CALCIUM MODULATION OF THE SPONTANEOUS ACTIVITY AND THE IONIC CURRENTS IN RABBIT ATRIO-VENTRICULAR NODAL CELLS

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Abstract: Modulation by changing the extracellular Ca^{2+} concentration ($[Ca]_o$) of the electrophysiological activity in isolated rabbit atrio-ventricular (AV) nodal cells was investigated using the two-microelectrode voltage-clamp technique. Low $[Ca]_o$ (0.9 mM or Ca^{2+}-free) decreased the rate of spontaneous beating, but the maximum rate of depolarization was significantly enhanced. Simultaneously, the $[Ca]_o$ decline tended to increase the action potential amplitude, prolong the action potential duration (at 50 % repolarization), and shorten the cycle length. The maximum diastolic potential was hyperpolarized. But these factors were not significant. In contrast, elevation of $[Ca]_o$ to 5.4 or 10 mM (from 1.8 mM) transiently stimulated, but then inhibited the spontaneous activity. The effects on the action potential parameters were rather depressant. In voltage-clamp experiments, the $[Ca]_o$ elevation increased the maximum conductances for the slow inward current and the delayed outward K^+ current, whereas the $[Ca]_o$ decline decreased them. An arrhythmia occurred in 4 out of 6 preparations only at 10 mM $[Ca]_o$. These results indicate that $[Ca]_o$ would modulate the electrical activity of the AV nodal cells due to conductance changes in the ionic channels, but high $[Ca]_o$ induces rather the depressant effects.

Index Terms

Ca^{2+} concentration, spontaneous activity, ionic currents, voltage-clamp, atrio-ventricular nodal cells

INTRODUCTION

An initial electrical activation of the action potentials of supraventricular muscles is more dependent on the Ca^{2+} current (I_{Ca}) than on the fast Na^+ current (I_{Na}) across the membrane. The maximum diastolic potential (MDP) of sino-atrial (SA) and atrio-ventricular (AV) nodal cells is approximately -60 to -70 mV[12),15–17),19),21)]. At this potential, I_{Na} is almost inactivated by its h kinetic[4),5),14),21)]. The activation of AV nodal cells is influenced by many extracellular and intracellular ions, and the activity is modulated by complex mechanisms to maintain the steady state (homeostasis) due to autonomic nervous systems[1)–3),15)]. The maintenance could also be carried out by cellular regulation such as Na-Ca and Na-H exchanges[10),11)]. The AV nodal activity reflects the P-R period on ECG, and clinically plays a most important role for regulation of the cardiac conduction system.

Of many factors regulating its activity, the AV nodal cell would be regulated strongly by extracellular Ca^{2+} concentration ($[Ca]_o$). In the present experiments, therefore, the modula-

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tion by changes in $[Ca]_o$ levels (from free to 10 mM) of the spontaneous activity and the ionic currents underlying the mechanisms were examined by the two-microelectrode voltage-clamp technique.

METHODS

Preparations of atrio-ventricular node

Rabbits of either sex, weighting 1.5-2.0 kg, were used. The preparations were made by the same method as described previously[18),19),22),23)]. The rabbits were anaesthetized with pentobarbital (30 mg/kg, i. v.), and exsanguinated. The chest was opened, and the heart was quickly removed. Both the right and left atria, with the AV node region left intact, were dissected in the bath solution. The preparation was made smaller by dissection to a final dimension of approximately 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 bath solution oxygenated by 100 % O_2 at 36°C, and were left spontaneously beating.

Recording of membrane currents

Two-microelectrode voltage-clamp technique used has already been described[18),19),23)], and 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. The membrane 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 pen-recorder (Nihon Kohden RJG-4124). The ionic currents and voltages were displayed on an oscilloscope (SONY Tektoro, Tokyo, Japan) and photographed with a polaroid camera. The amplitude of the slow inward current was determined an the difference between the peak current and the current level method developed by McDonald and Trautwein[9)]. 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.

Solutions

The bath sulution had the following composition (mM): NaCl 137. KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, and HEPES [N-(2-hydroxyethy) piperazine-N'-2-ethansulfonic acid] (Wako Pure Chemical Industries, Ltd., Osaka, Japan) 5.0. The pH was adjusted to 7.4 with NaOH. Since the bath solution was completely exchanged with solutions having different $[Ca]_o$ within at least 3 min, the data were obtained 7 to 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

Effects on the AV nodal action potentials

In normal Tyrode's solution ($[Ca]_o$=1.8 mM), the mean value of the spontaneously beating rate was 133.5±3.3 beats/min (n=8)(Fig. 1). The maximum diastolic potential (MDP) was −65±2 mV. Increasing $[Ca]_o$ to 10 mM caused a positive chronotropic effect (Figs. 1 A and B). Simultaneously, the MDP was depolarized, and the maximum rate of depolarization ($\dot{V}_{max}$) was finally decreased after an early transient and small enhancement. In contrast, the fall of $[Ca]_o$ to 0 mM from the normal level greatly depressed the electrical activity (Figs. 1 C and D). The sinus rate was markedly reduced, and the $\dot{V}_{max}$ was depressed. Since the change to Ca^{2+}-free solution usually caused an arrest, the solution was exchanged to normal solution within 10 min. Average values of the action potential parameters in different $[Ca]_o$ solutions are summarized in Table 1. The $[Ca]_o$ decline to 0.9 mM caused a negative chronotropic effect by 6.1±1.8 % (n=7, P>0.05). The $\dot{V}_{max}$ was increased by 78.5±2.7 % (P<0.001). The $[Ca]_o$ elevation caused a positive chronotropic effect at both 5.4 and 10 mM, but the increase at 10 mM(by 6.4±1.7 %, n=6, P>0.05) was less than that at 5.4 mM(by 6.6±2.2 %, n=8, P>0.05). At 10 mM(n=6), the APA and $\dot{V}_{max}$ were decreased by 8.5±1.0 % (P<0.05) and

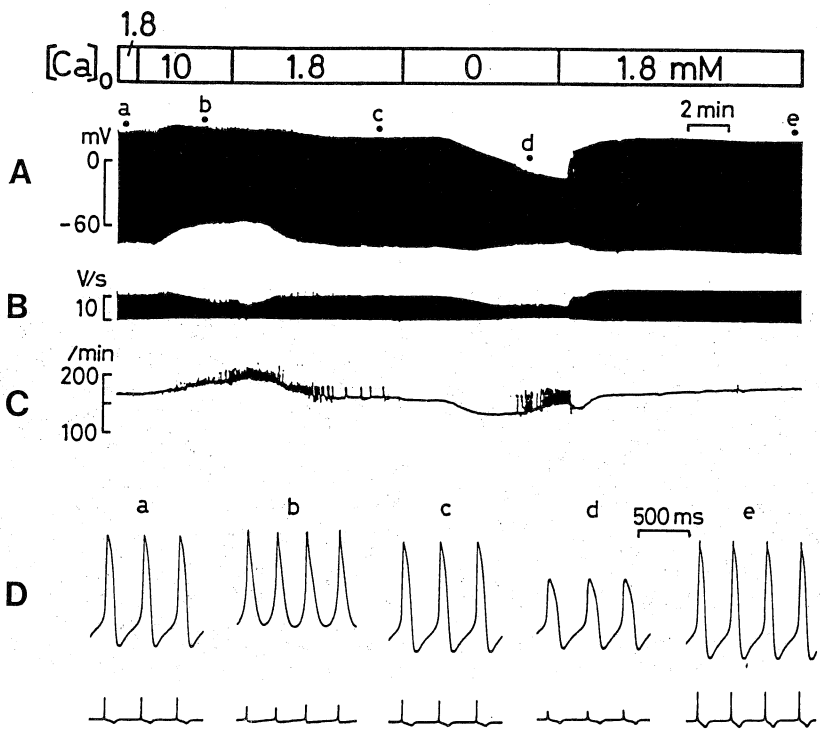

Fig. 1. Modulations of the action potentials by extracellular Ca^{2+} concentrations in rabbit spontaneously beating atrio-ventricular nodal cells. A : Changes in the action potentials. B : Maximum rate of rise of depolarization ($\dot{V}_{max}$). C : sinus rate. D : Action potentials and $\dot{V}_{max}$ at fast time scale at points (a-e) above the action potential recording in panel A.

by 50.0±2.2 %($P<0.001$), respectively. The $\dot{V}_{max}$ at 5.4 mM $[Ca]_o$ was −35.7±1.4 %($n=8$, $P<0.01$).

In 4 out of 6 preparations, an arrhythmia occurred only at 10 mM $[Ca]_o$. On return to normal Tyrode's solution, the regular rhythm was recovered.

Effects on the ionic currents

To examine the underlying ionic currents of the AV nodal action potentials, voltage-clamp experiments were performed. Test pulses were applied with an increment of 10 mV. The

Table 1. Modulation by the low and high extracellular Ca^{2+} solutions of the action potential parameters in the rabbit atrio-ventricular nodal cells

$[Ca]_o$	0.9 mM	1.8 mM	5.4 mM	10 mM
n	7	8	8	6
APA (mM)	96±2	94±3	93±3	86±4*
APD (msec)	123±4	113±4	102±3	107±5
MDP (mV)	−75±2	−73±2	−70±2	−69±2
$\dot{V}_{max}$ (V/S)	25±5***	14±2	9±3**	7±3***
CL (msec)	481±10	453±9	423±9	424±8

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 depolarization. CL: Cycle length. * : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$, with respect to control value (at 1.8 mM $[Ca]_o$).

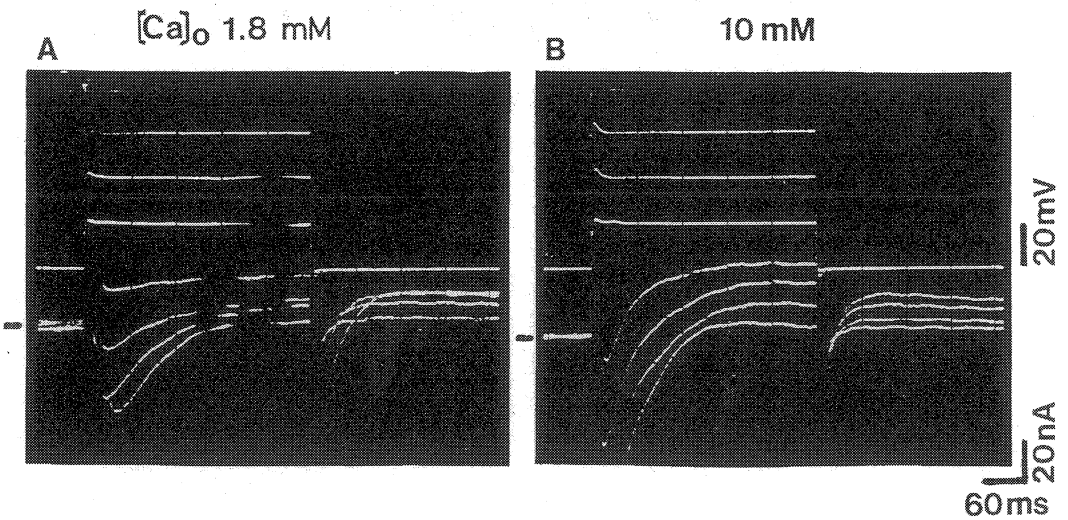

Fig. 2. Voltage-dependency of the ionic currents in rabbit atrio-ventricular nodal cells. Superimposed voltage traces and ionic current traces (a slow inward and a delayed outward K^+ currents). Test pulses were applied with an increment of 20 mV from a holding potential of −40 mV for a duration of 300 msec. A: At normal Ca^{2+} concentration (1.8 mM). B: At high Ca^{2+} concentration (10 mM). Short line at the left of the current trace represents zero current level.

holding potential was −40 mV. The depolarizing test pulse activated the slow inward current (I_{si}) followed by a time-dependently activated outward K^+ current (I_K), as shown in Figs. 2 A and B. In high $[Ca]_o$ solution (10 mM), there were the striking changes in the I_{si} and I_K currents (Fig. 2 B). The current-voltage (I-V) relationships are represented in Figs. 3 A and B. The $[Ca]_o$ elevation enhanced both the currents and the $[Ca]_o$ decline inhibited both. The increases in I_{si} (at 0 mV) and I_K (at+40 mV) were 31.7±2.0 % (n=5, P<0.001) and 34.3±3.3 % (n=5, P<0.001) by the elevation from 1.8 to 5.4 mM, and they were 75.2±2.2 % (n=6, P<0.001) and 78.8±3.4 % (n=6, P<0.001) by the elevation to 10 mM, respectively. On the other hand, the $[Ca]_o$ decline to 0.9 mM in 7 preparations decreased I_{si} at 0 mV by 64.1±2.7 %(P<0.001), and I_K at+40 mV by 40.5±4.3 % (P<0.01), The slope conductance of I_{si} was increased with an increase in $[Ca]_o$ (Fig. 3 B). These responses to the different $[Ca]_o$ solutions were completely reversible.

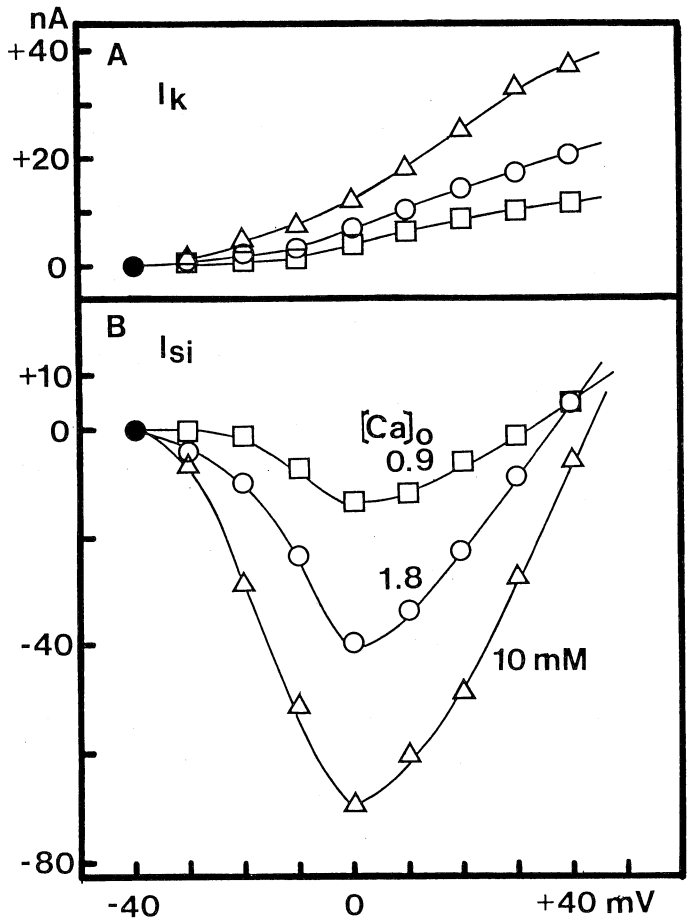

Fig. 3. Current-voltage relationships for the membrane ionic currents. A : I-V curves for the delayed outward K^+ current (I_K). B : I-V curves for the slow inward current (I_{si}). Symbols used are control at 1.8 mM (open circles), at 0.9 mM (triangles), and at 10 mM (squares).

DISCUSSION

The present experiments in rabbit AV nodal cells showed the following results. (a) The $\dot{V}_{max}$ was enhanced at 0.9 mM $[Ca]_o$, and was depressed at 5.4 and 10 mM $[Ca]_o$. (b) Low $[Ca]_o$ caused a negative chronotropic effect, whereas high $[Ca]_o$ caused a positive chronotropic effect, albeit statistically insignificant. (c) Elevation of $[Ca]_o$ caused potent depressions in the action potentials. (d) Both I_{si} and I_K currents were enhanced with an increase in $[Ca]_o$ level.

Increasing $[Ca]_o$ did not affect any significant chronotropic effects of AV nodal cells, although there was a tendency to increase the activity in high $[Ca]_o$ solution, and to decrease it in low $[Ca]_o$ solution. The less sensitivity to the changes in $[Ca]_o$ level would result from the physiological and anatomical functions of AV node[6]. Supraventricular muscles are mainly regulated by Ca^{2+} ion[7),12),14)]. However, the regulation of AV node is different from SA node dependent on only Ca^{2+} influx. The AV node is supplied with an anterior septal artery (Ca^{2+}−sensitive) from anterior descending artery, and also with a posterior septal artery (Na^+−sensitive) from posterior descending artery. Thus, AV node is also regulated strongly by Na^+ ion[1),15)].

Major effect of changes in $[Ca]_o$ was the modulation of the $\dot{V}_{max}$. The changes in $[Ca]_o$ exhibit a sigmoidal relationship between the $\dot{V}_{max}$ and the membrane potential in rabbit SA nodal cells[15)] and in Purkinje fibers[26)]. The sigmoidal curve was shifted toward the positive potential with an increase in $[Ca]_o$ level. This phenomenon was also observed in this study (see Fig. 1), but was produced early after exchanging to high $[Ca]_o$ solution. The initial increase was transient and small, and subsequently, the $\dot{V}_{max}$ amplitude was reduced. With an increase in $[Ca]_o$ (to 5.4 and 10 mM) in 6 to 8 AV nodal preparations, the APA and $\dot{V}_{max}$ were significantly decreased. The $\dot{V}_{max}$ and APA of the AV nodal cells are dependent on the I_{si} in voltage-clamped cells at a holding potential of −40 mV, different from those in the Purkinje fibers and the ventricular muscle (dependent on the fast Na^+ current). In addition, the amplitude may be in part attributed to the level of MDP; the hyperpolarization enhances the $\dot{V}_{max}$ and APA, whereas the depolarization inhibits them[17),20),23),25)].

At 10 mM, the spontaneous activity was not stimulated more than at 5.4 mM, and the action potential configuration was greatly depressed. As a result, in some preparations, arrhythmias occurred, presumably resulting from a development of the cellular calcium overload. Under the calcium overload conditions, triggered activities are elicited and lead to induce arrhythmias[8),13),17),18),21)].

On the other hand, the decrease in APD in high $[Ca]_o$ solution may be due to stimulation of I_K, which is activated by elevation of intracellular Ca^{2+} concentration (Ca^{2+}−activated K^+ current). Thus, it is possible that high $[Ca]_o$ shortened the APD. It also appears that the time course of inactivation for I_{si} may attribute to the APD regulation. It's inactivation is Ca^{2+} concentration-dependent, and is stimulated by higher cellular Ca^{2+} concentration[18),21)]. Therefore, increasing $[Ca]_o$ would shorten the APD due to the I_K enhancement and the stimulation of I_{si} inactivation.

Clinically, plasma Ca^{2+} level would not change so much as the high or low $[Ca]_o$ concentration used in this study. The slight alteration might not affect any important electrical activity of the AV node. But the $[Ca]_o$ elevation causes the depressant effects on the cardiac functions

(especially on P-R period), and arrhythmias often occurred, as well as in low $[Ca]_o$ solution.

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