PROTON MODULATIONS ON THE IONIC CURRENTS IN RABBIT ATRIO-VENTRICULAR NODAL CELLS

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Abstract : Modulations of changing the extracellular pH on the electrophysiological activity of isolated rabbit atrio-ventricular (AV) nodal cells were investigated using the two microelectrode voltage-clamp technique. Increasing pH from 7.4 to 9.5 enhanced spontaneous activity. The action potential amplitude and the maximum rate of depolarization were decreased. The action potential duration at 50 % repolarization and the cycle length were shortened. The maximum diastolic potential was hyperpolarized. The pH elevation increased the maximum conductances for both the slow inward current and the delayed outward current systems. In contrast, a decline of pH from 7.4 to 5.5 inhibited the activity and the ionic currents. The effects on the action potential parameters were reversed. However, both acidification and alkalinization failed to affect the gating kinetics of the channels. These results suggest that H^+ would modulate the electrical activity of the AV nodal cells, due not to an alteration of the membrane surface charge, but to a direct protonation of the ionic channels.

Index Terms

INTRODUCTION

It is well known that changes in pH alter cardiac performance under the conditions of ischaemia and cardiac failure. It has been reported that acidosis decreased myocardial contraction[22][31][32]. Low pH solution inhibited the contractile force of frog cardiac muscle, whereas high pH solution enhanced it[8]. In sheep spontaneously firing Purkinje fibers, acidification or alkalinization of the bathing solution also decreased or increased the firing rate, respectively[1][3].

An inhibitory effect of extracellular protons on fast sodium current has already been shown in nerve and skeletal muscle[4][12][36]. It is generally agreed that increasing the extracellular proton concentration would exert two actions[37] : (1) protons cause a shift in channel gating to more positive potentials, and (2) protons reduce the maximal conductance of the sodium channels.

We have also studied for the effects of protons on cardiac muscle cells. In isolated puppy spontaneously beating sino-atrial (SA) node preparations perfused with Tyrode's solution

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through the sinus node artery, the spontaneous activity was depressed by low pH, whereas it was stimulated by high pH solution[24]. In addition, Satoh and Seyama[25] have demonstrated the effects of different pH solutions on the kinetics of the ionic channels in rabbit voltage-clamped SA nodal cells; low pH inhibited the ionic currents, whereas high pH enhanced them. Still, little is known about the effect of pH on atrio-ventricular (AV) node, which has higher sensitivity to Na^+ channels than the SA nodal cells[27][28]. In the present experiments, we attempted to examine the changes in the AV node action potential configuration in different pH solutions. Also, we examined the effects of changes in extracellular pH on the ionic currents underlying the action potentials.

METHODS

Preparations of atrio-ventricular node

Rabbits of either sex, weighing 1.5-2.0 kg, were used. The preparations were made by the same method as described previously[23][25]. The rabbits, anesthetized with pentobarbital sodium (30 mg/kg), were killed by a blow to the neck, and exsanguinated. The chest was opened, the heart was quickly removed, and both the right and left atria with the AV node region left intact were dissected in the bathing solution. The preparation was made smaller by dissection to a final dimension of about 0.25 x 0.25 mm. The preparations were usually smaller than one-half of the length constant (0.3-0.8 mm). The preparations were superfused with the bathing solution oxygenated by 100 %O_2 at 36°C, and were left spontaneously beating.

Recording of membrane currents

The two-microelectrode voltage-clamp technique used has already been described[23][25], and is similar to the method developed by Noma and Irisawa[20]. Conventional glass microelectrodes filled with 3 M KCl were used, and their resistances were 20-30 MΩ. The memdrane potential was held at −40 mV, where little or no net current flowed, by using a feedback amplifier (Dia Medical DP 100, Tokyo, Japan). Test pulses were applied with increment of 10 mV from the holding potential. The interval between test pulses was 1 min or more. Between applications of the voltage-clamp, the preparations invariably showed spontaneous rhythmicity. The membrane potential, the maximum rate of depolarization($\dot{V}_{max}$), and the sinus rate were recorded with a penrecorder (Nihon Kohden RJG-4124). The ionic currents and voltages were displayed on an oscilloscope (SONY Tektoro, Tokyo, Japan) and photographed by a POLAROID camera. The amplitude of the slow inward current was determined as the difference between the peak current and the current level measured at 100 ms after the onset of the step, according to the method developed by McDonald and Trautwein[17]. The magnitudes of the delayed outward K^+ current were determined by taking the difference between the value of the current at the end of a long clamp pulse and zero current level. The tail current was measured as the difference between its peak amplitude and the steady state current value between the test pulses.

Solutions

The bathing solution had the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, and HEPES [N-(2-hydroxyethyl)piperazine-N′-2-ethansulfonic acid] (Wako Pure

Chemical Industry, Ltd., Osaka, Japan) 5.0. The pH was adjusted to 7.4 with NaOH. Since the bathing solution was completely exchanged with solutions of different pH within at least 3 min, the data were obtained 10 min after changing to a different solution.

Statistical analysis

Values are represented as arithmetical mean ± S. E. M. The significance of differences between mean values was assessed by Student's t-test for paired data, and were considered significant when P values were less than 0.05.

RESULTS

When a superfusing solution having a normal pH (7.4) was switched to pH 6.5, the spontaneous activity of AV nodal cells was markedly reduced (Fig. 1 A). The maximum diastolic potential(MDP)was depolarized, and the maximum rate of depolarization ($\dot{V}_{max}$) was decreased. The sinus rate was shortened. In contrast, when the pH was elevated to 8.5 from 7.4, the electrical activity of the AV nodal cells was enhanced (Fig. 1 B). The MDP was hyperpolarized, and the sinus rate was increased. The $\dot{V}_{max}$ was enhanced. Average values of the action potential parameters in three different pH solutions are summarized in Table 1. The action potential amplitude (APA) was reduced by 24.4±2.3 % (n=8, P<0.01) at pH 6.5, and was not significant (2.2 %) at pH 8.5. The MDP was depolarized by 20.3±1.2 % (n=8, P< 0.01) at pH 6.5, and hyperpolarized by 7.8±1.5 % (n=9, P>0.05) at pH 8.5. The $\dot{V}_{max}$
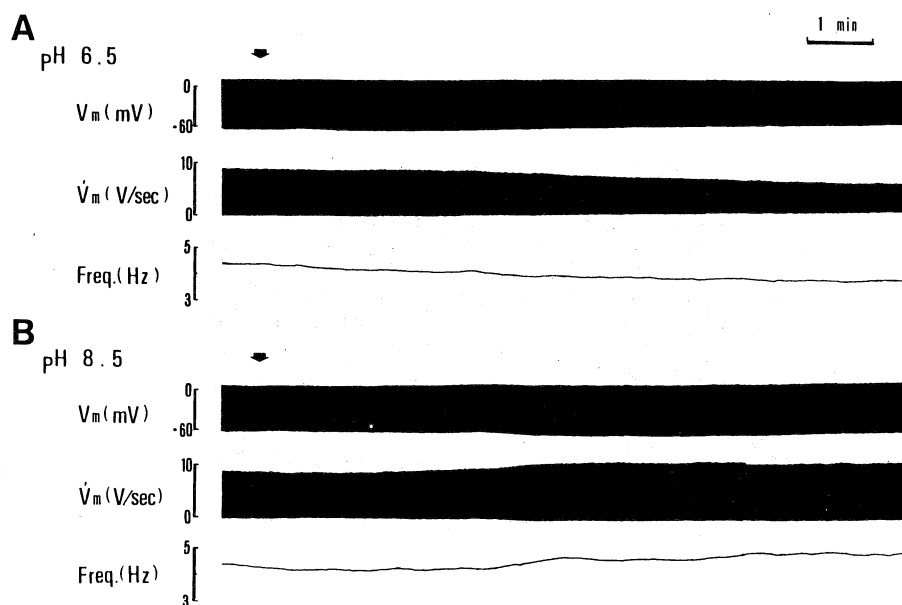

Fig. 1. Modulations of the action potentials by changes in extracellular pH in rabbit spontaneously beating atrio-ventricular nodal cells. Arrow indicates the time point switched to a different pH solution. A : Changes in the action potentials in pH 6.5 solution. B : Changes in the action potentials by elevation to pH 8.5. Vm : Spontaneous action potentials. $\dot{V}$m : Maximum rate of depolarization. Freq. : Sinus rate.

was inhibited by 49.9 ± 3.4 % (n=8, P<0.01)at pH 6.5, and was enhanced by 16.7 ± 2.6 (n=9, P<0.05) at pH 8.5. In addition, the pH 6.5 solution prolonged the cycle length (CL) by 10.2 ± 1.9 (n=8, P<0.05), and the pH 8.5 solution shortened the CL by 11.3 ± 1.3 % (n=9, P<0.05). The action potential duration at 50 % repolarization (APD) was increased at pH 6.5, and was decreased at pH 8.5, but not to a significant extent. In quiescent AV nodal cells(which were often obtained with approximately 30 % incidence), the resting membrane potential was -34 ± 1 mV (n=5) at pH 6.5, -41 ± 1 mV (n=6) at pH 7.4, and -46 ± 1 mV (n=6) at pH 8.5.

On the slow inward current

To examine the underlying ionic currents of the AV node action potentials, voltage-clamp experiments were performed. Test pulses for 300 msec duration were applied with an incre-

Table 1. Modulation of the low and high extracellular pH solutions on the action potential parameters in the rabbit atrio-ventricular nodal cells

		pH 6.5	pH 7.4	pH 8.5
n		8	10	9
APA	(mV)	68 ± 8	90 ± 9	92 ± 8
APD	(msec)	63 ± 6	60 ± 6	58 ± 6
MDP	(mV)	-51 ± 4	-64 ± 3	-69 ± 4
$\dot{V}_{max}$	(V/s)	6 ± 2	$12+3$	$14+4$
CL	(msec)	302 ± 8	274 ± 7	243 ± 6

Values represent mean $\pm$ S.E.M. n: Number of experiments. APA: Action potential amplitude. APD: Action potential duration at 50% repolarization. MDP: Maximum diastolic potential. $\dot{V}_{max}$: Maximum rate of rise of depolarization. CL: Cycle length.
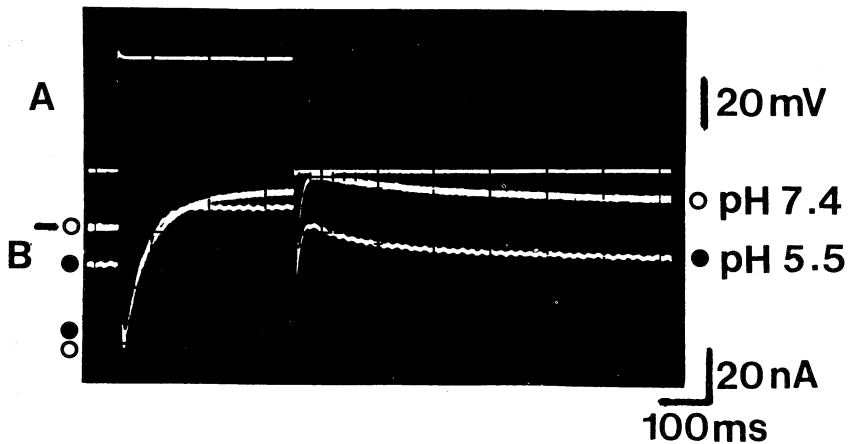
Fig. 2. Effects of low pH solution on the ionic currents in rabbit atrio-ventricular nodal cells. A: Voltage step to 0 mV from a holding potential of -40 mV for a duration of 300 msec. B: Current traces (a slow inward current and a delayed outward current) in pH 7.4 (open circles) and pH 5.5 (filled circles) solutions. Note a shift of the holding current level in the inward direction. Short line at the left of the current trace represents zero current level.

ment of 10 mV. The depolarizing test pulse activated the slow inward current followed by a time-dependently activated outward current, as shown in Figs. 2 and 3. In altering extracellular pH, the most striking change is that the slow inward current decreased with acidification (Fig. 2) and increased with alkalinization of the perfusing solution (Fig. 3). The slow inward current (at 0 mV) was inhibited by 43.1 ± 3.4 % (n=5, P<0.001) at pH 5.5, and was enhanced by 78.3 ± 3.5 % (n=5, P<0.001) at pH 9.5. The holding current level was shifted by 14.7 ± 2.5 mV (n=5) in the inward direction at pH 5.5, and by 10.2 ± 2.3 mV (n=5) in the outward
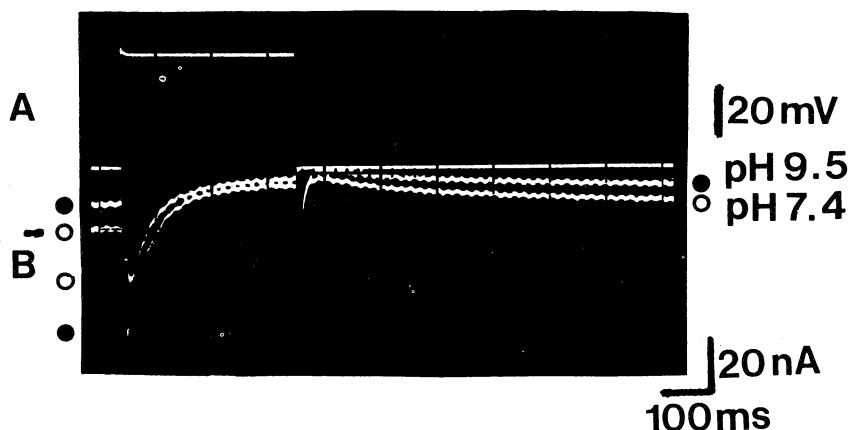

Fig. 3. Effects of high pH solution on the ionic currents in rabbit atrio-ventricular nodal cells. A : Voltage step to 0 mV from a holding potential of −40 mV for 300 msec duration. B : Current traces (a slow inward current and a delayed outward current) in pH 7.4 (open circles) and pH 9.5 (filled circles) solutions. Note a shift of the holding current level in the outward direction. Short line at the left of the current trace represents zero current level.
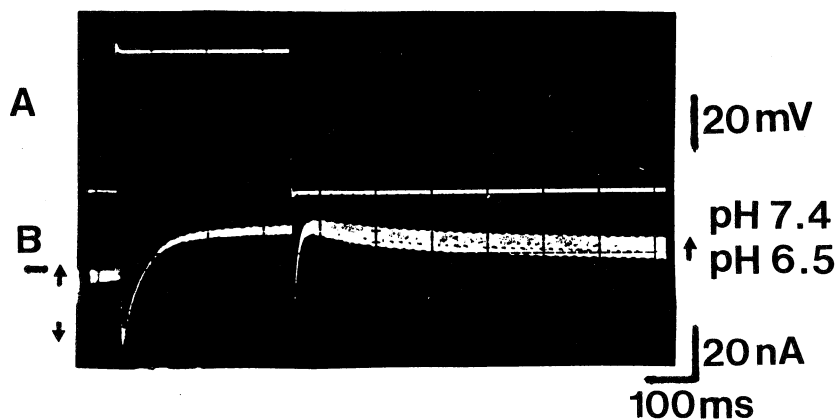

Fig. 4. Time-dependent changes in the ionic currents by changing from extracellular pH 6.5 to 7.4 in rabbit atrio-ventricular nodal cells. A : Voltage step to 0 mV from a holding potential of −40 mV for 300 msec. B : Superimposed current traces (a slow inward current and a delayed outward current) during exchanging from pH 6.5 to pH 7.4 solutions. Note a shift of the holding current level in the inward direction. Short line at the left of the current trace represests zero current level.

direction at pH 9.5.

Figure 4 shows the recovery of both the ionic currents from pH 6.5 to 7.4. The responses to the different pH solutions were completely reversible. The inactivation curve was not significantly affected by low and high pH solutions (but increasing pH tended to shift in the negative potential). The voltage of half-maximum inactivation from 4 preparations was -20.5 ± 1.1 mV at pH 5.5, -21.3 ± 1.2 mV at pH 7.4, and -21.9 ± 0.9 mV at pH 9.5. The relative value of the slope conductance (taking a value of pH 7.4 as 1.0) was 0.8 ± 0.1 (n=5) at pH 6.5, and 1.1 ± 0.1 (n=8) at pH 8.5.

On the delayed outward current

Figures 2 and 3 show the effects on the delayed outward current in the low and high pH solutions. The current was time-dependent, and was activated by a depolarizing pulse from the holding potential of -40 mV. With an increase in extracellular pH from 6.5 to 8.5, the magnitude of the outward current was enhanced. The outward current was decreased by 40.2 ± 3.2 % (n=5, P<0.01) at pH 5.5, and was increased by 33.5 ± 2.8 % (n=5, P<0.001) at pH 9.5. The activation curve for the outward current is obtained by the peak magnitude of the tail current (on repolarization of the membrane to -40 mV) (data not shown). This curve was not affected by changing pH. Thus, these results indicate that the changes in extracellular pH alter the maximum conductance of the delayed outward current system.

DISCUSSION

Biologically important reactions involve the participation of protons. Proton movement through membranes by the gradients is involved in energy transduction[18][37]. Many membrane processes like carrier-and channel-mediated transport have been found to be directly and indirectly modulated by protons (e. g. $Na^+ - H^+$ exchange). The present experiments showed the following: (1) the spontaneous activity was stimulated by high pH, but was depressed by low pH; (2) elevating pH from acidic to alkalinic had excitatory actions on the action potential parameters; (3) the slow inward current was enhanced in the high pH solution, whereas it was inhibited in the low pH solution; (4) the delayed outward current was also increased at high pH, and was decreased at low pH; (5) the kinetics of the ionic channels were not affected by the pH changes. These results are consistent with previous reports in rabbit atrium[32], frog atrium[6][34], cat papillary muscle[14], chick embryo heart[33], and canine SA nodal cell[24]. Especially, the pH actions on the characteristics of ionic channels are consistent with our previous report of SA nodal cells (in which the effect on I_h was measured)[25][26], and of frog skeletal muscles and neurons[4][36].

Two major mechanisms can be proposed to explain the effects of altering pH on the ionic currents of the cardiac membane: (1) an alteration in the surface potential (or surface charge) of the membrane, and (2) a change in conductance due to protonation of the channels. It has been shown in myocardial cells that acid pH shifts the kinetics of the Na^+ and Ca^{2+} currents in their voltage dependence to more positive potentials. A prominent effect of changing pH is an alteration of the surface potential, involving either the screening or binding of negatively charged groups associated with the membrane[2][3]. These are well in accord with previous

reports on the frog node of Ranvier[12], frog skeletal muscles[5], and Myxicola and crayfish giant axons[29][30].

On the other hand, some evidences support the second mechanism. Kohlhardt and colleague[14] showed in cat papillary muscles that lowering extracellular pH suppresses the slow inward current without shifts of the current-voltage relationships and the steady-state inactivation curves. Their observations are in good harmony with the present results and those in rabbit SA nodal cells. Study of the SA nodal cells has demonstrated that the suppression of g_s and g_k by acid pH is voltage-independent. These results suggest that the gating systems for these channels would be electrically isolated from the surface charge, and that the binding site (at which protons block the channels) should be located outside the transmembrane field. This is consistent with the proposal of Campbell[4] that the proton binding site in Na^+ channels is located at or near the mouth of the channel on the face of the membrane. The membrane potential ($V_{1/2}$), at which the peak inward current attained one-half of its value, remained nearly constant in pH 6.5 and 8.5 solutions. A change in $V_{1/2}$ can be regarded as a good indication of a difference in surface potential[11][21]. These results suggest for little or no contribution of a change in the surface potential to the changes in membrane currents. In contrast, Kurachi[15] examined the electrical modulations by changes in intercellular H^+ concentration in guinea-pig ventricular cells. The effects resemble those observed in the present study, suggesting that there might be a proton binding site located on the membrane intracellular surface.

Ellis & Thomas[10] showed that intracellular pH remains relatively constant even during changing from pH 7.4 to 6.4, suggesting that the intracellular medium has a high buffering capacity. The mechanisms to regulate the intracellular H^+ concentration are present in heart cells. This would result from $Na^+ - H^+$ exchange mechanism[9]. In addition, the H^+ flux is promoted by protein kinase C to neutralize the activity of electrogenic enzymes[13][19].

Thus, the SA and AV nodal cells play an important role for a chemoreceptor. It is possible that there would be distinct binding sites for external H^+. Recently, proton modulations on the other channels have also shown. The pH change does not affect the open-closed reaction, but rather weaken Ca^{2+} binding to all the conformational states of the Ca^{2+}-activated K^+ channel[16]. Protons compete with Ca^{2+} for the Ca^{2+}-binding sites and increased the closed times[7]. Further extensive experiments are required to elucidate the pH-induced complex mechanisms.

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