INTERNAL CATIONS, MEMBRANE CURRENT, AND SODIUM INACTIVATION GATE CLOSURE IN *MYXICOLA* GIANT AXONS

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ABSTRACT Steady state to peak Na current ratio ($I_{Na_\infty}/I_{Na_{peak}}$) in *Myxicola* is greater, under some conditions, in internal Cs than in *K*, indicating less steady state inactivation in Cs_i. Cs_i effects are selective for steady state inactivation, with negligible effects on single-pulse inactivation time constants (τ_h). Mean τ_h ratios (Cs_i to K_i) were 1.04 and 1.02 at 0 and 10 mV. Two pulse inactivation time constants were also little affected. Inactivation is blocked in an all or none manner. K_i has little effect on steady state inactivation in the presence of inward I_{Na}, with $I_{Na_\infty}/I_{Na_{peak}}$ often declining to zero at positive potentials and independent of external Na concentration from ¼ to ⅔ artificial sea water (ASW). Cs also has little effect at more negative potentials, but more with either more positive potentials or Na reduction, both reducing inward I_{Na}. *K* effects are evident when Na channel current is outward. A site in the current pathway when occupied selectively blocks inactivation gate closure. As occupancy does not depend significantly on potential, the site must not be very deep into the membrane field. Inactivation gates may associate with these sites on closure. The inactivated state may consist of a positively-charged structure occluding the inner channel mouth.

INTRODUCTION

Recently an effect was identified in Cs glutamate perfused *Myxicola* giant axons (Goldman, 1986). Na currents recorded in the presence of internal Cs (Cs_i) display a larger steady state to peak current ratio ($I_{Na_\infty}/I_{Na_{peak}}$) than do those recorded under *K* glutamate perfusion, i.e., in Cs_i less steady state inactivation develops during a step in potential. This effect of Cs_i may be a selective one. In observations pooled from a number of axons, Cs_i had no other obvious effects on inactivation, although a definitive answer was not possible from these preliminary studies.

Steady state inactivation in Cs_i decreases further with more positive steps in potential, where the inward I_{Na} magnitude will also decrease. And, steady state inactivation in K_i decreases on moving from potentials where Na channel current is inward to those where it is outward. Both these observations could be accounted for if the inactivation blocking site were located in the current pathway and inward I_{Na} displaced both *K* and (less effectively) Cs from this site. However, direct evidence as to the location of the site was not provided by the preliminary studies.

A site in the current pathway which, when occupied by a cation, selectively blocks inactivation gate closure is very likely to be a site that the inactivation gate normally associates with or latches to on closure. Moreover, the Cs binding site may be in or near the internal (axoplasmic) channel mouth, consistent with the site of action of some pharmacological blockers of inactivation (e.g., Armstrong et al., 1973; Oxford et al., 1978), as Cs is not measurably permeant through the Na channel in *Myxicola* (Ebert and Goldman, 1976). Hence, the inactivation gate latch may be experimentally accessible owing to a higher affinity for Cs over *K*, and it is of interest, then, to characterize its properties.

Experiments reported here were done using paired observations on the same axons. The preliminary conclusions (Goldman, 1986) that the effects of Cs_i on the Na current time course might be accounted for simply by an increase in the fraction of inactivation gates that do not close, with the rest closing in a normal way, and that the Cs site is located in the current pathway are here established in an unambiguous way. Both *K* and Cs occupancy of the inactivation blocking site are strongly dependent on the Na channel current magnitude with apparently little direct effect of membrane potential. The inactivated state may consist of a positively charged structure occluding the inner channel mouth, as earlier proposed by Armstrong and Bezanilla (1977; see also Oxford and Yeh, 1985)

METHODS

Preparation and Electrical Recording

Myxicola infundibulum were obtained either from R. Bosien, Cummings Cove, Deer Island, New Brunswick, Canada, or from the Station d'Oceanologie et de Biologie Marine, Roscoff, France. No significant differences in these experiments were found between animals from the two sources. Methods for preparing and electrically recording from the giant axons were as described by 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. 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

Internal perfusion was initiated 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-ethane sulfonic acid; Calbiochem-Behring Corp., 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 equivalent per equivalent with Cs glutamate and CsF. Osmotic pressure was checked and compared with that of ASW (osmette *A*; Precision Systems Inc., Natick, MA). Internal perfusates were freshly prepared at one to two week intervals.

Data Acquisition

All voltage clamp observations were made using compensated feedback to reduce errors produced by the series resistance, R_s, as described by Goldman (1986). To reduce R_s errors further, observations were made in bathing media with the Na concentration reduced by substitution with Tris to either ⅔, ½, or ¼ of that in ASW. With the current densities encountered in these experiments displacements in membrane potential produced by any residual, uncompensated R_s will generally be well <1 mV, and in no case >2 mV (see details in Goldman, 1986).

Pulses sent to the voltage clamp were formed by a PDP 11/34 computer (Digital Equipment Corp., Marlboro, MA). Membrane currents were filtered occasionally at 50 but generally at 25 KHz with a four pole Bessel filter (model 4302; ITHACO Inc., Ithaca, NY), digitized at a 20 or 25 μs sampling interval with a 12-bit analog to digital converter (model ADC-EH12B3; Datel Systems Inc., Canton, MA) and stored on hard disks for later analysis.

Extraction of I_{Na}

For all experiments Na currents were extracted by repeating the entire voltage clamp protocol in either 1 or 2 μM tetrodotoxin (TTX; Calbiochem-Behring Corp.) and subtracting the two sets of records with the aid of the PDP-11/34. In addition, for all $I_{Na_\infty}/I_{Na_{peak}}$ and single-pulse inactivation time constant determinations linear leak and capacitative current components for both normal and TTX control currents were first subtracted by summing each depolarizing step in potential with four hyperpolarizing steps, each ¼ of the depolarizing step (divided pulse procedure), and then proceeding with TTX subtraction. Divided pulses were used as all holding potentials were at −100 mV. However, each Na current record is now the sum of five individual current traces from which the sum of five additional current traces has been subtracted, and so records are clearly noisier than those obtained by Goldman (1986). For experiments in which the same axon was exposed to more than one perfusate or external bathing medium, TTX controls were run separately for each experimental condition. To minimize any effects of slow inactivation (Rudy, 1981) the preparation was held at the fixed −100 mV holding potential for 2 min before the start of each voltage clamp run, and 15 s were allowed between each voltage clamp pulse.

Both K and Cs perfusates contained 40 mM tetraethylammonium Br (TEA; Eastman Kodak Co., Rochester, NY) in all experiments. In addition, all experiments were done in the presence of 2 mM 3,4-diaminopyridine (Aldrich Chemical Co., Milwaukee, WI) in the external bathing medium to suppress the K conductance. 3,4-diaminopyridine solutions were freshly prepared each day and the pH checked.

Analysis

Both $I_{Na_{peak}}$ and I_{Na_∞} values were read with the aid of the PDP 11/34 after digital filtering as described by Goldman and Chandler (1986). Inactivation time constants as determined both with one (τ_h) and two (τ_c) pulse methods were also determined with the aid of the PDP 11/34 using patternsearch, a nonlinear least squares minimization procedure (see Colquhoun, 1971).

RESULTS

Effects of Cs_i as Compared with K_i

Effects on Na Channel Current Time Course. Fig. 1 presents two families of Na channel current records from the same axon recorded at the potentials indicated at the left. The left hand column of records were obtained in K_i and the middle in Cs_i perfusate. The external solution was ½ Na ASW throughout. No record is presented for a step to 30 mV in K_i as this was nearly at the reversal potential, E_{Rev}, and the current was

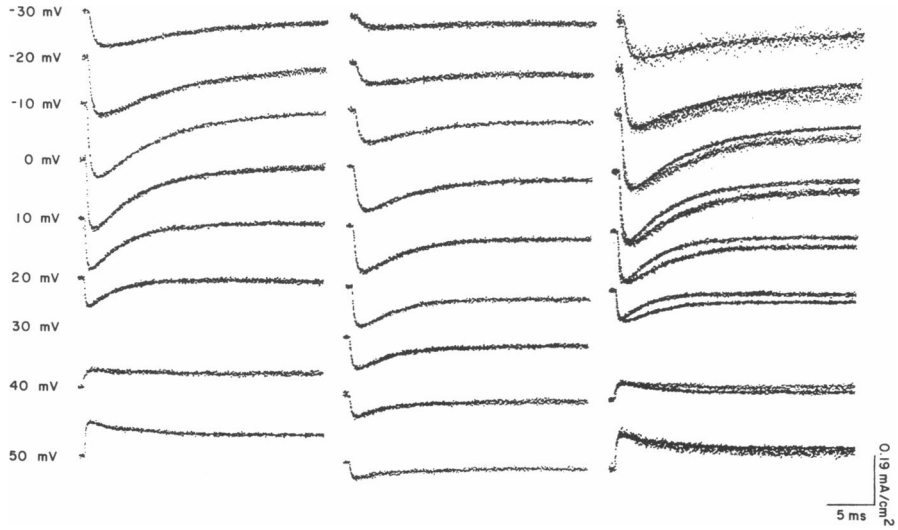

FIGURE 1 Na channel current records from the same axon in K_i (*left column*) and Cs_i (*middle column*), at the clamp potentials indicated at the left. The right hand column again presents the records in K_i with those in Cs_i superimposed and scaled so that their peak currents match those for the corresponding K_i records. Where different, currents in Cs_i always fall below those in K_i at the end of the trace. Scaling factors are: 3.40 (−30 mV), 3.00 (−20 mV), 2.20 (−10 mV), 1.60 (0 mV), 1.10 (10 mV), 0.75 (20 mV), −0.75 (40 mV), −2.23 (50 mV). ½ Na ASW. Axon 7MB4.

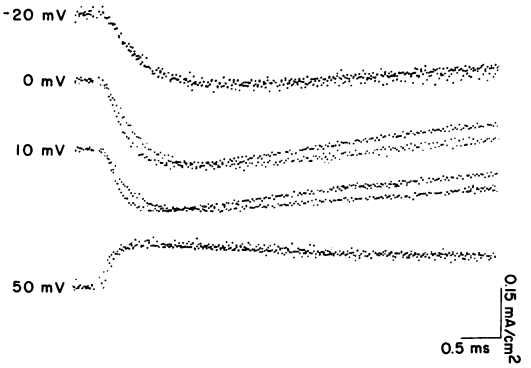

FIGURE 2 First 5 ms of four of the superimposed traces of Fig. 1 (*right column*) now shown at very expanded time and somewhat expanded current scales. Where different, currents in Cs_i are below at the end of the 5 ms of record illustrated.

too small for analysis. The right hand column presents again the records in K_i with those in Cs_i at the corresponding potentials now superimposed. The Cs_i records have all been scaled so that their peak currents match those in K_i with the Cs_i records at 40 and 50 mV also inverted. Where the current time courses differ, in each case the Cs_i trace falls below that for K_i at the end of the record. Records in Cs_i were taken 2 h after those in K_i and the difference in amplitudes is due to run down of the currents over this interval.

At -30 mV the I_{Na} time courses are not obviously different in the two perfusates. The Cs_i record is very noisy. It has been scaled up 3.40 fold, and the procedure used generates rather noisy records to begin with. However, it is clear that the inactivation time courses are essentially the same. With more positive steps in potential the inactivation time courses increasingly diverge, at first most clearly at the end of the pulse, but with more positive stps in potential strongly throughout inactivation. At potentials where inactivation is strongly reduced, there is also some slowing of the time to peak, as expected. At the most positive potentials used, where Na channel current is outward in K_i, the time courses again become very similar and are not distinguishable over the early part of the traces at 50 mV.

Differences in the time to peak I_{Na} in Cs_i and K_i (Goldman, 1986) are only seen where $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i is substantially larger than that in K_i. This is shown more clearly in Fig. 2. The first 5 ms of four of the superimposed traces of Fig. 1 are shown on expanded time and current scales. At -20 mV I_{Na} time courses in K_i and Cs_i are indistinguishable for about the first 3 ms of the traces, but are diverging by 5 ms. At 0 and 10 mV, time courses are substantially different in the two perfusates and the time to peak is slowed. At 50 mV I_{Na} time courses in the two perfusates are essentially identical for the whole 5 ms of record illustrated. Correspondingly, $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i is only 1.70 fold greater than that in K_i at -20 mV and 0.75 of that in K_i at 50 mV, but 3.76 and 6.77 fold greater at 0 and 10 mV respectively, consistent with the previous suggestion (Goldman, 1986) that the slowing of the time to peak I_{Na} seen in Cs_i can be attributed to the decreased steady-state inactivation produced. In comparing Na current time courses in Cs_i and K_i it is necessary to insure that differences have not been generated by run down of the residual unblocked delayed currents between experimental and TTX control runs during K perfusion. The results of Fig. 2 address this question. In the 0 and 10 mV records clear differences in I_{Na} time course are evident by 300 μs, and current in K_i has peaked by 1 ms. At such times the delayed conductance will not have begun to develop with -100 mV holding potentials. These early time course differences cannot be attributed, then, to any degree to possible changes in the residual delayed currents, and must arise from genuine effects of Cs_i. Correspondingly, the substantial changes in steady state inactivation needed to generate these effects on time to peak also cannot be attributed just to changes in the residual delayed current. It will be shown in Fig. 9 *A* that these results have not in fact been affected to any significant degree by run down of the residual delayed currents and that the differences in time course of Fig. 1 have been reliably determined.

Close agreement at large positive potentials between the inverted inward I_{Na} time course in Cs_i and the outward Na channel (K) current time course in K_i was generally found, and suggests that the increase in $I_{Na_\infty}/I_{Na_{peak}}$ produced by replacing K_i with Cs_i and that produced by stepping from potentials where current is inward to those where it is outward, in K_i, may be generated by similar mechanisms. This close agreement between inward and outward current time courses also indicates that the decrease in steady-state inactivation seen with outward currents is a genuine Na channel effect and not attributable to any significant degree to residual delayed currents.

Fig. 3 presents superimposed I_{Na} records at two potentials from another axon with the records in Cs_i scaled so that their peaks match those in K_i. These records have been selected for illustration because the peak inward current density in K_i was about the same at the two potentials. At -30 mV I_{Na} time courses are essentially identical through the rising and early part of the inactivation phases in both perfusates, and clearly diverge only towards the end of the trace. At 0 mV current time courses differ substantially.

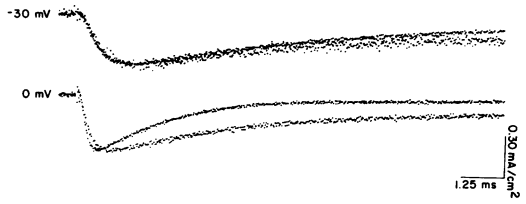

FIGURE 3 Superimposed I_{Na} records from the same axon at the two potentials indicated. Records in Cs_i have been scaled so that their peaks match those in K_i. Scaling factors are 3.05 at -30 mV and 1.18 at 0 mV. Records in Cs_i fall below at the end of each superimposed pair of traces. ½ Na ASW. Axon 7MA19.

At −30 mV peak current density in Cs_i was much less than that in K_i. The Cs_i record has been scaled 3.05 fold. At 0 mV peak current densities were very similar in the two perfusates, owing to the approach to E_{Rev} in K_i, and the Cs_i record at this potential has been scaled only 1.18 fold. Any R_s errors ought, then, to have altered current time courses in the two perfusates more at −30 mV and have a negligible effect at 0 mV. In fact, I_{Na} time courses in the two perfusates are substantially different only at 0 mV, indicating that the changes in I_{Na} time course in Cs_i cannot be attributed to R_s or any other voltage clamp errors associated with the current magnitude. Further, the −30 mV pulse falls on the negative conductance limb of the current-voltage relation where R_s and spatial stability problems are most severe (Taylor et al., 1960), while the 0 mV pulse is on the positive limb. Note that identical conclusions are reached even if R_s is an element in series with each individual Na channel rather than a lumped component in series with the membrane as a whole, as single-channel current magnitude will be greater at −30 than at 0 mV in 10 mM external Ca (Goldman and Hahin, 1978).

Effects on $I_{Na_\infty}/I_{Na_{peak}}$. Fig. 4 shows $I_{Na_\infty}/I_{Na_{peak}}$ values, from the same axon as for Fig. 1, as a function of membrane potential. $I_{Na_\infty}/I_{Na_{peak}}$ for inward I_{Na} in K_i decreases with more positive potentials, with I_{Na} inactivating completely at 20 mV. For potentials positive to E_{Rev}, $I_{Na_\infty}/I_{Na_{peak}}$ increases substantially, reaching 0.67 at 40 mV, and declining again with potentials increasingly positive to E_{Rev}. $I_{Na_\infty}/I_{Na_{peak}}$ values in Cs_i are very similar to those in K_i at the most negative potentials examined. However, with increasingly positive potentials $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i becomes increasingly greater than that in K_i (when I_{Na} is inward), and reaches fairly large values at very positive potentials.

Essentially identical results were obtained in five additional axons. Fig. 5 shows the collected results. In each case the same axon was exposed to both K_i and Cs_i perfusates and $I_{Na_\infty}/I_{Na_{peak}}$ was determined at the same potentials. ½ Na ASW was used throughout. The vertical dashed line indicates the mean value of E_{Rev} (27.6 mV with a range of 25 to 33 mV). All values positive to the dashed line are taken from outward Na channel currents. Circles indicate mean values of $I_{Na_\infty}/I_{Na_{peak}}$ in K_i relative to that in Cs_i, and the bars show the total range of variation. The mean ratio of $I_{Na_\infty}/I_{Na_{peak}}$ values is near unity at the most negative potentials and decreases over the potential range where I_{Na} is inward in K_i, owing to the decrease in $I_{Na_\infty}/I_{Na_{peak}}$ in K_i and its relative insensitivity to membrane potential in Cs_i. Immediately positive to E_{Rev} the ratio is fairly large and declines somewhat on further depolarization to fall near unity at the most positive potentials examined.

The results of Figs. 4 and 5 do not indicate that the increase in $I_{Na_\infty}/I_{Na_{peak}}$ in K_i is discontinuous at E_{Rev}, but only that it is steep in this region. Currents were recorded at 10

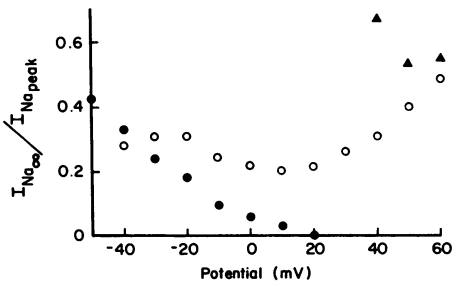

FIGURE 4 $I_{Na_\infty}/I_{Na_{peak}}$ as a function of membrane potential. Values in Cs_i are indicated by the open circles. Those in K_i are indicated by filled circles for inward and filled triangles for outward currents. Same axon as for Fig. 1.

mV increments in potential. In a single experiment an increase in $I_{Na_\infty}/I_{Na_{peak}}$ with potential was detected in K_i in the presence of inward I_{Na}. Fig. 6 shows two records from the same K perfused axon in ½ Na ASW. In this experiment $I_{Na_\infty}/I_{Na_{peak}}$ decreased smoothly from 0.206 at −50 mV to 0.097 for the record at 10 mV illustrated. At the next potential tested (20 mV) $I_{Na_\infty}/I_{Na_{peak}}$ increased again to 0.373. E_{Rev} was 25 mV. For most experiments test pulses presumably fell either too negative to E_{Rev} to produce this effect, or were so close to reversal that currents were too small for analysis. The $I_{Na_\infty}/I_{Na_{peak}}$ value from the 20 mV record of Fig. 6 has not been included in the means of Figs. 5 or 9 *A*.

Cs_i and Inactivation Kinetics

Na activation kinetics do not seem to be appreciably affected by Cs_i. Previous studies (Goldman, 1986) based

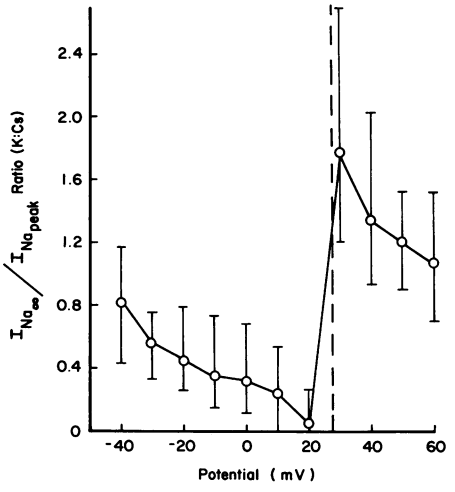

FIGURE 5 Mean values of $I_{Na_\infty}/I_{Na_{peak}}$ as a function of membrane potential. Means are of the ratios of $I_{Na_\infty}/I_{Na_{peak}}$ in K_i to that in Cs_i from the same axon at the same potential. The vertical dashed line indicates the mean value of E_{Rev} in K_i for these six axons. ½ Na ASW throughout. Means of four determinations at 30 mV, five determinations at −40, 20, and 60 mV, and six at all other potentials. Bars indicate the range of variation.

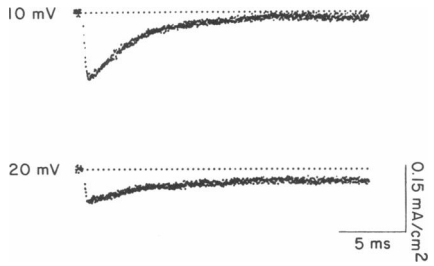

FIGURE 6 Two I_{Na} records from the same K perfused axon at the indicated potentials. Dotted lines indicate zero current. $I_{Na_\infty}/I_{Na_{peak}}$ is 0.097 at 10 and increases to 0.373 at 20 mV. This is the only sequence of inward currents observed in these experiments for which $I_{Na_\infty}/I_{Na_{peak}}$ increased with more positive potential. ½ Na ASW. Axon 7MB3.

on pooled data showed no significant effect of Cs_i compared with K_i on the time to half maximum I_{Na} ($t_{1/2}$) over a broad range of potentials. Deactivation kinetics were also unaffected by Cs_i. Na tail currents, recorded from the same axons in both Cs_i and K_i under the same conditions superimposed. Correspondingly, effects of Cs_i on I_{Na} time to peak are seen only when effects on $I_{Na_\infty}/I_{Na_{peak}}$ are large. Cs_i effects on I_{Na} seem to be inactivation effects.

Previous studies, based on pooled data, also showed no obvious effects of Cs_i on inactivation time constants. However, for those measurements there could have been an effect of Cs_i on τ_h or τ_c that was within the variance between preparations encountered and still appreciable. Therefore, the effects of the two perfusates on inactivation kinetics were compared in the same axons.

Single-Pulse Inactivation Determinations. Fig. 7 presents superimposed I_{Na} records at two potentials, all from the same axon. At each potential records in Cs_i fall below those in K_i at the end of the traces. Cs_i records have been scaled so their peaks match those in K_i, and ½ Na ASW was used throughout. Superimposed on the current traces are single exponential fits to the inactivation time course (*thin lines*). For the inactivation phases, the current records and fitted exponentials agree quite closely.

FIGURE 7 Superimposed I_{Na} records at the two potentials indicated. These records are the first 20 ms of those from the right hand column of Fig. 1 shown on a somewhat expanded time scale. Superimposed on each of the four records is a single exponential fit to the inactivation time course (*thin lines;* extended to the start of the potential step). Fits were generated with the aid of the PDP 11/34 using a nonlinear least squares procedure. During the inactivation phase, the fitted exponentials are not distinguishable from the current records. Fitted τ_h values are 5.38 ms in K_i and 5.49 ms in Cs_i at 0 mV, and 4.42 ms in K_i and 4.78 ms in Cs_i at 10 mV.

The fitted τ_h values at 0 mV are 5.38 ms in K_i and 5.49 ms, or only 1.02 fold greater, in Cs_i. At 10 mV values were 4.42 ms in K_i and 4.78 ms, or 1.08 fold greater, in Cs_i. At both potentials, then, τ_h can be taken as nearly identical in the two perfusates while the overall time course of inactivation clearly differs. The difference in each case is attributable essentially entirely to the increase in $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i. At 0 mV, $I_{Na_\infty}/I_{Na_{peak}}$ in this experiment was 0.059 in K_i and 0.221 in Cs_i. At 10 mV the corresponding values were 0.030 and 0.204.

τ_h values were determined for each of the six axons exposed to both perfusates. As at both very negative and very positive potentials inactivation time courses were very similar or even identical in the two perfusates, τ_h values were extracted for each experiment only at 0 and 10 mV, potentials where I_{Na} time courses differed substantially in every case. Results are presented in Table I. In no case does τ_h differ significantly in the two perfusates. Mean ratio of τ_h in Cs_i to that in K_i was 1.04 at 0 mV and 1.02 at 10 mV. Cs_i has no significant effect on single-pulse inactivation kinetics. Its effect is to increase the fraction of inactivation gates that do not close, the rest closing in a normal way. At the level of a single channel, then, inactivation is blocked by Cs_i in an all or none manner.

The close agreement of inactivation time constants in the two perfusates again indicates that the effects of Cs_i on I_{Na} time course cannot be attributed to R_s or spatial

TABLE I
τ_h IN Cs_i AND K_i

0 mV	Axon	τ_h		$\tau_{h_{Cs}}/\tau_{h_K}$
		Cs	K	
		ms	*ms*	
	7MA21	6.09	5.76	1.057
	7MB4	5.49	5.38	1.020
	7MB2	5.33	4.76	1.120
	7MA19	5.24	5.23	1.002
	7MA18	5.19	5.29	0.981
	7MB3	4.30	4.05	1.062
				Mean 1.040
10 mV	Axon	τ_h		$\tau_{h_{Cs}}/\tau_{h_K}$
		Cs	K	
		ms	*ms*	
	7MA21	5.65	5.97	0.946
	7MA18	4.98	4.71	1.057
	7MA19	4.96	4.80	1.033
	7MB4	4.78	4.42	1.081
	7MB2	4.43	4.76	0.931
	7MB3	3.85	3.60	1.069
				Mean 1.020

½ Na ASW throughout.

stability errors. Similarly, this close agreement also indicates that the time course differences are not generated by any significant inclusion of the residual delayed current in the records taken under *K* perfusion. If this were the case then the kinetics should have changed.

Two Pulse Inactivation Determinations. Cs_i also has no appreciable effect on τ_c. Fig. 8 presents a two pulse inactivation determination in K_i and again in Cs_i from the same axon. Peak I_{Na} during a −10 mV test pulse, normalized to that with no conditioning pulse, is shown as a function of the duration of a −20 mV conditioning pulse. Determinations were done as described by Goldman and Kenyon (1982), and included a 5-ms gap between conditioning and test pulses. Values in K_i and Cs_i agree closely at all conditioning durations even though the absolute current density was more than twice as large in Cs_i than in K_i. The delay in inactivation development in this experiment (Goldman and Kenyon, 1982; Goldman, 1987) was too small for patternsearch to reliably fit a time constant to, and so the fit was begun at a conditioning duration of 1.5 ms. τ_c in K_i (*solid curve,* Fig. 8) was 9.14 ms, and that in Cs_i was 9.47 ms or 1.036 fold greater. Cs_i has no appreciable effect on τ_c in this experiment.

Similar results were obtained in three additional axons. Results are presented in Table II. Two determinations at −30 mV and two at −20 mV yielded mean ratios of τ_c in Cs_i to that in K_i of 1.001 and 0.929 respectively. These determinations were all done on North American *Myxicola.* Conditioning potentials of −30 and −20 mV were selected because the $\tau_c - \tau_h$ difference in North American *Myxicola* is substantial in this region (Goldman and Chandler, 1986), and Schauf and Bullock (1978) had previously reported that internal dialysis with Cs_i as compared with K_i abolished the $\tau_c - \tau_h$ difference by selectively speeding τ_c. I find no such effect on Cs perfused North

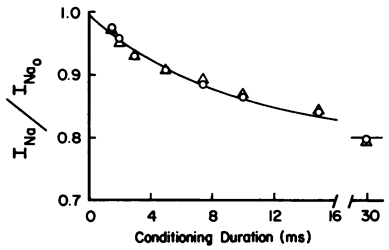

FIGURE 8 Peak I_{Na} during a −10 mV test pulse relative to that with no conditioning pulse (I_{Na}/I_{Na_0}) as a function of the duration of a −20 mV conditioning pulse. Values are from the same axon in K_i (*circles*) and Cs_i (*triangles*). Each conditioning pulse was followed by a 5 ms gap (step back to the holding potential) before presenting the test pulse. Each conditioning pulse, gap, test pulse sequence was both preceded and followed by an unconditioned test pulse. Means of these bracketing values were used to determine I_{Na}/I_{Na_0} for each conditioning duration. The solid curve is a single exponential fit to the inactivation time course again fitted with the aid of the PDP 11/34 as described in the legend to Fig. 6. The fitted, τ_c, value is 9.14 ms. ½ Na ASW. Axon 7MA2.

TABLE II
τ_c IN Cs_i AND K_i

−30 mV	Axon	τ_c		τ_{cCs}/τ_{cK}
		Cs	K	
		ms	*ms*	
	7MA1	7.99	7.91	1.010
	7MA6	6.28	6.34	0.991
				Mean 1.001
−20 mV	**Axon**	**τ_c**		**τ_{cCs}/τ_{cK}**
		Cs	K	
		ms	*ms*	
	7MA2	9.47	9.14	1.036
	7MA3	9.26	11.28	0.821
				Mean 0.929

½ Na ASW throughout.

American preparations consistent with the findings on Plymouth *Myxicola* (Goldman, 1986).

$I_{Na_\infty}/I_{Na_{peak}}$ and External Na Concentration in *K* Perfused Axons

Inward Na Current. When compared in the same *K* perfused axons $I_{Na_\infty}/I_{Na_{peak}}$ values for inward I_{Na} recorded in ⅔ and in ¼ Na ASW could agree quite closely over a range of potentials. One example is seen in the −10 mV records of Fig. 10. The right hand column of this figure presents superimposed current records from the same K perfused axon obtained in ⅔ and ¼ Na. The time courses of these inward currents are essentially identical although that recorded in ¼ was 3.75-fold smaller. This close agreement indicates that these records do not include any significant contribution from the residual delayed current as any such effects should have been more evident in the much smaller current in ¼. A second conclusion from these results is that there is little or no effect of inward Na channel current magnitude on $I_{Na_\infty}/I_{Na_{peak}}$ in K_i.

Collected results are shown in Fig. 9 *A*. Mean values from four preparations in ¼, six in ½, and five in ⅔ Na ASW are presented. There is no appreciable effect of inward I_{Na} magnitude on $I_{Na_\infty}/I_{Na_{peak}}$ in K_i, with the data generally displaying a rather smooth decline towards zero. The mean in ¼ Na at 0 mV falls below this general trend. No steady state I_{Na} was detected for these determinations. However, I_{Na} magnitude is very small here. Mean E_{Rev} for these four axons in ¼ Na was 6.7 mV. It would have required an I_{Na_∞} of only 3–7 $\mu A/cm^2$, a value within the noise level, to bring the mean $I_{Na_\infty}/I_{Na_{peak}}$ in ¼ Na at 0 mV up to that for the other two solutions, and so it is not possible to attach much significance to this point. Of note is the close agreement between values in ½ and ⅔ Na. These Na concentrations are not very different. However, for the experiments in ⅔ TTX controls were recorded before the determinations in the absence of TTX, and for those in ½

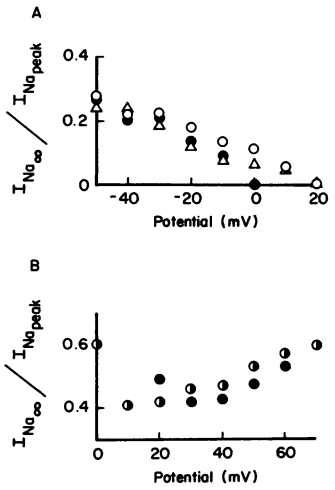

FIGURE 9 (*A*) Mean values of $I_{Na_\infty}/I_{Na_{peak}}$ under *K* perfusion as a function of membrane potential. All values are from inward Na channel currents in 1/4 Na (*filled circles*), 1/2 Na (*triangles*), or 2/3 Na ASW (*open circles*). Means of two determinations at −50, three at 0 mV, and four at all other potentials in 1/4 Na. Means of five determinations at 20 mV and six at all other potentials in 1/2 Na. Means of two determinations at −50 and 20 mV, four at 10 mV and, five at all other potentials in 2/3 Na ASW. (*B*) Mean values of $I_{Na_\infty}/I_{Na_{peak}}$ under *K* perfusion as a function of membrane potential. All values are from outward Na channel currents, in 1/10 Na (*half filled circles*) or 1/4 Na ASW (*filled circles*). Means computed from four determinations at 0 mV and five determinations at all other potentials in 1/10 Na. Means computed from five determinations at 60 mV and four at all other potentials in 1/4 Na.

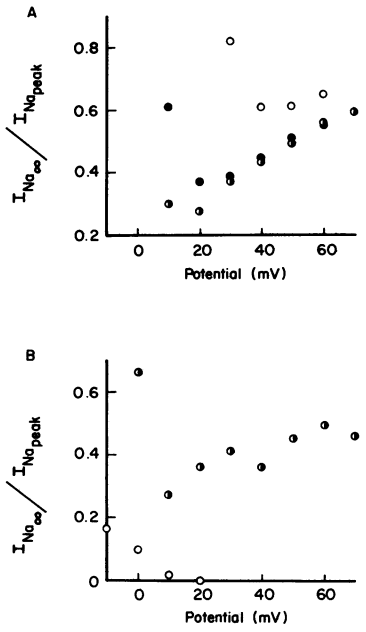

FIGURE 11 (*A*) $I_{Na_\infty}/I_{Na_{peak}}$ values, all from the same *K* perfused axon, as a function of membrane potential. Values are from outward Na channel currents in 1/10 Na (*half filled circles*), 1/4 Na (*filled circles*) and 2/3 Na ASW (*open circles*). Same axon as for Fig. 10. (*B*) $I_{Na_\infty}/I_{Na_{peak}}$ values from the same *K* perfused axon as a function of membrane potential. Values are from inward currents in 2/3 Na (*open circles*) and outward currents in 1/10 Na ASW (*half filled circles*). There is an overlapping range of potentials where $I_{Na_\infty}/I_{Na_{peak}}$ decreases with potential for inward and increases for outward currents. Axon 7MB5.

they were run after. Hence any errors produced by run down of the residual delayed currents will increase $I_{Na_\infty}/I_{Na_{peak}}$ in one case and decrease it in the other. In fact values in the two bathing media are essentially the same with the means at 10 and 20 mV, where residual delayed current effects should be greatest in the inward current experiments, found to be nearly identical. The determinations in 1-2- Na are from the same axons used for the Cs-*K* comparisons of Figs. 1–5, and the Cs effects then seem to be reliably determined.

Over the range of conditions tested $I_{Na_\infty}/I_{Na_{peak}}$ under *K* perfusion is insensitive to the inward Na channel current magnitude. The decline in $I_{Na_\infty}/I_{Na_{peak}}$ seen with positive potentials for inward current must then be due to the normal voltage dependence of steady-state inactivation seen over this potential range (Goldman and Schauf, 1972). I_{Na_∞} for inward current in K_i was often found to be undetectable at more positive potentials. In eleven determinations from eleven different axons, in 1/2 and 2/3 Na only, I_{Na_∞} was measured as zero at 10 or 20 mV in seven cases indicating that Na channels can inactivate completely in K_i in the presence of inward I_{Na}.

Outward Na Channel Current. $I_{Na_\infty}/I_{Na_{peak}}$ increases substantially in *K* perfused axons at potentials just positive to E_{Rev}, suggesting a current dependent effect. Another possibility is a potential dependent effect that just happens to fall near E_{Rev} in 1/2 Na. That this latter explanation is not the case is illustrated by the results of Fig. 10. I_{Na} records from the same *K* perfused axon in 2/3

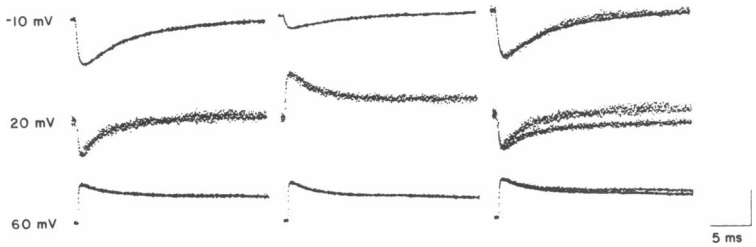

FIGURE 10 Na channel current records from the same *K* perfused axon at the indicated potentials. Records obtained in 2/3 Na (*left column*) and 1/4 Na ASW (*middle column*). Right hand column again shows the records in 2/3 Na now with those in 1/4 superimposed. At −10 and 20 mV in the right hand column records in 1/4 Na have been scaled (factors of 3.75 and −0.795 respectively) so that their peaks match those of the corresponding records in 2/3 Na. The record at 60 mV is unscaled. Current scale is 0.30 mA/cm² at −10 and 60 mV, and 0.075 mA/cm² at 20 mV. $I_{Na_\infty}/I_{Na_{peak}}$ is 0 at 20 mV in 2/3 Na, and 0.370 in the same axon at the same potential in 1/4 Na where Na channel current is outward. Axon 7MB7.

(*left hand column*) and again in 1/4 Na ASW (*middle column*) are shown at three potentials. Records at 20 mV (near E_{Rev} in 2/3 Na) are presented at four times the current scale of that for the other two potentials. The right hand column again shows the records in 2/3 Na with those in 1/4 now superimposed. At −10 and 20 mV the records in 1/4 Na have been scaled so that their peak currents match those in 2/3 at the corresponding potentials. At −10 mV where both currents are inward, I_{Na} time courses are essentially identical in the two concentrations as previously noted. 20 mV was the most positive potential tested in this experiment for which I_{Na} was still inward in 2/3 Na. At this potential $I_{Na_\infty}/I_{Na_{peak}}$ was measured as 0 in 2/3 while in 1/4 Na at the same potential but with current now outward it was 0.370, indicating that the increases in $I_{Na_\infty}/I_{Na_{peak}}$ seen positive to E_{Rev} in K_i are in fact current and not voltage dependent effects.

$I_{Na_\infty}/I_{Na_{peak}}$ values for this experiment are shown as a function of membrane potential in Fig. 11 *A*. Values are shown in 2/3, 1/4, and 1/10 Na ASW, all from outward current records. For each Na concentration $I_{Na_\infty}/I_{Na_{peak}}$ is particularly large for test potentials just positive to E_{Rev}, and in each case values first rapidly decrease with potential. The rapid decline in $I_{Na_\infty}/I_{Na_{peak}}$ with potential just positive to E_{Rev} is clearly a current dependent effect as the potential range over which it is observed shifts with E_{Rev}.

A second effect is evident in these outward current data. In the experiment of Fig. 10, $I_{Na_\infty}/I_{Na_{peak}}$ values in the two bathing media are approaching at positive potentials. Records at 60 mV have been superimposed without scaling. Even closer agreement is seen when values in 1/4 and 1/10 Na ASW are compared (Fig. 11 *A*). Sufficiently positive to E_{Rev}, $I_{Na_\infty}/I_{Na_{peak}}$ increases in an identical way with potential in these two bathing media. A similar effect is seen in the collected outward current results. Mean values of $I_{Na_\infty}/I_{Na_{peak}}$ in 1/4 and 1/10 Na ASW are shown as a function of membrane potential in Fig. 9 *B*. Values were obtained from five axons in 1/4 and four axons in 1/10 Na. No value is indicated at 10 mV in 1/4 Na as in only a single experiment were currents large enough for analysis at this potential. A large $I_{Na_\infty}/I_{Na_{peak}}$ value positive to E_{Rev}, and an initial rapid decline with potential are evident. In both 1/10 and 1/4, $I_{Na_\infty}/I_{Na_{peak}}$ then increases again with potential in a similar way.

The similar dependency of $I_{Na_\infty}/I_{Na_{peak}}$ on potential in the two concentrations could indicate a voltage dependent decrease in steady state inactivation, subsequent to its increase at more negative potentials (Chandler and Meves, 1970b). Alternatively, this too could be a current dependent effect, as at potentials sufficiently positive to E_{Rev} outward Na channel current magnitude will be increasing in a similar way with potential under both conditions as the perfusates are the same. That this effect depends on the current direction is illustrated by the data of Fig. 11 *B*. $I_{Na_\infty}/I_{Na_{peak}}$ values from the same *K* perfused axon are shown as a function of membrane potential in 2/3 (*inward currents*) and again in 1/10 Na ASW (*outward currents*). $I_{Na_\infty}/I_{Na_{peak}}$ for inward currents decreases with potential, reaching 0 at 20 mV, due to the voltage dependent increase in steady state inactivation. Overlapping part of this same potential range $I_{Na_\infty}/I_{Na_{peak}}$ for outward currents increases with potential, after first showing the initial rapid decrease. The increase in $I_{Na_\infty}/I_{Na_{peak}}$ with potential cannot, then, be attributed to a voltage-dependent decrease in steady state inactivation.

$I_{Na_\infty}/I_{Na_{peak}}$ and External Na Concentration in Cs Perfused Axons

Fig. 12 shows mean values of $I_{Na_\infty}/I_{Na_{peak}}$ from Cs perfused axons in 1/4, 1/2, and 2/3 Na. These data are the means of three experiments in 1/4, six in 1/2, and four in 2/3 Na. $I_{Na_\infty}/I_{Na_{peak}}$ increases substantially with positive potentials in 1/4 Na. In 2/3 Na, $I_{Na_\infty}/I_{Na_{peak}}$ is smaller than in 1/4 over nearly the whole of the potential range studied with the increase seen at positive potentials strongly suppressed. Results in 1/2 Na are intermediate, being much like those in 2/3 over most of the potential range, but also showing an increase in $I_{Na_\infty}/I_{Na_{peak}}$ at positive potentials, now to a lesser extent and at more positive potentials than seen in 1/4 Na. The increase in $I_{Na_\infty}/I_{Na_{peak}}$ seen in 1/4 Na, then, does not define a potential dependency of Cs occupancy of its site of action. It can be supressed by increasing the current magnitude.

$I_{Na_\infty}/I_{Na_{peak}}$ values in Cs_i are clearly dependent on the magnitude of the inward Na current. The inactivation blocking site occupied by Cs is, then, located in or exposed to the current pathway as previously suggested (Goldman, 1986). The increase in $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i seen at positive potentials in 1/4 and 1/2 Na must, then, be due at least in part to an increased occupancy of the inactivation blocking site by Cs owing to the reduced inward current magnitude encountered over this potential range and a less effective displacement of Cs from the sites.

Note that the effect of external Na concentration on Cs occupancy of the inactivation blocking sites is itself apparently potential dependent. Effects of Na are substantial at

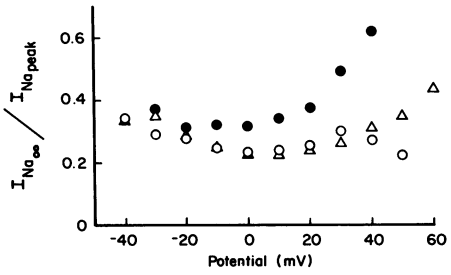

FIGURE 12 Mean values of $I_{Na_\infty}/I_{Na_{peak}}$ under Cs perfusion as a function of membrane potential, recorded in 1/4 Na (*filled circles*), 1/2 Na (*triangles*), or 2/3 Na ASW (*open circles*). Means of two determinations at 40 mV and three at all other potentials in 1/4 Na. Means of five determinations at −40 and 60 mV and six determinations at all other potentials in 1/2 Na. Means of three at −40, two at 50 mV and four determinations at all other potentials in 2/3 Na ASW.

the most positive potentials tested, but are small or moderate over the negative range, including potentials where Cs occupancy of these sites is substantial (Figs. 4 and 5). Considering only potentials at or positive to 20 mV where voltage dependent inactivation is largely complete, mean $I_{Na_\infty}/I_{Na_{peak}}$ in ¼ Na is 0.303 at 20, 0.490 at 30, and 0.616 at 40 mV. Doubling the Na concentration to ½ Na ASW yielded corresponding mean values of 0.238, 0.265, and 0.310. The ratio of mean $I_{Na_\infty}/I_{Na_{peak}}$, values in these two concentrations, then, increased from 1.57 at 20 to 1.99 at 40 mV.

DISCUSSION

Summary and Interpretation of Results

There is a site located in or exposed to the current pathway of the Na channel which can be occupied by Cs ions. When occupied the sole effect is to increase the fraction of inactivation gates that do not close. This site need not be located deeply within the pore, but could be in a widened vestibule near the channel mouth. Cs was presented internally. The Na channel is not measureably permeable to Cs in *Myxicola* (Ebert and Goldman, 1976), and the site is presumeably located in or near the inner, axoplasmic mouth of the channel. Outward Na channel currents, carried by *K* ions under *K* perfusion, can show nearly identical $I_{Na_\infty}/I_{Na_{peak}}$ values to those seen in Cs_i. It seems probable that *K* too binds to this same site, but only appreciably in the absence of inward current. A simple explanation for the inward current results would be that *K* binds only loosely, and is substantially displaced by inward current. TEA was also present internally. Goldman (1986) found increased steady state outward currents in both the presence and absence of TEA. It seems likely that the occupancy of the Cs binding site seen with outward currents is at last largely by *K* (present at 410 mM) rather than TEA (40 mM). However, a possible small contribution from TEA binding during outward current cannot be excluded.

Inactivation during inward currents under *K* perfusion can often be complete by 10 or 20 mV under these experimental conditions, suggesting that these sites can often be swept entirely, or nearly entirely free of *K* ions. At more negative potentials occupancy of this site by *K* (or TEA) should only be less frequent, as the open channel Na current increases with more negative potentials at least down to −50 mV in 10 mM external Ca (Goldman and Hahin, 1978; see also Yamamoto et al., 1985). At the most negative potentials tested these sites must often be swept nearly free of Cs ions also, as $I_{Na_\infty}/I_{Na_{peak}}$ in the two perfusates is not very different in this region. With increasingly positive potentials the single channel Na current magnitude decreases and Cs will be less effectively displaced from the inactivation blocking sites producing an $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i increasingly larger than that in K_i. The voltage dependent increase in steady state inactivation will be opposed by an increased block of inactivation produced by increased Cs occupancy yielding $I_{Na_\infty}/I_{Na_{peak}}$ values that are relatively insensitive to potential in Cs_i. At very positive potentials where voltage dependent steady state inactivation is essentially complete inward current continues to decrease producing the increase in $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i. Hence the results for inward currents of Figs. 4 and 5 can all be accounted for if the inactivation blocking site is located in the current pathway, and inward current displaces *K* ions more effectively than it does Cs from this site. An alternative possibility, that *K* binds only when there is outward *K* current through the Na channel, seems less likely as an increased $I_{Na_\infty}/I_{Na_{peak}}$ can be observed during inward currents in K_i when the inward single-channel current magnitude is sufficiently low (Fig. 6).

This site binds cations and so is presumeably negatively charged. When occupied by an ion the effect is an all or none block of inactivation gate closure. Its location in the current pathway, an effective site for the inactivation gate, and the selective block of inactivation gate closure make it seem likely that Cs and K bind to a site that the inactivation gate normally associates with or latches to on closure. The inactivated state may consist, then, of a positively-charged structure occluding the inner channel mouth either electrostatically or sterically, as has been proposed by Armstrong and Bezanilla (1977), based on an entirely different line of evidence.

These results do not exclude the possibility of some altered potential dependency, in addition to the clear current dependency, of steady state inactivation in Cs_i, i.e., that internal Cs somehow alters the voltage dependency of the inactivation gates. Arguing against this possibility is the finding that time courses for outward Na channel currents in K_i can be very similar to the (inverted) I_{Na} time courses in Cs_i.

The effect of external Na concentration on Cs occupancy of the inactivation blocking site apparently is potential dependent, however. Increasing external Na from ¼ to ⅔ of that in ASW was not very effective in displacing Cs from these sites at more negative potentials, in contrast to the clear effect at the most positive potentials. At more negative potentials Cs remaining on the blocking sites can be displaced by driving Na ions inward more vigorously with stronger electric fields, but apparently not by increasing the density of current carriers. It may be that only very tightly bound ions remain at more negative potentials even in ¼ Na.

In K_i, sufficiently positive to E_{Rev}, $I_{Na_\infty}/I_{Na_{peak}}$ increases with potential. This is not due to a decrease in voltage-dependent steady state inactivation. Possibly *K* binding increases when the magnitude of the outward *K* current through the Na channel increases. Alternatively there may be some direct voltage dependency of *K* binding, although such effects may be expected to be small (see below). Just positive to E_{Rev}, $I_{Na_\infty}/I_{Na_{peak}}$ decreases with potential. This current-dependent effect must arise from a condition par-

ticular to reversal. Increases in $I_{Na_\infty}/I_{Na_{peak}}$ with potential negative to E_{Rev} might be expected owing to the decrease in the inward current, but increases positive to E_{Rev} require an additional explanation.

The effect just positive to E_{Rev} could be accounted for if the outward portion of the Na channel current, carried by K, fell rapidly to small values at and negative to E_{Rev} with the inward, Na, portion also falling rapidly to small values at and positive to E_{Rev}. The point is that at E_{Rev} (and also negative to it) the K flux would be very small. At potentials positive to E_{Rev} the outward Na channel current would be carried exclusively by K while that through the internal perfusate would be partitioned among all the ions present according to their concentrations and mobilities. Therefore K locally at the channel mouth, and at the inactivation blocking site, would be depleted. This is the identical sort of argument used to account for K accumulation extracellularly (Frankenhaeuser and Hodgkin, 1956; Binstock and Goldman, 1971). However, at E_{Rev} little depletion would occur, the K concentration at the blocking site would be maximal, and site occupancy would be high. Moving positive to E_{Rev} depletion would increase with increasing outward current magnitude at first dominating site occupancy, but on further depolarization an increase in site occupancy overtakes the depletion effect, generating the nonmonotonic $I_{Na_\infty}/I_{Na_{peak}}$ versus potential curves of Figs. 9 *B*, and 11. A requirement of this suggestion is that ions do not transit the Na channel independently as is already well established (Hille, 1975*a*; Hille, 1975*b*; Danko et al., 1986). For independence models neither the partial outward K or the partial inward Na currents will display discontinuities at E_{Rev}.

Comparison with Previous Work

Chandler and Meves (1970*a*; see also Oxford and Yeh, 1985) described large steady state Na currents in squid axons internally perfused with 300 mM NaF. However, the effects seen with Na_i differ in several respects from those described here. In addition to decreasing steady state inactivation, Na_i also slowed τ_h as compared with that recorded either in K_i (Chandler and Meves, 1970*c*) or in Cs_i (Oxford and Yeh, 1985). And, the voltage dependence of steady state inactivation, especially at large positive potentials, was dramatically altered by large internal Na concentrations (Chandler and Meves, 1970*b*, *c*), indicating that high Na_i produces more profound effects on the inactivation gating machinery than a simple all or none block of inactivation gate closure. Moreover, the site(s) of action of Na_i may not be located in the current pathway. Oxford and Yeh (1985) presented clear evidence that the increased $I_{Na_\infty}/I_{Na_{peak}}$ seen with elevated Na_i in squid does not depend on the current direction. Schauf and Bullock (1979) found no break in the $I_{Na_\infty}/I_{Na_{peak}}$ versus potential curve at E_{Rev} in *Myxicola* axons dialyzed with high Na_i. The high Na_i effect seems then to be different from that described here. To avoid any confounding of effects none of these experiments were done in the presence of Na_i.

Steady state inactivation can also be reduced by internal tetramethylammonium (TMA; Oxford and Yeh, 1979; Schauf, 1983; Oxford and Yeh, 1985) which seems to act much like elevated Na_i (Oxford and Yeh, 1985). Based on TMA effects on steady state inactivation both Schauf (1983) and Oxford and Yeh (1985) suggested that the inactivation gate might associate with a site not far into the internal channel mouth.

In squid, Chandler and Meves (1970*a*) and Oxford and Yeh (1985) both reported similar values for $I_{Na_\infty}/I_{Na_{peak}}$ in Cs_i as compared with K_i. However, Oxford and Yeh compared values only at 80 mV where Na channel currents are outward in K_i. $I_{Na_\infty}/I_{Na_{peak}}$ values in Cs_i and K_i were also found to be similar here under these conditions. Chandler and Meves, moreover, did not use TTX subtraction in their determinations, and it is not yet clear if the inactivation gate latch in squid displays the higher affinity for Cs over K described here. In fact, Oxford and Yeh (1985) did find that steady state inactivation in Cs_i was not monotonic with potential, but decreased again at very positive potentials, and Chandler and Meves (1970a) presented inward I_{Na} records in Cs_i showing clear steady state currents, as did Adelman and Senft (1966).

Some Implications for the Inactivation Gate

Both K and Cs occupancy of the inactivation blocking sites are dependent on channel current magnitude. There is, however, no definite evidence from these results of any appreciable potential dependency of site occupancy. A contribution from potential dependent binding is not excluded, but any such effects cannot be very large in these experiments. There is no indication of an increased occupancy by Cs at very positive potentials in ⅔ Na, and K occupancy declines steeply with potential, just positive to E_{Rev}, even at more positive potentials (Figs. 4 and 11 *A*). The inactivation gate latch, then, probably does not lie very deeply within the membrane field. This suggestion is interesting in the light of results from single channel studies which indicate relatively little inherent voltage dependency of inactivation rate constants (Vandenberg and Horn, 1984; Aldrich and Stevens, 1987).

τ_h is unaffected by Cs_i even under conditions where $I_{Na_\infty}/I_{Na_{peak}}$ is substantially increased and occupancy of the inactivation blocking site is high. An implication is that an inactivation gate attempting to close is unable to displace Cs from the latch. Should this have happened for any appreciable fraction of channels τ_h should have slowed owing to the increased energy barrier generated by the work of electrostatic repulsion. On the other hand, Cs is readily displaced by inward Na channel current, and it is necessary to ask why inactivation gate closure is unable to do the same. One possible way that displacement of Cs by the inactivation gate would be unlikely could be the

presence of a steric barrier. For example, the latch could be recessed in a well or groove that allowed no exit for Cs when the gate approached. This same sort of structure could account for why inwardly moving Na ions on displacing Cs or *K* do not themselves bind. In any case, the experimental result is that inactivation gate closure is not sufficiently energetic to displace Cs from the sites. A second implication is that the dwell time of Cs on these sites is not short relative to the channel open time. If it were, then τ_h would have slowed.

If the inactivation blocking site is always accessible to the internal medium then at least some fraction of the sites would normally be occupied by K at rest. This could in part provide a mechanism for the delay in inactivation development (Goldman and Schauf, 1972; Gillespie and Meves, 1980; Bean, 1981; Goldman and Kenyon, 1982; Goldman, 1987), which is particularly prominent in *Myxicola,* as a conducting channel would be necessary to clear the inactivation gate latch of blocking ions. For channels that inactivate without conducting (Gillespie and Meves, 1980; Bean, 1981; Horn et al., 1981; Aldrich and Stevens, 1983; Horn and Vandenberg, 1984) something else would be needed.

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