Sensitivity of the Sodium and Potassium Channels of *Myxicola* Giant Axons to Changes in External pH

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**ABSTRACT** *Myxicola* giant axons were studied using standard voltage-clamp techniques in solutions whose pH values ranged from 3.9 to 10.2. Buffer concentrations of 50 mM or greater were necessary to demonstrate the full effect of pH. In acidic solutions the axon underwent a variable depolarization, and both the sodium and potassium conductances were reversibly depressed with approximate pKa's of 4.8 and 4.4, respectively. The voltage dependence of $G_s$ was only slightly altered by acidic conditions, whereas there occurred large shifts in $G_k$ along the voltage axis consistent with a substantial decrease in net negative surface charge in the vicinity of the K⁺ channels. The sodium and potassium activation rate constants were decreased by acidic conditions, but the results could not be described as a simple translation along the voltage axis.

**INTRODUCTION**

Alteration of the external pH basically has two distinct effects on membrane parameters. Various reports (Hille, 1968, 1973; Drouin and The, 1969; Mozhayeva and Naumov, 1970, 1972; Ehrenstein and Fishman, 1971; Stillman et al., 1971; Woodhull, 1973; Drouin and Neumcke, 1974; Shrager, 1974) have shown that under acidic conditions both the maximum sodium and potassium conductances are reversibly depressed in a manner more or less consistent with the titration of a weak acid. In frog nerve there is general agreement that the pKa for the sodium conductance is around 5.2, whereas for potassium the pKa is near 4.5. In squid the pKa for the sodium channel was found to be 6.5, while $G_k$ was nearly unaffected by pH values above 5 (Stillman et al., 1971). However, in crayfish Shrager (1974) found a pKa of 6.3 for the potassium conductance, and commented that the sodium conductance was relatively less sensitive to changes in pH.

In addition to this effect on channel conductance, a reduction in external pH also has a marked effect on the voltage dependence of a variety of membrane parameters, and here also the results depend on the preparation employed. In frog nerve both the sodium and potassium conductance-voltage relations are shifted in the depolarized direction as the solution becomes increasingly acidic, although the effect may be somewhat greater for the potassium channel (Hille,
1968; Drouin and Tey, 1969). The rate constants generally appear to be shifted by amounts comparable to the conductances. In crayfish axons, however, the effect of pH on the rate constant for potassium activation is much larger than the effect on the conductance, and combined with a relative insensitivity of the sodium channel to pH, this results in a nearly complete temporal separation of the sodium and potassium currents (Shrager, 1974). Detailed data have not been reported for squid axons.

Because of these variations in responsiveness to pH from one system to another, and the existence of complete data for both channels only in node, we felt it appropriate to carry out similar studies in voltage-clamped *Myxicola* giant axons. In addition, such studies tend to complement the previous investigation of membrane surface charge in this preparation (Schauf, 1975).

**METHODS**

*Myxicola* giant axons were voltage clamped and the resulting data analyzed by methods described in detail elsewhere (Binstock and Goldman, 1969; Goldman and Schauf, 1972, 1973; Schauf, 1973, 1975). Compensated feedback was used in all experiments. The reference artificial seawater (ASW) solution had the composition: 430 mM NaCl, 10 mM KCl, 10 mM CaCl₂, 50 mM MgCl₂. In a few cases Mg²⁺ free seawater having various Ca²⁺ concentrations was used. Temperature was maintained at 5.0 ± 0.5°C.

In the initial experiments 5 mM Tris maleate had been used as the buffer system. At this buffering capacity no changes in membrane parameters could be observed after changes in solution pH. A nearly comparable insensitivity was found at 10 mM buffer. Only when the concentration of Tris maleate (or Tris acetate in experiments at the most acidic pH values) was raised to 50 mM was the effect of pH readily apparent. A further increase in buffer concentration to 100 mM proved no more effective than 50 mM. Thus all the data reported here were obtained using 50 mM Tris maleate or Tris acetate with the pH adjusted by addition of HCl or NaOH just before an experiment, and remeasured immediately after its use on an axon. The high temperature coefficient of Tris was taken into account. It should be pointed out that there was no detectable effect on membrane parameters when the buffer concentration was increased from 5 to 50 mM at the standard pH of 7.8, only when buffer concentration was increased under acidic conditions.

In the majority of cases tetrodotoxin (TTX) was not routinely used to accurately obtain nonsodium currents. Rather peak transient and steady-state currents were corrected for the leak conductance measured during a 100-mV hyperpolarization. However, in those experiments involving measurements of the rate of potassium activation, or steady-state sodium inactivation, TTX (10⁻⁶ M) was used. In addition, a few experiments were done with TTX to ensure that there was in fact no significant difference in the conductance shifts measured using either method.

A solution of ASW at a pH of 7.8 ± 0.05 served as the control for all measurements. The effects of pH were usually complete within a few minutes, but we normally allowed the system to equilibrate for 15 min before recording data. Each experiment at an acidic or basic pH was bracketed by runs at the reference pH of 7.8. The maximum sodium and potassium conductances, as well as the half-maximal potentials (Vₙₕ and Vₖₕ) were averaged for the bracketing runs at 7.8, and the relative conductance or relative voltage shift calculated using these averaged values. Axons in which there were very large differences between the bracketing values were assumed to have deteriorated during the course of the experiment and the data were not used.
RESULTS

Fig. 1 illustrates the effect of an acidic solution on the membrane currents in *Myxicola*. Reducing the pH from 7.8 to 4.6 causes an inhibition of both the peak transient and steady-state currents, though the former is slightly more sensitive. There is a large decrease in the rate of activation of the potassium current which is better shown in Fig. 2 where $10^{-8}$ M TTX has been used to eliminate membrane sodium current. Although it may be difficult to discern from the records in Fig. 1, the time to peak inward current is significantly increased as well. However, in contrast to the results of Shrager (1974) on crayfish axons, the sodium and potassium currents do not become temporally separated under acidic conditions.

Membrane current-voltage relations at normal and acid pH are shown in Fig. 3, after correction for a linear leak. The sodium $I(V)$ curve is decreased with little sign of a voltage shift and with no significant change in the sodium equilibrium potential ($E_{Na}$). In contrast, the potassium $I(V)$ shows a marked translation in the depolarized direction along the voltage axis. In both cases reversibility is excellent.

It should be noted that in *Myxicola* axons a reduction of the solution pH below 6.0 causes an appreciable membrane depolarization (range 4–12 mV), which is however completely reversible. The data show a large amount of scatter, however, and it is impossible to demonstrate a monotonic pH dependence of membrane potential.

**FIGURE 1.** Voltage-clamp currents recorded in ASW at a pH 7.8 (top) and 4.6 (bottom) in response to depolarizations of 40, 50, 60, 70, 80, 90, and 100 mV from a holding potential of $-60$ mV. Current and time scales are 0.3 mA/cm² and 2.0 ms, respectively. Temperature 5°C.
Effects of pH on Channel Conductance

In Fig. 4 we have plotted the maximum sodium and potassium conductances as a function of solution pH, normalized to the conductances observed at the reference pH of 7.8. Data are only shown over the range 3.9–7.8, but were obtained up to a pH of 10.2. When we averaged the values of relative conductance at pH values above 7.8 we obtained 1.01 ± 0.03 for potassium (± SEM) and 1.03 ± 0.04 for sodium with no evidence for any systematic dependence on pH (six experiments). The solid lines are calculated from the dissociation of a weak acid with a pKa of 4.8 for \( G_{Na} \) and 4.4 for \( G_K \).

Although the solid lines more or less describe the pH dependence of the maximum channel conductance, there seems to be some systematic deviation. Points at pH values larger than the pKa are below the line, while those at a pH more acidic than the pKa are above. This deviation could be due to the existence of a finite range of dissociation constants near the assumed pKa. Measurements below pH 3.9 could help to clarify this but unfortunately more acidic solutions begin to cause very large depolarizations and the decreases in membrane conductances tend to be poorly reversible.

Effects of pH on Membrane Surface Charge

In addition to decreasing the maximum sodium and potassium conductances, acidic solutions also alter their voltage dependence. However, in this case the differences between the effects of pH on \( G_{Na} \) and \( G_K \) is striking. Fig. 5 shows normalized conductance-voltage data for a typical experiment in which the pH
was reduced from 7.8 to 4.2. $G_N(V)$ is shifted by only 4 mV in the depolarized direction, while the shift in $G_K(V)$ is approximately 24 mV (note the difference in scale on the ordinate). The presence of two points at each potential for the pH 7.8 solution is due to the fact that we have shown computations for the bracketing runs to indicate the magnitude of experimental uncertainty.

![Graph showing current-voltage relations for an axon at pH 7.8 and 5.5. Data were leak corrected assuming a constant leak conductance equal to that measured using a 100-mV hyperpolarizing clamp step. Solid symbols were obtained both before and after exposure to the acidic solution. Holding potential was −65 mV. Temperature 5°C.]

Fig. 6 shows voltage shift data pooled from eight experiments. The sodium conductance is relatively insensitive to pH, shifting by little more than 1 mV per pH unit. In contrast, the potassium conductance is shifted by 4-mV/pH unit in the range 6.5–10.2 and by 9-mV/pH unit below pH 6.5. This difference in the pH sensitivity of $G_K(V)$ is clearly outside the limits of experimental error, and is similar to experiments of Hille (1968) and Drouin and Neumcke (1974) on the sodium conductance in frog nerve.
FIGURE 4. Relative sodium and potassium conductances as a function of solution pH. Unnormalized $G_{Na}(V)$ and $G_{K}(V)$ curves similar to those shown in Fig. 5 were constructed for the data in each solution and the maximum values determined. The conductances at a particular pH are expressed as a fraction of the average of the maximum conductances at pH 7.8 before and after exposure to acidic solutions. The solid lines are calculated assuming the dissociation of a single weak acid with the indicated pK$_a$. Data pooled from eight axons.

FIGURE 5. Relative sodium and potassium conductances as a function of membrane potential at pH 7.8 and 4.2. Unnormalized values of $G_{Na}(V)$ and $G_{K}(V)$ were calculated by the relation $G(V) = I(V-E_i)$ and were subsequently divided by the maximum conductances. Note the difference in scale on the voltage axis. The solid line was drawn by eye to fit the data at pH 7.8 (solid symbols) before and after exposure to acidic conditions, and then translated by 4 and 24 mV to fit the data for $G_{Na}(V)$ and $G_{K}(V)$ at pH 4.2. Holding potential -70 mV. Temperature 5°C.
We should point out that the potentials at which the sodium and potassium conductances reach their half-maximum values, $V_{\frac{1}{2}}^{Na}$ and $V_{\frac{1}{2}}^{K}$, are quite consistent from axon to axon under constant experimental conditions. For 10 axons at pH 7.8, $V_{\frac{1}{2}}^{Na}$ averaged $-21.3 \pm 2.4$ mV and $V_{\frac{1}{2}}^{K}$ was $-3.4 \pm 2.2$ mV (± SEM).

These results suggest that although the surface charge densities in the vicinity of the sodium and potassium channels are comparable at pH 7.8 (Schauf, 1975), the charges near the sodium channel are not very titratable over the range 3.9–10.2, while those near the potassium channel titrate readily. In order to provide further confirmation of this we attempted to use changes in $[Ca^{++}]$ to estimate membrane surface charge density under acidic conditions in three experiments.

Translations in $G_{Na}(V)$ and $G_{K}(V)$ produced by increases in $[Ca^{++}]$ from 10 to 100 mM at pH 7.8 agreed with the calculations based on the surface charge density of $-0.013$ charges/A² determined previously in more extensive experiments (Schauf, 1975). When the experiment was repeated at pH 5.0, $G_{Na}(V)$ was shifted by amounts only slightly smaller than the shift at pH 7.8. However, the translation of $G_{K}(V)$ was 5–6 mV smaller than that measured at pH 7.8. The data are not sufficiently extensive to provide an accurate value of surface charge density, but assuming that there is no binding of Ca⁺⁺ (Schauf, 1975), the surface charge in the vicinity of the K⁺ channel seems to have been reduced to approximately 0.007 charges/A² at pH 5.0. This value is quite close to that which could be obtained from the calculated surface potential at 60 mM divalent ion concentration, pH 7.8, and the assumption that the data in Fig. 6 simply represent changes in this surface potential. Thus the entire set of data seems completely self-consistent.

Effects of pH on Membrane Rate Constants

Figs. 7 and 8 illustrate the effects of acidic solutions on the time to peak sodium current and time to half-maximum potassium current, respectively. In the
Figure 7. Time to peak inward current as a function of membrane potential in ASW at pH 7.8 and 5.5. The solid symbols were obtained during several bracketing runs at pH 7.8 and indicate the magnitude of the experimental error. Holding potential -65 mV. Temperature 5°C.

Figure 8. Time to half-maximum steady-state current as a function of membrane potential at pH 7.8 and pH 4.6. Measurements were made in the presence of 10⁻⁶ M TTX. The solid symbols were obtained during bracketing runs at pH 7.8. Holding potential -65 mV. Temperature 5°C.
experiment shown in Fig. 7 the shift in $G_{Na}(V)$ was only 1 mV, nevertheless there are substantial increases in time to peak. The data are also not particularly well described by a simple voltage shift, rather the effect is small for negative potentials, and becomes appreciable beyond 0 mV. The effects on the potassium kinetics are even more striking where for this axon the shift in $G_{K}(V)$ was 18 mV. For positive potentials there is at least a shift of 40 mV in the kinetics, but again the effect is not as large at negative potentials so that a simple translation cannot fit all the data. (Again note the duplication of data at pH 7.8 as an indication of experimental error.) These data resemble the behavior reported by Shrager (1974) on crayfish axons, though detailed comparison is difficult, and suggests another, more complex, effect of pH on excitable channels.

Effects of pH on Sodium Inactivation

In several experiments steady-state sodium inactivation curves were measured using large test pulses. Protocols and procedures were similar to those described elsewhere (Goldman and Schauf, 1972). When the axons were exposed to solutions with a pH in the range 4.5–5.0, no significant effect was seen. The steady-state inactivation curve was translated in the depolarized direction by 1–4 mV, amounts comparable to the magnitude of the pH effect on $G_{Na}(V)$.

Discussion

In *Myxicola* axons reducing the solution pH from neutrality to acidic values has three different effects. The maximum conductances for both the Na$^+$ and K$^+$ channels is reduced in a manner nearly consistent with the titration of a single weak acid (pK$^*_a$'s of 4.8 and 4.4 for $G_{Na}$ and $G_{K}$, respectively). The membrane surface charge density in the vicinity of the K$^+$ channel is reduced, but its value near the Na$^+$ channel remains relatively constant. Finally, there are significant effects of acidic conditions on the activation processes in both channels beyond those expected on the basis of the alteration in surface potential.

Certain aspects of this behavior are seen in other systems. The relatively large effect of pH on the rate of potassium activation compared to the effect on $G_{K}(V)$ is similar to Shrager's (1974) findings using crayfish. Although the pK$^*_a$ for the inhibition of $G_{K}$ is larger (approximately 6.3), the shift of $G_{K}(V)$ of 25 mV for a reduction in pH from 7.5 to 5.8 is not very much larger than that seen in *Myxicola* over the pH range 4–6. *Myxicola* differs from crayfish primarily in the absolute value of the pK$^*_a$ for potassium, and in the high sensitivity of $G_{Na}$ to acidic conditions.

In myelinated nerve (Hille, 1968, 1973; Drouin and The, 1969; Mozhayeva and Naumov, 1970, 1972; Woodhull, 1973; Drouin and Neumcke, 1974) the sodium conductance is also more sensitive to acidic conditions than $G_{K}$. The pK$^*_a$ for $G_{Na}$ is generally found to be 5.2 (but see Drouin and Neumcke, 1974), slightly higher than the value in *Myxicola*, while the pK$^*_a$ for $G_{K}$ is quite close to the value of 4.4 determined in these experiments. Where the systems differ, however, is in the way in which the voltage dependence of the ionic conductances depends on pH. In node both the sodium (Hille, 1968; Drouin and The, 1969; Drouin and Neumcke, 1974) and potassium (Mozhayeva and Naumov, 1970, 1972) conduct-
ances are shifted in acidic solutions, whereas in *Myxicola* $G_{\text{Na}}(V)$ is relatively insensitive to pH. It is interesting to observe that the relative effects of pH on $G_{\text{Na}}(V)$ and $G_{\text{K}}(V)$ may differ for *R. pipiens* (Hille, 1968) and *R. esculenta* (Drouin and The, 1969). Finally, Mozhayeva and Naumov (1970, 1972) report that $G_{\text{K}}(V)$ in frog nerve exhibits large shifts consistent with there being a second titratable group with a $pK_a$ near 9.2. No evidence for the presence of a specific titratable group with $pK_a < 10.2$ can be found in *Myxicola*.

Woodhull (1973) has observed in node yet another effect of acidic solutions. At a pH of 5, $G_{\text{Na}}(V)$ was clearly changed in shape from that at pH 7 in that it did not quickly reach a plateau at positive potentials, but showed a continuing, gradual increase. This was interpreted as a voltage-dependent block of Na$^+$ channels by H$^+$. Such behavior does not seem to consistently exist in *Myxicola* axons. In Fig. 5, for example, the slight deviation of the $G_{\text{Na}}(V)$ curve at pH 4.2 from a perfect translation is much less than in Woodhull's data, and was not clearly seen in many cases. However, the data are certainly not compelling since the major effect in node occurs at potentials where membrane potassium currents are large, and we did not carry out experiments using TEA$^+$ internally.

The $pK_a$ for a carboxyl side chain in a protein molecule is given by Tanford (1961) as 4.7. Presumably carboxyl groups in slightly different local environments would have dissociation constants which differed slightly from one another, possibly introducing some “splay” in the titration curve. The data in *Myxicola* would certainly be consistent with the presence of such a dissociable group in the sodium channel as has been argued for the node of Ranvier (Hille, 1975).

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