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AUTOMATIC ACTIVITY OF PACEMAKER CELLS OF THE ATRIOVENTRICULAR VALVES OF THE RABBIT HEART

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Automatic activity of pacemaker cells with slow diastolic depolarization was found by means of a microelectrode technique in the cusps of the atrioventricular valves taken from the hearts of 34 rabbits. Electrophysiological characteristics of the action potentials of these cells were studied. Inhibitors of the slow sodium–calcium channel (Mn^{++}, Co^{++}, and Mg^{++} ions) were found to abolish automatic activity of the pacemaker cells, whereas a three- to fourfold decrease in the potassium ion concentration in the perfusion solution did not depress it. The automatic activity of the pacemaker cells of the atrioventricular valves is considered to be due to the function of the slow sodium–calcium channel.

KEY WORDS: atrioventricular valves; automatic activity; action potentials; inhibitors of ionic permeability.

Potential pacemakers have recently been found in the atrioventricular valves of the dog's [11, 12] and monkey's [8] heart. The cells of these pacemakers are characterized by slow diastolic depolarization, by action potentials of relatively low amplitude, and by a low level of the threshold and maximal diastolic potentials As regards the ionic mechanisms of automatic activity of the cells of the atrioventricular valves all that is known is that verapamil, which blocks the slow calcium channel, abolishes it [8]. The writers found pacemaker cells in the atrioventricular valves of the rabbit heart and investigated their electrophysiological characteristics as well as the effect of inhibitors of the slow sodium–calcium channel and of substances selectively modifying potassium permeability on them.

EXPERIMENTAL METHOD

The heart was removed from 34 rabbits under urethane anesthesia and placed in oxygenated Tyrode solution at 36–37°C, and the cusps of the atrioventricular valves were excised. The spontaneously contracting preparations were perfused with Tyrode solution of the following composition (in mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 12.0, NaH_2PO_4 10.4, and glucose 5.5. The solution was oxygenated with a mixture of 95% O_2 and 5% CO_2; the pH of the solution was 7.4 and its temperature 36–37°C. Membrane potentials of the pacemaker cells were derived by means of glass microelectrodes filled with 3 M KCl, with a tip 0.5 μ in diameter and a resistance of 20–40 MΩ. To amplify and record the potentials a cathode follower (Biofizpribor Special Engineering Design Office), an S1-19 cathode-ray oscilloscope, and an N041 loop oscillograph were used. The spontaneous excitation rate of the pacemaker cells, the levels of their critical and maximal

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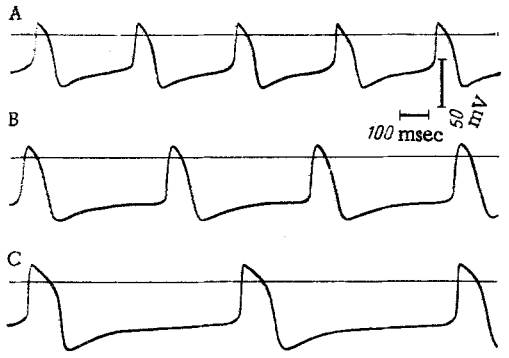

Fig. 1. Automatic activity of pacemaker cells of atrioventricular valves taken from hearts of three rabbits. Spontaneous APs with generation frequency of 170 (A), 110 (B), and 75 (C) per minute recorded.

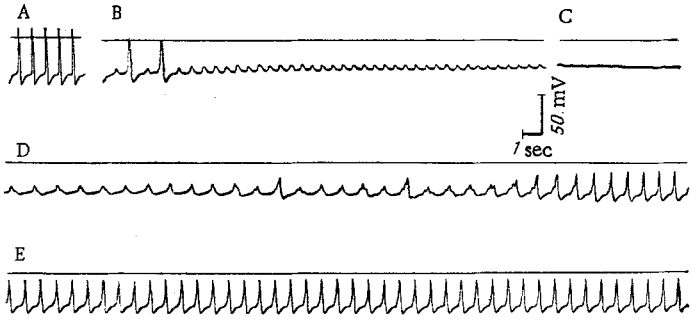

Fig. 2. Effect of Co^{++} ions on automatic activity of pacemaker cell of atrioventricular valve: A) spontaneous APs; B, C) gradual disappearance of APs and subthreshold potentials at seventh minute of action of Co^{++} ions (4 mM); D, E) gradual recovery of automatic activity during perfusion with ordinary Tyrode solution.

diastolic potentials, the amplitude of their action potential (AP) and its duration at the level of the critical potential, and the mean rate of rise of the slow diastolic depolarization (SDD) were determined. In 18 experiments the effect of manganese, cobalt, or magnesium ions, inhibitors of the slow sodium–calcium channel [1, 5-7, 9, 10], on automatic activity of the valve cells was studied. For this purpose one of the following substances was added to the Tyrode solution: $MnCl_2$ (2-4 mM), $CoCl_2$ (4 mM), or $MgCl_2$ (10-20 mM). In nine experiments the action of a three- to fourfold increase in the potassium ion concentration in the Tyrode solution (8.1-10.8 mM) was investigated. In seven experiments the action of tetraethylammonium chloride (TEA, 10-20 mM), which inhibits potassium permeability [7, 10], was investigated. Before and during the action of the test substances the microelectrode remained in the same pacemaker cell.

EXPERIMENTAL RESULTS AND DISCUSSION

The atrioventricular valves of the rabbit heart contain potential pacemakers with stable spontaneous automatic activity. This is manifested chiefly in the cusps of the valves of the right ventricle. Action potentials of different frequency, amplitude, and shape, with slow diastolic depolarization, were recorded in the pacemaker cells (Fig. 1, Table 1). In the course of 5-15 min Mn^{++} (2-4 mM), Co^{++} (4 mM), and Mg^{++} (10-20 mM) ions abolished the automatic activity of the valve cells. The activity disappeared most rapidly during the action of Mn^{++} ions. Before the automatic activity ceased as a result of this treatment the amplitude of the AP fell on average by 17% of its initial value ($P < 0.01$), whereas the other electrophysiological parameters were not significantly changed. During the action of Co^{++} ions the frequency of excitation decreased (on average by 27%), the rate of rise of SDD was reduced (on average by 31%), and the level of the critical potential (on average by 26%) and amplitude of the AP (on average by 28%) fell. Individual spikes of the AP then disappeared, leaving subthreshold potentials, oscillations of which, gradually diminishing in amplitude, preceded the disappearance of electrical activity (Fig. 2).

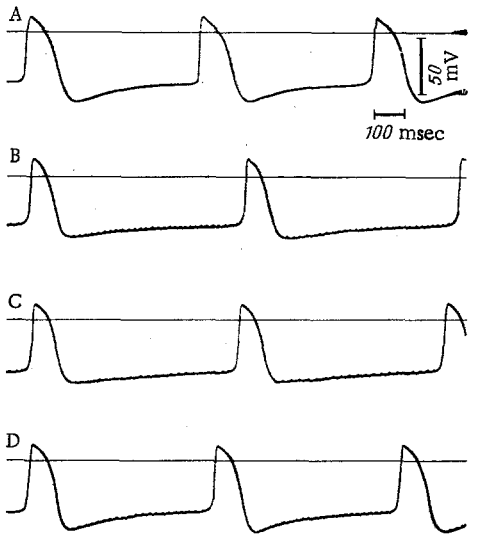

Fig. 3. Effect of increased concentration of K^+ ions on automatic activity of pacemaker cell of atrioventricular valve: A) spontaneous APs; B, C) APs during perfusion with Tyrode solution with threefold increase in concentration of K^+ ions at 15th and 30th min respectively of action of K^+ ions; D) APs during perfusion with ordinary Tyrode solution.

TABLE 1. Electrophysiological Characteristics of Pacemaker Cells of Atrioventricular Valves of the Rabbit Heart (data from 39 cases)

	Freq. of excitation, per min	Duration of AP, in msec	Amplitude of AP	Critical potential	Maximal diastolic potential
			in mV		
Limits of variations	52—170	80—130	41—73	—32—60	—46—93
$M \pm m$	82±4,76	105±2,5	56±1,2	—42±1,4	—64±1,8

During the action of Mg^{++} ions the frequency of excitation fell considerably (on average by 64%), the rate of rise of SDD was reduced (on average by 66%), and the amplitude of the APs also fell (on average by 22%). In some experiments the extinction of automatic activity during the action of Mg^{++} ions passed through a stage of low-amplitude potentials, just as during the action of Co^{++} ions. The effect of extinction of automatic activity by bivalent cations was reversible following perfusion with Tyrode solution of normal composition.

A three- to fourfold increase in the concentration of K^+ ions in the Tyrode solution did not abolish the automatic activity of the preparations. Not until the 10th-15th min of action of an excess of K^+ ions was the excitation frequency reduced (on average by 19%), the rate of rise of SDD slowed (on average by 55%), and the level of the maximal diastolic potential lowered (on average by 19%). The duration of the APs was slightly shortened (Fig. 3). After the action of TEA in a concentration of 10-20 mM for 10-15 min the excitation frequency of the pacemaker cells increased (on average by 40%), SDD rose more rapidly (on average by 52%), and the level of the maximal diastolic potential (on average by 25%) and the amplitude of AP (on average by 31%) were raised. The duration of the AP was 1.5-2 times longer than initially.

The electrophysiological characteristics of cells of the potential pacemakers in the cusps of the atrioventricular valves of the rabbit hearts were thus similar to those of pacemaker cells of the atrioventricular valves of other mammals [3, 8, 11, 12] and very similar to the characteristics of the automatic cells of the sinoatrial node [2, 4]. The limits of variations in the critical potential of rabbit heart valve cells (from −32 to −60 mV) differed significantly from those established for Purkinje fibers of the ventricles of warm-blooded animals (from −60 to −80 mV) [2, 4, 12].

In the opinion of most workers, a leading factor in AP generation at low levels of membrane potential is the slow inward current, carried by sodium and calcium ions [7, 10]. Inhibitors of the slow sodium−potassium, channel, including Mn^{++}, Co^{++}, and Mg^{++} ions as used in the present experiments and varapamil, as other workers have found [8], abolish the automatic activity of cells of the atrioventricular valves. Co^{++} and Mg^{++} ions inhibit not only the generation of AP spikes, but also the subthreshold spontaneous low-amplitude potentials.

The generation of pacemaker activity in the valves still continues during the action of an excess of potassium ions. The TEA inhibitor of potassium permeability causes an increase in the rate of SDD and in the frequency of spontaneous excitation, evidently on account of a shift in the ionic equilibrium toward an increase in

sodium–calcium permeability of the membrane and a decrease in its potassium permeability. On the grounds mentioned above it can be postulated that the automatic activity of the pacemaker cells of the atrioventricular valves of the rabbit heart is due principally to the functioning of the slow sodium–calcium channel.

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EFFECT OF ANGIOTENSIN II ON THE HEMODYNAMICS AFTER SYSTEMIC AND PORTAL ADMINISTRATION

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Changes in the general hemodynamics were studied in healthy unanesthetized dogs after injection of angiotensin II for 60 min into the superior vena cava and portal vein at the rate of 27 ng/kg/min. Portal administration of the peptide was found to induce a weaker pressor effect. After systemic injection of angiotensin II the arterial pressure rose as the result of an increase in peripheral vascular resistance, and the minute volume of the circulation was reduced. After portal injection of angiotensin the increase in arterial pressure was due chiefly to an increase in the minute volume of the circulation. The differences in the hemodynamic responses cannot be explained entirely by metabolism of the peptide in the liver. After portal injection of angiotensin II it is possible that depressor substances from the liver enter the blood stream.

KEY WORDS: angiotensin II; hemodynamics; peptide metabolism in the liver.

Directing blood from the kidneys and adrenals into the liver leads to an increase in the metabolism of humoral factors of the renin–angiotensin–aldosterone system [3, 5], and produces a hypotensive effect in vasorenal hypertension [5, 6].

To analyze this effect changes in the hemodynamics were compared after infusion of angiotensin II into the systemic and portal circulation.

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