EVIDENCE FOR NEGATIVE GATING CHARGES IN *MYXICOLA* AXONS

C. L. SCHAUF
Department of Physiology, Rush University, Chicago, Illinois 60612

ABSTRACT In *Myxicola* giant axons, the total amount of intramembrane charge available to move over the range −80 to +120 mV decreases by 23% when the external pH is reduced from 7.3 to 5.5. The remaining charge moves more slowly at the onset of a depolarizing pulse, but the rate of charge movement at the end of the pulse is unchanged. In contrast to acidic external pH, intramembrane charge movements are insensitive to alkaline external pH or any change in the internal pH. The results are consistent with a hypothesis in which a portion of the initial outward gating current consists of titratable negative charges moving inward.

INTRODUCTION

Determination of the sign of the mobile charges that gate Na^+ channels in nerve membranes is a matter of considerable interest. One proposal, based on experiments using external Zn^{++}, is that part of the ON gating current consists of negative charge(s) moving inward from the external surface (Armstrong and Gilly, 1979; Gilly and Armstrong, 1982). If so, then the magnitude of this charge could, in principle, be sensitive to external pH (pH_o). However, in myelinated nerve fibers, although decreasing pH_o from 7.0 to 5.2 slowed the gating currents observed at the onset of a depolarizing step (consistent with a surface charge effect), the total charge movement was actually increased (Neumcke et al., 1980). It was thus suggested that the gating charges in nodal membranes might bear a net positive charge. When tested some years ago in squid giant axons, changes in pH_o were not reported to change the magnitude or rate of intramembrane charge movements (Keynes and Rojas, 1974). Clearly, more experiments would be useful, particularly considering the extensive data that is available on the pH_o dependence of the Na^+ conductance in a variety of preparations (Hille, 1968; Drouin and The, 1969; Mozhayeva and Naumov, 1972; Woodhull, 1973; Drouin and Neumcke, 1974; Shrager, 1974; Campbell and Hille, 1976; Schauf and Davis, 1976; Ohmori and Yoshii, 1977; Carbone et al., 1978; Mozhayeva et al., 1981).

In contrast to pH_o changes, studies of the effects of internal pH (pH_i) are less frequent,and, in particular, no data concerning the effects of pH_i on charge movements have been reported. In squid axons, a reduction in pH_i produces a voltage-dependent block of Na^+ channels (Ehrenstein and Fishman, 1971), and when $[Na^+]$ is high, inactivation is accelerated and becomes more complete under acidic conditions (Wanke et al., 1980). In muscle cells, on the other hand, block of Na^+ channels at low pH_i is not seen; rather, inactivation is selectively eliminated (Nonner et al., 1980). Internal alkaline pH has generally been found to slow inactivation (Brodwick and Eaton, 1978; Carbone et al., 1981). The present experiments on *Myxicola* axons were undertaken in an attempt to provide further information on the way in which both external and internal pH alters Na^+ channel gating currents.

MEHODS

Myxicola giant axons were voltage clamped, internally dialyzed, and their series resistance compensated for by methods described in previous publications (Bullock and Schauf, 1978, 1979). Leak and capacity currents were eliminated by an appropriate analog circuit. The voltage clamp system used in these studies had a settling time (to 90% of the final voltage) of 1.5 μs. Internal solutions contained 600 mM Cs^+ glutamate and were buffered with 20 mM HEPES, morpholinoethanesulfonic acid, or phosphate. The nature of the internal buffer was not a significant factor. For measurements of Na^+ currents, the external solutions contained 86 mM NaCl, 10 mM, $CaCl_2$, 50 mM $MgCl_2$, and 360 mM Tris (i.e., 20% normal Na^+), to avoid possible series resistance errors. For asymmetry current measurements Tris was the sole external monovalent cation, and, in addition, 10^{-6} M tetrodotoxin was present. Asymmetry currents were generally measured using a $P/4$ protocol (Armstrong and Bezanilla, 1977). The initial holding potential from which 16 depolarizing steps ($+P$) were applied was −80 mV. Linear charge movement was subtracted by summing the currents during 64 steps of amplitude $-P/4$ from a reference potential of −160 mV. Total charge movement as a function of membrane potential was calculated by integrating the asymmetry currents following pulse onset and termination over a time interval of at least 5 ms to ensure that all charge movement was being accounted for. All experiments were done at 5°C. Experiments in which the effects of pH_o were studied were carried out at an internal pH of 7.30 ± 0.05.

Data from a total of 25 axons are reported in this study. In six axons both gating and ionic currents were obtained at normal and acidic external pH over the entire voltage range from −120 mV (tails) to +120 mV. These data are given in Table I. In an additional nine axons, the major goal was to assess either the effects of alkaline pH or Zn^{++} and charge movement as a function of external acidic pH. Measurements were taken only over a limited voltage range. Thus, $Q(V)$ curves could not be calculated. Data from these axons were, however, included in calculating the overall averages in Table II. Experiments on the remaining 10

TABLE I
EFFECTS OF ACIDIC EXTERNAL pH ON I_g AND I_{Na} IN *MYXICOLA* GIANT AXONS

Axon	t_{pk}	$\bar{g}_{Na}$	τ_{ON}	Q_{Total}
81M4	1.35 (1.06)	0.68 (0.97)	1.38 (1.04)	0.74 (0.97)
81M5	1.32 (0.97)	0.65 (0.94)	1.30 (1.01)	0.66 (0.95)
81M6	1.38 (1.02)	0.75 (0.96)	1.49 (1.04)	0.80 (0.98)
81M7	1.20 (1.05)	0.88 (0.99)	1.22 (1.03)	0.83 (1.01)
81M10	1.17 (0.98)	0.87 (0.93)	1.29 (0.99)	0.79 (0.96)
81M11	1.24 (1.01)	0.79 (0.98)	1.18 (0.96)	0.82 (0.94)
Average	1.28 ± 0.09	0.78 ± 0.10	1.31 ± 0.11	0.77 ± 0.06

Because of the variation in quantitative values from one axon to another, all of the above data have been normalized by the following procedure. Data were obtained at pH 7.3, then at pH 5.5, and again at pH 7.3. For Q_{Total} and $\bar{g}_{Na}$ the values at pH 5.5 (not in parentheses) were simply divided by the average of the bracketing data at pH 7.3. In the case of τ_{ON} and t_{pk}, the effect of external pH was essentially independent of voltage (Figure 2), and the relative increases were obtained from the vertical shifts necessary to make the semilogarithmic plots of the rate constants as a function of voltage superimpose at normal and acidic pH. In the above table, the values of the various parameters given in parentheses were obtained by dividing the measurement determined during the second exposure to the control pH by the initial value at pH 7.3.

Abbreviations: $\bar{g}_{Na}$, maximum Na^+ conductance; t_{pk}, time-to-peak Na^+ current; Q_{Total}, maximum charge movement for large depolarizations; τ_{ON}, time constant of asymmetry current decline following pulse onset.

axons were directed toward assessment of Zn^{++} effects or internal pH alone. In six of the Zn^{++}-treated axons, we were again able to obtain complete charge movement and ionic current data in the same axon. Values for the maximum sodium conductance were calculated using the relation $\bar{g}_{Na} = I_{Na}/(V - E_{Na})$, and thus the comparisons made in this study implicitly assume that changes in pH do not alter single-channel conductance.

TABLE II
COMPARISON OF EXTERNAL pH AND Zn^{++} EFFECTS ON GATING IN Cs^+-DIALYZED *MYXICOLA* AXONS

Parameter	pH 5.5	30 mM Zn_o^{++}
$\bar{g}_{Na}$	0.78 ± 0.10 (6)	0.55 ± 0.11 (6)
t_{pk}	1.24 ± 0.06 (15)	1.61 ± 0.05 (16)
τ_{Tail}	0.98 ± 0.06 (10)	0.97 ± 0.08 (8)
Q_{Total}	0.77 ± 0.06 (6)	1.04 ± 0.09 (6)
τ_{ON}	1.33 ± 0.07 (14)	1.34 ± 0.06 (12)
τ_{OFF}	0.99 ± 0.08 (6)	1.02 ± 0.07 (6)

For the pH experiments, all data at pH 5.5 is expressed relative to the bracketing control data at pH 7.3 (see footnote to Table I). For the Zn^{++} experiments the data are given relative to control data obtained in the absence of Zn^{++} at pH 7.3. Recovery from exposure to Zn^{++} was generally comparable to the reversibility seen with external pH changes. Data are given as mean ± SD. τ_{Tail}, time constant of Na^+ tail current following repolarization; τ_{OFF}, time constant of asymmetry current at termination of pulse.

For both the pH and Zn^{++} experiments, the general experimental procedure was to obtain bracketing data under control conditions (pH = 7.3, no Zn^{++}) before and after exposure of the axon to pH changes or Zn^{++}, and to normalize the data to these values. In most axons, both the effect of pH, and that of external Zn^{++} were readily reversible, with recovery during the bracketing run at pH 7.3 to within 10% of the initial control parameters (see footnote to Table I, and Fig. 2). Data from axons failing to fully recover to their original condition were not used.

RESULTS

Fig. 1 illustrates the ON and OFF gating currents recorded during a 5 ms depolarization from −80 to +20 mV at the control external pH of 7.3 and 10 min after changing the bathing solution to one buffered at pH 5.5. At the acidic pH, the asymmetry current immediately following the start of the depolarizing pulse (ON response) was slowed, and the total charge moved at this voltage (obtained by direct integration of the records) was reduced. Similar records at neutral and acidic pH were obtained in this axon at test voltages between −60 and +120 mV. The ON response could always be fitted with a single exponential, and the time constant for its decline (τ_{ON}) was increased by ~38% over the entire voltage range (Fig. 2). The $Q(V)$ curves obtained by integrating the ON response at each test voltage in this axon at normal and acidic pH are also illustrated in Fig. 2. The total charge (Q_{Total}) that was able to move over the range −80 to +120 mV decreased by 26% when the external pH was reduced from 7.3 to 5.5, with no detectible change in the position of the $Q(V)$ curve along the voltage axis.

These effects of acidic pH_o on intramembrane charge movement can be compared with the alterations in the Na^+ conductance produced by the same decrease in pH_o (Fig. 3). At acidic pH the peak Na^+ conductance is reduced, and the rate of Na^+ activation is slower. (In our experiments, as in those of Carbone et al., 1978, the time course of inactivation of conducting channels seemed relatively insensitive to the effects of acidic external pH. Corresponding determinations of the time course of charge immobilization revealed a similar pH insensitivity, but the latter

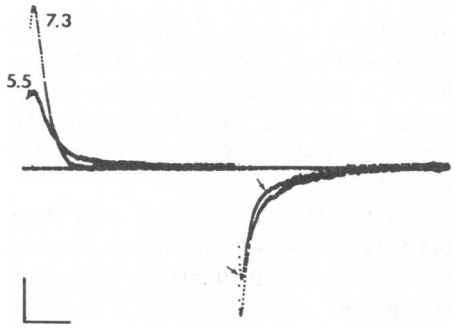

FIGURE 1 Experimental records of intramembrane charge movement in *Myxicola* illustrating the effect of a reduction in external pH from 7.3 to 5.5. The test potential was +20 mV. Current and time calibrations are 25 μA/cm² and 1.0 ms, respectively. The record following the end of the depolarizing pulse obtained at pH 5.5 is indicated by the arrows. Temperature was 5°C.

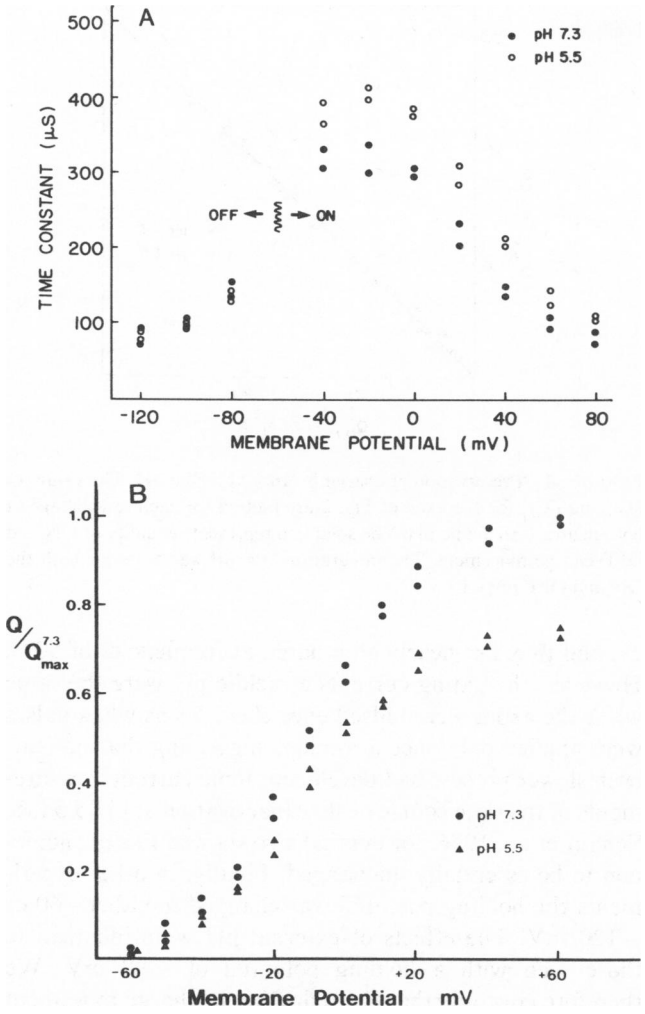

FIGURE 2 (*A*) Effects of acidic external pH on the time constant of intramembrane charge movement in *Myxicola*. For test voltages more positive than −60 mV, the data were obtained from an exponential curve fit to the falling phase of the ON response during a 5 ms depolarization. Thus, τ_{ON} was measured. For more negative voltages, the time constants are those that describe the falling phase of the OFF response following a test pulse of 1–2 ms (short enough that charge immobilization would not be significant). Thus, τ_{OFF} is defined. Temperature was 5°C. These data are from a single axon in which the external pH was lowered in two separate trials, separated by measurements made at pH 7.3. They illustrate both the extent of the experimental variability in such determinations and the degree of reversibility typically encountered. (*B*) Effects of increasing external [H^+] on the charge-voltage curve. The asymmetry current ON responses at pH 7.3 and 5.5 (two trials in each solution as in *A*) obtained using a *P*/4 protocol and 5 ms depolarizations between −60 and +120 mV were integrated, normalized to the total charge moved at +120 mV and the initial exposure to pH 7.3, and plotted as a function of membrane potential. Total charge movement at +120 mV was the same as that at +60 mV, and thus for clarity only data to +60mV are plotted. The total intramembrane charge movement in this axon at pH 7.3 was 26.8 nC/cm^2.

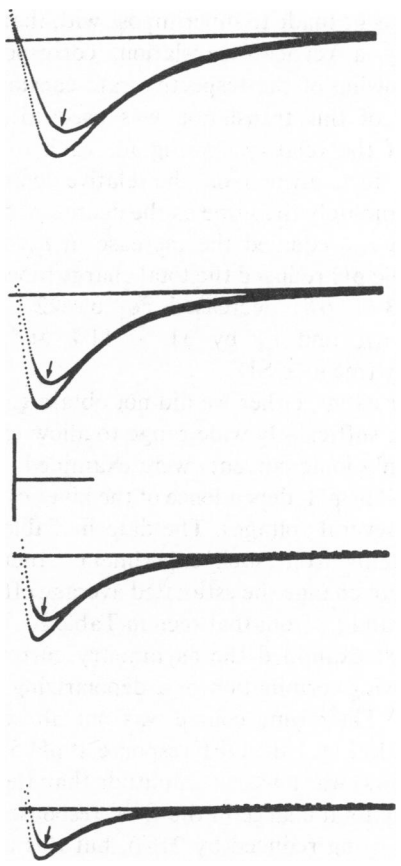

FIGURE 3 Membrane Na^+ currents as a function of external pH in *Myxicola* axons. Records are shown for depolarizations to −20, 0, +20, and +40 mV. In each case, the trace indicated by the arrow was obtained at pH 5.5, and the unmarked trace at neutral pH. The temperature was 5°C. Calibrations are 1.0 ms and 0.5 mA/cm^2, respectively.

measurements were not sufficiently accurate to allow further interpretation). As in previous studies (Schauf and Davis, 1976), the $g_{Na}(V)$ curve was not shifted along the voltage axis to any significant degree. The overall results of our analysis of the effect of external pH on Na^+ currents in the axons used in this study were not measureably different from those we previously described (Schauf and Davis, 1976). We therefore have chosen to simply summarize the ionic current data without presenting additional figures.

Absolute values of total charge movement in these experiments ranged from 19.5 to 29.0 nC/cm^2, with a corresponding variation in $\bar{g}_{Na}$. Thus, to quantitatively compare the relative effects of pH_o on the ionic and gating currents, data from each axon at acidic pH were normalized to those at neutral pH. In those six axons in which gating and ionic currents were available over the full voltage range (Table I), the $Q(V)$ and $g_{Na}(V)$ curves were constructed for the bracketing runs at pH 7.3, and for the run(s) at pH 5.5, and the values of Q_{Total} and $\bar{g}_{Na}$ were estimated for each. The relative effect of acidic pH was calculated by dividing the values at pH 5.5 by the average of those at pH 7.3. Next the time constant of the falling phase of the ON response (τ_{ON}), and the time required for the Na^+ conductance to reach its peak value (t_{pk}), were determined for each test voltage at both control and acidic pH (the former by least-squares methods), and then plotted semilogarithmically as a function of membrane potential. Within experimental error, the data at pH 5.5

could always be made to superimpose with that obtained at pH 7.3 by a vertical translation, corresponding to a uniform slowing of the respective rate constants, and the magnitude of this translation was used to generate an estimate of the relative slowing for each axon. Table I shows that, for a given axon, the relative decrease in Q_{Total} was approximately the same as the decrease in $\bar{g}_{Na}$, and the increase in τ_{ON} equaled the increase in t_{pk}. In these six axons, acidic pH reduced the total charge movement by an average 23 ± 6%, decreased $\bar{g}_{Na}$ by 22 ± 10%, and increased τ_{ON} and t_{pk} by 31 ± 11% and 28 ± 9%, respectively (mean ± SD).

In other axons, either we did not obtain gating current data over a sufficiently wide range to allow calculation of Q_{Total}, or only ionic currents were examined. However, in many cases the pH_o dependence of the kinetics was characterized at several voltages. The data in Table II includes time constants from such experiments. Including these axons did not change the estimated average effect of acidic pH on τ_{ON} and t_{pk} from that seen in Table I.

We next examined the asymmetry currents immediately following termination of a depolarizing pulse (OFF responses). Their time course was not altered by acidic conditions. In Fig. 1 the OFF response at pH 5.5 (indicated by the arrows) was lower in amplitude than the response at pH 7.3 (the total charge in the OFF response in this axon at low pH being reduced by 21%), but this is completely consistent with the magnitude of the decrease in ON charge movement. If the records were scaled so that the peaks coincided, the time course of the OFF response, although clearly complex for a 5 ms pulse owing to the presence of charge immobilization, was quite unchanged. For test pulse durations of 1–2 ms, the OFF response is not complicated by charge immobilization and declines as a single exponential. τ_{OFF} is plotted in Fig. 2 at several voltages.) Table II demonstrates that at pH 5.5 τ_{OFF} following short pulses was unchanged, averaging 99 ± 8% of that at pH 7.3.

It is important to note that charge balance was preserved in all the axons we examined (Fig. 4). Thus, not only was $Q_{OFF}(V)$ equal to $Q_{ON}(V)$ at pH 7.3, but when Q_{ON} was reduced at acidic pH, Q_{OFF} was reduced by an equal amount. This implies, not only that the integration interval was sufficiently long to include all the charge movement, but also that acidic pH was not artifactually reducing the apparent asymmetry current via some change in a nonspecific ionic leakage current. Such effects would not, in general, preserve charge balance.

We also considered whether acidic pH might be altering slow Na^+ inactivation, since this process reduces both the Na^+ conductance and charge movement (Bullock and Schauf, 1979). This did not seemto be the case. One way of revealing slow inactivation is to pulse *Myxicola* axons repetitively. At frequencies >0.1/s the ionic and gating currents get progressively smaller with incresing frequency, and they are nearly eliminated at frequencies of 50/s. However, the gating currents at acidic pH were the same when the axons were pulsed once every 5 s as when pulses were applied only once a minute, suggesting that no new, even slower process had developed. Ionic current measurements of the time course of slow inactivation at pH 5.5 (see Schauf et al., 1976, for details) also showed this phenomenon to be essentially unchanged. Finally, in other experiments the holding potential was changed to either −60 or −120 mV. The effects of external pH were identical to those seen with a holding potential of −80 mV. We therefore conclude that the reduction in charge movement was not the result of a confounding slow inactivation process.

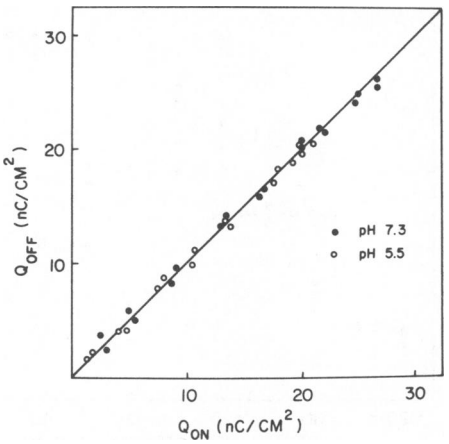

FIGURE 4 Preservation of charge balance at acidic pH. The values of Q_{ON} and Q_{OFF} for the axon of Fig. 2 are plotted for each test voltage at both neutral and acidic pH. The solid line represents equality of ON and OFF charge movement. The integration interval was 5 ms for both the ON and OFF responses.

In four experiments the effects of an alkaline external solution (pH_o = 9.5) on intramembrane charge movement were examined. In contrast to acidic pH, no significant changes were seen. Both the ON and OFF responses were identical at all test voltages. The total charge moved at pH 9.5 averaged 98 ± 6% of that at pH 7.3, and the rate of charge movement was not altered. The ionic currents were similarly insensitive to this degree of alkalinity, as previously noted by others (e.g., Carbone et al., 1978).

One might be tempted to interpret the ionic and asymmetry current changes as arising from alterations in membrane surface potential (Schauf, 1975). However, this is unlikely. As noted above, the positions of both the $Q(V)$ and $g_{Na}(V)$ curves along the voltage axis were unchanged at acidic pH. (The upper limit permitted by the experimental scatter would be a translation in the depolarizing direction of no more than 2–3 mV.) This implies that the surface charge density in the vicinity of the Na^+ channel is relatively constant over this pH range (in contrast to that near the K^+ channels, see Schauf and Davis, 1976). More importantly, just as τ_{OFF} was unchanged at pH 5.5, sodium

tail currents were also not altered (data obtained in the present study). If a surface charge effect were responsible, both the rate of deactivation of Na^+ channels and the associated charge movement would have been accelerated. The data (Fig. 2) do not seem to allow for an appreciable acceleration, even though the rate of change of these time constants with voltage is not very steep in this voltage range.

In some respects, the observed changes in the Na^+ kinetics and the rate of intramembrane charge movement at low pH resemble the effects produced by external Zn^{++} in squid axons (Armstrong and Gilly, 1979; Gilly and Armstrong, 1982). We have repeated many of these measurements in *Myxicola* to compare the two interventions in the same species. Overall, the effects of Zn^{++} that we observed were nearly identical to those reported for squid, and we have thus chosen to simply summarize our data without presenting original records (Table II). Charge movement experiments revealed that external Zn^{++} increased τ_{ON} by 34 ± 6% with no effect on τ_{OFF} but, unlike acidic pH, Zn^{++} had no detectible effect on the total charge moved. Analysis of the ionic currents revealed a Zn^{++}-dependent reduction in the maximum Na^+ conductance of 45 ± 11%, a shift of the $g_{Na}(V)$ curve by 6–10 mV in the depolarizing direction, and a slowing of Na^+ activation by 61 ± 5%. As with acidic pH, there was no detectible effect of Zn^{++} on the time course of the Na^+ tail currents. The effects of external Zn^{++} were fully reversible upon returning to control conditions. These data confirm those of Gilly and Armstrong (1982),and as these authors concluded, surface charge effects do not appear to be the major origin of the Zn^{++}-induced changes in Na^+ channel gating.

In contrast to the effects of acidic external pH, a reduction of the internal pH to 5.5 produced no significant changes in intramembrane charge movements. In six axons where both ionic and asymmetry current data were available over the full voltage range, the average total charge movement at pH 5.5 was 98 ± 6% of the control value at pH 7.3; τ_{ON} was 0.94 ± 0.04 times control; and τ_{OFF} was 1.04 ± 0.06 times control.The kinetics of the Na^+ ionic currents (including tail currents) were similarly unchanged. The only consequence of acidic internal pH was a reduction in the maximum Na^+ conductance by 52 ± 8%, and a 6–8 mV shift of the $g_{Na}(V)$ curve in the hyperpolarizing direction.

A few experiments were also performed in which the internal pH was increased to 10.0 ± 0.05. Although $\bar{g}_{Na}$ was unchanged, as was the rate of Na^+ activation, inactivation of open Na^+ channels during a maintained depolarization was slowed by a factor of 2.92 ± 0.44 at membrane potentials between 0 and +40 mV, in agreement with other published data (Brodwick and Eaton, 1968; Carbone et al., 1981). There was no change in the time constant of the Na^+ tail currents compared with those measured in the same axons at neutral pH. Intramembrane charge movement was unaffected by internal alkaline pH.

DISCUSSION

This study demonstrates that, in *Myxicola*, the total amount of intramembrane charge available to move over the range −80 to +60 mV decreases by 20–25% when the external pH is reduced. The magnitude of the reduction in total charge is equal to the observed decrease in $\bar{g}_{Na}$. If the effect of acidic pH on the maximum Na^+ conductance were, in part, the result of a decrease in single channel conductance, it might have been expected that the pH-induced decrease in $\bar{g}_{Na}$ would have exceeded the decrease in Q_{Total}. In addition to these effects, decreases in external pH slow both the asymmetry current and the activation of g_{Na}, again by comparable amounts. In contrast to the effects on channel activation, acidic external pH does not alter the rate of intramembrane charge movement or the Na^+ tail currents following membrane repolarization. External alkalinity has no significant effect on either the ionic or gating currents.

The effects of external pH can be compared with those produced by Zn^{++}. As with acidic pH_o, 30 mM external Zn^{++} slows ON charge movement and Na^+ activation, but does not affect OFF charge movement or Na^+ tail currents. Whereas increased $[H^+]_o$ decreases both the maximum Na^+ conductance and total mobile charge, external Zn^{++} specifically decreases the Na^+ conductance, without affecting the total charge moved. The effects of Zn^{++} and external pH are not only distinct with respect to their influence on total charge movement, but they show another essential difference. External Zn^{++} slows Na^+ activation and concommitantly shifts the $g_{Na}(V)$ curve. Thus, the lack of an effect on Na^+ tail currents can be explained in terms of a simple two-state model in which Zn^{++} slows the forward transition without affecting the backward rate constants. However, acidic external pH does not seem to shift the $Q(V)$ curve to any appreciable extent (a 33% increase in τ_{ON} would require a $Q[V]$ shift of 6–8 mV in the simplest scheme and this is clearly in excess of the experimental uncertainty in Fig. 2). Thus, the fact the pH changes produce a specific increase in τ_{ON} may require a more complex model of channel activation.

It has been suggested (Armstrong and Gilly, 1979; Gilly and Armstrong, 1982) that Zn^{++} might reversibly bind (externally)to a negatively charged group that migrates inward upon depolarization, thus increasing the energy barrier for activation, without having access to the reverse charge movement following repolarization. Within this scheme, the effects ofexternal pH might, at least in part, be to decrease the magnitude of this gating charge. Thus, our results lend credence to the hypothesis that, in *Myxicola*, a portion of the initial outward gating current may consist of a negative charge moving inward. The reason for the

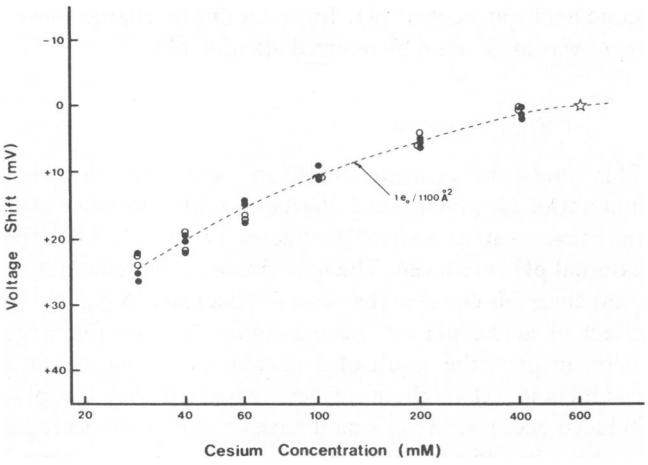

FIGURE 5 Dependence of the internal surface potential on ionic strength in Cs^+-dialyzed *Myxicola* axons. The membrane potential at which g_{Na} was 50% of $\bar{g}_{Na}$ (•), and the membrane potential at which 50% of the Na^+conductance is inactivated (○; points obtained from a standard h_∞ curve), were measured as a function of internal [Cs^+] over the range 40 to 600 mM (using isosmotic sucrose to replace Cs^+). The shifts of the two parameters are plotted relative to the values obtained with 600 mM Cs^+ as a function of internal [Cs^+]. The solid line was calculated from the Grahame equation assuming an internal surface charge density of 0.0009 $e_o/Å^2$ (equivalent to 1 e_o/1,100 $Å^2$). The procedures used were identical to those followed by Chandler et al. (1965) and in previous studies of external surface charge density (Schauf, 1975).

increase in charge movement at low pH_o in myelinated nerve (Neumcke et al., 1980) is uncertain. Perhaps the gating charges indeed have the opposite sign. However, it should be noted that recent experiments in frog muscle (Hahin and Campbell, 1982) have also revealed a decrease in total charge movement at pH 5.0 (as well as a slowing of the ON response).

Of course, instead of indicating the sign of the intramembrane gating charges, the changes produced by acidic external pH could be interpreted as arising from some allosteric change in the state of the channel proteins that reduce, and in part eliminate, their ability to move in response to an applied electric field. We obviously cannot eliminate this possibility, but it is difficult to imagine a direct experimental test of such a mechanism.

Within the framework of the first hypothesis, the absence of an effect of pH_i on gating currents and the concommitant insensitivity of the ionic kinetics to internal acidic conditions suggest that the gating charges may not move all the way to the internal membrane surface. However, one must be cautious in this interpretation, since it is possible that the dialysis technique can be overwhelmed by an active H^+ extruding mechanism, as has been observed in barnacle muscle (Boron et al., 1978). Although we could demonstrate using a longitudinal pH electrode (attached to the dialysis tubing) that the internal pH appeared to be controlled, the electrode was located some distance away from the membrane, and thus the pH at the membrane surface might have been reduced by less than we thought. The fact that internal alkaline pH alters Na^+ gating in *Myxicola* in the same manner as in squid axons (Brodwick and Eaton, 1978; Carbone et al., 1981) may not be relevant, since the barnacle pump is only activated by acidic pH.

However, some experimental evidence suggests that control of internal acidity might not have been a significant problem. In the experiments reported here, the Na^+, conductance was reduced at pH_i = 5.5 and there was a concommitant 6–8 mV shift in the $g_{Na}(V)$ curve along the voltage axis. Several years ago we carried out a series of studies designed to measure the internal surface charge density in *Myxicola* following the low ionic strength perfusion procedures of Chandler et al. (1965). These experiments yielded an internal charge density in the vicinity of 1 e_o/1,100$Å^2$ (Fig. 5). If we assume that most of the internal negative surface charge is titrated at an internal pH of 5.5, then a 6–8 mV shift of $g_{Na}(V)$ would be expected on the basis of the calculated internal surface potential, and this could be interpreted as evidence of adequate control of internal pH by the dialysis procedure.

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