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Effect of Pinacidil on the Electrophysiological Properties in Guinea-pig Papillary Muscle and Rabbit Sino-atrial Node

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Abstract—The electrophysiological effect of the antihypertensive drug pinacidil has been examined in preparations of guinea-pig papillary muscle and rabbit sino-atrial node using a standard microelectrode method. In papillary muscle preparations, pinacidil (> 30 μM) shortened the action potential duration (APD), whereas it did not affect the maximum rate of rise (V_{max}). Pinacidil (> 1 μM) also decreased APD of slow action potentials evoked by high K^+ (27 mM) solution containing 0·2 mM Ba^{2+}. At 30 μM, the drug reduced the V_{max} of slow action potentials. In the spontaneously beating sino-atrial node, pinacidil (> 30 μM) shortened APD. At 100 μM, it also decreased the heart rate, V_{max}, action potential amplitude and the rate of diastolic depolarization. It is concluded that pinacidil modifies the electrical activity of myocardial cells probably due to an increase in the potassium conductance although in high concentrations the compound might also reduce Ca^{2+} influx through the cell membrane, which would contribute to an obvious negative chronotropic action.

Pinacidil, a new antihypertensive agent, has been shown to inhibit the contraction of rabbit and rat aorta elicited by noradrenaline, 5-hydroxytryptamine or potassium chloride (Arrigoni-Martelli et al 1980; Nielsen & Arrigoni-Martelli 1981; Mikkelsen & Pedersen 1982). The mechanism of its vasodilating action is mainly explained by an increase in the potassium permeability of vascular smooth muscle (Bray et al 1987). Recently, pinacidil has also been found to increase the potassium conductance in myocardial cells (Iijima & Taira 1987), and thus would be expected to alter the duration of the action potential of cardiac tissues and to modify cardiac pacemaker activity.

We have studied the electrophysiological effects of pinacidil in preparations of guinea-pig papillary muscle and rabbit sino-atrial node by means of a standard microelectrode technique to clarify whether the drug modifies the electrical activity of fast- and slow-response fibres of the heart.

Material and Methods

Guinea-pig papillary muscles

Guinea-pigs, 200–300 g, were stunned by a blow to the neck, the heart quickly removed and papillary muscles (less than 1 mm thick) excised from the right ventricle. The preparation was mounted and perfused in a recording chamber with oxygenated (95% O_2, 5% CO_2), warmed (36 ± 0·5°C) Tyrode solution of composition (mM): NaCl 132·0, KCl 4·0, $MgCl_2$ 1·0, $NaHCO_3$ 12, NaH_2PO_4 0·4, $CaCl_2$ 1·8, glucose 10·0. The pH of all solutions was 7·4. The preparation was then driven at 1·0 Hz for more than 1 h by stimulating electrodes consisting of a pair of Ag-AgCl wires (diameter 1 mm, length 5 mm) placed near the preparation. The stimulus threshold was determined in experiments before drug perfusion; the current pulse intensity was set 1·5 times the threshold. The effects of pinacidil on the slow action potentials were examined using 0·2 mM Ba^{2+}-containing Tyrode solution in which $[K^+]o$ was increased to 27 mM.

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Rabbit sino-atrial nodes

Rabbits, 1·5–2·0 kg, were killed by a blow to the neck and the hearts quickly isolated. After removing the right atrium, strands of sino-atrial node tissue (1 mm long and 0·3 mm wide) were obtained by dissection in the direction perpendicular to the crista terminalis. These were placed in a recording chamber and were ligated at two sites with a fine silk fibre to give a final dimension of about 0·3 × 0·3 mm. The specimens were prepared by the method of Noma & Irisawa (1976). The Tyrode solution was composed as follows (mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·0, NaH_2PO_4 0·6. The pH was adjusted to 7·4 by adding Na_2HPO_4.

Pinacidil was kindly supplied by Shionogi Pharmaceutical Ltd.

Recording of transmembrane action potential

Transmembrane action potentials were recorded with conventional glass microelectrodes filled with 3 M KCl having resistances of 10–30 Ω. The electrodes were connected to an amplifier (Nihon Kohden, MEZ 7101). The membrane potential was monitored on an oscilloscope (Nihon Kohden, VC-10), photographed by Nihon Kohden, RLG-6201 camera, and recorded simultaneously on a chart recorder (Nihon Kohden, RJG-4122). The bath temperature was continuously monitored with an electric thermometer (Nihon Kohden, MGA-III).

Statistical analysis

The electrophysiological measurements were performed 20 min after changing to a new solution. Values are expressed as mean ± s.e. Statistical analysis was performed using Student's *t*-test for paired data and the difference was considered significant when *P* values were less than 0·05.

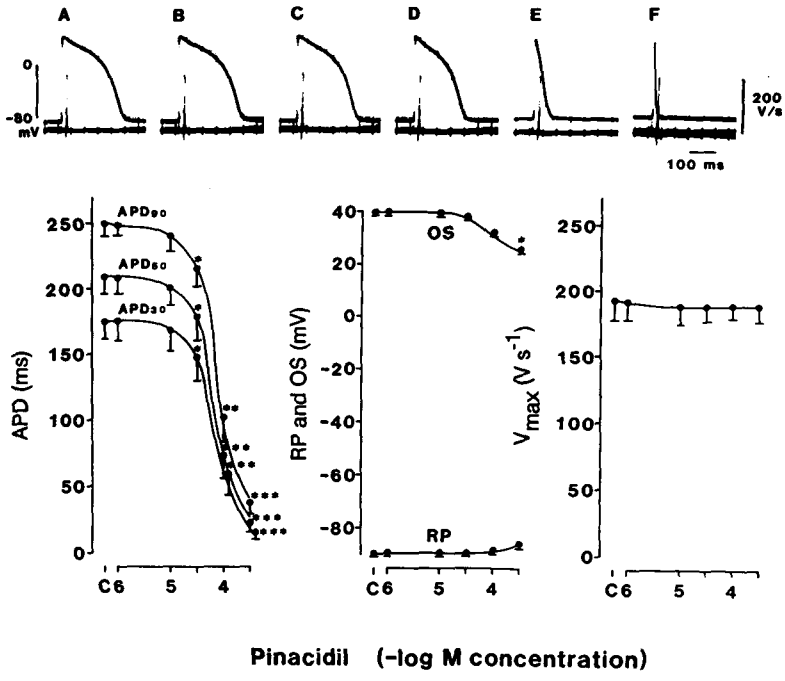

FIG. 1. Effect of pinacidil on action potential and its various parameters in guinea-pig papillary muscle. Upper panel: Action potential (upper trace) and its first derivative (lower trace) are recorded before (A) and after exposure to 1 (B), 10 (C), 30 (D), 100 (E), and 300 μM (F) pinacidil. Lower panel: Action potential duration at 30, 50, 90% repolarization (APD30, APD50, APD90), overshoot potential (OS), resting potential (RP) and maximum rate of rise (v_{max}) are measured with and without pinacidil. C = control. $*P<0.05$; $**P<0.01$, $***P<0.001$ with respect to control values. Values are mean ± s.e. (n = 5).

Results

Guinea-pig papillary muscle preparations

The effects of pinacidil on the fast-response transmembrane action potentials were examined in preparations constantly driven at 1·0 Hz.

Fig. 1 shows the effect of pinacidil on the action potential (upper panel) and its various parameters (lower panel). Of the variables tested, the action potential duration (APD) appeared to be the most sensitive to pinacidil, showing a statistically significant decrease at 30 μM. Above 30 μM, the drug reduced APD at 30, 50 and 90% repolarization in a dose-related manner. The overshoot potential (OS) was also decreased by pinacidil 300 μM, whereas the resting potential (RP) and the maximum rate of rise (V_{max}) of the fast-response action potential were not significantly altered. These electrophysiological findings suggest that pinacidil did not affect the fast Na^+ inward current (I_{Na}) and that the drug induced an increase in K^+ conductance (g_K) and/or a decrease in Ca^{2+} conductance (g_{Ca}).

To inactivate the cardiac Na^+ channel, the preparation was perfused with a high K^+ solution (27 mM K^+ with 0·2 mM Ba^{2+}) before the experiment (Ehara & Inazawa 1980; Inazawa et al 1982). In this condition, the resting potential is depolarized to approximately −40 mV, so that the Na^+ channel is almost completely inactivated (slow action potential). Therefore, g_{Ca} plays a major role in the initiation of slow action potentials, while a repolarizing phase is mainly explained by a combination of g_{Ca} and g_K. Fig. 2 shows the cumulative effect of pinacidil on the slow action potential (upper panel) and its various characteristics (lower panel). Above 1 μM, pinacidil shortened APD at 30, 50 and 90% repolarization in a dose-dependent fashion. OS was slightly but significantly reduced, while RP was not obviously changed after drug application. Pinacidil also tended to reduce V_{max} of the slow action potential, but its reduction became statistically significant at 30 μM. Considering the evidence that relatively low concentrations (1–10 μM) of pinacidil obviously shortened APD without a significant decrease in V_{max}, it is likely that the compound increased K^+ conductance. However, a higher concentration (30 μM) of pinacidil markedly and significantly depressed V_{max} of the slow action potential, namely, the slow Ca^{2+} inward current (I_{si}), which would induce further shortening of APD.

Rabbit sino-atrial node preparations

To investigate the pinacidil action on the chronotropism, we studied the drug effect on the action potential of spontaneously beating sino-atrial node pacemaker cells (Fig. 3). Pinacidil (100 μM) induced a negative chronotropic effect associated with a reduction of the rate of diastolic (phase 4) depolarization. Pinacidil, 300 μM, produced sinus arrest, the preparation recovering after being returned to drug-free control Tyrode solution. The effect of pinacidil on various action potential parameters is summarized in Fig. 4. Although there were no significant changes at less than 10 μM, 30 μM pinacidil shortened APD at 50% repolarization. At 100 μM, the drug also increased the spontaneous cycle length (SCL), and decreased V_{max}, the action potential amplitude (APA) and rate of the diastolic depolarization (RDD).

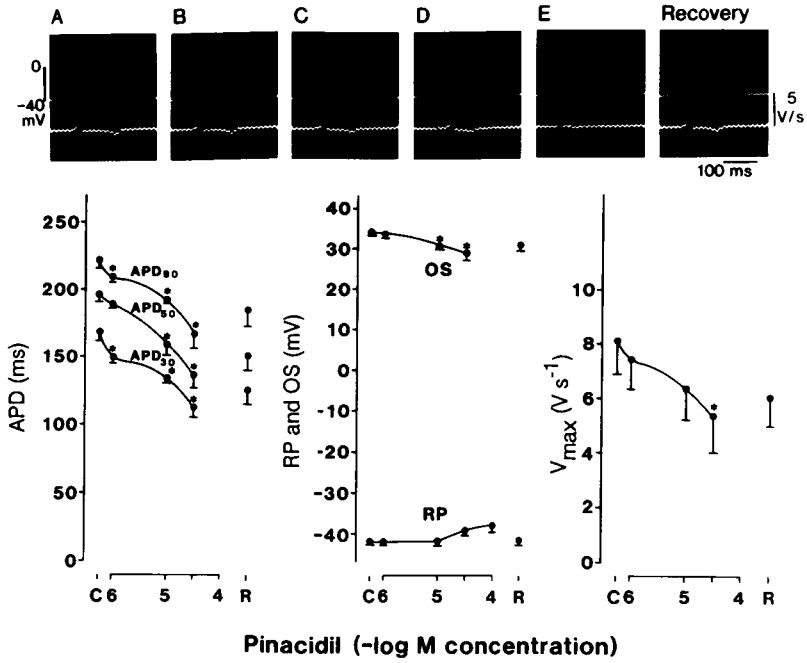

FIG. 2. Effect of pinacidil on slow action potential and its various characteristics in K^+-depolarized (27 mM, K^+, 0·2 mM Ba^{2+}) papillary muscle. Upper panel: Upper trace shows action potential and lower shows its first derivative. Measurements are made before (A) and after exposure to 1 (B), 10 (C), 30 (D), 100 μM (E) pinacidil. 100 μM pinacidil did not induce an electrical activity, so that only RP was obtained. C = control, R = recovery. Lower panel: Values are mean ± s.e. $*P < 0{\cdot}005$ (n = 4).

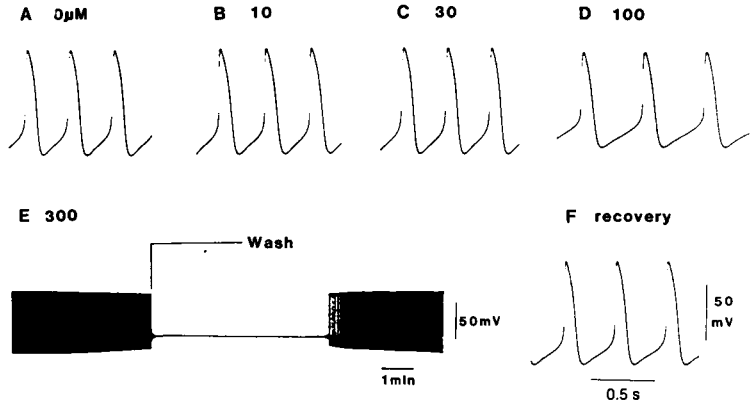

FIG. 3. Effect of pinacidil on action potential in rabbit sinoatrial node preparation. The drug concentration was cumulatively increased to 10 (B), 30 (C), 100 (D) and 300 μM (E). (E): 300 μM pinacidil abolished the spontaneous discharge, which resumed after washing out (Wash) with Tyrode solution. (F): 20 min after washing out, the action potential almost recovered.

Discussion

Pinacidil, a new antihypertensive agent increases the permeability of vascular smooth muscle membrane to potassium ions (the potassium conductance, g_K, Bray et al 1987). Pinacidil also increases the background potassium current in guinea-pig ventricular cells (Iijima & Taira 1987). Since an efflux of positive charges repolarizes the membrane, a similar increase in g_K in cardiac tissue would lead to a shortened APD and probably altered cardiac automaticity.

The present study has demonstrated that pinacidil produced a statistically significant shortening of APD in guinea-pig papillary muscle, in slow action potentials evoked by high K^+ solution and in rabbit sino-atrial node. These findings are in accord with a previous study observed in canine Purkinje fibres and ventricular muscle (Smallwood & Steinberg 1988). Pinacidil also reduced V_{max} of slow action potentials of papillary muscle induced by high $[K^+]o$ and in sino-atrial node, whereas it did not affect V_{max} of fast responses of guinea-pig papillary muscle.

In fast-response fibres, shortening of APD is generally due to an increase in outward K^+ current and a decrease in slow inward Ca^{2+} current and/or partially inactivated sodium current that flows during the plateau phase of the action potential (the window current) (Colatsky 1982). However, pinacidil did not affect V_{max} of fast-response action potentials, implying that the compound induced no significant change in I_{Na}. Therefore, changes in g_K and g_{Ca} are considered

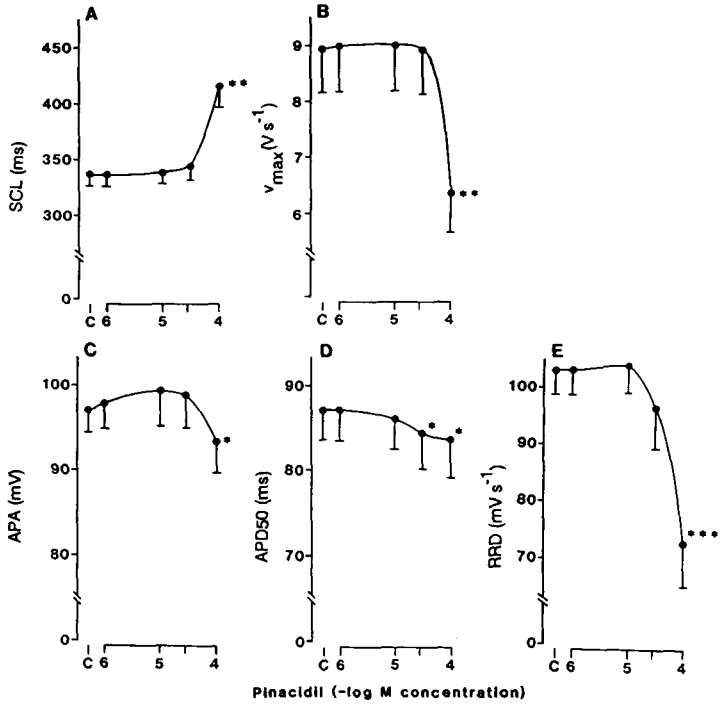

FIG. 4. Effect of pinacidil on various action potential parameters of sino-atrial node. SCL = spontaneous cycle length, V_{max} = maximum rate of rise, APA = action potential amplitude, APD50 = action potential duration at 50% repolarization, RDD = rate of diastolic depolarization. C = control. Values are mean ± s.e. $*P<0.05$, $**P<0.01$, $***P<0.001$ (n = 7).

to be mainly responsible for a decrease in APD. To investigate the drug action of g_K and g_{Ca}, we studied slow-response fibres such as K^+ depolarized papillary muscle and spontaneously beating sino-atrial node. In these preparations, the threshold concentration for inducing APD shortening is obviously lower than that for inducing a reduction of V_{max}, indicating that pinacidil decreased APD mainly by increasing g_K, and that the compound might also depress I_{si} at relatively high concentrations.

Changes in g_K would also alter the cardiac automaticity. The results obtained in sino-atrial node preparations revealed that a high concentration (100 μM) of pinacidil caused a negative chronotropic effect. Such a bradycardia is mainly due to a decrease in the rate of phase 4 depolarization. Since the decaying outward K^+ current is responsible for the pacemaker depolarization (Noma et al 1980; Brown 1982), it is likely that the pinacidil-induced increase in g_K would decelerate the phase 4 depolarization and, concomitantly, decrease the heart rate. However, the activation of I_{si} also plays a role in slow diastolic depolarization (Noma et al 1980; Yanagihara et al 1980), and we previously demonstrated that drugs which reduce Ca^{2+} influx through the cell membrane induce a negative chronotropic action (Kotake et al 1986, 1987, 1988). In the present experiment, pinacidil (100 μM) decreased V_{max} and RDD significantly suggesting that the drug might depress I_{si} only at high concentrations.

Considering these electrophysiological observations, it is concluded that pinacidil, which increases g_K of vascular smooth muscle membrane, induces APD shortening of the action potential in isolated mammalian myocardium probably by a similar mechanism to that observed in smooth muscle, namely, an increase in g_K. The compound also decreases I_{si} at relatively high concentrations, which might also contribute to the APD shortening and the decreased heart rate.

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