Altered Sodium and Gating Current Kinetics in Frog Skeletal Muscle Caused by Low External pH

DONALD T. CAMPBELL and RICHARD HAHIN

From the Department of Physiology and Biophysics, School of Medicine, University of Iowa, Iowa City, Iowa 52242

ABSTRACT The effect of low pH on the kinetics of Na channel ionic and gating currents was studied in frog skeletal muscle fibers. Lowering external pH from 7.4 to 5.0 slows the time course of Na current consistent with about a +25-mV shift in the voltage dependence of activation and inactivation time constants. Similar shifts in voltage dependence adequately describe the effects of low pH on the tail current time constant (+23.3 mV) and the gating charge vs. voltage relationship (+22.1 mV). A significantly smaller shift of +13.3 mV described the effect of pH 5.0 solution on the voltage dependence of steady state inactivation. Changes in the time course of gating current at low pH were complex and could not be described as a shift in voltage dependence. τ_g, the time constant that describes the time course of the major component of gating charge movement, was slowed in pH 5.0 solution by a factor of ~3.5 for potentials from −60 to +45 mV. We conclude that the effects of low pH on Na channel gating cannot be attributed simply to a change in surface potential. Therefore, although it may be appropriate to describe the effect of low pH on some Na channel kinetic properties as a "shift" in voltage dependence, it is not appropriate to interpret such shifts as a measure of changes in surface potential. The maximum gating charge elicited from a holding potential of −150 mV was little affected by low pH. In 12 fibers, the gating charge measured at +45 mV in pH 5.0 solution was 91 ± 7% of the peak charge determined at pH 7.4. This implies that protons exert their effects on Na channel kinetics without altering the charge on the portion of the channel structure that moves through the membrane field during gating, or that they unbind before a significant fraction of the field is traversed.

INTRODUCTION

Low pH and elevated divalent cation concentration alter the kinetics of Na channels in nerve and muscle (Frankenhaeuser and Hodgkin, 1957; Hille, 1968; Woodhull, 1973; Drouin and Neumcke, 1974; Hille et al., 1975; Begenisich,

1975; Schauf and Davis, 1976; Campbell and Hille, 1976; Carbone et al., 1978; Neumcke et al., 1980; Schauf, 1983; Hahin and Campbell, 1983). Typically, these altered kinetics have been described as a shift in the voltage dependence of channel gating. To account for such shifts, it has been suggested that both divalent ions and protons act by altering the surface potential that arises from fixed negative charges on the membrane surface (Frankenhaeuser and Hodgkin, 1957; Hille, 1968; Woodhull, 1973; Drouin and Neumcke, 1974; Hille et al., 1975). In a previous paper (Hahin and Campbell, 1983), we reported that elevated Ca causes identical shifts in eight parameters describing the kinetics of Na ionic and gating currents in frog muscle, results that are entirely consistent with the surface charge hypothesis. In the present paper, we report the effects of low pH on the kinetics of Na ionic and gating currents. Although we find that many of the kinetic effects of low pH can be described as shifts in voltage dependence, we also find more complicated kinetic changes that cannot be attributed to such shifts.

In addition to altering the kinetics of Na channel gating, it has variously been reported that lowering external pH increases or decreases the total gating charge. In frog myelinated axon, Neumcke et al. (1980) reported an increase in total gating charge. In contrast to this result, Schauf (1983) reported that lowering external pH decreases the total gating charge in *Myxicola* giant axon. In experiments designed to eliminate the complicating effects of charge immobilization on the maximum charge determined at normal and low pH, we find little change in the total gating charge in frog skeletal muscle.

METHODS

Single fibers from semitendinosus muscles of large (11–17 cm) bullfrogs (*Rana catesbiana*) were studied under voltage-clamp conditions using the vaseline-gap method (Hille and Campbell, 1976). Data acquisition and analysis were as described in Campbell (1983) and Hahin and Campbell (1983). Briefly, membrane current was measured with a current-to-voltage converter, filtered with a four-pole Bessel filter at 30–50 kHz, amplified, filtered again at the same frequency and digitized by a 12-bit A/D converter, and stored on floppy diskettes by a laboratory minicomputer. The preliminary subtraction of linear leakage and capacity currents was accomplished using an analog transient generator, which permitted the residual currents to be recorded at high gain without exceeding the voltage range of the A/D converter. The final subtraction of linear components of leakage and capacity currents was accomplished digitally. Linear capacity and leakage currents were recorded for control voltage steps between −180 and −150 mV. These control currents were scaled appropriately and then subtracted from test currents, leaving only nonlinear ionic and gating currents (Campbell, 1983). To improve the signal-to-noise ratio in gating current experiments, 8–32 sets of records were averaged.

Series resistance was estimated from the "hop" in voltage elicited by a sudden step of current. This series resistance was typically in the range of 1–2 $\Omega \cdot cm^2$ and was compensated electronically. In the standard vaseline-gap preparation, only a thin film of fluid bathes the upper surface of the membrane in the artificial node, resulting in a small area of membrane that has a higher and variable resistance in series with it. To eliminate this source of error, we routinely applied a small flap of plastic film (Saran Wrap, Dow Chemical Corp., Indianapolis, IN) across the artificial node, which by capillarity brought the solution level well up over the top of the fiber.

Low pH causes significant block of Na channels. To minimize the disparity in peak ionic currents between the test and control solutions, and thereby minimize possible errors from residual uncompensated series resistance, the control solution for the ionic current experiments generally contained one-half the normal Na concentration. In several experiments, full Na Ringer was used for one of the pH 7.0 bracketing controls and 1/2 Na for the other. In these experiments, the kinetics determined from the two different control records were typically in good agreement.

The volume of the chamber pool containing the artificial node was 0.2 ml. Solution changes consisted of flushing 30–50 vol of the new solution through the pool in 2–4 stages, with about a 30-s pause between each stage. Following the solution change, analog leak compensation was readjusted and 1–2 min was allowed for the temperature to equilibrate. Measurements were typically made 3–5 min after the first introduction of new solution.

Except where noted, the holding potential was set at −150 mV in order to eliminate the effects of long-term inactivation and charge immobilization. From this holding potential, the entire charge vs. voltage relationship could be determined using depolarizing test steps (Campbell, 1983). The control pulses used for the digital subtraction of leak and capacity currents were depolarizing steps from −180 to −150 mV. At pH 7.4, these hyperpolarized potentials were well tolerated by most preparations. At pH 5.0, however, deterioration of preparations seemed to be greatly accelerated by holding at −150 mV. This deterioration, signaled by an abrupt increase in leakage current, was cause for ending the experiment. Frequently this occurred after only two or three changes to low pH solution and for this reason it was not practical to make measurements of more than one or two kinetic parameters in most fibers.

Integration of Gating Current

Integration of gating current records requires that the appropriate baseline be chosen. Horizontal or sloping baselines were fitted to the current records as illustrated in Fig. 3 of Campbell (1983). For control currents obtained at pH 7.4, this correction is slight. However, at low pH, where the time course of gating current is greatly slowed, this correction makes a greater difference in the resulting integral. Long test pulses (13–20 ms) were used so that the final slope of the baseline could be more accurately determined. We also sought to minimize the difficulty of choosing baselines by working at relatively elevated temperatures in order to speed the gating current decay, thereby increasing the peak current and shortening the necessary integration interval. Unfortunately, the size and slope of the nonlinear "pedestal" currents was also increased at higher temperatures. Temperatures between 13 and 15°C provided a reasonable trade-off between these conflicting effects. In addition to these measures, it was still necessary to select fibers with very little pedestal. Thus, although it was nearly always possible to accurately integrate the pH 7.4 records, only about half of the fibers had sufficiently small and flat pedestal currents for us to be confident in the integrations of records obtained for steps between −60 and −15 mV at pH 5.0.

Solutions

The 1/2 Na Ringer contained (in mM) 55 NaCl, 55 tetramethylammonium (TMA)-Br, 5 CsCl, 2 CaCl$_2$, and 10 HEPES adjusted to pH 7.4 with Tris base. This solution was used as the control for most of the ionic current experiments, including all of those illustrated. The full Na control solution that was used in a few experiments for one of the bracketing controls was identical except that it contained 110 NaCl and 0 TMA-Br. The low pH solution for ionic current experiments contained 110 NaCl, 5 CsCl, 2 CaCl$_2$, and 10 MES

adjusted to pH 5.0 with Tris base. A similar low pH solution adjusted to pH 6.0 was used in a few early experiments. Gating current solutions contained 110 tetraethylammonium (TEA)-Br, 5 CsCl, 2 CaCl$_2$, and 10 HEPES (pH 7.4) or 10 MES (pH 5.0 or 6.0). In addition, gating current solutions contained either 1 (pH 7.4) or 4 μM (pH 5 and 6) tetrodotoxin (TTX) to block Na channels. More TTX was used in low pH solutions since low pH decreases the potency of TTX (Ulbricht and Wagner, 1975). The ends of the muscle fiber were cut in a solution containing 115 CsF and 5 NaF buffered to pH 7.4 with 10 HEPES.

RESULTS

Low External pH Shifts Ionic Current Kinetics

Fig. 1 illustrates ionic currents recorded at normal and low pH in a single muscle fiber. On the left, control currents recorded for steps to −45 and −15 mV are shown superimposed on test currents recorded in pH 5.0 solution for steps 30 mV more depolarized. To facilitate a visual comparison of the time courses, the pH 5.0 records have been scaled so that their peaks approximately coincide with the peaks of the control records. The close coincidence of the control and the scaled pH 5.0 records for the two sets of membrane potential confirms previous reports of an apparent shift in the voltage dependence of Na channel gating at
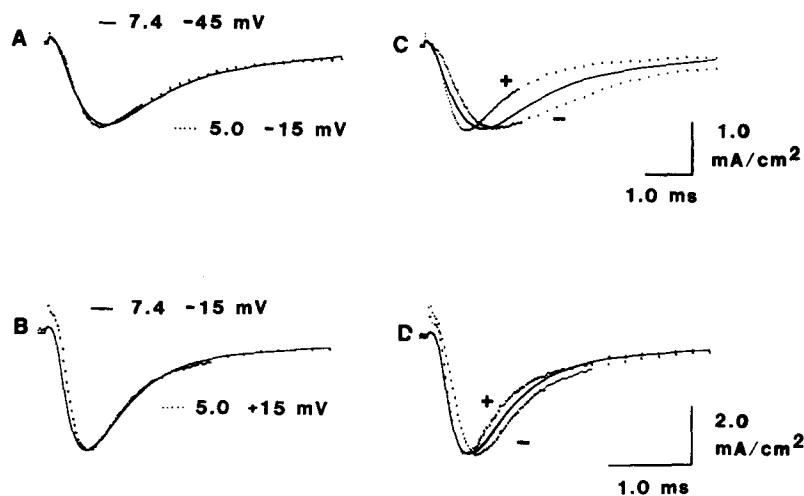

FIGURE 1. Low pH shifts the voltage dependence of Na current activation and inactivation. (A) The solid trace is a control Na current recorded in a single muscle fiber at pH 7.4 for a step depolarization to −45 mV. The dotted trace was recorded in the same fiber, for a step to −15 mV, after changing to pH 5.0 solution. The pH 5.0 record has been scaled so that its peak approximately coincides with the peak of the control. (B) A similar pair of records elicited by steps to −15 (control) and +15 (pH 5.0) mV in the same fiber. (C) The same control record shown in A is displayed superimposed on pH 5.0 records elicited by test potentials of −7.5 mV (+) and −22.5 mV (−). (D) The control record of B is displayed superimposed on pH 5.0 records elicited by steps to +22.5 mV (+) and +7.5 mV (−). Current calibrations are for the control records. Fiber 209; 12°C; holding potential, −150 mV.

low pH. The sets of records on the right side of Fig. 1 illustrate the sensitivity of the "fits" by demonstrating that small increases or decreases in the "shifted" voltage result in substantial mismatches in ionic current time course.

The shift in ionic current kinetics that is qualitatively apparent in Fig. 1 is illustrated quantitatively in a different fiber in Fig. 2. Frame A shows time constants of inactivation fitted to the falling phase of ionic current. The curve drawn through the pH 7.4 measurements has been drawn again after shifting it 26 mV to the right, where it provides a reasonable fit to the time constants determined at pH 5.0. Fig. 2B shows steady state inactivation curves determined in the same fiber. Again, the curves drawn through the points are identical, except that in this case the curve through the pH 5.0 points was shifted 16 mV in the depolarizing direction. The greater shift in the voltage dependence of τ_h was a consistent finding. In five determinations from four fibers, h_∞ was shifted an average of 13.0 ± 2.5 mV (SD). In four fibers, the average shift in the voltage dependence of τ_h, determined in the fashion illustrated in Fig. 2A, was 25.0 ± 3.6 mV. In three fibers in which both h_∞ and τ_h were measured, the average shifts were 13.5 ± 2.6 mV for h_∞ and 26.7 ± 1.5 mV for τ_h.
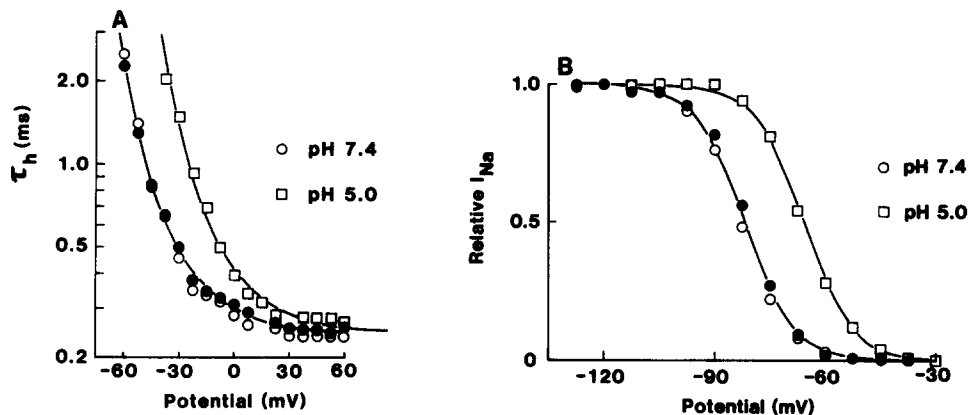

FIGURE 2.   Low pH shifts the voltage dependence of inactivation. (A) τ_h obtained by fitting a single exponential to the falling phase of Na current. An arbitrary curve was drawn by eye through the time constants obtained from currents recorded in the control pH 7.4 solution before (closed circles) and after (open circles) bathing the fiber in the pH 5.0 test solution. The same curve shifted 26 mV in the depolarizing direction is drawn through the time constants obtained from currents recorded in pH 5.0 solution (squares). (B) Steady state inactivation determined from the same fiber. The relative amplitude of the peak Na current elicited by a step to 0 mV is plotted against the variable potential during 100-ms prepulses. As in A, the symbols represent the values measured before (closed circles), after (open circles), and during (squares) bathing the fiber in pH 5.0 solution. The curves are computed according to the function:

$$h_\infty = 1/(1 + \exp[(E - E_h)/k]),$$

with k = 6.7 mV, E_h = −81 mV (control), and −65 mV (pH 5.0). Fiber 211; 13°C; holding potential, −150 mV.

In Fig. 2, the before-control points taken prior to exposing the fiber to the pH 5.0 solution are represented by the filled circles, and the after-control points are represented by the open circles. In both frames, the before and after controls are nearly identical over the entire voltage range, which illustrates the reversibility of the effects of low pH on ionic current kinetics. In many experiments, changing to the low pH solution seemed to cause drastic changes in channel kinetics that could not be reversed by returning the fiber to the control pH 7.4 Ringer. However, such irreversible effects were typically accompanied by large increases in leakage current and thus appeared to be due to nonspecific damage to the preparation. In some early experiments, currents measured in the before- and after-control runs exhibited moderately different kinetics in fibers that otherwise seemed healthy. This appeared to result from allowing an insufficient period at the beginning of the experiment for the preparation to stabilize. These fibers have not been included in the averages. When fibers were allowed a 30–40-min "settling in" period (see Campbell, 1983), the effects of low pH were found to be reversible.

Low pH Speeds Tail Current Decay

Fig. 3 shows time constants obtained from Na current tails recorded in a muscle fiber bathed in Ringer at pH 7.4 and 5.0. The Na conductance was first activated by a prepulse to +90 mV, and then at the peak of conductance the membrane potential was stepped to various test potentials. An arbitrary curve was drawn by eye through the control points. The same curve shifted 28 mV in the depolarizing direction provides a good fit to tail currents elicited by test potentials below about −60 mV. In four fibers, the average shift determined by this method was 23.3 ± 3.6 mV.

Low pH Alters Gating Current Kinetics and Shifts Q vs. V

If, as hypothesized, gating currents represent the movement of the Na channel gating mechanism, then it is reasonable to look for alterations of gating current kinetics consistent with the alteration of ionic current kinetics at low external pH. We have previously shown that elevated external divalent cations alter ionic and gating current kinetics in a parallel manner, as if there were a simple shift in the voltage sensed by the channel gating mechanism (Hahin and Campbell, 1983). Fig. 4 shows gating currents recorded at pH 7.4 and 5.0, for steps to three different test potentials from a holding potential of −150 mV. It can be seen that at each test potential the gating current recorded in pH 5.0 solution has a smaller amplitude and slower rate of decay than does the control trace recorded at pH 7.4.

Fig. 5 shows charge vs. voltage (Q vs. V) relationships obtained by integrating the gating currents recorded in the experiment of Fig. 4. In order to eliminate the effects of low pH on the amount of charge immobilized at the holding potential (discussed below), fibers were held at −150 mV. The smooth curve drawn through the pH 7.4 points represents a two-state Boltzmann distribution. The same curve shifted 27 mV in the depolarizing direction provides a good fit to the points obtained at pH 5.0. In nine fibers, the average shift in the midpoint of Q vs. V was 22.1 ± 4.7 (SD) mV.
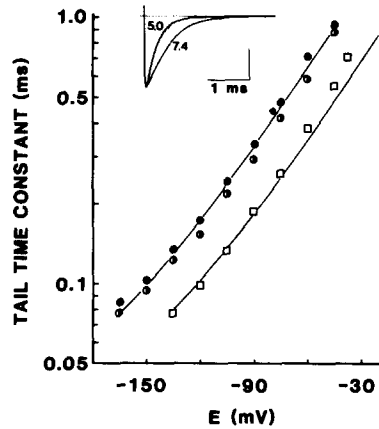
FIGURE 3.   Low pH shifts the voltage dependence of the tail current time constant. 0.5-ms prepulses to +90 mV to activate the Na conductance were followed by test steps to varying potentials. The resulting current tails decay in an approximately exponential fashion at a rate that depends on the test potential. The inset shows a pair of such tail currents recorded at pH 7.4 and pH 5.0, for a test potential of −75 mV. The pH 5.0 record has been scaled so that its peak coincides with the peak of the control. The symbols plot time constants determined from single-exponential fits to tails measured in the same fiber, against the potential during the test step. An arbitrary curve is drawn through the control time constants determined before (closed circles) and after (half-closed circles) bathing the fiber in pH 5.0 solution. The same curve, shifted 28 mV in the depolarizing direction, is drawn through points determined at pH 5.0. The vertical calibration for the inset is 2.0 mA/cm^2 for the pH 7.4 trace and 0.93 mA/cm^2 for the pH 5.0 trace. Fiber 171; 3.5°C; holding potential, −120 mV.

It has been reported that low pH causes an increase in the maximum gating charge in frog myelinated axons (Neumcke et al., 1980) and a decrease in the maximum charge in invertebrate giant axons (Schauf, 1983). Fig. 5 demonstrates that in frog muscle, low pH has little effect on the maximum charge. In this experiment, the charge determined for a step to +45 mV at pH 5.0 was 96% of the average of the maximum charge determined in the two control runs. In 12 fibers, the charge measured at +45 mV in pH 5.0 solution was 91 ± 7% of the maximum charge measured at pH 7.4. In three fibers studied at pH 6.0, the maximum charge was 97 ± 4% of the control charge. For several reasons, we think it likely that the charge determined at +45 mV in pH 5.0 solution slightly underestimates the maximum charge. First, because of the shift in Q vs. V, it appears that the gating charge at pH 5.0 has not saturated at +45 mV. Therefore, to measure the true maximum charge, it would be necessary to step to potentials more positive than +45 mV. Unfortunately, in most preparations, time-dependent ionic currents are activated for voltage steps above about +30 mV (Campbell, 1983). These ionic currents make an accurate determination of gating charge at potentials above +45 mV difficult. In addition, such currents may cause the charge at +45 mV in pH 5.0 solution to be underestimated. Thus, despite the observation that the charge at +45 mV is slightly smaller at pH 5.0 than at pH
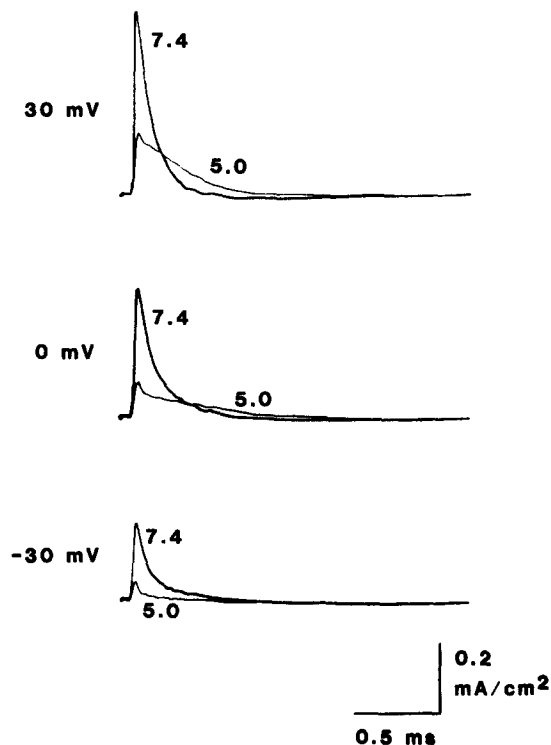
FIGURE 4. Low pH slows the time course of gating current. Gating currents recorded in a single fiber bathed in pH 7.4 solution are shown superimposed on gating currents recorded in the same fiber at pH 5.0. Fiber 220; 15°C; holding potential, −150 mV.

7.4, we conclude that low pH does not greatly affect total Na channel gating charge.

Low pH Removes Charge Immobilization

Two previous studies (Neumcke et al., 1980; Schauf, 1983) have reported large changes in the maximum gating charge measured at low pH when holding potentials between −80 and −100 mV were used. As stated above, we have measured charge vs. voltage relationships and maximum charge from a holding potential of −150 mV. The purpose of this very negative holding potential was to eliminate the complicating effects of pH on charge immobilization. This is important, because at −90 mV, the normal resting potential for frog skeletal muscle, ~40% of the gating charge is "immobilized" (Campbell, 1983). Fig. 6 shows the effect of low pH on gating currents measured in frog muscle from such intermediate holding potentials. Frame A shows gating currents elicited by a 120-mV depolarization from −90 to +30 mV in a fiber bathed first in pH 7.4 solution and then in pH 5.0 solution. The pH 5.0 trace has a smaller amplitude and declines more slowly than the control current. The integrals of these records are shown in Fig. 6B. Although the pH 5.0 current is smaller in amplitude, it
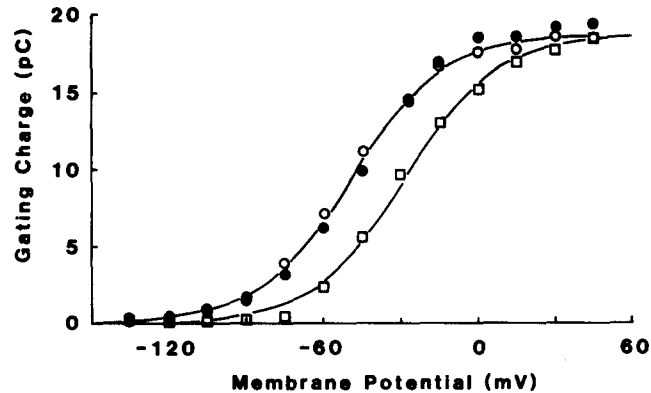
FIGURE 5.   Low pH shifts Q vs. V. The symbols represent the charge determined by integrating gating current records from the fiber illustrated in Fig. 4. The curve drawn through the pH 7.4 points (closed circles are before-control data, open circles are after-control data) represents a two-state Boltzmann distribution with a midpoint of -45 mV and for a charge with an effective valence of 1.35. The same curve with a midpoint of -18 mV is drawn through the charge determined at pH 5.0.
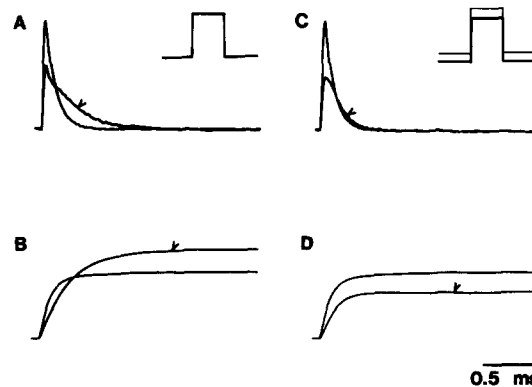

FIGURE 6.   Low pH shifts charge immobilization. (A) Current records obtained from a fiber bathed in pH 7.4 solution and pH 5.0 solution (arrow) are shown superimposed. The inset illustrates the point that the identical pulse protocol, a step to $+30$ mV from a holding potential of -90 mV, was used to elicit both gating currents. (B) Integrals of the gating current records shown in A. The slower pH 5.0 transient (arrow) carries 39.6 pC and the faster pH 7.4 transient carries 29.0 pC. (C) The same control gating current record from frame A is shown superimposed on a pH 5.0 gating current elicited by the offset pulse protocol illustrated in the inset. The same 120-mV depolarization was applied as a step from a holding potential of -65 mV to a test potential of $+55$ mV. The offset is close to the average shift in Q vs. V when determined from a holding potential of -150 mV. In this fiber, the Q vs. V relationship was shifted by $+31$ mV. (D) Integrals of the current records shown in C. Shifting the holding potential from -90 to -65 mV decreased the peak charge in pH 5.0 solution from 40.3 pC to 20.4 pC. The vertical calibration bar represents 0.2 mA/cm^2 for A and C, and 24 pC for B and D. Fiber 206; 13°C.

nonetheless carries 37% more charge. From a holding potential of −90 mV, the maximum charge measured in this preparation was 40.3 pC at pH 5.0 (+60 mV), 39% more than the 29.0 pC measured at pH 7.4 (+30 mV). When the holding potential was changed to −150 mV, the maximum charge in this fiber was 49.0 pC at pH 7.4 and 45.0 pC at pH 5.0. Thus, the total charge was not increased by the pH 5.0 solution. The increased charge seen at the −90 mV holding potential is due to two factors. First, at normal pH, ∼15% of the gating charge moves between −120 and −90 mV. For this reason, an increase of 10–15% in the charge moved from a holding potential of −90 mV is expected to arise from the 20–30-mV shift in the Q vs. V relationship at low pH. The remaining increase appears to be due to removal of immobilization at low pH. Thus, at pH 5.0, only ∼10% of the gating charge is immobilized at −90 mV, whereas at pH 7.4, ∼40% is immobilized at −90 mV.

The result illustrated in Fig. 6, C and D, suggests that the voltage dependence of charge immobilization is not shifted by as much as the Q vs. V relationship. In this fiber, the Q vs. V relationship measured from a holding potential of −150 mV was shifted by 31 mV at pH 5.0. The control current shown in Fig. 6C is the same pH 7.4 gating current record shown in Fig. 6A, elicited by a 120-mV depolarization from −90 to +30 mV. If the voltage dependence of charge immobilization was shifted by the same amount as the shift in the Q vs. V relationship, then at pH 5.0, a 120-mV depolarization from −59 mV should have elicited the same amount of charge. However, as shown in Fig. 6, C and D, a smaller shift of 25 mV (a 120-mV depolarization from −65 mV) results in 30% less charge than moved in the control. The shift of 25 mV was selected as a
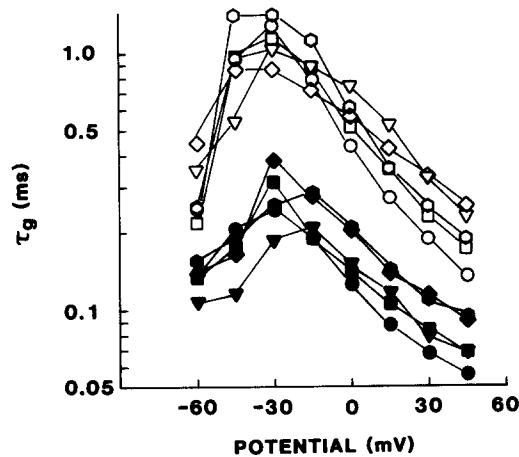

FIGURE 7. Low external pH slows the decay of gating current. The symbols represent τ_g, the slower of the two time constants determined by fitting the sum of two exponentials to the gating current (Campbell, 1983). Filled symbols were obtained from five different fibers bathed in the control pH 7.4 gating current solution. The corresponding open symbols were obtained from the same fibers bathed in pH 5.0 gating current solution. Temperatures, 13.2–18.7°C; holding potential, −150 mV.

reasonable approximation to the average shift of Q vs. V observed in all fibers. Linear interpolation between the 39% increase in charge seen at a holding potential of -90 mV and the 30% decrease in charge seen at a holding potential of -65 mV suggests that shifting the holding potential by about $+14$ mV (from -90 to -76 mV) would have approximately compensated for the effect of low pH on charge immobilization in this experiment.

Low pH Slows Gating Current

As seen in Fig. 4, the decay of gating current is slowed at low pH. In contrast to "shifts" in the voltage dependence of ionic current kinetics and of the Q vs. V relationship, Fig. 7 shows that the kinetics describing the time course of charge movement are not simply shifted in voltage. The sum of two exponentials was fitted to gating current traces as previously described (Campbell, 1983). In muscle, the time constant (τ_g) of the slower of these exponentials describes the movement of the major component of the gating charge. Fig. 7 shows τ_g determined from gating currents recorded from five fibers at pH 7.4 and pH 5.0. Over the potential range of -60 to $+45$ mV, the effect of lowering the pH from 7.4 to 5.0 is to increase τ_g by a factor of ~ 3.5.
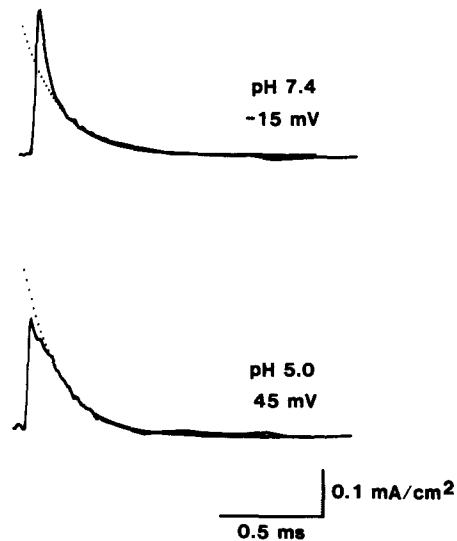

FIGURE 8. The effect of low external pH on gating current time course is complex. Displayed are gating current transients obtained from a fiber bathed first in the control pH 7.4 solution and then in the pH 5.0 test solution. At the two different pH's, traces were chosen on the basis of having similar τ_g's determined from fits as described in Fig. 7. Top: an exponential with a time constant of 200 μs (dotted trace) is shown superimposed on a gating current elicited by a step to -15 mV in the control pH 7.4 solution. Bottom: an exponential with a time constant of 187 μs is shown superimposed on a gating current elicited by a step to $+45$ mV when the same fiber was bathed in pH 5.0 solution. Although similar τ_g's result from the two-exponential fit, the time courses of the two current transients are quite different. Fiber 220; 15°C; holding potential, -150 mV.
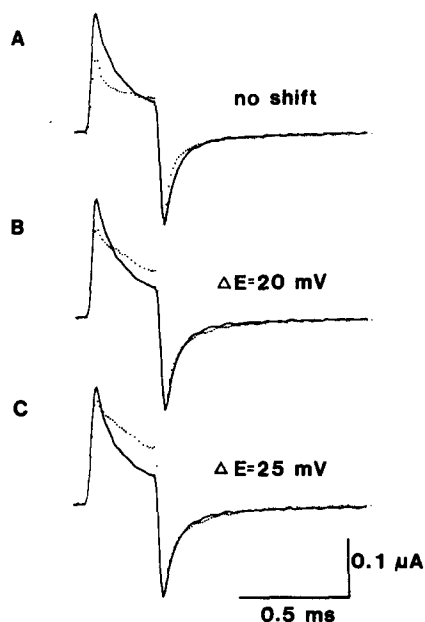
FIGURE 9. Low external pH speeds the decay of OFF gating current. The identical control current recorded for a step to 0 mV from a holding potential of −90 mV is shown three times, superimposed on test gating currents (dotted traces) elicited by the same 90-mV depolarization from three different holding potentials of −90, −70, and −65 mV. To permit a visual comparison of the time courses of OFF gating currents, the sizes of the pH 5.0 records have been scaled so that the peak OFF gating currents coincide with the peak of the control. Fiber 206; temperature, 13.2°C.

For test potentials above −30 mV, the τ_g vs. voltage relationships shown in Fig. 7 are roughly parallel. Thus, it is possible to find gating current pairs, recorded at pH 7.4 at one potential and at pH 5.0 at a more depolarized potential, that have similar τ_g's. Fig. 8 shows such a pair of records. Above, a single exponential with a time constant of 200 μs is shown superimposed on a gating current trace recorded for a voltage step of −15 mV in pH 7.4 solution. Below, an exponential with a time constant of 187 μs is shown superimposed on a gating current trace recorded in the same fiber for a step to +45 mV in pH 5.0 solution. Although the final decay of current occurs at nearly the same rate in both records, the kinetics at early times are quite different. The pH 7.4 record has a rapid early transient not seen in the pH 5.0 record. In short, the detailed kinetics of charge movement fail to exhibit a true shift in voltage dependence, even for potentials beyond about −30 mV, where the control τ_g vs. voltage curve at pH 7.4 can be "shifted" to the right to overlie the pH 5.0 curve.

Low pH Speeds OFF Gating Current

Fig. 9 shows three pairs of traces that illustrate the effect of low pH on the kinetics of OFF gating currents. In each pair, the solid trace is the same control

record obtained in pH 7.4 solution in response to a 90-mV step from a holding potential of −90 mV to 0 mV for a duration of 0.3 ms. Upon returning to the holding potential, an inward OFF gating current transient flows. The rate of decay of this OFF gating current is dependent on the OFF voltage, which in the case of the simple pulse protocol of this example is also the holding potential. The dotted traces are gating currents recorded in the same fiber at pH 5.0. In A, the holding and test potentials for the pH 5.0 record were the same as for the control. The decay of the OFF gating current recorded in pH 5.0 solution is significantly faster than that of the control current elicited by the same voltage protocol. The dotted traces in Fig. 9, B and C, are gating currents elicited by similar 90-mV steps from holding potentials of −70 and −65 mV to test potentials of +20 and +25 mV. Shifting the OFF voltage by 20–25 mV in the depolarizing direction slows the decay rate of the pH 5.0 OFF gating current to approximately that of the control OFF gating current. Similar results were obtained from two other fibers. Although complicated by recovery from immobilization, the decay of OFF gating currents at a holding potential of −150 mV is also faster at pH 5.0.

DISCUSSION

Previous studies on a variety of excitable cells have reported that low pH alters the kinetics of Na ionic (Hille, 1968; Woodhull, 1973; Hille et al., 1975; Drouin and Neumcke, 1974; Schauf and Davis, 1976; Campbell and Hille, 1976; Carbone et al., 1978; Pooler and Valenzeno, 1979; Courtney, 1979) and gating currents (Neumcke et al., 1980; Schauf, 1983). In most of these studies, one or two parameters that describe Na current kinetics were measured, often at several different pH's. We have chosen the alternative approach of characterizing the changes caused by a single value of low pH, in a wider range of Na ionic and gating current kinetic parameters. In agreement with most previous studies, we have found the voltage dependence of individual kinetic parameters to be shifted in the depolarizing direction by low external pH. We have found similar shifts of 25 mV for τ_h, 23.3 mV for the tail current time constant, and 22.1 mV for Q vs. V. These values are close to the 22-mV shift in the permeability vs. voltage relationship reported in frog node of Ranvier (Hille et al., 1975), and are also similar in magnitude to the changes in τ_m and τ_h found in squid giant axons (Carbone et al., 1978).

We found a smaller shift of ∼13 mV in the voltage dependence of steady state inactivation. Consistent with this observation is the similar small shift in charge immobilization. Previous studies on squid giant axon (Carbone et al., 1981) and frog node of Ranvier (Courtney, 1979) concluded that the voltage dependence of τ_h is not simply shifted. It is not possible to compare these results directly with ours, since the inactivation time course in the previous studies was measured in part (Carbone et al., 1981) or entirely (Courtney, 1979) using the two-pulse protocol of Hodgkin and Huxley (1952), whereas we determined τ_h from the decay of Na currents elicited by single voltage steps. Nevertheless, the failure of inactivation time constants measured with two pulses to exhibit a simple voltage shift is consistent with our conclusion (discussed below) that low pH exerts a complicated pharmacological effect on Na channel gating.

The Time Course of ON and OFF Gating Current

We find that the τ_g's of ON gating currents are slowed by a factor of ~3.5 at pH 5.0. This is somewhat more than the slowing reported by Neumcke et al. (1980) in frog node at pH 5.2 and considerably greater than the ~38% increase in the ON gating current time constant observed in *Myxicola* (Schauf, 1983). Rojas (1976) has reported that varying pH from 4.5 to 8.5 has no effect on ON gating current kinetics in squid giant axons. We find the decay of OFF gating currents to be faster at low pH. This finding also differs from previous results from squid (Rojas, 1976) and *Myxicola* (Schauf, 1983) giant axons in which low external pH does not affect OFF gating current. The relatively small effect of low pH on invertebrate axons may reflect species differences. Alternatively, the effect of low pH may be smaller for these invertebrate preparations because seawater has far greater ionic strength than does frog Ringer. The second possibility appears to be supported by the results of Hille et al. (1975), which show that the effect of low pH is considerably reduced when the divalent ion concentration is raised from 2 to 20 mM.

Do Shifts in Kinetics Imply Altered Surface Potential?

Shifts in the voltage dependence of channel kinetics at low pH have frequently been interpreted as being due to decreased surface potential resulting from the binding of protons to negative surface charges. However, there are difficulties with such a simple interpretation. As mentioned above, Courtney (1979) and Carbone et al. (1981) found the altered kinetics of inactivation to be inconsistent with a simple shift in potential. In addition, the altered kinetics of gating current are also inconsistent with such an interpretation. Consistent with previous results (Neumcke et al., 1980; Schauf, 1983), we find that low pH slows the dominant decay of the gating current transient at all potentials, an effect inconsistent with a simple shift. Moreover, gating currents are distorted to an even greater extent than the approximately three- to fourfold slowing suggested by Fig. 7. Fig. 8 demonstrates that when a pair of low pH and control gating currents (measured at different potentials) can be found which have a similar τ_g, their overall time courses are nevertheless quite different. One must conclude that low pH exerts a pharmacological effect on Na channel gating that cannot be simply accounted for by a change in surface potential. Gilly and Armstrong (1982) reached a similar conclusion for the effect of zinc on Na channel gating in squid axon. Although they found that the voltage dependence of some kinetic parameters could be described as shifted by external zinc, the shifts were different for different parameters as well as being accompanied by more complex kinetic changes.

It is possible that the effect of low pH on Na channel kinetics may be mediated partly by a change in surface potential. We have previously shown (Hahin and Campbell, 1983) that elevated external Ca causes a simple, identical shift in the voltage dependence of eight different kinetic parameters of Na and gating currents, which is consistent with the hypothesis that Ca acts entirely by decreasing surface charge. If this surface charge hypothesis is correct, then it seems likely that protons may also bind to these surface charges, thereby altering

surface potential. However, the more complicated pharmacological effects of low pH make it impossible to estimate the extent to which the surface potential sensed by the Na channel is altered at low pH. Such a determination will require a measure of surface potential that is independent of channel gating. Thus, although it may be appropriate to describe the effect of pH on Na channel kinetics as "shifts" in the voltage dependence of various kinetic parameters, it is not appropriate to interpret these shifts as a measure of changes in surface potential.

Maximum Charge at Low pH

At pH 5.0, we find the maximum charge to be 91 ± 7% of the control charge measured at pH 7.4. Although there may be a reduction of charge at low pH, we think that the decrease may instead represent a systematic underestimation of the maximum charge at low pH. The Q vs. V relationship is shifted in the depolarizing direction at low pH, requiring a stronger depolarization to elicit the maximum charge. In most muscle fibers, it appears that the Q vs. V at pH 5.0 has not yet saturated for steps to +45 mV. However, in most preparations, the nonlinear ionic currents activated at strong depolarizations limit the maximum useful depolarization to about +45 mV. Thus, the maximum charge that can be measured at low pH may be slightly smaller than the true saturating amount of charge. For example, if we shift the typical Boltzmann relationship fitted to the Q vs. V determined at pH 7.4 by the average 22-mV shift determined at pH 5.0, then the charge calculated at +45 mV is ~4% smaller than the saturating charge. In addition, the slowed time course of gating current at pH 5.0 makes the fitting of the baseline for integration subject to error. Comparison of pH 7.4 control records elicited for saturating potentials of +30 mV with control records elicited at +45 and +60 mV suggests that such errors also lead to a systematic underestimation of charge.

Our finding that low pH has little effect on maximum charge is the major difference between our results and previous results. Neumcke et al. (1980) reported a 46% increase in the gating charge of frog node at pH 5.2. They hypothesize that this increase may be due to protonation of the channel gating structure. According to this hypothesis, protons bind to a portion of the gate that is already positively charged at neutral pH. At the resting potential, most of this charge is presumed to be located near the inside of the membrane, and upon depolarization its outward movement is measured as gating current. Thus, protonation, by increasing the charge on this channel structure, would increase the maximum charge moved. On the other hand, Schauf (1983) has reported that gating charge in *Myxicola* giant axons is decreased by 23% at pH 5.5. He has also interpreted his results as evidence that low pH leads to protonation of the gating structure, which, however, he hypothesizes is negatively charged at neutral pH. At the resting potential, most of this negative charge is presumed to be located near the outside of the membrane. Upon depolarization, the inward movement of this negative charge would be measured as an outward gating current. Thus, by this theory, protonation would decrease the charge on the gating structure, decreasing the maximum charge moved.

Interpretation of these results is complicated by differences in method, as well as by differences in species and tissue. In particular, in both of the previous studies, holding potentials (−80 to −100 mV) were not sufficiently negative to remove all charge immobilization. At pH 7.0, depolarizing a frog muscle from a holding potential of −90 mV moves only about half of the total gating charge. Fig. 6 demonstrates that lowering external pH shifts the voltage dependence of immobilization, making more charge available to move at −90 mV. However, this additional charge does not represent an increase in the total gating charge since lowering the pH does not increase the charge elicited from a holding potential of −150 mV, a potential where no charge is immobilized at pH 7.4.

Neumcke et al. (1980) sought to correct for effects of low pH on charge immobilization by shifting the holding potential for fibers bathed in pH 5.2 solution. The amount of the shift was chosen to be equal to the shift in the permeability vs. voltage relationship obtained in previous ionic current experiments (Drouin and Neumcke, 1974). Our results suggest that for changes in pH it is not appropriate to equate shifts in voltage dependence of kinetic parameters with changes in surface potential, and thus it is possible that the shift applied by Neumcke et al. (1980) was not sufficient to compensate for the change in charge immobilized at pH 5.2. Another possibility is that with the 300 nM TTX used by Neumcke et al., not all Na channels have been blocked at low pH. Ulbricht and Wagner (1975) found that low pH affects the rates of binding and unbinding of TTX to Na channels. From the rates given in their Table II, we calculate that the K_D of toxin binding at pH 5.6 is between 22 and 34 nM, ~10 times the K_D at normal pH. Thus, at pH 5.6, 300 nM TTX will leave ~7–10% of the channels free of toxin. Although some of these channels will be blocked by protons, there may be significant contamination of the gating currents by ionic current. In the absence of external Na, this ionic current will be outward, contributing to the charge determined from the total asymmetric current.

The decreased gating charge observed by Schauf (1983) at low pH is also difficult to reconcile with our results. For positive displacements from a holding potential of −80 mV, he found a 23% decrease in charge at pH 5.5. The discrepancy seems large when this decrease is compared with the nearly 40% increase in charge at pH 5.0 that we measured from a holding potential of −90 mV. To determine to what extent this discrepancy represents a significant difference between the Na channels of frog and *Myxicola*, it would be useful for the experiments to be repeated in *Myxicola* using a protocol that eliminates the effects of charge immobilization.

Our results suggest that low pH does not alter Na channel kinetics by simply changing surface charge nor does low pH greatly alter the charge that moves during channel gating. Carbone et al. (1981) have suggested that low pH may act indirectly by altering membrane fluidity. Alternatively, protons may act by binding to a site on the channel that does not move through the membrane field during gating. It is also possible that protons exert their kinetic effects by directly binding to the channel gate, but by doing so without altering the amount of charge moved. For example, the proton binding may be an ion exchange reaction

that does not alter the charge on the gate, or the bound protons may unbind before the binding site moves through the field.

REFERENCES

Begenisich, T. 1975. Magnitude and location of surface charges of *Myxicola* giant axons. *J. Gen. Physiol.* 66:47–65.

Campbell, D. T. 1983. Sodium channel gating currents in frog skeletal muscle. *J. Gen. Physiol.* 82:679–701.

Campbell, D. T., and B. Hille. 1976. Kinetic and pharmacological properties of the sodium channel of frog skeletal muscle. *J. Gen. Physiol.* 67:309–323.

Carbone, E., R. Fioravanti, G. Prestipino, and E. Wanke. 1978. Action of extracellular pH on Na^+ and K^+ membrane currents in the giant axon of *Loligo vulgaris. J. Membr. Biol.* 43:295–315.

Carbone, E., P. L. Testa, and E. Wanke. 1981. Intracellular pH and ionic channels in the *Loligo vulgaris* giant axon. *Biophys. J.* 35:393–413.

Courtney, K. R. 1979. Extracellular pH selectively modulates recovery from sodium inactivation in frog myelinated nerve. *Biophys. J.* 28:363–368.

Drouin, H., and B. Neumcke. 1974. Specific and unspecific charges at the sodium channels of the nerve membrane. *Pflügers Arch. Eur. J. Physiol.* 351:207–229.

Frankenhaeuser, B., and A. L. Hodgkin. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol. (Lond.).* 137:218–219.

Gilly, W. F., and C. M. Armstrong. 1982. Slowing of sodium channel opening kinetics in squid axon by extracellular zinc. *J. Gen. Physiol.* 79:935–964.

Hahin, R., and D. T. Campbell. 1983. Simple shifts in the voltage dependence of sodium channel gating caused by divalent cations. *J. Gen. Physiol.* 82:785–805.

Hille, B. 1968. Charges and potentials at the nerve surface. Divalent ions and pH. *J. Gen. Physiol.* 55:221–236.

Hille, B., and D. T. Campbell. 1976. An improved vaseline-gap voltage-clamp for skeletal muscle fibers. *J. Gen. Physiol.* 67:265–293.

Hille, B., A. M. Woodhull, and B. I. Shapiro. 1975. Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 270:301–318.

Hodgkin, A. L., and A. F. Huxley. 1952. The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo. J. Physiol. (Lond.).* 116:497–506.

Neumcke, B., W. Schwarz, and R. Stampfli. 1980. Increased charge displacement in the membrane of myelinated nerve at reduced extracellular pH. *Biophys. J.* 31:325–332.

Pooler, J. P., and D. P. Valenzeno. 1979. Titration of sodium channel sites for hydrogen block and sensitized photochemical modification of lobster axons. *Biochim. Biophys. Acta.* 555:307–315.

Rojas, E. 1976. Gating mechanism for the activation of the sodium conductance in nerve membranes. *Cold Spring Harbor Symp. Quant. Biol.* 40:305–320.

Schauf, C. L. 1983. Evidence for negative gating charges in *Myxicola* axons. *Biophys. J.* 42:225–231.

Schauf, C. L., and F. A. Davis. 1976. Sensitivity of the sodium and potassium channels of *Myxicola* giant axons to changes in external pH. *J. Gen. Physiol.* 67:185–195.

Ulbricht, W., and H.-H. Wagner. 1975. The influence of pH on the rate of tetrodotoxin action on myelinated nerve fibres. *J. Physiol. (Lond.).* 252:185–202.

Woodhull, A. M. 1973. Ionic blockage of sodium channels. *J. Gen. Physiol.* 61:687–708.