Increased Dependency of Cardiac Pacemaker Activity on Extracellular Ca after Adrenergic Blockade in the Frog Heart

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Summary The frog sinus venosus shows spontaneous regular pacemaker activity, even in the absence of extracellular Ca^{2+} . When an α -adrenergic blocking agent (phentolamine) is applied, the rate of pacemaker activity, height of action potential, rate of slow diastolic depolarization, and the maximum diastolic potential become strongly dependent upon the extracellular Ca^{2+} concentration.

Key words: cardiac pacemaker activity, extracellular Ca, adrenergic blocking agents.

The initiation of spontaneous action potential in the cardiac pacemaker tissues depends, at least partly, upon Ca^{2+} movement through slow membrane channels (CARMELIET and VEREECKE, 1979; FLECKENSTEIN, 1981). Not only Ca entry blockers but also decreases in the extracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_e$) are able to effectively suppress pacemaker action potentials of the heart (BROOKS and LU, 1972; KOHLHARDT and FLECKENSTEIN, 1976; SEIFEN *et al.*, 1964; WIT and CRANEFIELD, 1974). In the frog heart, however, superfusing the sinus venosus with a Ca^{2+} -free solution failed to arrest the regular pacemaker activity (FUKUDA, 1984). Our previous report suggested that Ca^{2+} released from stored site(s) may contribute to the source of Ca^{2+} movement during depolarization in the absence of extracellular Ca^{2+} (FUKUDA, 1984). Since catecholamines increase the slow inward (Ca^{2+}) currents and augment Ca stores in the cardiac muscle (FLECKENSTEIN, 1981; NOMA *et al.*, 1980), and the pacemaker tissues are densely innervated by sympathetic (and vagal) fibers, the effects of autonomic blocking agents on the relationship between the pacemaker activity and $[\text{Ca}^{2+}]_e$ were examined in the present study.

The sinus venosus-atrial preparation obtained from the Japanese bullfrog (*Rana catesbeiana*) was placed with its internal surface upwards in an incubation chamber and was continuously superfused by a Ringer solution equilibrated with

air. Normal Ringer solutions have the following composition, in mmol/l: NaCl 111.0, KCl 1.9, CaCl_2 1.1, NaHCO_3 2.4, NaH_2PO_4 0.08, and glucose 5.6. Intracellular potentials were recorded from the right side of the ventral sinus venosus close to the sino-atrial junction with a floating glass microelectrode, and they were displayed on an oscilloscope for photography. Effects on pacemaker activities of a "nominally" Ca^{2+} -free Ringer solution and Ringer solutions containing the following autonomic blocking agents were studied: phentolamine mesylate, 2×10^{-7} – 5×10^{-6} g/ml; propranolol hydrochloride, 3×10^{-7} – 2×10^{-6} g/ml; atropine sulfate, 1 – 3×10^{-6} g/ml. In order to prevent possible interactions between drugs, only one drug was used in each preparation.

Figure 1A illustrates the effect of superfusing the sinus venosus with a Ca^{2+} -free solution on the rate of spontaneous firing of pacemaker tissues (sinus rate). The sinus venosus was still able to generate regular pacemaker activity even under Ca^{2+} -free circumstance. After 60 min of Ca^{2+} washout, the sinus rate decreased to

Fig. 1. Effect of Ca^{2+} -free solution on spontaneous pacemaker activity of frog sinus venosus. A: changes in the rate of spontaneous firing of sinus venosus (sinus rate) during superfusing with Ca^{2+} -free solutions; control, preparations were superfused with a Ca^{2+} -free Ringer solution; propranolol, propranolol hydrochloride was added to superfusing solutions (2×10^{-6} g/ml); phentolamine, phentolamine mesylate was added to superfusing solutions (5×10^{-7} g/ml). Values of sinus rates are expressed as the percentage of value measured during superfusing with a normal Ringer solution ($[\text{Ca}^{2+}] = 1.1$ mmol/l) (mean $\pm$ S.E., $n = 11$). B: typical example of the effect of a Ca^{2+} -free solution on pacemaker activity in the presence of phentolamine (5×10^{-7} g/ml).

$64 \pm 5\%$ (mean $\pm$ S.E., $n=11$) of the control value measured in normal $[\text{Ca}^{2+}]_e$ (1.1 mmol/l). The maximum diastolic potential decreased from -63 ± 3 (control, mean $\pm$ S.E., $n=11$) to -44 ± 6 mV (Ca^{2+} -free 60 min, mean $\pm$ S.E., $n=11$). The height of action potential in control and Ca^{2+} -free (60 min) conditions was 76 ± 2 (mean $\pm$ S.E., $n=11$) and 42 ± 6 mV (mean $\pm$ S.E., $n=11$), respectively. The mean rate of rise of slow diastolic depolarization decreased from 32 ± 1 (control, mean $\pm$ S.E., $n=11$) to 15 ± 4 mV/s (Ca^{2+} -free 60 min, mean $\pm$ S.E., $n=11$). When a β -adrenergic blocking agent (propranolol) was applied, changes in pacemaker activity resulted from Ca^{2+} washout were slightly augmented, whereas under application of α -adrenergic blocker (phentolamine), the Ca^{2+} -free solution abolished the initiation of spontaneous activity within 20 min (Fig. 1A, B). Before complete cessation of activity, there was a small oscillation in the membrane potential which decreased progressively with time. In some experiments, the recording electrode was inserted into other cells in the sinus venosus after complete cessation of spontaneous activity in one cell. Penetration, however, failed to detect any remaining spontaneous oscillation of potentials in adjacent cells, which showed that a pacemaker shift accompanying conduction block within the sinus venosus did not occur. A cholinergic blocking agent, atropine, did not affect the sinus rate and its reduction by Ca^{2+} washout.

A more quantitative relationship between the $[\text{Ca}^{2+}]_e$ and various indices of pacemaker activity is shown in Fig. 2A and B. In the control preparation, the height of action potential decreased by about 25 mV with the lowering of $[\text{Ca}^{2+}]_e$ from 1.1 (control) to 0.07 (1/16 of control) mmol/l though the sinus rate and the rate of slow diastolic depolarization changed only slightly. When phentolamine (5×10^{-7} g/ml) was applied, the sinus rate, the height of action potential and the rate of slow diastolic depolarization sharply decreased with a reduction in $[\text{Ca}^{2+}]_e$. At 1/16 of control $[\text{Ca}^{2+}]_e$ level, most preparations ceased their spontaneous firing. The reduction in the maximum diastolic potential brought about by low $[\text{Ca}^{2+}]_e$ was also enhanced by phentolamine.

As previously described (FUKUDA, 1984) spontaneous pacemaker activity of the frog's heart could be maintained, even in the absence of extracellular Ca^{2+} , by the Ca^{2+} possibly derived from store-site(s) in the control preparation. The present study showed that under the influence of phentolamine the extracellular Ca^{2+} plays important roles not only in the generation of spontaneous depolarization but also in the maintenance of diastolic potential in the frog sinus venosus. Possible mechanisms of this action of phentolamine, however, deserve careful consideration. Although phentolamine was used with the intention of blocking adrenergic receptors, the drug may have direct action on Ca^{2+} movement, for instance, suppressing Ca^{2+} entry into the cell interior from surface store-sites that are independent of adrenergic receptors. This possibility remains to be verified. Another possibility is that the drug blocked adrenergic receptors might have been activated by catecholamine (epinephrine in the case of frog hearts (AZUMA *et al.*, 1965)) released spontaneously from sympathetic nerve terminals, at least in an early phase

Fig. 2. Effects of phentolamine on the relationship between $[Ca^{2+}]_e$ and spontaneous pacemaker activity. A: the sinus rate is expressed as the percentage of value measured during superfusing with a normal Ringer solution. ECF $[Ca^{2+}]$, extracellular Ca^{2+} concentration expressed as a fraction of $[Ca^{2+}]$ in the normal Ringer solution (1 $[Ca^{2+}]$ = 1.1 mmol/l); phentolamine, phentolamine mesylate was added to superfusing solutions (1×10^{-6} g/ml). B: typical example of the effect of decreasing $[Ca^{2+}]_e$ on the pacemaker activity in the presence of phentolamine. A and B were obtained from the same preparation.

after isolating the preparation. The result is, however, peculiar in that the relationship between $[Ca^{2+}]_e$ and pacemaker activity was affected by phentolamine (α -blocker) but not by propranolol (β -blocker). It is generally believed that the increase in slow inward (Ca^{2+}) current by catecholamines in the mammalian pacemaker tissues is brought about by increases in the concentration of cyclic AMP due to activation of β -receptors (NOMA *et al.*, 1980; BROWN, 1982; IRISAWA and KOKUBUN, 1983; REUTER, 1984). There is, however, evidence that the α -receptor activation by catecholamines causes an increase in the intracellular $[Ca^{2+}]$ in many tissues including the liver, vascular smooth muscles and heart, though the population of α -receptors is sparse in the cardiac tissue (EXTON, 1980; HOFFMAN and LEFKOWITZ, 1982). Subtypes of adrenergic receptors responsible for the chronotropic action of epinephrine have not yet been identified in the frog sinus venosus.

In conclusion, phentolamine clearly enhances the roles of extracellular Ca^{2+} in the modulation of pacemaker activity of frog sinus venosus although the

mechanism of action of the drug on stored Ca or on adrenergic receptors is unknown.

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