MODULATIONS BY EXTERNAL PH ON ANODAL BREAK EXCITATION IN RABBIT SINO-ATRIAL NODAL CELLS

HIROYASU SATOH
Department of Pharmacology, Nara Medical University
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Abstract: Effects of extracellular pH on anodal break excitation in rabbit sino-atrial (SA) nodal cells were investigated using the two-microelectrode voltage-clamp technique. The method of anodal break excitation was developed by Weidmann[28], and is regarded as a measurement of the activity of the fast Na^+ channel. Increasing external pH from 7.4 to 8.5 enhanced the maximum rate of depolarization by anodal break excitation, as compared with the values of control (at pH 7.4). In contrast, a decline of pH from 7.4 to 5.5 inhibited the maximum rate of depolarization, accompanied with depression in the activity. Acidification shifted the inactivation curves (h_∞) of the fast Na^+ current in the depolarizing direction, and alkalinization shifted it in the hyperpolarizing direction. These results suggest that proton would modulate the membrane surface charge of the SA nodal cells, resulting in alteration of the gating kinetics of ionic channels.

Index Terms

extracellular pH, fast Na^+ channel, anodal break excitation, voltage-clamp, sino-atrial nodal cells

INTRODUCTION

Acidification under the conditions of ischaemia and cardiac failure alters cardiac performance. Low pH decreased myocardial contraction[18),26),27)], whereas high pH enhanced it[8)]. The authors have also reported on the effects of protons on cardiac muscle cells. In puppy sino-atrial (SA) node preparations perfused with Tyrode solution through the sinus node artery, the spontaneous activity was decreased by low pH solution, whereas it was increased by high pH solution[20)]. In addition, we have demonstrated the effects of different pH solutions on the kinetics of the ionic channels in rabbit voltage-clamped SA and atrio-ventricular (AV) nodal cells; low pH inhibited the ionic currents, whereas high pH enhanced them[21-23)].

External proton inhibits the fast Na^+ current (I_{Na}) in nerve[1),11),29)], skeletal muscle[4)], and cardiac muscles[31)]. It is generally agreed that increasing the extracellular proton concentration would exert two actions[30)]: (1) protons cause a shift in channel gating to more positive potentials, and (2) protons reduce the maximal conductance of the Na^+ channels. However, the alteration of pH did not affect the kinetics of the ionic channels (the Ca^{2+} and delayed K^+ currents, I_{Ca} and I_K) of the pacemaker cells of the SA and AV nodes. The nodal cells also possess the fast Na^+ channels, which may make a minor contribution to normal spontaneous beating. In the present experiments, we attempted to examine the modulation of the I_{Na} in

Send correspondence to: Dr. Hiroyasu Satoh, Department of Pharmacology, Nara Medical University, Kashihara, Nara 634, Japan

rabbit SA nodal cells in different pH solutions using anodal break excitation, since it is too fast to measure the precise values of the activation of I_{Na}.

METHODS

Preparations of sino-atrial node

Rabbits of either sex, weighing 1.5-2.0 kg, were used. The preparations were made by the same method as described previously[19-21]. The rabbits were anesthetized with pentobarbital sodium (30 mg/kg, *i. v.*), and exsanguinated. The chest was opened, the heart was quickly removed, and the right atria with the SA node region left intact was dissected in the bath solution. The preparation was made smaller by dissection to a final dimension of about 0.25 ×0.25 mm. The preparations were usually smaller than the length constant (0.3-0.8 mm). The preparations were superfused with the bath solution oxygenated by 100 % O_2 at 36 ℃, and were left spontaneously beating.

Procedure of anodal break excitation

The two-microelectrode voltage-clamp technique used has already been described[19),21),22)], and it is similar to the method developed by Noma and Irisawa[16]. Conventional glass microelectrodes filled with 3 M KCl were used, and their resistances were 20-30 MΩ. According to the methods developed by Weidmann (1955) as shown in Fig. 1A, the test pulses of voltage-clamp (for 300 msec) were applied from −50 to −120 mV, with increment of 10 mV, by using a feedback amplifier (Dia Medical DP 100, Tokyo, Japan). 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 and the maximum rate of depolarization ($\dot{V}_{max}$) were recorded with a pen-recorder (Nihon Kohden RJG-4124), and were also displayed on an oscilloscope (SONY Tektono, Tokyo, Japan) and photographed with a camera (Nihon Kohden, RLG-6201).

Solutions

The bath solution had the following composition (mM) : NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES [N-2-hydroxy ethylpiperazine-N'-2-ethansulfonic acid] (Wako Pure Chemical Industry, Ltd., Osaka, Japan) 5.0, and glucose 5.0. The pH in each solution was adjusted with NaOH. Since the bath solution was completely exchanged with solutions of different pH within at least 3 min, the data were obtained 5-7 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

The experimental procedure is illustrated in Fig. 1 A. Voltage-clamp pulses for 300 msec

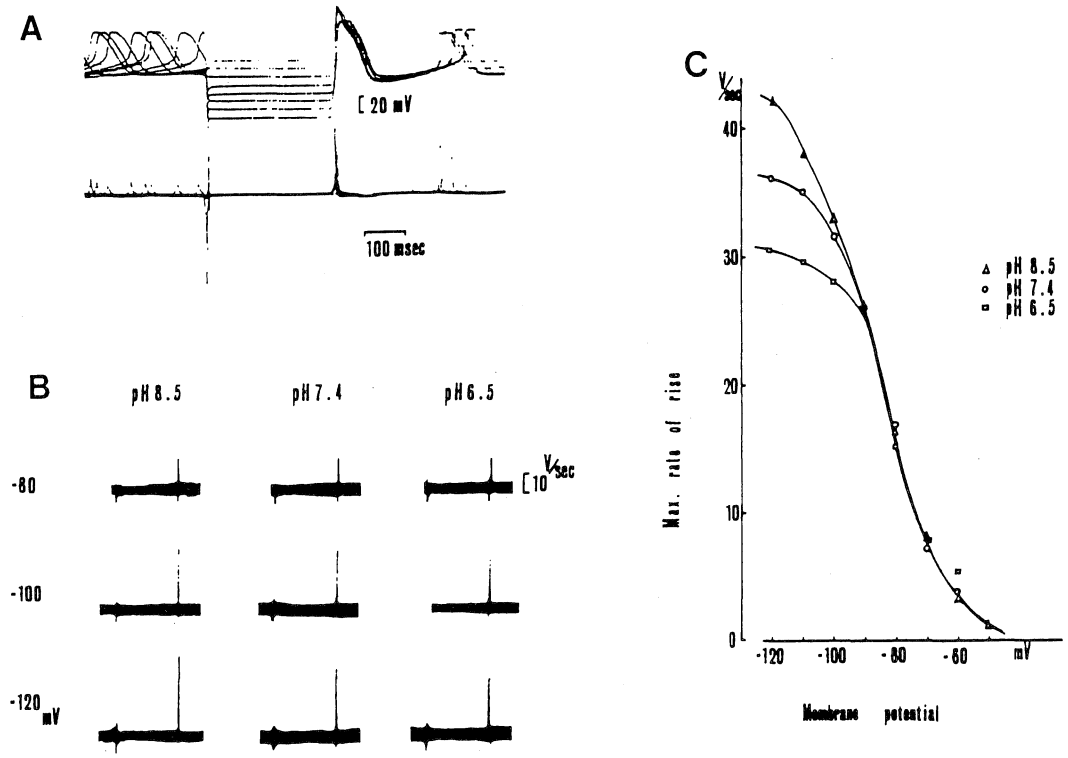

Fig. 1. Modulations of the anodal break excitation by changes in extracellular pH in rabbit spontaneously beating sino-atrial nodal cells. A : Protocol of the anodal excitation. The preparation was applied from −50 to −120 mV by voltage-clamp method. The values of the maximum rate of depolarization was measured, when the voltage-clamp was released. B : The maximum rate of depolarizations at different potentials of voltage-clamp in three external pH solutions. C : Inactivation curves at differnt pH solutions. The values were plotted from the panel B. Symbols are pH 8.5 (triangles), pH 7.4 (open circles) and pH 6.5 (squares).

were applied from −50 to −120 mV. At the end of test pulses, the feedback circuit was broken. The courses of the membrane potential and the maximum rate of rise of depolarization ($\dot{V}_{max}$) at the end of clamp period were measured. The stronger hyperpolarizing pulses produced larger amplitude of $\dot{V}_{max}$, but the values at more negative voltages than −120 mV reached a steady state.

On switches of a superfusing solution having a normal pH (7.4) to one of pH 6.5 or 8.5, the spontaneous activity of SA nodal cells was depressed in acidic solution and was stimulated in alkaline solution. Changes in the action potential parameters are summarized in Table 1. Increasing external pH shortened the cycle length (CL), and the action potential duration at 50 % repolarization (APD) (but not significantly). The action potential amplitude (APA) and the maximum rate of depolarization ($\dot{V}_{max}$) were increased. The maximum diastolic potential (MDP) was hyperpolarized by elevating pH.

On the other hand, the $\dot{V}_{max}$ at the end of release of feedback voltage-clamp was examined at the various hyperpolarizing steps (Fig. 1 B). When the more negative potentials were applied, larger $\dot{V}_{max}$ was elicited. The $\dot{V}_{max}$ at −120 mV was inhibited by 19.1±3.4 % (n=6,

Table 1. Modulation by different extracellular pH solutions on the action potential parameters in the rabbit sino-atrial nodal cells

	pH 6.5	pH 7.4	pH 8.5
n	10	14	11
APA (mV)	65±8**	81±9	90±8*
APD (msec)	63±6	61±6	58±6
MDP (mV)	−52±4**	−62±3	−69±3*
$\dot{V}$max (V/s)	6±2***	11±2	14±3**
CL (msec)	294±4*	273±6	246±5*

Values represent mean ± 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. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, with respect to control values (at pH 7.4).

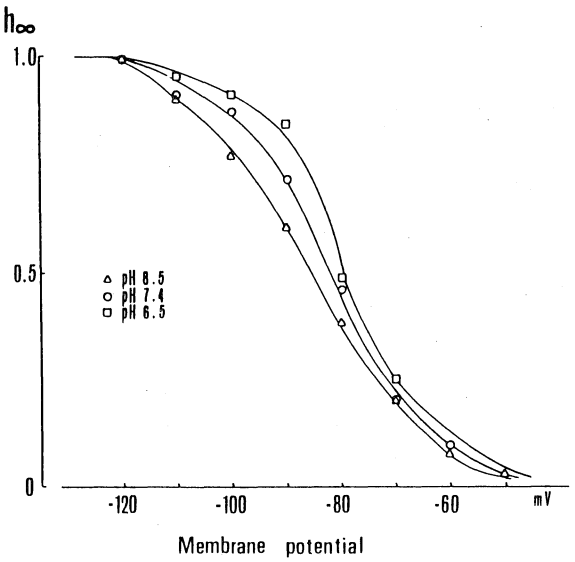

Fig. 2. Normalized inactivation curves of the Na^+ current in different pH solutions in rabbit sino-atrial nodal cells. Symbols are pH 8.5 (triangles), pH 7.4 (open circles) and pH 6.5 (squares).

$P<0.01$) at pH 6.5, and was enhanced by 18.5±2.6 ($n=6$, $P<0.01$) at pH 8.5, as compared with the values at pH 7.4. Figure 1 C shows the inactivation curves in different pH solutions in which the values (obtained from Fig. 1 B) are plotted against the membrane potentials. In addition, the inactivation curves were normalized by taking the value at −120 mV as 1.0 (h_∞), as shown in Fig. 2. The half-maximal potential was −86.1±2.5 mV ($n=6$) at pH 8.5, −81.7 ±2.1 mV ($n=8$) at pH 7.4, and −78.9±2.9 mV ($n=6$) at pH 6.5.

DISCUSSION

The present experiments showed the following: (1) the pacemaker activity was stimulated by high pH, but it was depressed by low pH; (2) the $\dot{V}_{max}$ induced by the anodal break excitation was enhanced in high pH solution, whereas it was inhibited in low pH solution; and (3) the inactivation kinetic (h_∞) of the I_{Na} was shifted in the hyperpolarizing direction with an increase in the external pH.

According to the ionic theory of electrical activity, a change in membrane potential depends on the displacement of charged particles across the membrane[13]. The upstroke of the cardiac action potential (from the resting potential at more negative potentials than −80 or −90 mV) is due to Na ions entering the cells[7),9)]. Since the driving force made up by membrane potential and Na^+ concentration difference would be similar, the $\dot{V}_{max}$ may thus be regarded as a measure for the inward Na^+ current[28]. Therefore, the present experimental data are recognized as an indicator of the I_{Na} current, and could be fitted satisfactorily by the following equation[14]:

$$h=1/\{1+\exp(V_h-V)/5\}$$

where h is the fraction of the highest value observed for the $\dot{V}_{max}$, V is a clamp potential (mV), and V_h is the potential at which h is half-maximal inactivation.

Two mechanisms can be proposed to explain the effects of altering pH on the ionic currents of the cell 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 is generally agreed that decline of external pH causes a shift in channel gating to more positive potentials and reduces the maximal Na^+ conductance, similar to the results of this study. The shift in gating is commonly thought to result from titration of external negative surface charges[2),3),12)]. These are in accord with previous reports on the frog node of Ranvier[11)], frog skeletal muscles[5)], and Myxicola and crayfish giant axons[24),25)].

On the other hand, the second mechanism is also supported by some evidences. In rabbit SA and AV nodal cells (from my laboratory), the decreases in g_s and g_k by low pH are 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 should be located outside the transmembrane field. This is consistent with the proposal of Campbell[4),15)] that the proton bining site in Na^+ channels is located at or near the mouth of the channel on the surface of the membrane. These results suggest for little or no contribution of a change in the surface potential to the changes in membrance currents.

In the present experiments, the $V_{1/2}$ of $\dot{V}_{max}$ was shifted by approximately 7 mV in the hyperpolarizing direction with an increase in the external pH from 6.5 to 8.5. A change in $V_{1/2}$ can be regarded as a good indication of a difference in surface potential[10),17)]. Therefore, the shift may be considered to be due to the alteration of the surface charge, although the kinetics of I_{Ca} and I_K currents were not affected, as reported previously[19–23)]. The SA and AV nodal cells as pacemaker cells also play a chemoreceptive role. There would be distinct binding sites for external H^+ (which might cause the direct action). Protons compete with Ca^{2+} for the Ca^{2+}-binding sites and reduce the open times[6)]. Further extensive experiments are required to

elucidate the mechanisms of pH-induced complex phenomena.

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