INTERNAL CESIUM AND THE SODIUM INACTIVATION GATE IN *MYXICOLA* GIANT AXONS

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ABSTRACT Internal cesium (Cs_i), relative to internal potassium (K_i), alters Na current (I_{Na}) time course in internally perfused *Myxicola* giant axons. Cs_i slows the time to peak I_{Na}, slows its decline from peak and increases the steady state to peak current ratio, $I_{Na\infty}/I_{Na peak}$. Neither activation nor deactivation kinetics are appreciably affected by Cs_i. Na current rising phases, times to half maximum and tail current time courses are similar in Cs_i and K_i. Inactivation time constants determined by both one (τ_h) and two (τ_c) pulses are also little changed by Cs_i. The Cs_i effects are due largely or entirely to an increased $I_{Na\infty}/I_{Na peak}$. Cs_i decreases the steady level of inactivation reached during a step in potential, preventing some fraction of inactivation gates from closing at all, the rest apparently closing normally. Inactivation block in Cs_i decreases with increasing inward current magnitude and in K_i inactivation block is appreciable only for outward Na channel current, suggesting the site of action is located somewhere in the current pathway. If this site mediates the normal operation of the inactivation gate, then a possible mechanism for gate closure could involve a positively charged structure moving to associate with a negative site near or into the inner channel mouth.

INTRODUCTION

There are a number of studies demonstrating that internal or external inorganic cations can alter the time course of membrane Na and K currents in ways other than can be accounted for solely by screening or neutralization of membrane surface fixed charges, which is expected to produce only a simple translation of kinetic parameters along the voltage axis (Frankenhaeuser and Hodgkin, 1957; Chandler et al., 1965; Gilbert and Ehrenstein, 1969; Hahin and Campbell, 1983). For example, in squid, Chandler and Meves (1970*b*) found a faster time constant of Na current inactivation (τ_h) with internal KF than with internal NaF. Swenson and Armstrong (1981) found that elevated external K or Rb produced a slowing of deactivation of the delayed conductance, as if the K activation gates closed less readily when the channel was occupied with an ion. And, for both the Na and K channels Gilly and Armstrong (1982*a*, *b*) found that external Zn slowed activation, but not deactivation, as if Zn stabilized the occupancy of the resting state(s).

Here the effects of internal cesium (Cs_i), as compared to internal potassium (K_i), on Na currents in *Myxicola* are described. The Cs_i effect was found to be fairly specific, and suggests a possible mechanism for the operation of the inactivation gate.

METHODS

All experiments were done on internally perfused giant axons of *Myxicola infundibulum*. Animals were obtained from the Laboratory of the Marine Biological Association of the United Kingdom (Citadel Hill, Plymouth, United Kingdom). Methods for preparing and electrically recording from the axons were as in Binstock and Goldman (1969). Normal artificial sea water (ASW) had the following composition: 440 mM Na, 10 mM Ca, 50 mM Mg, 560 mM Cl, 5 mM Tris (tris [hydroxymethyl] aminomethane; Sigma Chemical Co., St. Louis, MO), pH 8.0 ± 0.1. Temperature was 5 ± 0.5°C. All potentials are reported as absolute membrane potential (inside minus outside) and have been corrected for liquid junction potentials as described by Ebert and Goldman (1975).

Internal perfusion was established with the KCl-axoplasm dispersal method of Goldman and Kenyon (1979). Standard (K) internal perfusate had the following composition: 410 mM K, 50 mM F, 360 mM glutamate, 1 mM HEPES (*N*-2- hydroxyethylpiperazine *N'*-2-ethanesulfonic acid; Calbiochem-Behring, San Diego, CA), 4 mM EGTA (ethyleneglycol-bis-(β-amino-ethyl ether) *N*, *N'*-tetra acetic acid; Sigma Chemical Co.), 145 mM sucrose, pH 7.30 ± 0.05. Cs perfusate was identical except that K glutamate and KF were replaced with Cs glutamate and CsF, respectively, on an equivalent per equivalent basis. All internal perfusates had an osmotic pressure within 5% of that of ASW as determined by osmometer (osmette A; Precision Systems Inc., Sudbury, MA). Internal perfusates were freshly prepared every few days.

All voltage clamp observations were made using compensated feedback to reduce errors produced by the series resistance, R_s. The new, faster, voltage clamp used in these experiments also included an adjustable differentiating circuit in the R_s compensation loop to reduce the phase shift produced by R_s compensation and so improve the clamp settling time. In practice feedback compensation was first adjusted to the maximum possible without the clamp going into oscillation, to ~2/3 of the measured R_s (Goldman and Schauf, 1972), the differentiating circuit was then activated and R_s compensation increased somewhat further. This will leave ~1–3 Ωcm^2 of residual uncompensated R_s (Ebert and Goldman, 1976; Binstock et al., 1975). Clamp settling time was 20 μs. To reduce R_s errors further all observations were made in bathing media with the Na concentration reduced, by substitution with Tris, to 1/2 that in ASW. The largest net inward Na currents encountered in these experiments were ~0.65 mA/cm^2 for a maximum displacement in membrane potential produced by the R_s of 2, and generally well <1 mV.

Pulses sent to the voltage clamp were formed by a PDP 11/34 computer (Digital Equipment Corp., Maynard, MA). Membrane current records were lightly filtered (100 KHz) with a four pole Bessel dual electronic filter (model 4302; ITHACO Inc., Ithaca, NY), digitized at a

10 or 20 μs sampling interval with 12-bit accuracy using a Datel analog to digital converter (model ADC-EH12B3; Datel Systems Inc, Canton, MA), and stored on floppy disks for later analysis. Na currents were extracted by repeating, for each axon, the entire voltage clamp protocol in the presence of 1 μM tetradotoxin (TTX; Calbiochem-Behring Corp.) and subtracting the two sets of records with the aid of the PDP 11/34. In experiments where the same axon was exposed to both K and Cs perfusates, the TTX controls were repeated separately for each perfusate.

All experiments were done in the presence of 2 mM 3,4-diaminopyridine (Aldrich Chemical Co., Milwaukee, WI) in the external bathing medium to supress the K conductance (Kirsch and Narahashi, 1978). The 3,4-diaminopyridine solutions were made fresh each day and the pH carefully checked. In addition, for some experiments 40 mM tetraethylammonium Br (TEA; Eastman Kodak Co., Rochester, NY) was added to the internal perfusate. There were no differences in any of the results presented here with or without TEA present.

Holding potentials were always about −100 mV, a potential that produces an approximately maximal delay in the development of the delayed conductance (Goldman and Schauf, 1973). In all axons the membrane was held at the holding potential for 2 min before the start of each voltage clamp run, and 15 s were allowed between each voltage clamp pulse to minimize any effects of slow inactivation (Rudy, 1981). τ_c determinations were done as described by Goldman and Kenyon (1982), and always included a 5-ms gap between conditioning and test pulses. This was sufficient to allow Na tail currents to decay to zero well before the start of the test pulse (Goldman and Kenyon, 1982; see also Figs. 1 *C* and 8, *bottom* trace).

RESULTS

Cs_i and Na Current Time Course

The effects of Cs_i, as compared to K_i, on the Na current time course are illustrated by the records of Fig. 1. Each part of Fig. 1 presents a pair of Na current records corrected for leak and capacitative currents as described in Methods. Both records of each pair are taken from the same axon at the same or nearly the same potential. One record of each pair was obtained in K perfusate (Figs. 1 *A* and *B*, *left* traces, Fig. 1 *C*, *upper* trace) and the other in Cs perfusate (Figs. 1 *A* and *B*, *right* traces; Fig. 1 *C*, *lower* trace). Records in Cs_i have all been scaled up so that their peak currents match those of the corresponding records in K_i.

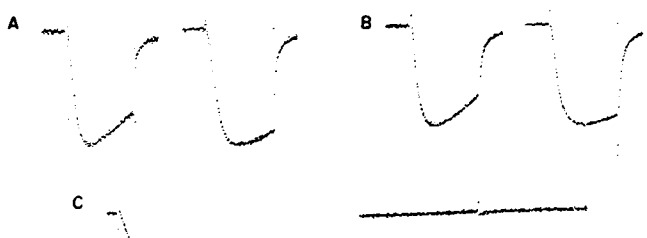

FIGURE 1 Na current records from internally perfused *Myxicola* giant axons. (*A*) Records in K (*left*) and Cs (*right*) internal perfusate, both at 8 mV. The Cs record has been scaled up by a factor of 1.22. Axon 84MP27. (*B*) Records in K (*left*) and Cs (*right*) perfusate at 11 mV (K perfusate) and 8.5 mV (Cs perfusate). Cs record scaled by 1.09. Axon 84MP28. (*C*) *Left* traces are the first 10 ms and the right the last 5 ms of the current records during a 30-ms step in potential. *Top* traces in K perfusate, at −34 mV, and the *bottom* in Cs at −36.5 mV. Cs record scaled by 1.96. Same axon as for *B*. 1/2 Na ASW and 2 mM 3,4-diaminopyridine throughout. 5°C. Scale: 0.30 mA/cm², 2 ms.

Cs_i produces three effects. First, Na currents rise to a peak more slowly in Cs_i as compared to K_i. This is seen best at more positive potentials where the kinetics are rapid. In Fig. 1 *A* (both records at 8 mV) peak Na current is reached at 0.85 ms in K_i but not until 1.40 ms in Cs_i. A similar effect on another axon is shown by the records of Fig. 1 *B*. Here Na current peaks at 1.00 ms in K_i, at 11 mV, and at 1.55 ms in Cs_i, at a nearly identical potential of 8.5 mV. This effect is less evident at more negative potentials (Fig. 1 *C*) owing to the flatness of the records. Second, Na currents decline from their peak value more slowly in Cs_i. This is seen in each of the pairs of records of Fig. 1.

The third effect of Cs_i is shown in Fig. 1 *C* (same axon as for Fig. 1 *B*). The *left-hand* part of this figure shows the first 10 ms and the *right-hand* part the last 5 ms of the currents during a 30-ms step in potential. Potential was −34 mV for the K_i determination and −36.5 mV for that in Cs_i. For both perfusates there is still inward Na current at the end of the 30-ms pulse, but that in K_i is very much smaller than that recorded in Cs_i. The current at 30 ms relative to that at peak, I_{Na30ms}/I_{Napeak}, is 0.308 in Cs_i and only 0.039 in K_i.

Analysis of these Cs_i effects on the Na current time course is presented in the following three sections.

Cs_i and Na Activation Kinetics

Cs_i effects are evident very early in the current records of Fig. 1. Na current time course clearly differs in Cs_i as compared to K_i well before 1 ms, at times short relative to that needed for any appreciable activation of the K conductance. However, Na activation kinetics seem to be little affected by Cs_i.

Time-to-peak Na current can be slowed just by a decrease in the rate or extent of inactivation even when activation kinetics are unchanged. Cs_i does slow the time to half maximum current, $t_{1/2}$, less than it does the time to peak. For example, in the experiment of Fig. 1 *A* the ratio of times to peak in Cs_i as compared to K_i is 1.65, while the ratio of $t_{1/2}$'s is only 1.24. That Cs_i has little effect on activation is suggested by the results of Fig. 2. The *left-hand* panel of Fig. 2 *A* presents a pair of superimposed Na current records from the same axon at −29 mV in K_i and −31.5 mV in Cs_i. The records are presented on an expanded time scale, and that for Cs_i has been scaled up so that a comparison of their time courses can be made. In K_i (*upper* trace) current has reached peak and has even begun to decline by the end of the trace, while in Cs_i it is still rising by the end of the trace, again showing the increased time to peak in Cs_i. However, the curving rising phases superimpose, suggesting little effect on activation. The *right* panel presents a simulation of this experiment using Hodgkin-Huxley (1952) kinetics as an empirical descrip-

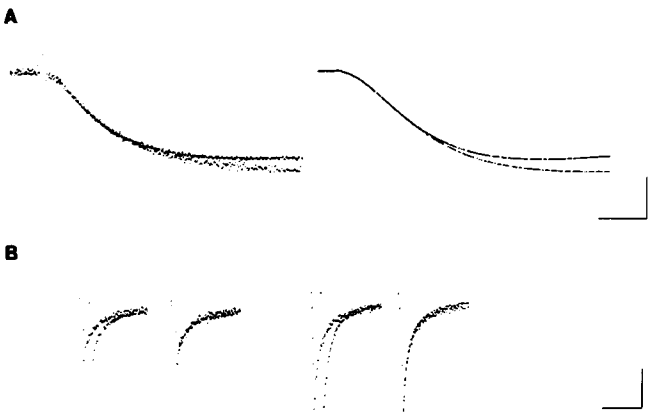

FIGURE 2 (*A*) *Left* panel: Superimposed Na current records from an axon perfused with K (*upper* trace) and Cs (*lower* trace) perfusate at −29 mV (K) and −31.5 mV (Cs). Cs record scaled by 3.15. Axon 84MP28. *Right* panel: Simulation of this experiment using Hodgkin-Huxley kinetics. τ_m is 0.564 and τ_h 9.744 ms for both simulated records. *Lower* trace has an increased h_∞ and has been scaled by the same factor as for the experimental records. Scale: 0.30 mA/cm², 0.5 ms. (*B*) *Left* two panels: Na tail currents at −102 mV after the two potential steps of Fig. 1 *A*. Far *left* panel shows the tail currents in K_i (*left*) and in Cs_i (*right*) displaced by 250 μs so that their time courses may be compared. Second panel presents the traces superimposed. Record in K_i scaled by 1.025. The two *right* panels present the tail currents at −99 mV (K_i) and −101.5 mV (Cs_i) after the two potential steps of Fig. 1 *B*. Again, the third panel shows the record in Cs_i displaced 250 μs to the right while they are superimposed in the far *right* panel. Record in K_i scaled by 1.21. Scale: 0.15 mA/cm², 1 ms.

tion of the currents. The simulation reproduces the essential features of the experimental records, the *lower* trace showing a slowed time to peak while the rising phases superimpose. However, both simulated records were computed with the identical activation time constant, τ_m, and differ only in that the *lower* trace inactivates less extensively.

Deactivation also seems to be little affected by Cs_i. Fig. 2 *B* shows again, now at higher gain and on expanded time scale, the tail currents following each of the two steps in potential of Fig. 1 *A* (*left* three traces) and also those following the two steps in potential of Fig. 1 *B* (*right* three traces). Tail currents in K_i have been scaled up so that their amplitudes match those for the corresponding currents in Cs_i. Tail currents for both experiments are presented first with the records in Cs_i displaced from these in K_i 250 μs to the right along the time axis (first and third panels) and then superimposed (second and fourth panels). Na tail current time courses are indistinguishable in the two perfusates.

Fig. 3 presents the pooled results on Na activation (89 determinations from 18 axons). $t_{1/2}$ is shown as a function of membrane potential both in K_i (filled circles) and Cs_i (open circles). $t_{1/2}$ (V) is not appreciably different in the two perfusates. Inactivation delay, which is produced by the activation process (Goldman and Kenyon, 1982), is also similar in K_i and Cs_i (not illustrated). When $t_{1/2}$ values are compared in the two perfusates over a range of potentials in the same axon those in Cs_i are somewhat larger as expected from the increased times to peak. The effect is small and not clearly distinguishable in the pooled data of Fig. 3. The effect of Cs_i on $t_{1/2}$, then, is small relative to the variance between axons, and overall Cs_i seems to have little effect on Na activation.

Cs_i and Na Inactivation Kinetics

Na currents decline more slowly from their peak value in Cs_i than in K_i. The overall rate of decline in the Na current is determined both by the time constant of inactivation, τ_h, and by the steady level of inactivation reached during the potential step. The decline can be slowed, then, either by reducing the rate constant governing the transition from the conducting to the inactivated state or by reducing the fraction of channels that make this transition. Cs_i seems to have little effect on inactivation time constants, both as determined with one-pulse (τ_h) and two-pulse (τ_c) methods.

Fig. 4 presents two τ_c determinations from the same axon in K_i (*upper* curve, conditioning potential of −23 mV) and in Cs_i (*lower* curve, conditioning potential of −19 mV). τ_c was 12.45 ms in K_i and 10.05 ms at a similar potential in Cs_i. For this same experiment τ_h was 11.46 ms

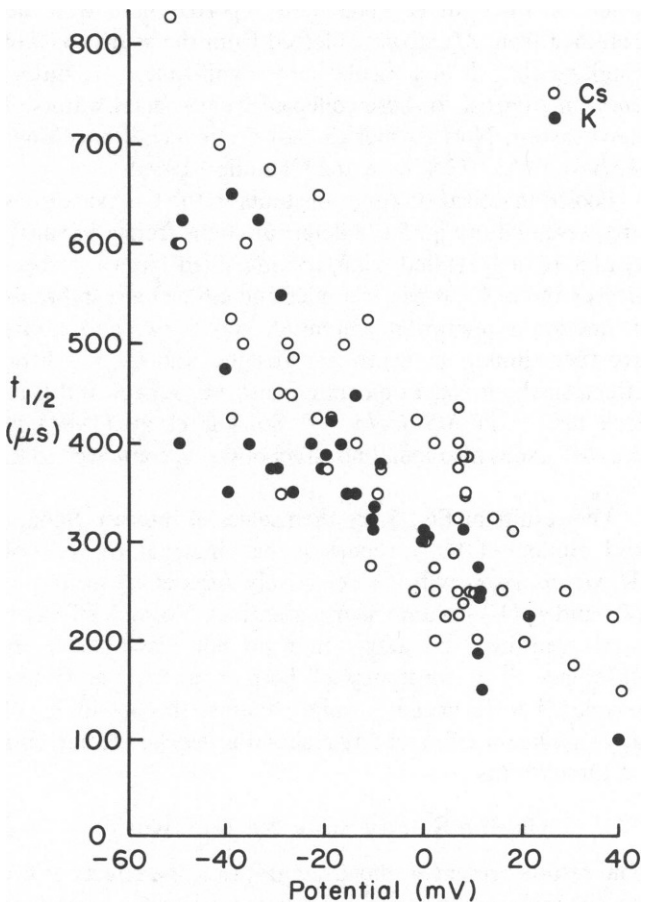

FIGURE 3 Time to half maximum Na current, $t_{1/2}$, as a function of membrane potential in K_i (filled circles) and in Cs_i (open circles).

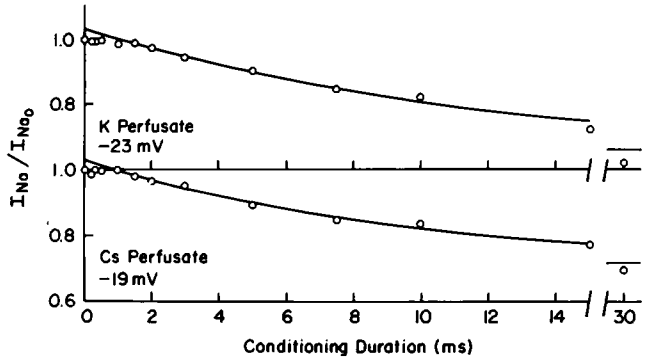

FIGURE 4 Time course of inactivation development. Peak I_{Na} during 12 mV (*top*, K_i) and 6 mV (*bottom*, Cs_i) test pulses are shown as functions of the duration of −23 mV (*top*) and −19 mV (*bottom*) conditioning pulses. Solid curves are simple exponentials with time constants (τ_c) of 12.45 ms (*top*) and 10.05 ms (*bottom*). Holding potentials −98 mV (*top*) and −104 mV (*bottom*). 5-ms gap. 1/2 Na ASW and 2 mM 3,4-diaminopyridine throughout. Axon 84MP26. 5°C.

in K_i and 14.54 ms in Cs_i, indicating little effect of Cs_i on inactivation kinetics. These small effects of Cs_i as compared to K_i contrast sharply with the findings of Chandler and Meves (1970*b*) in squid of up to a twofold slowing of τ_h when K_i is replaced by Na_i.

Note that in this experiment τ_c and τ_h are similar even when recorded in K_i. The data reported here were all obtained from *Myxicola* collected from the south coast of England that do not display any significant τ_c–τ_h difference, in contrast to those collected from coastal waters of northeastern North America that do under all conditions we have tested (Goldman and Chandler, 1986).

Pooled inactivation time constants in the two perfusates are presented in Fig. 5 (78 determinations from 18 axons). τ_c and τ_h in K_i (filled triangles and filled circles, respectively) and in Cs_i (open triangles and circles) are shown as a function of membrane potential. Both τ_c (V) and τ_h (V) are very similar in the two perfusates, and Cs_i has little effect on the inactivation time constant. Schauf and Bullock (1978) in *Myxicola* and Starkus et al. (1984) in crayfish axons also found no effect of Cs_i as compared to K_i on τ_h.

The results of Fig. 5 are themselves of interest. Schauf and Bullock (1978) reported that internal dialysis of *Myxicola* axons with Cs selectively speeded τ_c so that τ_c (V) and τ_h (V) became nearly identical. No such effect on τ_c is seen here on axons that do not display a τ_c–τ_h difference. The constancy of both τ_c and τ_h in Cs_i as compared to K_i argues strongly against the possibility of any significant effect of any residual unblocked K currents on these results.

Cs_i and Steady State Na Inactivation

The results presented above suggest that the effects of Cs_i on the Na current time course seen in Fig. 1 may be attributed largely to a reduction in the steady level of inactivation reached during a potential step. Steady state

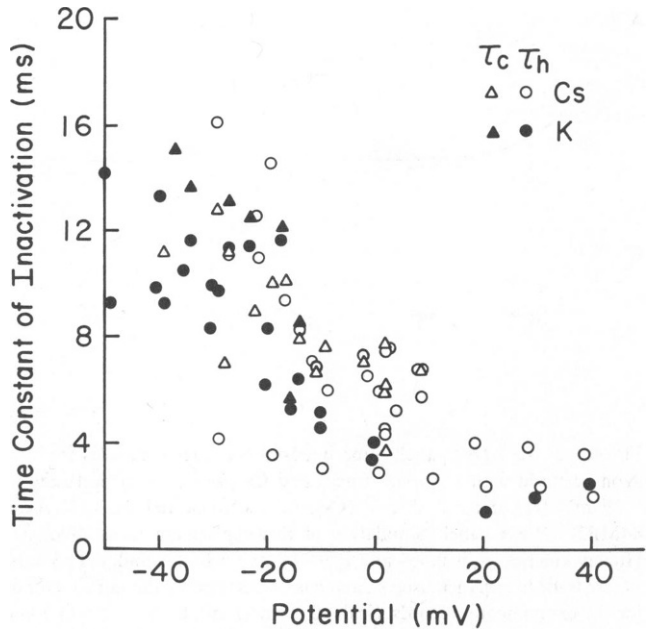

FIGURE 5 Inactivation time constants as determined by one pulse (τ_h, circles) and two pulse (τ_c, triangles) methods in K_i (filled symbols) and Cs_i (open symbols) as a function of membrane potential.

inward Na current, relative to that at peak, $I_{Na_\infty}/I_{Na_{peak}}$, both in K_i (filled circles) and in Cs_i (open circles) is shown as a function of membrane potential in Fig. 6 (57 determinations from 17 axons). I_{Na_∞} values were obtained by fitting inactivation time constants to single pulse current records or in a few cases at large positive potentials where

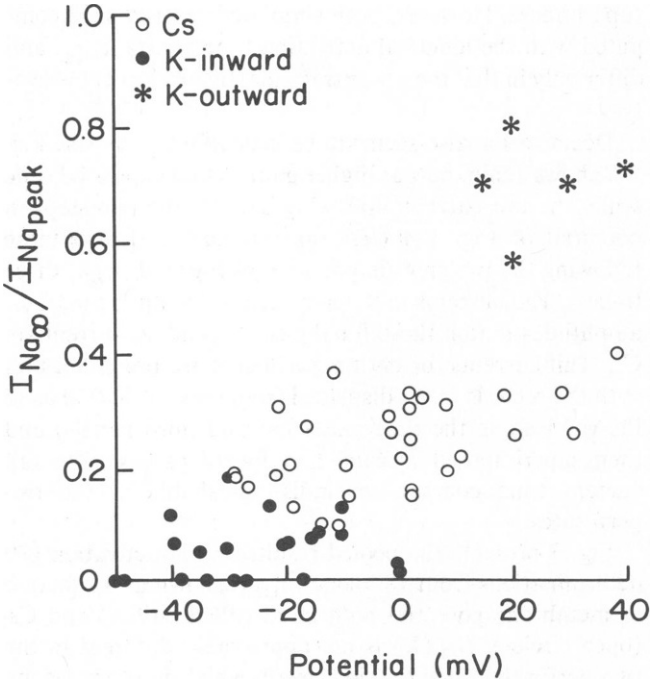

FIGURE 6 Steady state to peak Na channel current ratio, $I_{Na_\infty}/I_{Na_{peak}}$, as a function of membrane potential in Cs_i (open circles) and in K_i (filled circles for inward and stars for outward currents).

τ_h is rapid read directly from the record. For all inward currents $I_{Na_\infty}/I_{Na_{peak}}$ is larger in Cs_i than in K_i. Cs_i increases the steady state value of the Na current and therefore decreases the steady state level of inactivation during a step in potential. A similar effect is suggested in the records of Schauf and Bullock (1978). As Cs_i has little effect on the inactivation time constant, these results suggest that at the level of a single channel inactivation is blocked in an all or none manner. Some inactivation gates fail to close at all, the rest apparently closing normally.

$I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i increases somewhat with increasing membrane potential, especially at positive potentials (Fig. 6). This is consistent with a weak potential dependency of the Cs_i-induced blockade of inactivation. An alternative possibility is that inactivation is blocked more effectively when the inward current magnitude through the Na channel is less. That this second possibility is at least partly the case is suggested by the following two observations.

Fig. 7 (open circles) shows the peak Na current voltage relation in an axon perfused with Cs. Plotted on the same axes (x's) are the steady state inactivation values from this experiment. The x's are the reciprocal of the $I_{Na_\infty}/I_{Na_{peak}}$ values normalized to that for the maximum inward current (at -1.5 mV in this experiment) and plotted on an inverted scale for comparison with the peak currents. Note that peak inward current values, now normalized to the maximum value at -1.5 mV, can also be read directly from the right-hand ordinate of Fig. 7. For the positive limb of the current voltage relation peak inward current density and steady state inactivation decrease in a parallel way, as if inward Na current displaces Cs and so relieves the block of inactivation. If this were the only factor, then steady state inactivation should increase for potentials negative to that at which the maximum current is seen, as current through a single channel should increase at more negative potentials. The decreased steady state inactivation at -11.5 mV as compared to that at -1.5 mV is consistent with some voltage dependency of steady state inactivation at negative potentials. $I_{Na_\infty}/I_{Na_{peak}}$ values could not be reliably determined for currents at more negative potentials in this experiment owing to the flatness of the records. This finding, that the degree of inactivation block is affected by the inward Na current magnitude, is supported by a comparison of the ratio of steady state to peak current values for inward vs. outward Na channel currents in K perfused axons.

Fig. 8 presents two current records from the same axon, in K_i. The *bottom* trace shows the first 10 ms (*left*) and the last 5 ms (*right*) of the inward Na current during a 30-ms pulse at a potential of -15.5 mV. Note the change in time scale for the last 5 ms of the record. Steady state current is reached by the end of the 30-ms pulse at -15.5 mV. $I_{Na_\infty}/I_{Na_{peak}}$ determined from the τ_h value (5.31 ms) fitted to the current decay was 0.081, in good agreement with the directly measured value of 0.077. The *upper* trace shows outward K current through the Na channel at a potential of 29.5 mV. τ_h is 2.06 ms and steady state current is clearly reached by the end of the 10-ms pulse at 29.5 mV, yielding a steady state to peak current ratio of 0.696.

Collected values of steady state to peak current ratios for outward currents in K perfused axons are shown as the stars in Fig. 6. Steady state inactivation is strongly supressed in K_i, but only in the absence of inward Na current. Again, as if inward current displaces, now K, and so relieves the block of inactivation. The clear difference in the steady state to peak current ratios for inward as compared to outward currents in K_i, and the change in steady state inactivation with inward current magnitude in Cs_i are both consistent with a location for the site of action of these ions somewhere in the current pathway.

DISCUSSION

Summary of Results

The central finding here is that Cs_i decreases steady state Na inactivation in a fairly selective way with little effect on either activation or inactivation kinetics. In the presence of Cs_i, then, an increased fraction of Na inactivation gates fail to close while the rest close in about a normal way. In fact, the simulated records of Fig. 2 *A* were both computed with

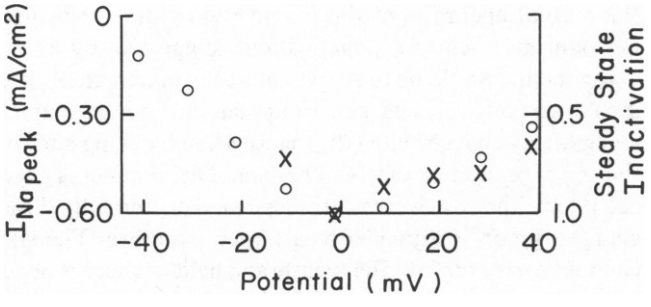

FIGURE 7 Comparison of peak I_{Na} current voltage relation (circles) and steady state inactivation (x's) in an axon perfused with Cs. Steady state inactivation determined as the reciprocal of $I_{Na_\infty}/I_{Na_{peak}}$, normalized to the value for the maximum inward current and plotted on an inverted scale for comparison with peak current values. Holding potential -101.5 mV. 1/2 Na ASW, 2 mM 3,4-diaminopyridine and 5°C. Axon 84MP28.

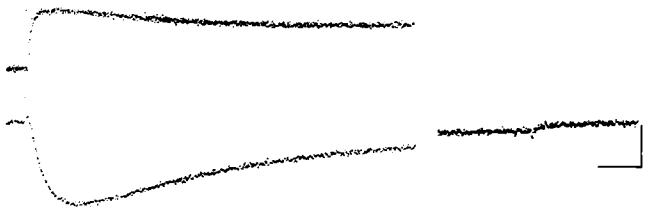

FIGURE 8 Na channel current records from an axon perfused with K. Holding potential -100.5 mV. Test potential 29.5 mV (*top*) and -15.5 mV (*bottom*). *Bottom* traces show the first 10 (*left*) and the last 5 ms of the current records during a 30-ms pulse. Current scale is 0.15 mA/cm². Time scale is 1 ms for the *top* and *left bottom* traces and 2 ms for the *right bottom* trace. 1/2 Na ASW, 2 mM 3,4-diaminopyridine externally and 40 mM TEA, internally. Temperature was 5°C. Axon 84MP31. $I_{Na_\infty}/I_{Na_{peak}}$ is 0.081 for the inward current record, and 0.696 for the outward.

an identical τ_m and τ_h and differ only in that steady state inactivation has been reduced for the *lower* trace. The site of action of Cs is located on the axoplasmic side of the membrane as Cs is not permeant through the Na channel in *Myxicola* (Ebert and Goldman, 1976).

K_i also strongly blocks Na inactivation, in the absence of inward Na channel current. The site of action of these ions may have a lower affinity for K as inward Na current appears to displace Cs less readily than it does K. Inactivation block is affected both by Na current magnitude (in Cs_i) and direction (in K_i) suggesting a location for this site in the current pathway, possibly into or near the inner mouth of the Na channel.

In principle, for those channels in which inactivation is blocked, activation kinetics should be altered to some degree as activation time constants are functions of all the rate constants, including those governing inactivation, of the kinetic process. In practice no effect on activation is detectable. This is not a surprising result as inactivation can even be suppressed entirely, with e.g., pronase (Armstrong et al., 1973) or *n*-bromoacetamide (Oxford et al., 1978), without detectable effects on activation kinetics. This is because the weight of any given rate constant in a kinetic scheme can be substantial in some and negligible in other of the various time constants of the process.

To illustrate this point consider a simple three state rest (R), conducting (C), inactivated (I) scheme with rate constants that are functions of membrane potential only. For an instantaneously rising step in potential, the time course of occupany of the C state is given by the sum of two exponentials and a steady state term. Goldman (1976) presented expressions for the time constants, steady state value, and initial conditions dependency for a fully generalized (i.e., 6-rate constant) three state scheme in terms of its elementary rate constants, and Goldman and Hahin (1979) presented analytical methods for determining the rate constants from experimental observations for such fully generalized schemes. Selecting values of 2.5, 0.286, 0.1275, and 0.0665 ms^{-1} for the rate constants k_{RC}, k_{CR}, k_{CI}, and k_{IC}, respectively, time constants of 0.357 and 5.555 ms are computed. Decreasing the rate constant for the transition from the conducting to the inactivated state, k_{CI}, 10-fold, increases the slower (inactivation) time constant to 12.837 ms, while the activation time constant is nearly unchanged (0.359 ms). Even with coupled activation and inactivation, inactivation may be slowed substantially with little effect on activation kinetics, and there is, then, no discrepancy between the data of Figs. 3 and 6.

Comparison with Other Work

Chandler and Meves (1970*a*) found large steady state Na currents in squid axons internally perfused with 300 mM NaF, and similar results were reported by Oxford and Yeh (1985) on replacing Cs_i with Na_i. However, the effects of Na_i differ in two respects from these reported here. First, the site of action of Na_i may not be in the current pathway. Chandler and Meves (1970*a*) found that $I_{Na_\infty}/I_{Na_{peak}}$ increased smoothly over the whole potential range examined, showing no sharp break at the reversal potential and providing, then no evidence for a current direction dependent effect. Recently, Oxford and Yeh (1985) have presented clear evidence that the increased $I_{Na_\infty}/I_{Na_{peak}}$ seen with elevated Na_i in squid does not, in fact, depend on current direction. Schauf and Bullock (1979) also found no break in the $I_{Na_\infty}/I_{Na_{peak}}$ curve at the reversal potential in high internal Na in dialyzed *Myxicola* axons. Second, substitution of Na_i for either K_i (Chandler and Meves, 1970*b*) or Cs_i (Oxford and Yeh, 1985) slowed τ_h as well as increasing I_{Na_∞}, although in neither case was activation appreciably affected. A simple explanation for the difference in effect on τ_h might be that inactivation gate closure can, with difficulty, displace Na from some of its sites. This would require more work to close the gate and so decrease the rate constant for the conducting to inactivated state transition. For the Cs site this apparently does not occur, inactivation gate closure having little effectiveness in displacing Cs, possibly owing to some steric hinderance.

In squid (Chandler and Meves, 1970*a*; Oxford and Yeh, 1979; Oxford and Yeh, 1985) $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i is the same as that recorded in K_i. There may still be an inactivation blocking site in squid, as the steady state inactivation curve is not monotonic in Cs_i (Oxford and Yeh, 1985), which, then, discriminates little between Cs and K. This difference in Na channel properties in squid and *Myxicola* axons stands in sharp contrast to the very similar properties of the Na channel selectivity filter in these two preparations. The Na to K selectivity ratio, P_{Na}/P_K, is an essentially identical function of K_i in squid and *Myxicola* over the whole (100–500 mM) concentration range studied (Cahalan and Begenisich, 1976; Ebert and Goldman, 1976).

A Possible Mechanism For Inactivation

There is a site near or into the inner mouth of the Na channel which, if occupied by an ion, can block closure of the inactivation gate. This site is ideally located to mediate the normal operation of the inactivation gate. A possible mechanism, then, for inactivation, suggested by these experiments, would be to suppose that the inactivation gate includes a positively charged group that on closing occupies a negatively charged site (the inactivation blocking site) in the inner portion of the Na channel. The channel is then occluded either by electrostatic repulsion or sterically if the charged group is attached to a larger structure. The site cannot be very far into the membrane field as there is not a steep potential dependency of the Cs_i block of steady state inactivation. An inner membrane location for this negatively charged site is consistent with an internal site of action for pharmacological blockers of inactivation (Armstrong et al., 1973; Oxford et al., 1978).

Essentially similar mechanisms for the operation of the inactivation gate have been proposed several times. Armstrong and Bezanilla (1977) pictured the inactivation gate as a tethered, positively charged particle that, on closure, moved to associate with a negatively charged membrane site. The negatively charged site is located only little into the membrane field, as no gating current relaxation with kinetics appropriate for the inactivation process was detected in squid (however, see Swenson, 1980 on crayfish axons). Schauf (1983), based on the ability of internal tetramethylammonium (TMA) ions to reduce steady state Na inactivation, suggested that there might be a site located near the internal channel mouth that must be free for inactivation to proceed. Recently, Oxford and Yeh (1985) again based on block of steady state inactivation with internal TMA suggested that there might be a positively charged natural inactivation particle that on closure associated with a site located at a shallow depth into the inner mouth of the channel.

Certainly other interpretations of the results presented here are possible. Specifically, the inactivation blocking site may not itself be part of the gate, but its occupancy might cause inactivation block secondarily by inducing some conformational change in the channel protein structure, or directly by either sterically or electrostatically impeding gate closure. Such two site models can never be excluded in the absence of detailed information as to the channel structure.

Independently of whether the inactivation blocking site is a component of the normal gating machinery or not, it is clear that if some fraction of resting channels have this site occupied, by e.g., K, then these channels inherently have inactivation coupled to activation in that a conducting channel is necessary to clear the sites and so permit the inactivation gates to close. The nature of the coupling between activation and inactivation certainly involves more just this effect, however, as immobilization of the gating charge (Armstrong and Bezanilla, 1977; Meves and Vogel, 1977; Neumcke et al., 1976; Nonner et al., 1978; Nonner, 1980; Bullock and Schauf, 1979; Swenson, 1980; Starkus et al., 1981) establishes that the operation of the activation gates is itself dependent on the inactivation state of the channel, and because channels can also inactivate without conducting (Gillespie and Meves, 1980; Bean, 1981; Horn et al., 1981; Aldrich et al., 1983; Horn and Vandenberg, 1984).

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