J. Membrane Biol. 78, 163–168 (1984)

The Journal of
Membrane Biology

Effect of Extracellular pH on Sodium Current in Isolated, Single Rat Ventricular Cells

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Summary. Effects of extracellular pH on the sodium current (I_{Na}) of single rat ventricular cells were examined under conditions of voltage clamp and internal perfusion. In this way, pH_i was controlled while pH_o was changed. The combined suction pipette-microelectrode method was used. The suction pipette passed current and perfused the cell's interior; the microelectrode measured membrane potential. Increasing extracellular H^+ depressed I_{Na} and slowed inactivation. The current-voltage curves for I_{Na} were shifted to positive and negative potentials at low and high pH_o, respectively. Similar potential shifts were observed in both the conductance voltage curve and the steady-state inactivation voltage curve (h_∞). Conduction was also depressed at low pH_o. The shifts were probably due to surface charge effects, while the impaired conduction was probably due to protonation of a site in the Na channel.

Key Words rat ventricle · isolated single cell · voltage-clamp · internal perfusion · Na current · extracellular pH

A change in pH of the external solution affects the sodium currents (I_{Na}) of various cell membranes. Part of the effect is due to the effects of pH_o on surface charge (Hille, 1968; Gilbert, 1971; Hille, Woodhull & Shapiro, 1975) and part may be due to an effect on conduction in Na channels (Woodhull, 1973).

Electrical effects of external pH are of importance in cardiac electrophysiology because variations in the plasma level of H^+ in cardiac tissue occur during myocardial ischemia. Voltage-clamp data are still limited with respect to the action of external pH on I_{Na} (Chesnais, Coraboeuf, Sauviat & Vassas, 1975; Brown & Noble, 1978; Bogaert, Verrecke & Carmeliet, 1978) because of the methodological difficulties of clamping cardiac syncytia (Johnson & Lieberman, 1971; Attwell & Cohen, 1977; Noble, 1979; Cohen & Kline, 1982).

Recently, a collagenase digestion technique has been developed to isolate mammalian heart cells (Powell, Terrar & Twist 1980). Properties of the macroscopic Na currents in these isolated myocytes have been analyzed by the suction pipette technique (Lee et al., 1979; Undrovinas, Yushmanova, Hering & Rosenshtraukh, 1980; Brown, Lee & Powell, 1981*a*, *b*), and, in general, the behavior is similar to Na currents in other excitable tissues, although important differences of detail are present. A similar statement may be made about circumscribed studies of Na currents in rabbit cardiac Purkinje fibers (Colatsky & Tsien, 1979) and chick embryonic heart cells (Ebihara, Sigeto, Liebermann & Johnson, 1980). This approach prompted us to examine the effect of external pH on I_{Na}, using isolated rat ventricular myocytes perfused internally by the suction pipette technique. This made the voltage clamp for measuring the fast ionic current of I_{Na} very effective and allowed regulation of the internal pH value.

Materials and Methods

Preparation

The experiments were conducted on individual rat ventricular cells dispersed by collagenase similar to that reported previously (Powell et al., 1980; Brown et al., 1981*a*).

Solutions

The solutions used had the following composition (in mM); (a) External solution: NaCl, 40; CsCl, 100; $MnCl_2$, 3; $MgCl_2$, 1.2; glucose, 10. The buffer consisted of 2 mM each of maleic acid, acetylglycine and Tris titrated to the final pH with HCl or NaOH. In a few experiments, a 5-mM maleic acid or a Tris buffer was used. (b) Internal solution: Na aspartate, 16; Cs aspartate, 140; glucose, 10; HEPES, 10, adjusted by Tris pH to 7.3. The solutions were superfused through the chamber at a rate of 2 ml/min. To secure the complete solution change, at least 10 to 20 volume of bath solution was exchanged with test solutions. The experiments were carried out at room temperature of 20 to 22 °C.

In the analysis of the experiments, the linear capacitive and leakage components of voltage-clamp currents were removed by adding two currents produced by identical voltage steps of opposite polarity, since the leakage current was linear

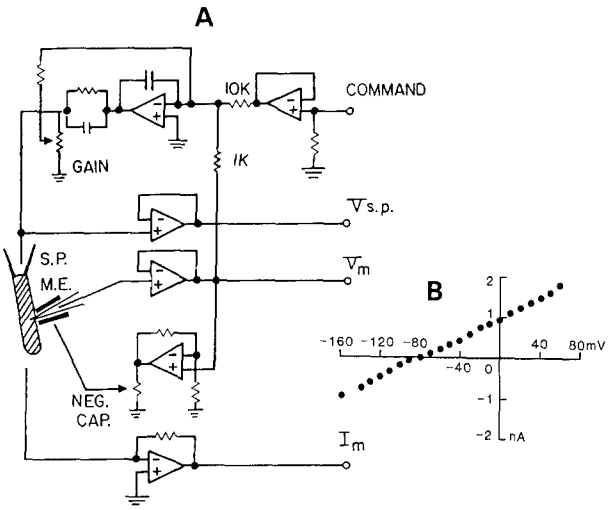

Fig. 1. (*A*): Schematic illustration of the experimental arrangement and voltage-clamp circuit. Suction pipette (*S.P.*) is used as a low resistance (500–800 kΩ) current passing electrode via a calomel half cell. Suction pipette potential ($V_{s.p.}$) is monitored only for checking the cell availability after sucking the myocyte. Membrane potential (V_m) is measured with a microelectrode (*M.E.*) connected to a preamplifier. V_m and command pulses were summed at the input stage of a feedback amplifier to pass the negative feedback current for voltage clamping. The stray capacitance to ground was compensated for by adjusting the negative capacitive feedback on the headstage amplifier. Membrane current (I_m) was measured by a current-to-voltage converter, one side of which was held at virtual ground and connected to the bath via 3-M KCl agar bridge. (*B*): *I–V* relationships of the leakage currents obtained either substituting 80 mM sucrose for 40 mM NaCl or adding 10^{-5} M TTX to normal medium. There is no significant difference between the *I–V* relationships recorded under both treatments. Note the leakage currents linearly changed between −160 to +80 mV

in the range explored (Fig. 1*B*; Fig. 12 of Brown et al., 1981*a*). In some cases peak I_{Na} was measured as the maximum inward current displacement from the steady background current that persisted at the end of the step. The measured reversal potential obtained from this analysis was in agreement with that calculated from the Nernst equation using the internal (16 mM) and external (40 mM) Na^+ concentrations, indicating that Na^+ is the principal charge carrier.

Electrophysiological Recordings

The suction pipettes used in the present experiments were made according to the method of Lee, Akaike and Brown (1980). They had tip diameters of 3 to 5 μm and when filled with internal solution had resistances of 500 to 800 kΩ. Conventional glass micropipettes filled with 3 M KCl has resistance of 5 to 15 MΩ. Two hydraulic manipulators (Narishige MO-2) were set at each side on the stage of an inverted phase-contrast microscope for positioning the suction pipette electrode and the glass microelectrode.

Figure 1*A* shows a schematic diagram of the experimental arrangement and the voltage-clamp circuit. The command pulses were generated by a signal generator (WPI Instruments, Model 830). The following points about the clamp are noteworthy. A variable compensation circuit has been added to the voltage-clamp loop, which allows stability of the clamp circuit while utilizing the full gain of the operational amplifier (approximately 10^4 at 10 kHz BW). Glass microelectrodes were shielded by painting them with conductive silver to within 100 μm of the tip and covering the paint with an insulating layer of lacquer. Insulated electrodes had the advantage of partially removing the stray capacitance to ground of the input voltage circuit and the coupling capacitance between the suction pipette and microelectrode. The stray capacitance to ground was compensated for by adjusting the negative capacitive feedback. Membrane current was measured by a current-voltage converter circuit, which was connected to the bath through a 3-M KCl agar bridge and calomel electrode (Fig. 1*A*).

Experimental Protocol and Data Analysis

After sucking the cell into the suction pipette, constant current (0.1 nA, 2 msec in duration) was passed through the suction electrode to check the viability of the cell. If the input resistance was greater than 50 MΩ, the glass microelectrode was inserted to measure membrane potential. The voltage response of the system to a small depolarizing command pulse was monitored on a storage oscilloscope, and the gain and capacitive feedback compensation were adjusted until the best response was achieved, i.e., the establishment of a new membrane potential as quickly as possible without oscillation.

Spatial control was considered adequate only if the following criteria were met: (i) potential measured by the microelectrode (V_m) and by the suction electrode (V_{sp}) were nearly identical, (ii) no abominable notches appeared in the current record, and (iii) graded increase in I_{Na} occurred with small depolarizations beyond threshold.

The current and voltage records in response to command pulses were photographed and digitized with sampling intervals at 2 μsec point using a signal averager (Nicolet 1170). The digitized records were stored on a digital tape recorder (Kennedy Model 9700). Experimental curve fitting was accomplished using a PDP 11/70 computer (Digital Equipment Corp., Marlboro, Mass.).

Results

General Effects

Figure 2 shows the effect of acidic solutions (pH 6.4 and 5.5) on the I_{Na} in response to a depolarization step of 55 mV from a holding potential of −85 mV. No correction for leak or capacitive currents has been made in this display. The test voltage steps were applied at a frequency of 0.1 Hz. Depolarization produced an initial outward surge of capacitive current, which subsided by almost 95% within 500 μsec. Low pH caused a decrease in the amplitude of I_{Na}. The holding current at −85 mV remained constant. Apart from the decrease in the amplitude of I_{Na}, the time course of inactivation was slowed after lowering the external pH. The effect of low pH on the time constants of inactivation will be elaborated later (Figs. 8 and 9).

Figure 3 shows the current-voltage (*I–V*) curves of I_{Na}, before and after changing the pH. The peak

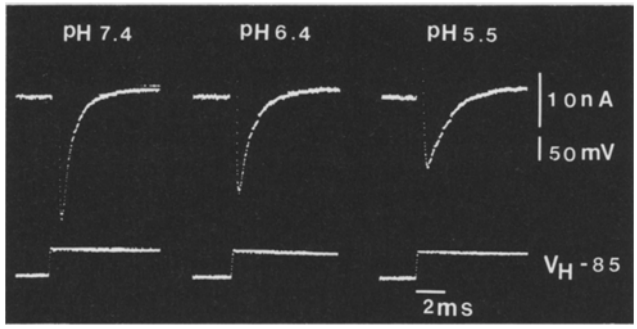

Fig. 2. I_{Na} in an isolated cell from the rat ventricle at different values of external pH in response to −30 mV from a holding potential of −85 mV. The currents are not corrected for capacitive or leakage current. The cell was exposed to 40 mM Na external solution and 16 mM Na internal solution

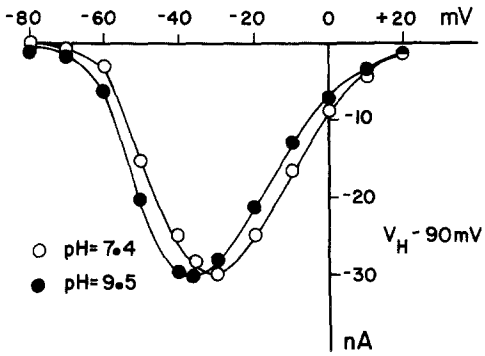

Fig. 4. Effect of pH (9.5) on the *I–V* relationships for I_{Na}

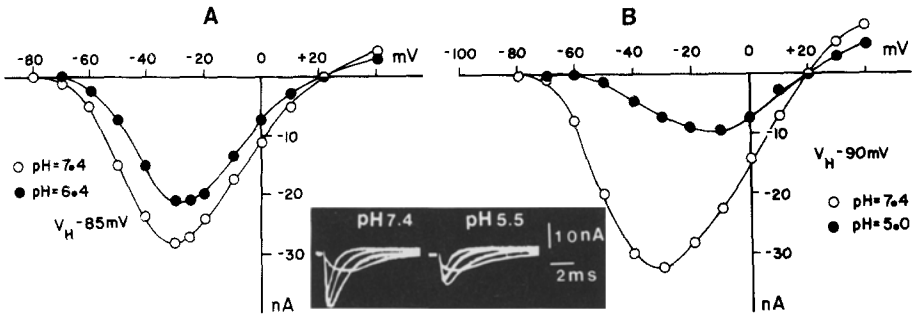

Fig. 3. Effect of pH 6.4 (*A*) and pH 5.0 (*B*) on the *I–V* relationships for I_{Na}. *Inset:* original current traces during 10-msec depolarizing voltage steps recorded from a holding potential of −80 mV in 10-mV increments from −50 to −20 mV in pH 7.4 and from −30 to 0 mV in pH 5.5. The currents are not corrected for capacitive or leakage current

I_{Na} was measured after removal of leakage and capacitive components by adding two currents produced by identical voltage steps of opposite polarity. At pH 6.4, I_{Na} was reduced and the *I–V* curve displaced a few mV to positive potentials. At pH 5.0, I_{Na} was greatly attenuated and the *I–V* curve was shifted approximately 20 mV to positive voltages. This was accompanied by a shift of the current threshold. The average depolarizing shift of the voltage of the maximum I_{Na} on the *I–V* curve was 2.5 ± 1.3 mV ($n=5$) at pH 6.4 and 16.9 ± 5.5 mV ($n=7$) at pH 5.0. In neither case was there a significant change in the reversal potential for the I_{Na} (E_{Na}).

Inset (Fig. 3) shows the I_{Na} traces in both normal pH 7.4 and pH 5.5, produced by 10-mV increments from −50 to −20 mV in the former and from −30 to 0 mV in the latter from a holding potential of −80 mV. To reiterate, low pH caused a reduction of I_{Na} and a shift in *I–V* relationships to positive potentials.

In contrast, as can be seen in Fig. 4, alkaline solutions produced a negative voltage shift in the *I–V* curve, although no significant change in I_{Na} amplitude was observed. The shift in the peak I_{Na}'s was -5.0 ± 3.3 mV.

The effects were independent of whether the buffer solution was made from a mixture of Tris-maleate-acetylglycine or from maleic acid or Tris. The effects of changes from normal pH to pH 5.0 or 9.5 were reversible. Depolarization of the resting membrane and deterioration of the cell often occurred at a pH of less than 4.5.

The cells responded very rapidly to pH changes. The pH effects reached a new steady-state condition within a few seconds and remained stable for 20 to 30 min, perhaps because protonation and deprotonation are very rapid reactions and because the hydrogen ion concentration in intra-and extracellular solutions was strongly buffered.

The *I–V* curve shows that decreased pH_o has complicated effects on the Na channel. Gating is shifted to more positive potentials, yet current is reduced as well as shifted. Measurements of tail currents were not possible due to limitations of the clamp, and in what follows a limited analysis is presented.

The pH dependence of the maximum I_{Na} of the *I–V* curve, referred to the value at pH 7.4 is an indication of the reduction in conductance since the shifts in gating are compensated for by the procedure. The results are plotted in Fig. 5. The

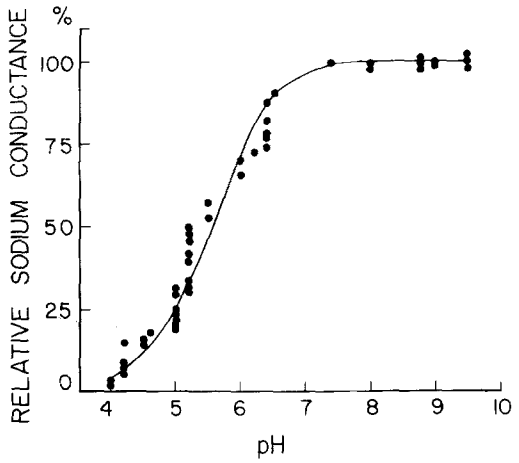

Fig. 5. The titration curve for the effect of extracellular pH on the maximum I_{Na} of the I–V curve. The relative amplitude of I_{Na}, referred to the value at pH 7.4 were plotted as a function of pH. The smooth curve has the shape of the dissociation curve of a weak acid with a pK_a of 5.4. The results of 46 experiments are plotted

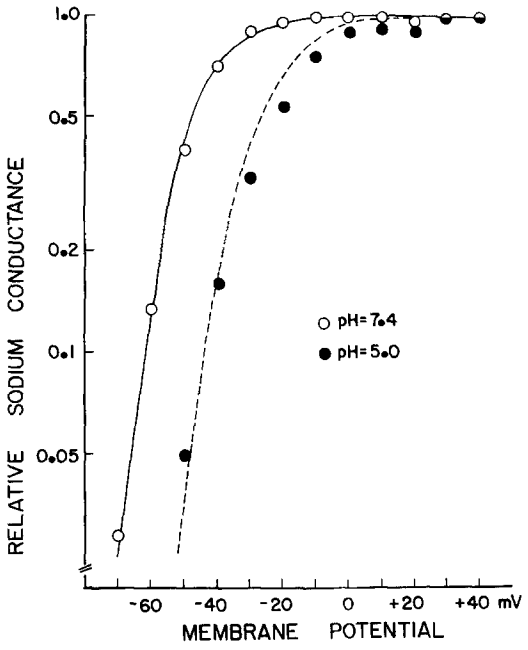

Fig. 6. Relative sodium conductance as a function of membrane potential at pH 7.4 and 5.0. Unnormalized value of $g_{Na}(V)$ was calculated by the relation $g_{Na}(V)=I_{Na}/(V-E_{Na})$ and was subsequently divided by the maximum conductance. The solid line was drawn by eye to fit the data at pH 7.4 and then translated by 20 mV to fit the data for $g_{Na}(V)$ at pH 5.0

smooth curve drawn through these points had the shape of the theoretical dissociation curve of a weak acid with a pK_a of 5.4.

The change of the chord conductance of $I_{Na}(g_{Na})$ in various external pH was also plotted as a function of pH. The conductance g_{Na} was calculated simply by diving I_{Na} by (E_m-E_{Na}), where E_m indicates membrane potential and E_{Na} is Na reversal potential. The g_{Na}'s were normalized to the maximum at pH 7.4. The obtained results were similar to those in Fig. 5 and fitted the dissociation curve with pK_a of 5.4. These observations suggest that Na channels were blocked when an acidic group associated with them was protonated.

Shifts in Gating Due to Low pH

Figure 6 shows conductance-voltage data for a typical experiment in which the external pH was reduced from 7.5 to 5.0. The I_{Na} conductance (g_{Na}) calculations were derived from current measurements similar to those shown in Fig. 3. The results were normalized by the peak g_{Na} for the relevant pH_o. Changing pH from 7.5 to 5.0 shifted the g_{Na}-V curve at its half-maximal value by 16.8 ± 7.0 mV ($n=7$). At the normal pH of 7.4, g_{Na} and voltage were related sigmoidally and $V_{1/2}g_{Na}$ was consistent from cell to cell and for 32 cells averaged -40 ± 6.4 mV.

Figure 7 shows the effect of pH on the steady-state inactivation (h_∞). The steady-state value of inactivation was measured by the usual method (Hodgkin & Huxley, 1952) of determining the peak I_{Na} from a test pulse applied after a 300-msec prepulse to various potentials as shown in the inset. As the prepulse was made more hyperpolarizing, inactivation was reduced and the evoked I_{Na} became larger until it finally saturated at extreme hyperpolarization (more negative than -100 mV). The saturated I_{Na} was then used to normalized the test I_{Na} resulting from other prepulse potentials. At a potential of -100 mV, sodium channels are less than 5% inactivated and the average potential for half-inactivation ($V_{1/2}$) was about -80 ± 4.2 mV for 16 cells at pH 7.4.

Changing the external pH from 7.4 to 5.0 caused a shift of the h_∞ curve in the less negative direction by 20 mV. The average shift in depolarizing direction was 16.0 ± 6.5 ($n=8$) at pH 5.0. This value was comparable to the magnitude of the pH effect on g_{Na}.

As shown in Fig. 2, the time course of I_{Na} was also affected by low pH. An analysis of the effect of low pH on the activation time course was not possible because the capacitive transient at potentials more positive than -20 mV prevented accurate measurement. This was due to uncertainties in the subtraction method.

The effect of low pH on the inactivation time constants for I_{Na} during a single voltage-clamp step was examined. The bottom records of Fig. 8 show the original current traces during changes of acidity. The linear component of capacitive and leakage currents have been subtracted. Inactivation as not-

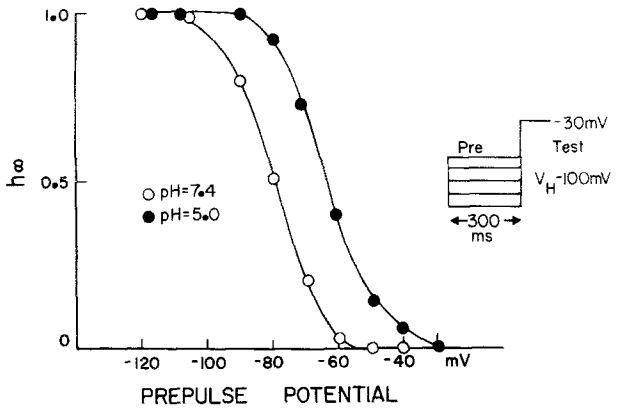

Fig. 7. The steady-state inactivation curves (h_∞) of I_{Na} at different pH values (7.4 and 5.0). Inset is the prepulse-test pulse program. The test pulse current amplitude was expressed as a fraction of the test pulse current at −120 mV (ordinate) and was plotted against the corresponding prepulse potential (abscissa)

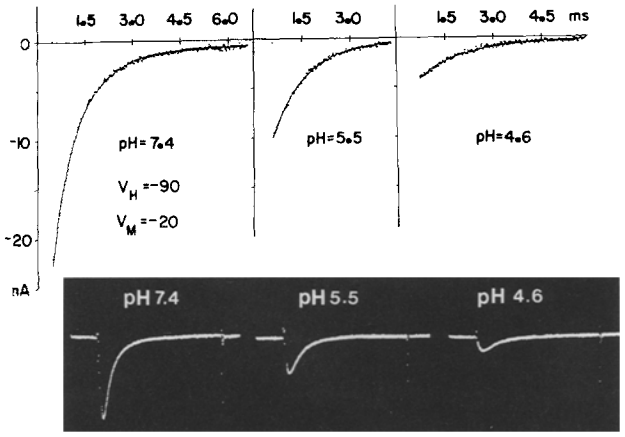

Fig. 8. Current time traces produced at potential of −20 mV from a holding potential of −90 mV at pH 7.4, 5.5 and 4.6. The relaxation phase of I_{Na} was plotted using nonlinear least-square fitting algorithm in the upper part of the figure. Dots are experimental current time records. Thin lines drawn through the points are the fits of the relaxation phase of I_{Na}. The inactivation fits the two time constants (τ_{h1}, τ_{h2}). pH 7.4, $\tau_{h1}=1.1$, $\tau_{h2}=4.27$ msec; pH 5.5, $\tau_{h1}=1.22$, $\tau_{h2}=6.0$ msec; pH 4.6, $\tau_{h1}=1.8$, $\tau_{h2}=6.8$ msec, respectively. The bottom part of the figure shows the original current traces used for curve fitting. The linear component of capacitive and leakage currents were substracted

ed was prolonged and the inactivating current was fitted by the sum of two exponentials, using a nonlinear least-squares algorithm (Marquardt as indicated in Bevington, 1969). The results are shown in the upper part of the figure. As previously reported by Brown et al. (1981b), the inactivation time constant for I_{Na} consists of two components, a fast τ (τ_{h1}) and a slow τ (τ_{h2}), with τ_{h1} dominating for over 90% of the relaxation; both were increased by lowering pH. The relationships between τ_{h1} and potential before and after changing the

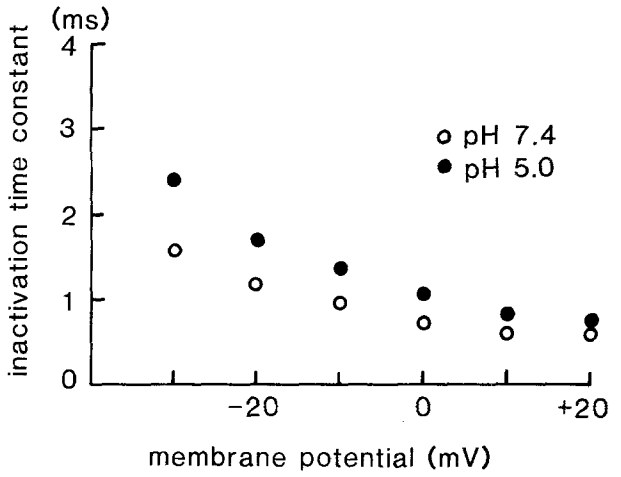

Fig. 9. Relationship between inactivation time constant and membrane potential before and after changing the external pH from 7.4 to 5.0

pH to 5.0 are shown in Fig. 9. τ_{h1} was voltage dependent and decreased as membrane potential became more positive. Low pH increased τ_{h1} over the voltage range examined and seemed to produce a shift of about +20 mV in the τ_h (V) relationship. A similar increase in the τ_{h1} was observed in all experiments.

Discussion

The present results show that a decrease in extracellular pH blocks I_{Na} in isolated single rat ventricular cells. The pK_a was approximately 5.4. Such a concentration-dependent blockage of I_{Na} by H^+ indicates that H^+ titrate acidic groups in the Na channel.

The changes in external pH also shifted gating of I_{Na}. This was true for inactivation, both steady and dynamic, and steady activation as well. The shifts for activation at pH 5.0 and 9.5 were $+16.9 \pm 5.5$ and -5.0 ± 3.3 mV, respectively. The $h_\infty(V)$ curve was shifted approximately +20 mV at pH_o 5.0, and $\tau_{h1}(V)$ appeared to show a similar shift. Both a decrease in I_{Na} and shifts in the voltage-dependent kinetics by H^+ have been previously reported in several other excitable cell membranes, such as nerve (Hille, 1968; Drouin & The, 1969; Woodhull, 1973; Hille et al., 1975), squid axon (Ehrenstein & Fishman, 1971), and tunicate egg (Ohmori & Yoshii, 1977). Low pH also decreased I_{Na} without a shift of the I–V curve in the frog atrial muscle (Chesnais et al., 1975). According to Brown and Noble (1978), the pK_a for Purkinje fiber I_{Na} threshold was approximately 5.3 and the corresponding maximum shift per pH unit was 15 mV, which was comparable to our results. The shift in threshold for I_{Na} was also re-

ported by Bogaert et al. (1978). Therefore, the effects of extracellular acidity on the properties of Na channels in isolated single ventricular cells agree in general with results reported for Purkinje fiber and other excitable cells.

In our experiments, low pH caused both a reduction of I_{Na} and a shift in the voltage-dependent kinetics. These changes occurred at similar pH's. It is possible that the reduction of surface potential caused by an increase of external H^+ may have reduced the Na concentration at the membrane surface, resulting in a decrease of I_{Na} (Brown & Noble, 1978). It is not clear, however, that the change in E_{Na} would be exactly offset by the change in surface potential. Alternatively, the shift in low pH may be produced by a decrease in the negative charge on the surface membrane, whereas the reduction in current would be related to protonation of an acidic group that is normally ionized. Further systematic observation on the interaction between the effects of pH change and the ionic strength of the external solution may distinguish between these possibilities.

Alterations of intracellular pH are known to affect Na channels in squid giant axon, frog skeletal muscle, and crayfish slow muscle (Ehrenstein & Fishman, 1971; Bass & Moore, 1973; Brodwick & Eaton, 1978; Nonner, Spalding & Hille, 1980). It is unlikely that internal pH mediated the results observed in the present experiments. The cells were perfused internally by strongly buffered solutions. The changes in I_{Na} after alteration of external pH occurred within a few seconds and remained steady up to 30 min. For a change in internal pH to produce our results an appreciable current of H^+ would be required. Such an H^+ inward current would depolarize the membrane, but no such depolarization was seen.

The authors wish to thank Dr. A. Noma for his valuable criticism on preparing the manuscript. This work was supported by N.I.H. Grant HL-25145.

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Received 29 July 1983; revised 25 October 1983