-clamped squid axons. J. Gen. Physiol. 63:675-689.

BENSON, L., and H. LECAR. 1969. Ammonium ion conductance in the squid giant axon. J. Gen. Physiol. 53:342-361.

CAHALAN, M., and T. BEGENISICH. 1975. Internal K* alters sodium channel selectivity. Biophysical Soc. Annu. Meet. Abstr. 15:261a.

CHAUDHURI, W. K., and H. MEYER. 1965. Voltage clamp experiments on internally perfused giant axons. J. Physiol. (Lond.). 186:788-820.

EISENMAN, G., J. SANDBLOM, and E. NEHER. 1976. Evidence for multiple occupancy of gramicidin A channels by ions. Biophys. J. 16:81.

GOLDMAN, D. E. 1943. Potential, impedance, and rectification in membranes. J. Gen. Physiol. 27:317-360.

HECKMANN, K. 1972. Single-file diffusion. In Biomembranes, Vol. 3, Passive Permeability of Cell Membranes. F. Kreuger and J. F. G. Slegers, editors. Plenum Press, New York. 127-153.

HILLE, B. 1967. Pharmacological modification of the sodium channels of frog nerve. J. Gen. Physiol. 51:199-219.

HILLE, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. J. Gen. Physiol. 58:599-619.

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

HILLE, B. 1975a. Ionic selectivity of Na and K channels in nerve membranes. In Membranes - A series of advances, Vol. 3. G. Eisenman, editor. Marcel Dekker, Inc., New York. 255-329.

HILLE, B. 1975b. Ionic selectivity, saturation and block in sodium channels: a four-barrier model. J. Gen. Physiol. 66:555-560.

HODGKIN, A. L., and A. F. HUXLEY. 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. J. Physiol. (Lond.). 116:449-472.

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 E. ROJAS. 1974. Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. J. Physiol. (Lond.). 239:395-434.

KIELAND, J. 1937. Individual activity coefficients of ions in aqueous solutions. J. Am. Chem. Soc. 59:1657-1678.

LAEGER, P. 1973. Ion transport through pores: a rate-theory analysis. Biochim. Biophys. Acta. 311:429-441.
Meyers, V. A., and D. A. Haydon. 1972. Ion transfer across lipid membranes in the presence of gramicidin A. II. The ion selectivity. *Biochim. Biophys. Acta.* 274:313–322.

Moore, J. W., N. C. Anderson, M. P. Blaustein, M. Takata, J. Y. Lettvin, W. F. Pickard, T. Bernstein, and J. Pooler. 1966. Alkali cation specificity of squid axon membrane. *Ann. N. Y. Acad. Sci.* 137:818–829.

Narahashi, T. 1974. Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* 54:814–889.

Woodbury, J. W. 1971. Eyring rate theory model of the current-voltage relationship of ion channels in excitable membranes. In *Chemical Dynamics: Papers in Honor of Henry Eyring.* J. Hirschfelder, editor. John Wiley and Sons, Inc., New York.