Negative Conductance Caused by Entry of Sodium and Cesium Ions into the Potassium Channels of Squid Axons

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ABSTRACT Internal Cs^+, Na^+, Li^+, and, to a lesser degree, Rb^+ interfere with outward current through the K pores in voltage clamped squid axons. Addition of 100 mM NaF to the perfusion medium cuts outward current for large depolarizations about in half, and causes negative conductance over a range of membrane voltages. For example, suddenly reducing membrane potential from +100 to +60 mv *increases* the magnitude of the outward current. Internal Cs^+ and, to a small extent, Li^+, also cause negative conductance. Na^+ ions permeate at least 17 times less well through the K pores than K^+, and Cs^+ does not permeate measurably. The results strongly suggest that K pores have a wide and not very selective inner mouth, which accepts K^+, Na^+, Li^+, Cs^+, tetraethylammonium ion (TEA^+), and other ions. The diameter of the mouth must be at least 8 A, which is the diameter of a TEA^+ ion. K^+ ions in the mouths probably have full hydration shells. The remainder of the pore is postulated to be 2.6–3.0 A in diameter, large enough for K^+ and Rb^+ but too small for Cs^+ and TEA^+. We postulate that Na^+ ions do not enter the narrower part of the pore because they are too small to fit well in the coordination cages provided by the pore as replacements for the water molecules surrounding an ion.

INTRODUCTION

The two major sets of channels that conduct ions through nerve membrane conduct preferentially either Na^+ ions (the Na channels) or K^+ ions (the K channels), but they are not perfectly selective. The Na channels are more permeable to Na^+ than to K^+ by a ratio of about 12:1 (Chandler and Meves, 1965; Rojas and Atwater, 1967; Hille, 1971 *a*). For the K channel the precise selectivity ratio of K to Na has not been worked out, but it is known that several ions can enter the K channels and interfere with flow (I_K) through them. This was first suggested by Chandler and Meves (1965) who found that substitution of a portion of the internal K^+ by Na^+, Rb^+, Cs^+, or choline decreased I_K below the values expected from the independence principle. Selec-

tive inhibition of inward I_K by external Cs^+ was later clearly demonstrated by Adelman and Senft (1968; see also Adelman et al., 1971). More recently Bergman (1970) has demonstrated in myelinated fibers that internal Na^+ inhibits the outflow of K^+ ions, and a similar action of internal Na^+ has since been demonstrated in squid axons (Adelman and Senft, 1971).

We were struck by the similarity between Bergman's (1970) Na-filled nodes and a squid axon containing tetraethylammonium ion (TEA^+). The mechanism of action of TEA^+ and several TEA^+ derivatives has been worked out in some detail (Armstrong, 1971), and the data suggest that a K^+ channel has a wide inner mouth capable of accepting a TEA^+ ion or a hydrated K^+ ion and a narrower outer portion that will accept a dehydrated K^+ ion but not TEA^+. If so, an ion passing outward through a K channel faces two successive selectivity barriers: the first is entry into the mouth, and the second is entry into the narrower portion (which, for convenience, will be called the tunnel). We report here a number of experiments with internal Li^+, Na^+, Rb^+, and Cs^+ which were designed to see if the effects of these ions could be reconciled with this picture of the K channel, and to determine the relative importance of the two selectivity barriers proposed in it. Our data are compatible with the model, and suggest that the inner mouth of the channel is not very selective.

METHODS

Experiments were performed at Woods Hole, Mass. on segments of isolated axon from the squid *Loligo pealei*.

Voltage Clamp

All of the axons were voltage clamped, with apparatus that has been described (Armstrong, 1969) except for two modifications. The first was the incorporation of an electronic switch similar to the one described by Bezanilla et al. (1970). The second was a safety device that automatically turned off the clamp by means of the switch whenever the membrane potential exceeded safe limits. The internal electrode was of the dual type, similar to the one described by Chandler and Meves (1965). This electrode consisted of a platinized platinum wire approximately 100 μm in diameter for passing current and a 75 μm pipette (approximately) for measuring voltage. The pipette contained an electrically floating platinum wire for lowering impedence. This electrode was introduced into the axon (either perfused or unperfused) through a cut in one end.

Internal Perfusion

Most of the axons were internally perfused by a modification of the method originally devised by Tasaki et al. (1962). Some of the details of our technique, and particularly the chamber, closely resemble the procedure described by Fishman (1970). The essential features of the chamber were (*a*) a central trough 15 mm in length, 2 mm

wide, and approximately 5 mm deep. Artificial sea-water (ASW) flowed through the trough. (*b*) At both ends of the trough was a 5 mm air gap, that extended to the support posts for the axon. A 250 μm cannula was introduced through a cut in the right end of the axon and a core of axoplasm was sucked into the cannula as it was advanced. When the cannula reached the left end of the segment another cut was made and the cannula tip was pushed out through this cut. The axoplasm in the cannula was then blown out, and perfusion was started through the cannula, with a solution that had added to it (usually) 1 mg/ml Pronase (Calbiochem, Los Angeles, Calif.). The wire of the dual electrode was inserted into the cannula and the dual electrode was advanced as the cannula was withdrawn. Withdrawal was halted about 5 mm from the cut in the left end, and this 5 mm segment (which was all within the left air gap) was treated for about 1 min with flowing perfusion solution containing Pronase. In some cases the cannula was then rapidly withdrawn with the flow stopped and the axon interior was treated for 2 or 3 min with Pronase. This procedure dissolved the axoplasm but had no effect on the axon's electrical properties provided the solution was not running. The perfusion solution was then changed to one that had no Pronase, and perfusion pressure was increased (usually to about 10–15 cm of water) to restart flow. In other cases only the part of the axon in the left air gap was Pronase treated, and the perfusion solution was made Pronase free before further withdrawal of the cannula. With either procedure flow through the axon was brisk and there were almost never flow stoppages due to clogging.

Conventions

Following the usual conventions, V (membrane potential) is the potential inside the axon minus the potential outside. Depolarization makes V more positive, polarization or hyperpolarization makes it more negative. Membrane current (I) is positive when outward, negative when inward.

Temperature was measured by means of a thermistor mounted in the chamber, and was usually maintained at 8°–10°C by continual flow through the chamber of cold external solution.

Standard Procedures

One commonly used procedure was to depolarize the axon to different voltages and to measure membrane current at a fixed time after the depolarizing step. The current voltage curve so obtained will be called the *isochronal I–V* curve. A second procedure involved turning on the potassium conductance by means of a first depolarizing pulse of fixed amplitude, and then determining the *instantaneous I–V* curve by stepping to another potential and measuring current immediately after subsidence of the capacity current. Table I gives the name and compositions of the solutions used.

RESULTS

Most of the experiments described in the following results are of two types. The first type was designed to determine the extent to which other cations interfere with K^+ movement through the K channels. For this purpose the internal K^+ concentration was held constant while other ions were added or

omitted. The second type was to determine how well other cations permeate through the K channels. In these experiments internal K^+ was replaced by the cation in question.

Na^+ Interference with Flow Through K Channels

In experiments with unperfused axons we noticed in many cases that the potassium current (I_K) was small relative to the sodium current (I_{Na}), and that I_K tended to saturate as the depolarization was made larger. This was particularly so for axons that were kept in the refrigerator for some time be-

TABLE I
SOLUTIONS AND COMPOSITIONS

	Li^+	Na^+	K^+	Rb^+	Cs^+	F^-	Cl^-	Ca^{++}	Mg^{++}	Sucrose
	mM	mM	mM	mM	mM	mM	mM	mM	mM	mM
550K			550			550				
375K			375			375				255
275K			275			275				400
275K 100Na		100	275			375				255
275K 100Cs			275		100	375				255
275K 50Cs			275		50	325				328
275K 100Rb			275	100		375				255
275K 100Li	100		275			100	275			255
275Rb				275		275				400
275Na		275				275				400
K-free ASW		440					560	10	50	
ASW		430	10				560	10	50	
440K SW			440				560	10	50	
340K 100Na SW		100	340				560	10	50	
340K 100Cs SW			340		100		560	10	50	

fore use. Fig. 1 *a* shows a family of current traces from such an axon. The axon was voltage clamped at a holding potential of −65 mv, and depolarized to the levels indicated in the figure next to each current trace. The perfusion procedure had been completed when these traces were taken, but perfusion pressure was so low that flow through the axon had not begun. From the size of the early outward current (I_{Na} predominantly) and the low value of the reversal potential for this current (+40 mv), it seems clear that the internal sodium concentration was quite high (75 mM if calculated from the reversal potential). The late outward current was smaller than the early outward current for large depolarizations and current amplitude saturated as V was made more positive: the top three traces superimpose or even cross. Shortly after recording these traces the perfusion pressure was increased to approximately 5–7 cm of water, and flow through the axon commenced. After a few minutes of perfusion the current pattern (Fig. 1 *b*) looked quite

different. The early outward current was much smaller and the reversal potential for the early current had increased to about +70 mv. The late outward current was now much larger (note that the maximum value of V in Fig. 1 *b* is less than in Fig. 1 *a*), and the late current, instead of saturating, grew steadily larger with depolarization.

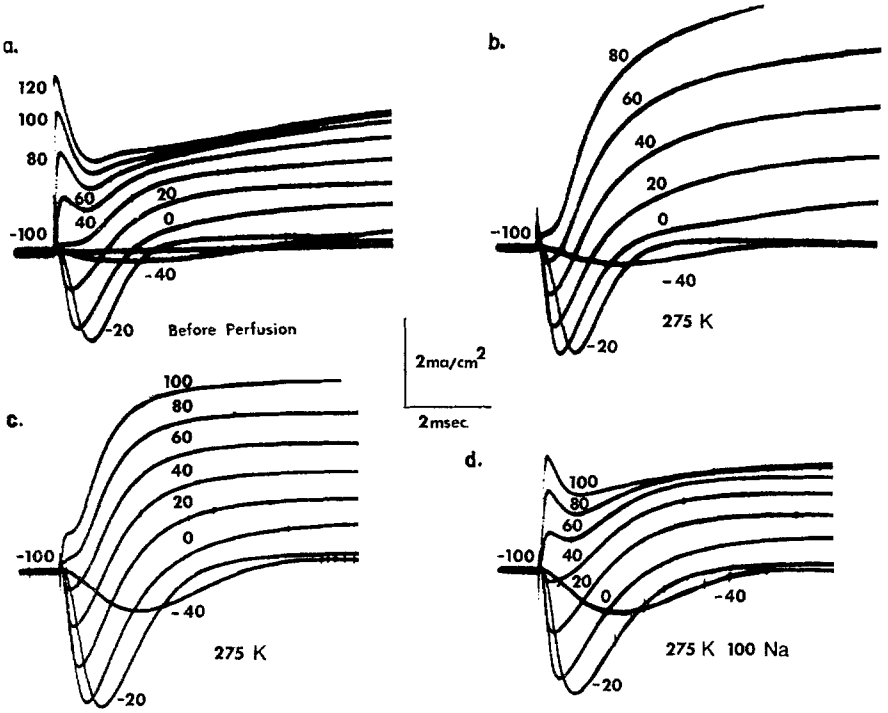

FIGURE 1. (*a*) Voltage clamp currents from an axon that was stored for 8 hr in t he re frigerator before use. Perfusion procedures had been completed, but no perfusion fluid had in fact flowed through the axon when this record was taken. Holding potential was −60 mv. The axon was hyperpolarized to −100 mv for 50 msec, then depolarized to the potentials shown in the figure. (*b*) The same axon after several minutes of internal perfusion with 275K. Outward Na^+ currents are smaller than in (*a*), indicating a decrease in internal Na^+, and outward K^+ currents are larger. (*c*) and (*d*) Another axon, perfused internally with 275KF in (*c*), and 275K 100Na in (*d*). 100 mM Na inside cuts I_K roughly in half for large depolarizations, and makes the isochronal (see text) I–V curve nonlinear. (*a*) and (*b*) 11°C; (*c*) and (*d*) 9.5°C.

To see if internal Na^+ ions can depress I_K as suggested by the results just mentioned, we internally perfused an axon with a solution containing sodium ions in addition to potassium. Fig. 1 *c* shows the traces recorded with 275K inside, and Fig. 1 *d* is from the same axon after changing to 275K 100 Na. The current pattern in Fig. 1 *d* is clearly quite similar to that of the unperfused axon in Fig. 1 *a*. Isochronal plots (see Methods) for another axon in 275K and

275K 100Na are shown in Fig. 2. Other experiments with 375K or even 550K inside demonstrated that the saturation of the late current with increasing depolarization is not due to an effect of ionic strength. We conclude that internal sodium ions depress I_K and cause saturation. Averaging several experiments, I_K at $V = +100$ mv in 275K 100Na was about half of I_K in the control solution, 275K.

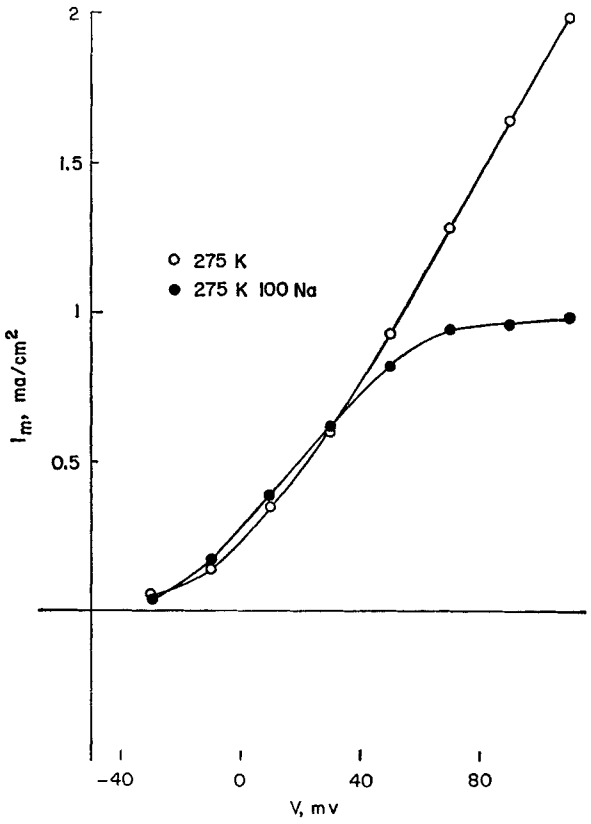

FIGURE 2. Depression of I_K by internal Na^+. The circles give the current at the end of a 7 msec pulse to the membrane potential shown on the abscissa. Each point on the 275K curve is the average of the current before and after perfusion with 275K 100Na.

The late current records in Fig. 1 *a* show an interesting anomaly called negative slope conductance (hereafter, negative conductance): at the end of the traces the current at 120 mv is smaller than at 80 or 100 mv. Negative conductance is much more evident in the instantaneous *I–V* curve, as illustrated in Fig. 3. In this figure a TTX (tetrodotoxin) poisoned axon was first depolarized to 115 mv to turn on g_K (the potassium conductance), and this first step was followed by another to the potential indicated in the figure. In Fig. 3 *a* the axon contained 275K, and nine different steps are superimposed.

(The curvature in the voltage trace is due to compensation for the series resistance.) All of the second steps are negative going, and all result in a current reduction which is roughly proportional to the size of the step. The remaining parts of the figure (Fig. 3 *b–f*) show currents from the same axon, but with 275K 100Na inside. Again the second steps are negative-going, but, for all

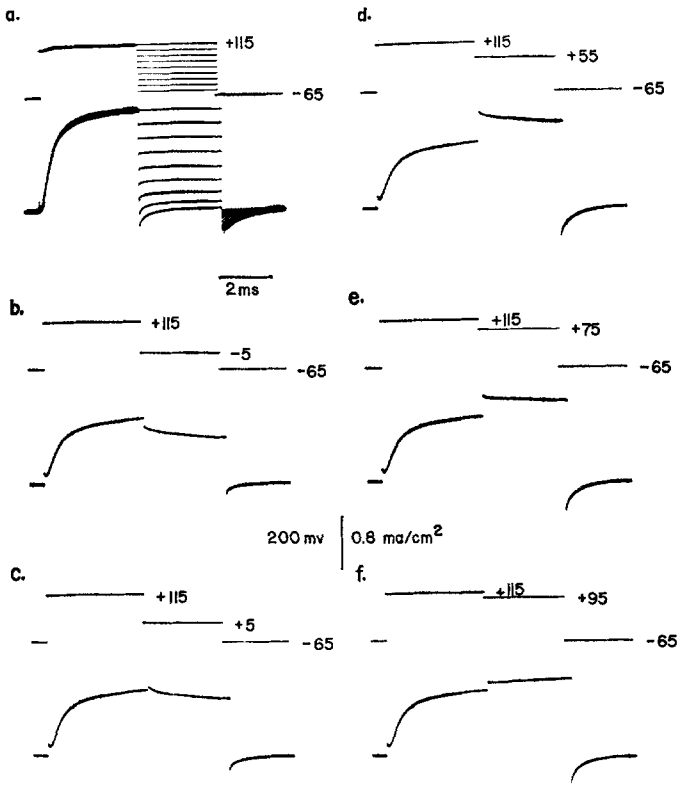

FIGURE 3. Negative conductance caused by internal Na^+. (*a*) Superimposed current traces when the axon contains 275K. The current just after the second step is an almost linear function of the potential during the second step. (*b*)–(*f*) Individual records from the same axon containing 275K 100Na. In (*c*)–(*f*), current immediately after the second step is increased even though the second step decreased V, i.e., there is negative conductance. The ASW contained 10^{-7} M tetrodotoxin (TTX) to eliminate Na current.

except Fig. 3 *b*, current is *larger* rather than smaller after the step. Thus, there is a very clear negative conductance that is caused by Na^+ ions.

During the second step in most parts of Fig. 3 the current diminishes with time. We explain this as follows. The shape of the current trace during the second step is determined by two factors. One is accumulation of K^+ ions in the Frankenhaeuser-Hodgkin space (Frankenhaeuser and Hodgkin, 1956) which occurs at a rate proportional to I_K, and tends to decrease it. The other is the

rate of change of g_K. During a large depolarization, g_K continues to rise for many milliseconds, much longer than is predicted by the Hodgkin-Huxley equations. If the second step is very positive, this g_K increase offsets K^+ accumulation, and I_K remains approximately constant during the step (Fig. 3 *f*). If the step is less positive, g_K increases slowly if at all, and K^+ accumulation causes a decline in the current (Fig. 3 *b–e*).

Instantaneous *I–V* curves for another axon containing 275K (open triangles) and 275K 100Na (filled triangles) are shown in Fig. 4. In the absence of internal Na^+ there is an almost linear relation between current and voltage, but in the presence of Na^+ the relation is curved and has a very definite region of negative slope. Because of K^+ accumulation in the Frankenhaeuser-Hodgkin space, the equilibrium potential for K^+ ion depends on the amount of current that has passed through the membrane (more accumulates with large currents), and for this reason the zero current intercept for the two curves is different.

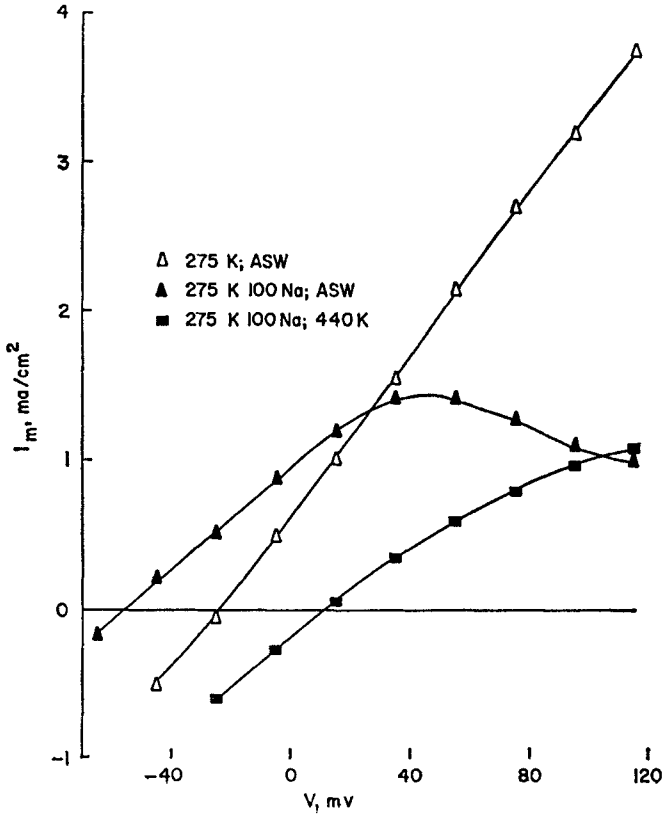

FIGURE 4. Instantaneous *I–V* curves for the conditions indicated. Raising the external K^+ concentration makes the nonlinearity caused by internal Na^+ much less pronounced. Curves are from a TTX poisoned axon at 10°C.

To see if external K^+ ion has the same clearing action for an Na^+ containing axon that it has for an axon containing TEA^+ (Armstrong and Binstock, 1965), we raised the external K^+ concentration of the medium surrounding the axon to 440 mM and determined the curve in Fig. 4 marked with filled squares. The negative conductance seen in ASW disappeared, even though the axon still contained 275K 100Na. The clearing effect of external K^+ could also be demonstrated simply by increasing the duration of the first pulse, allowing more time for K^+ accumulation in the Frankenhaeuser-Hodgkin space. In Fig. 5 an unperfused axon containing an unknown amount of Na^+ showed definite negative conductance after a short pulse (single arrow; the current increases even though the step is negative going) but after a longer pulse the negative conductance has disappeared (double arrow).

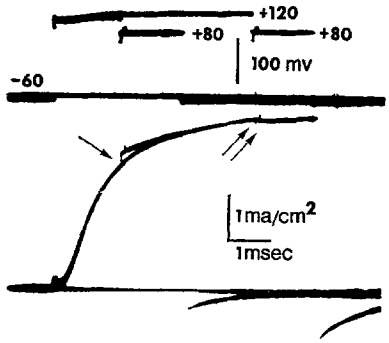

FIGURE 5. Abolition of negative conductance due to K^+ accumulation in the Franken haeuser-Hodgkin (F-H) space. Current just after the second (*negative* going) step is more *positive* when the second step is applied early (single arrow). Prolonging the time at +120 mv allows time for accumulation in the F-H space, and current immediately after the second step (to +80 mv) is then about the same as at the end of the first step (double arrow). Unperfused axon, poisoned with 10^{-7} M TTX; 9°C.

That K^+ is in fact accumulating and changing V_K is evident from the difference in amplitude of the inward current tails seen on repolarization.

Other Cations Also Interfere with Flow Through the K Channels

Cs^+ and Li^+ ions inside an axon affect the K current in the same way that Na^+ ions do, although there are quantitative differences. In Fig. 6 the open and filled circles mark the isochronal curves (7 msec) for an axon containing 275K and 275K 50 Cs, respectively. Cs^+ depresses the late current even more potently than Na ion, and produces a region of negative conductance in the isochronal curve. The instantaneous I–V curves are marked by triangles. With 275K inside the axon (open triangles) the instantaneous curve is an almost straight line. Addition of 50 mM Cs^+ to the 275K causes the same type of non-linearity seen with 100 mM internal Na^+, and from V = 20 mv to V = 100 mv, there is negative conductance. A representative trace taken in the negative

conductance region is given in the inset (Fig. 6). The equilibrium potentials of the two instantaneous curves are different, undoubtedly for the reason discussed in connection with Fig. 4.

Internal Li^+ has qualitatively the same effect as internal Na^+ or Cs^+. At a concentration of 100 mM, it causes saturation of the isochronal curve (not

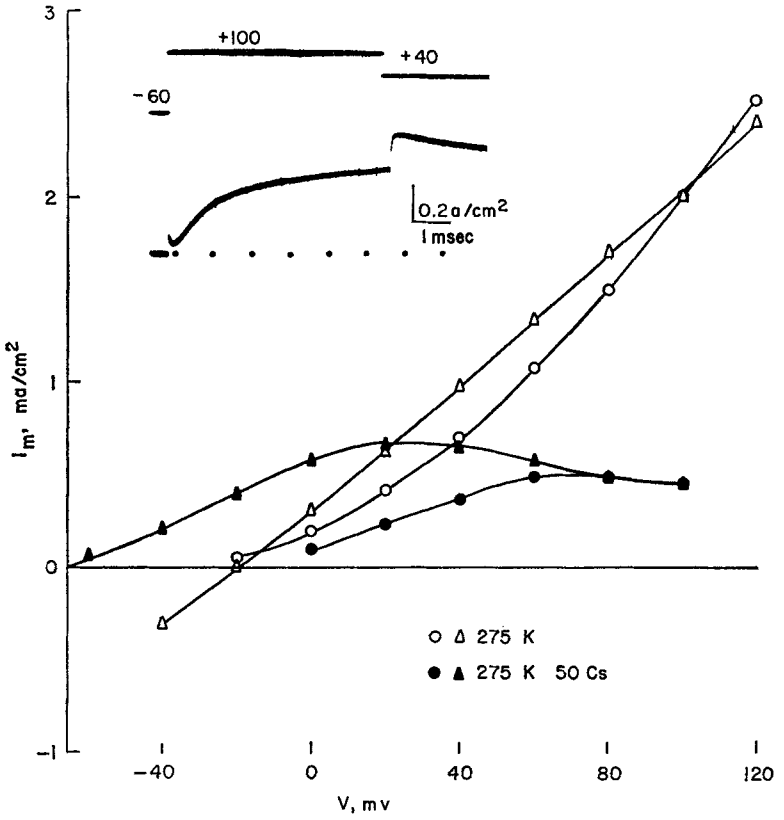

FIGURE 6. Negative conductance caused by internal Cs^+. Triangles denote the instantaneous curves, and circles denote isochronal curves. The inset shows the trace from which was derived the $V = +40$ mv point on the instantaneous curve for 275K 50Cs. TTX, 10^{-7} M; 9°C.

illustrated), nonlinearity of the instantaneous curve (Fig. 7), and negative conductance, although this last is not very prominent (Fig. 7, inset).

Internal Rb^+ added to a constant concentration of internal K^+ has much less effect on the K current than the other cations tested, and the effect is qualitatively different. The isochronal curve (6 msec) is only slightly depressed by the addition of 100 mM Rb (Fig. 8 *a*) and shows no sign of saturation. The instantaneous *I–V* curve (Fig. 8 *b*) with Rb^+ inside shows neither negative conductance nor a tendency to saturate at large depolarizations.

In fact, there is a slight curvature in the opposite direction. This curvature was observed in both of the two experiments where the instantaneous curve was determined with internal Rb, and we have no ready explanation for it.

Penetration of Cations Other Than K^+ Through the K Channels

To determine how well Na^+ penetrates through the K channels, we replaced all of the internal K^+ by Na^+, and measured the late current. Fig. 9 *a–c* illustrates the results of such an experiment. A comparison of part *b* of this figure

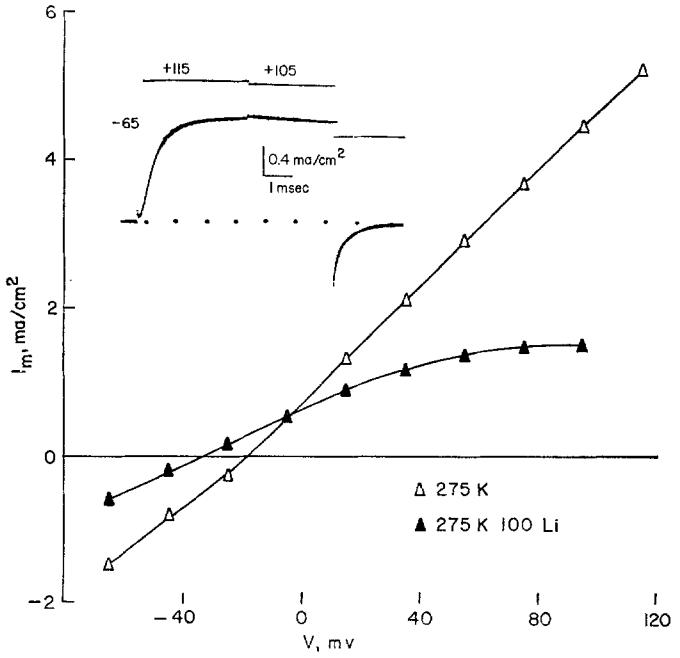

FIGURE 7. Curvature of the instantaneous *I–V* curve caused by internal Li^+. Negative conductance can sometimes by demonstrated with Li^+ (inset), but it is not very prominent. TTX, 10^{-7} M, 10°C.

(275Na) with parts *a* and *c* (275K) shows that the K channel is almost impermeable to Na^+. Exactly how impermeable is hard to say, because in axons perfused with NaF in the absence of K^+ ions, g_K is irreversibly reduced (Chandler and Meves, 1970). A lower limit for the P_K/P_{Na} ratio can be obtained by dividing the maximum outward current during 275Na perfusion by the maximum current on return to 275K (Fig. 9 *b* and *c*). This lower limit is about 17.

Rb^+ can carry substantial current through the K channels. Full replacement of internal K^+ by Rb^+ reduces the late current to about one-fourth of its amplitude with full potassium (Fig. 9 *d–f*).

Fig. 9 *g–i* illustrates that the K channels are essentially impermeable to

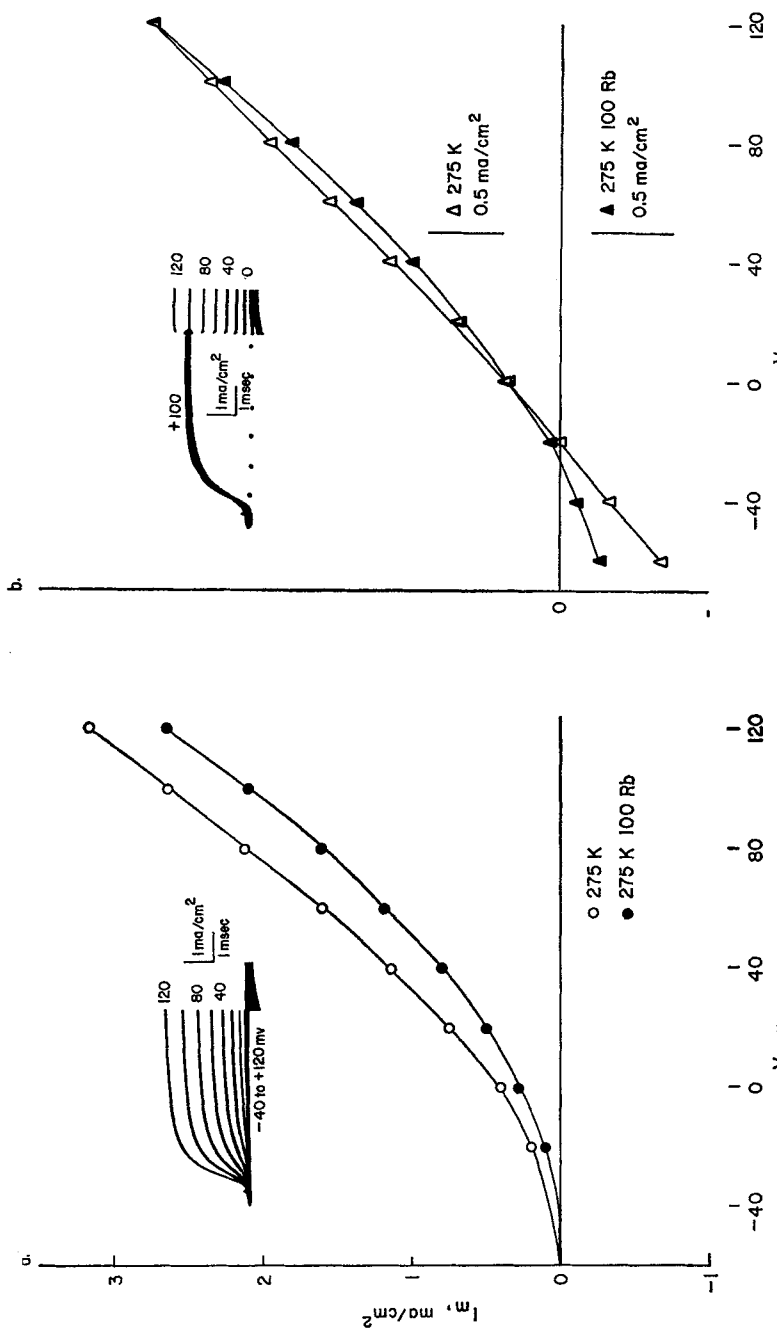

FIGURE 8. Addition of Rb^+ to the internal medium mildly depresses the isochronal curve (*a*) and does not cause negative conductance (*b*). The *insets* show the records from which the Rb curves were derived. TTX, 10^{-7} M; 7.5°C.

Cs^+. The procedure differed from that for the other two ions, in that 100 mM Cs^+ was added to the control solution (275K) instead of being substituted for K^+. There is no measurable current in Fig. 9 *h* that can be identified with the K channel. Axons in which K^+ was fully replaced by Cs^+ (not illustrated) also had no measurable K channel current.

External Cs^+ Blocks Inward K currents

Adelman and Senft (1968; see also Adelman et al., 1971) have shown that external Cs^+ ions interfere with the flow of inward K current, and we confirm

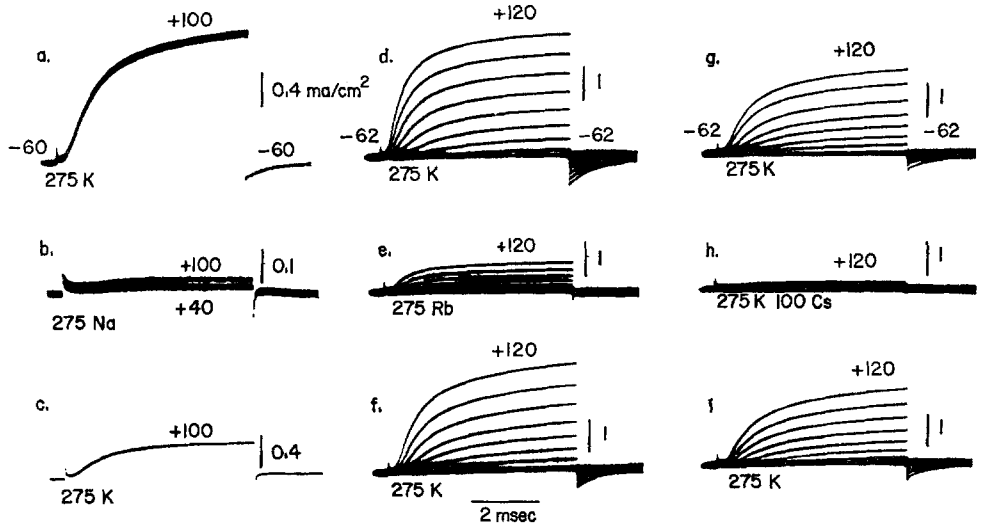

FIGURE 9. Permeation of Na^+, Rb^+, and Cs^+ through the K channels. Each column shows the records taken before, during, and after replacement of 275K with the solution indicated. Rb^+ is roughly one-fourth as permeable as K^+, while Na^+ and Cs^+ are much less permeable. g_K does not recover fully after internal perfusion with 275Na. TTX 10^{-7} M in all cases; 8°C.

this finding. Fig. 10 shows our results on an axon that was not perfused internally. With 340K 100Na outside (Fig. 10 *a*) the instantaneous *I–V* curve is almost linear. The outward currents (Fig. 10 *b*) are much the same with 340K 100Cs outside, but inward currents are smaller, and there is negative conductance, as is evident in Fig. 10 *c* and *d*. Two traces are superimposed in these parts of the figure, and the corresponding potential traces are shown above them. After the first step in Fig. 10 *c*, *V* was brought back either to the holding potential (−60 mv), producing the continuous current trace, or to a more *positive* potential (−40 mv) which caused a more *negative* (inward) current. Negative conductance is also seen in Fig. 10 *d*, in which the second step

was more negative than the holding potential, and the current during the step more positive than at the holding potential.

From this experiment it is evident that external Na^+ at a concentration of 100 mM does not produce nonlinearity of the *I–V* curve. In other experiments external Na^+ at a concentration of 190 mM was found to have no effect on the

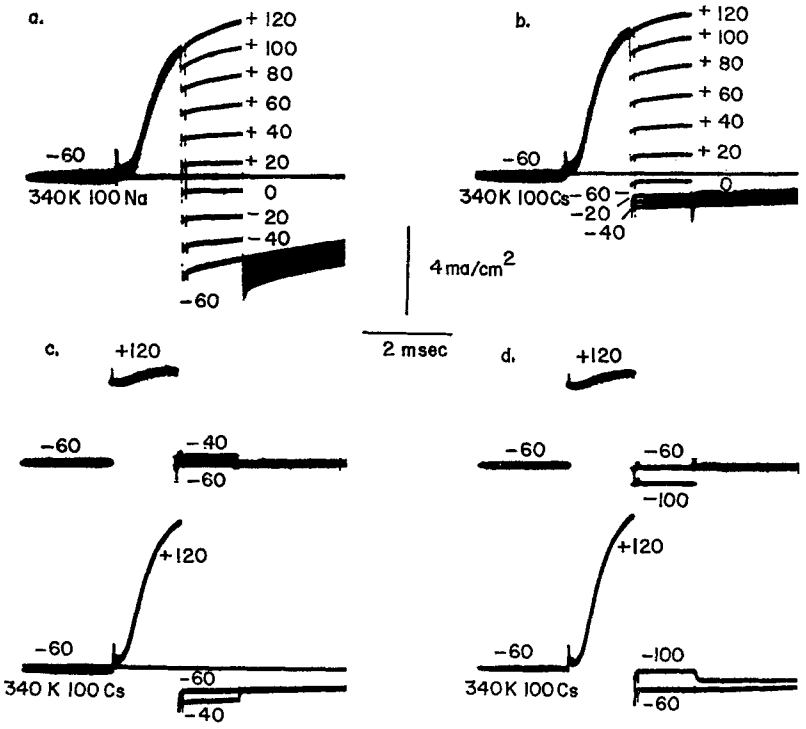

FIGURE 10. Negative conductance caused by external Cs. Records are from an unperfused axon in the external media indicated. In all records g_K was turned on by a step from −60 to +120 mv, and after 2 msec *V* was changed to the potentials shown. (*a*) In external 340K 100Na, the instantaneous *I–V* curve is almost linear. (*b*) External Cs reduces inward current without affecting outward current. (*c*) and (*d*) In addition to reducing inward K current, external Cs causes negative conductance. 8.5°C.

shape of this curve. Thus, although Cs^+ and Na^+ when inside affect outward current in a qualitatively similar way, their effects externally are quite different.

DISCUSSION

In terms of the Hodgkin-Huxley formulation (Hodgkin and Huxley, 1952), the potassium current is given by the formula

$$I_K = \bar{g}_K n^4 (V - V_K).$$

In this equation, n^4 is a time dependent gating factor, and V-V_K gives the driving force on the ion. $\bar{g}_K$ is a constant that expresses the total number of channels, the conductance of each, and the fact that the I–V plot of a single open channel is linear. One way of looking at our results is that the factor $\bar{g}_K$ has been replaced by a function that depends on V. Although mathematically this is a satisfactory proposal it is not easily identifiable with a physical picture of the action of Na^+ and Cs^+ ions on the channels. Another possibility is that n^4 is somehow altered by internal Na^+ or Cs^+. Since n^4 is a function that changes slowly with time, and we find that Na^+ and Cs^+ alter the instantaneous characteristics of the channel, it would be necessary to postulate that these ions in some way greatly speed up the kinetics of the n^4 process. Our results show that this cannot be the case, for the rise of I_K on depolarization, which is determined by n^4, has a normal time-course regardless of the presence of Na^+ or Cs^+. Likewise, the fall of I_K on repolarization, although not examined in detail, is not much altered.

A simple explanation that is consistent with the data is that Na^+ and Cs^+ ions can enter the channels and obstruct them, and that the fraction of the channels that are blocked in this way depends on membrane potential. To take account of this, the equation above can be altered to

$$I_K = y \cdot \bar{g}_K n^4 (V - V_K),$$

where y is the fraction of the channels that are not blocked by Na or Cs. For our purposes, y is an instantaneous function of V (or V-V_K), because its changes are too rapid to be resolved in our records. The other terms in the equation are not affected by the presence of Na^+ or Cs^+. To explain the shape of the instantaneous curve, y must decrease as V is made positive. For the isochronal curve, the decrease of y with V is offset by an increase of n^4, so there is saturation but little or no negative conductance.

In this scheme, y is determined by the rate that Cs^+ ions, for example, enter and leave the channels. We suppose that Cs^+ ions enter the channels at a rate proportional both to their axoplasmic concentration and to the rate of K^+ efflux through the open channels. That is, the Cs^+ entry rate varies with V in the same way as K^+ entry. Cs^+ ions can leave the channels either by passing on through or by returning to the axoplasm. Since Cs^+ does not permeate to an appreciable extent, we suppose that most of the Cs^+ ions that leave channels return to the axoplasm. Both negative conductance and the effects of external K^+ can be explained by saying that the majority of Cs^+ ions leaving the channels are knocked out of them by inmoving K^+ ions (see Armstrong, 1971). Clearing then slows as V is made positive, because K^+ influx decreases. Entry rate, on the other hand, increases with V, and y thus decreases with V, causing negative conductance. Raising the external K^+ concentration should help to clear the channels, and this is

observed experimentally. Na^+ behaves in qualitatively the same way as Cs^+. Rb^+, on the other hand, is relatively permeable, and does not obstruct the channels or cause negative conductance. In terms of our model, this is because most Rb^+ ions leave the channels by passing on through. Their leaving rate thus increases rather than decreases with V, just opposite to the requirements for negative conductance.

Li^+, Na^+, and Cs^+ thus might be said to have a TEA-like effect, but there are significant quantitative differences. TEA^+ and its derivatives (Armstrong, 1971) bind relatively firmly to their blocking sites and this has two consequences. First, the rate of dissociation from the blocking sites is slow, and can very easily be followed with our techniques. Second, a low concentration is sufficient to block virtually all of the channels in the steady state, and at these low concentrations the association of quaternary ammonium (QA) ions with blocking sites proceeds slowly. We postulate that Li^+, Na^+, and Cs^+ do not bind at all tightly in the channels, and it follows that a high concentration is necessary to depress the current measurably, and that the binding and dissociation rates are large.

These results with internal cations suggest that a K channel has a rather unspecific inner mouth that will accept many different cations, including Li^+, Na^+, Cs^+, Rb^+, TEA^+, and other QA ions. They further suggest that the remainder of the channel (the tunnel) is more specific, and that many of the ion species capable of entering the mouth do not pass readily through the tunnel. The mouth itself must be large enough to accept a TEA^+ ion, which has a diameter of about 8 A (or a bit more or less, depending on orientation). A cavity slightly larger than this would provide a good fit for a K^+, Rb^+, or Cs^+ ion with a full hydration shell. If hydrated diameters are estimated simply by adding twice the diameter of a water molecule (2×2.8 A) to twice the crystal radius, the diameters for K^+, Rb^+, and Cs^+ are 8.26, 8.54, and 8.94 A, respectively. NH_4^+, which also permeates (Binstock and Lecar, 1969), has the same crystal radius as Rb^+, and would therefore fit well with a complete hydration shell. Estimated in the same way, a hydrated Na^+ ion is 7.5 A. Possibly Na^+ enters the mouth with one complete hydration shell plus one or two additional water molecules. We conclude tentatively that the channel's inner mouth has a diameter of 8–9 A.

In our model, ions passing from the mouth to the tunnel must cross a second selectivity barrier that is probably more stringent than the first (entry into the mouth). It is tempting to think that the tunnel resembles the model proposed by Urry et al. (1971) for the gramicidin A transmembrane channel, and that passage through the tunnel requires dehydration of the ion. Selectivity for this step would then depend on the crystal diameter (twice the crystal radius) of the ion rather than its hydrated diameter. The most acceptable diameter by this supposition is approximately that of K^+,

2.66 A. NH_4^+ (2.96 A) and Rb^+ (2.96 A) are close enough to this figure to be relatively permeable, while Cs^+ (3.34 A) and QA ions (8 A for TEA^+) are above the limit of acceptability.

At first it seems paradoxical that Na^+ (1.90 A) should fail to permeate through a tunnel with a diameter of 2.66 A. This paradox by now is a familiar one, and its resolution is that the permeating ion must fit closely into the coordination cage provided by the tunnel, and it must be neither too large nor too small (Mullins, 1959). Figs. 11 *a* and *b* illustrate this schematically. Fig. 11 *a* shows a K^+ ion surrounded by four oxygen atoms (for convenience all are drawn in the same plane) which we first consider to be carbonyl oxygens (c.f. Hille, 1971 *b*) in the interior of the tunnel. The oxygens are fixed

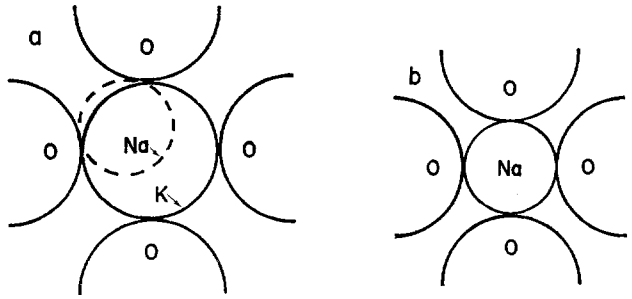

FIGURE 11. (*a*) Representation of a K^+ ion surrounded by four oxygen atoms of a postulated coordination cage in the narrow portion of a K^+ pore. For convenience, the oxygens are drawn as though all lie in the same plane. The same drawing can be used to represent a K^+ ion in water. The smaller circle shows an Na^+ ion in the cage, and it is distinctly a bad fit. (*b*) In water, oxygens can fit more closely around the Na^+ ion, and its potential energy is therefore lower than in the cage.

rigidly in positions that provide a good fit for a K^+ ion. Fig. 11 *a* could equally well represent the distribution of oxygens around a K^+ in water. Because the center-to-center distance from oxygen to K^+ in water and in the cage is the same, that portion of the coulombic energy of the K^+ ion which depends on interaction with the oxygens is the same in both situations. An Na^+ ion in the cage is represented by the smaller circle (dotted lines) in Fig. 11 *a*. The distance from Na^+ to two of the oxygens of the cage is greater than would be the case for Na^+ in water (Fig. 11 *b*), and the coulombic energy of an Na^+ ion in the cage is thus much higher than in water. Because of its relatively high energy in the cage, Na^+ would be unlikely to enter, but K^+ could enter readily. As pointed out in the Appendix, this mechanism is appropriate to selective ion permeation through a pore.

Our results are in generally good agreement with those of others regarding the effects of Cs^+ and Na^+ on I_K (Adelman and Senft, 1966, 1968; Adelman et al., 1971; Bergman, 1970). In an interesting series of experiments, Bergman

(1970) concluded that internal Na caused saturation of the late isochronal *I–V* curve for large depolarizations. He did not measure the instantaneous *I–V* curve for the K channel, but it can be inferred from our results that internal Na^+ causes negative conductance in this curve for large depolarizations in frog fibers as well as in squid. Bergman postulated that Na^+ ions enter and block the K channels, with which we fully agree, but we do not agree with his statement that block occurs only when the driving force on Na^+ ions is outward. The effect of internal Na^+ and internal Cs^+ are so similar that it is hard to believe the mechanism for the two ions is different, and in the Cs^+ experiments presented above the instantaneous *I–V* curve flattens and turns downward at potential well negative to V_{Na}. Also, the mechanism that we propose has the advantage that it can account for interference not only by Na, but by other cations as well, including TEA^+.

Moore (1967) has demonstrated an interesting negative conductance of unknown mechanism in the steady-state currents of myelinated fibers. The instantaneous *I–V* curve in his fibers is approximately linear, and the steady-state value of current after a potential step is approached with a time constant of roughly a millisecond. This is quite different from the phenomenon reported here, but it is similar in ways to the behavior of squid axons containing TEA^+ derivatives.

These results may shed light on an underlying cause of the heart arrhythmias seen in cases of digitalis intoxication. It is known that the internal Na^+ concentration in heart fibers is elevated in digitalis intoxication (Lee and Klaus, 1971). From the results presented here, it is clear that over a substantial range of voltages internal Na^+ ions reduce g_K in squid axons, and this may also be true for heart fibers. The potassium conductance helps to stabilize the membrane potential at a negative value, and a decrease of g_K would diminish this stabilizing influence, making arrhythmias more likely. Raising the external K^+ concentration helps to stop digitalis-induced arrhythmias, probably for two reasons. First, it clears obstructing Na^+ ions from K channels. And second, heart fiber membrane has the property of anomalous rectification, and thus g_K is probably increased by raising external K^+ (for a clear illustration of this in skeletal muscle, see Horowicz et al., 1968).

APPENDIX

It is possible to distinguish conceptually between two types of selectivity mechanism on the basis of kinetics. If the ions K^+ and Na^+ are competing for the same group of sites (S),

$$K^+ + S \underset{d_K}{\overset{a_K}{\rightleftharpoons}} KS$$

$$Na^+ + S \underset{d_{Na}}{\overset{a_{Na}}{\rightleftharpoons}} Na\ S$$

where a and d are association and dissociation rates, respectively, and the ratio of K^+ to Na^+ occupied sites is given by

$$\frac{[KS]}{[NaS]} = \frac{[K]a_K d_{Na}}{[Na^+]a_{Na}d_K}.$$

The site is K^+ selective if $a_K \cdot d_{Na}$ is larger than $a_{Na} \cdot d_K$. One limiting case for K^+ selectivity is that in which the association rate constants a_K and a_{Na} are the same for both ions, but the dissociation rates differ, with d_{Na} being greater than d_K. This might be called selectivity through selective binding. A potential energy diagram that corresponds to this case is shown in Fig. 12 *a*. The K^+ selectivity of nonactin is an example of this type of mechanism, as both Na^+ and K^+ apparently complex with nonactin at nearly the diffusion-limited rate, while K^+-nonactin dissociates more slowly than Na^+-nonactin (Winkler, 1969). Such a mechanism is suitable for a carrier but it is ill suited for selective permeation through a pore. In fact, if the pore contained

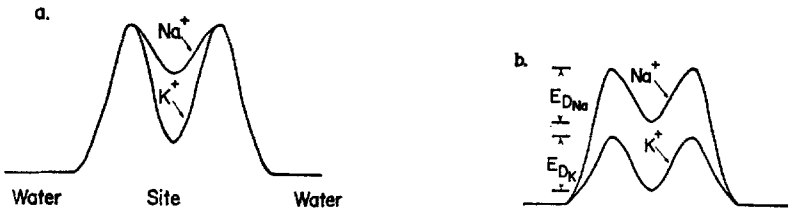

FIGURE 12. Free energy diagrams for two limiting cases of ion selectivity. In (*a*), the rate constants for Na^+ and K^+ ions entering the site from aqueous solution are the same, but the dissociation rate constants differ. K^+ is bound selectively. (*b*) The dissociation rate constants are equal ($E_{D_{Na}}$, the activation energy for dissociation of Na^+, equals E_{D_K}), but the association rate constants differ, since Na^+ faces a higher barrier when entering the site. Na^+ ion is thus selectively excluded.

a selectivity site which Na^+ and K^+ could enter with equal ease, but which bound K^+ selectively, movement of ions through the pore would be slower than in the absence of binding, but there would be no selectivity. Suppose that $a_K = a_{Na}$, and that $[Na^+] = [K^+]$ on both sides of a membrane containing many such pores. In these circumstances,

$$\frac{[KS]}{[NaS]} = \frac{d_{Na}}{d_K}.$$

The K^+ flux (J_K) through such a membrane from side 1 to side 2 would be

$$J_K = [KS] \cdot d_K \cdot q$$

and the Na^+ flux would be

$$J_{Na} = [NaS] \cdot d_{Na} \cdot q$$

where q is the fraction of the time that a K^+ ion (or Na^+ ion) in the site goes to side 2

after dissociating from the site. If binding to the site is the only factor which shows selectivity, then q is the same for Na and for K. Combining the three equations above then gives $J_K = J_{Na}$. In words, the slower dissociation of KS is just compensated by the greater K^+ occupancy of the sites. Thus, *selective binding is not an appropriate selectivity mechanism for a pore.*

The other limiting case is that in which the dissociation rates are equal and the association rates differ, with the association rate of the relatively permeant ion being greater. This might be called selectivity through *selective exclusion*, and an appropriate energy diagram is shown in Fig. 12 *b*. In this case,

$$\frac{[KS]}{[NaS]} = \frac{[K^+]a_K d_{Na}}{[Na^+]a_{Na}d_K}.$$

For $[Na^+] = [K^+]$,

$$\frac{[KS]}{[NaS]} = \frac{a_K}{a_{Na}}$$

and

$$\frac{J_K}{J_{Na}} = \frac{[KS]\cdot d_K \cdot q}{[NaS]\cdot d_{Na}\cdot q} = \frac{a_K}{a_{Na}}.$$

That is, when dissociation constants are equal, relative permeability is determined by the ratio of the association constants.

In summary, selective ion permeation through a pore requires a filter that operates by selective exclusion. The selectivity mechanism proposed in this paper operates in this way. The selectivity scheme of Eisenman (1962) provides for equilibrium selectivity, without specifying kinetic details. To the author, Eisenman's scheme seems to fit most closely in the selective binding category, and it is therefore not useful in explaining selective permeation through a pore.

The Appendix was written by Dr. Armstrong.

Dr. Bezanilla is a Rockefeller Foundation Fellow.

We are extremely grateful to Mrs. Lillian Peracchia for making the illustrations.
This work was supported by National Institutes of Health Grant No. NS 08951-04.

Received for publication 5 April 1972.

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