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Effect of *dl*-praeruptorin A on calcium current in ventricular cells of guinea pig[1]

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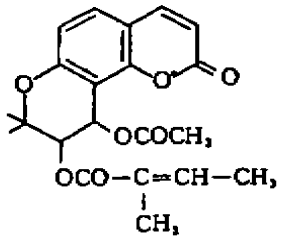
ABSTRACT With patch clamp technic (whole cell recording), the effect of *dl*-praeruptorin A (Pra), an ingredient of *Peucedanum praeruptorum* Dunn on calcium current (I_{Ca}) in the single ventricular cells of guinea pig was studied. Results showed that under Cs/Cs condition, when the holding potential was −40 mV and in the presence of Pra (1, 10, 100 $\mu mol \cdot L^{-1}$), I_{Ca} was decreased dose-dependently from 2.02 ± 0.24, 2.00 ± 0.12, 2.12 ± 0.33 nA (control) to 1.60 ± 0.24, 1.32 ± 0.08, 1.16 ± 0.43 nA, respectively, and their inhibitory rates were 21 %, 33.5 %, 45 %, respectively. The current-voltage relation curve showed that the reversal potential of I_{Ca} was +60 mV; the potential producing peak value of I_{Ca} was about 0 mV. The results indicated that Pra had a Ca^{2+} channel blocking effect.

KEY WORDS praeruptorin A; myocardium; cultured cells; calcium channel blockers; patch clamp technic

The crude extract of the root of *Peucedanum praeruptorum* Dunn, a traditional Chinese medicinal herb antagonized experimental arrhythmias induced by $BaCl_2$ and ligation of the left coronary arteries[1,2]. The ingredient *dl*-praeruptorin A (Pra, a white powder mp 156 — 8 ℃), is a nonglycosidic angular-type pyranocoumarin.



dl-Praeruptorin A
(3'-Angeloyloxy-4'-acetoxy-3',4'-dihydroseselin)

Pra, that is Pd-Ia, inhibited the influx of $^{45}Ca^{2+}$ into the smooth muscle cells[3]. We found that Pra reduced the APD_{30} and APD_{90} in the ventricular cells of guinea pig[4]. However, it is not known whether Pra can affect the membrane ionic channels of myocardial cells. In this study, the effects of Pra on calcium currents (I_{Ca}) in the single ventricular cells of guinea pig were investigated with patch clamp technic (whole cell recording) for exploring its anti-arrhythmic mechanisms.

MATERIALS AND METHODS

Preparation of myocardial cells Single ventricular cells of the guinea pig were prepared through an enzymatic dissociation method[5,6]. The heart was perfused through the coronary arteries with Ca^{2+}-free Tyrode solution containing collagenase 0.08 $mg \cdot ml^{-1}$ (Yakult, Japan) for 15 — 20 min at 36 ℃. A small piece of the ventricle was excised and agitated in the KB medium at 4 ℃ for at least 1 h. The recording chamber (0.3 ml) was perfused with normal Tyrode

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solution 2 — 3 ml·min^{-1}. Experiments were carried out at 36 — 37 ℃ on rod-shape quiescent single cells which exhibited distinct striation of sarcomeres.

Solutions and Pra Normal Tyrode solution contained NaCl 143, NaOH 24.4, KCl 5.4, $MgCl_2$ 0.5, NaH_2PO_4 0.3, $CaCl_2$ 1.8, glucose 5, and HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) 5 mmol·L^{-1}. Ca^{2+}-free Tyrode solution was normal Tyrode solutioo without $CaCl_2$. The KB medium contained KOH 70, *l*-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, GEDTA (glycoether-diaminetetraacetic acid) 0.5, glucose 10, and HEPES 10 mmol·L^{-1}. The internal solution of electrode contained CsCl 130, $MgCl_2$ 1, ATP-2K 5, HEPES 5, GEDTA 1, and CPK (creatine phosphate dipotassium salt) 5 mmol·L^{-1}. The extracellular solution was normal Tyrode solution containing CsCl 2 mmol·L^{-1}. Pra was isolated and identified [7].

Whole cell patch clamp technic The inside tip diameter was 2 — 3 μm and the resistance was 2 — 3 MΩ. For the current measurement, the holding potential was −40 mV, the stimulating frequency was 1 Hz, and the duration was 300 ms. Depolarizing or hyperpolarizing test pulses were applied once every 10 s.

RESULTS

The amplitudes of the peak inward I_{Ca} were decreased concentration-dependently by Pra. When cells were held at −40 mV, I_{Ca} reached the peak amplitudes and were decreased by Pra 1, 10, 100 μmol·L^{-1} from 2.02 ± 0.24, 2.00 ± 0.12, 2.12 ± 0.33 nA (control, n = 5) to 1.60 ± 0.24, 1.32 ± 0.08, 1.16 ± 0.43 nA, respectively, and their inhibitory rates were 21 %, 33.5 %, 45 %, respectively (Fig 1).

The isolated I_{Ca} were obtained at various potentials in control and after the administration of Pra. I_{Ca} reached a maximal value at 0 mV, and the apparent reversal potentials obtained by extrapolating the current-voltage relationship curves were approximately at +60 mV (Fig 2).

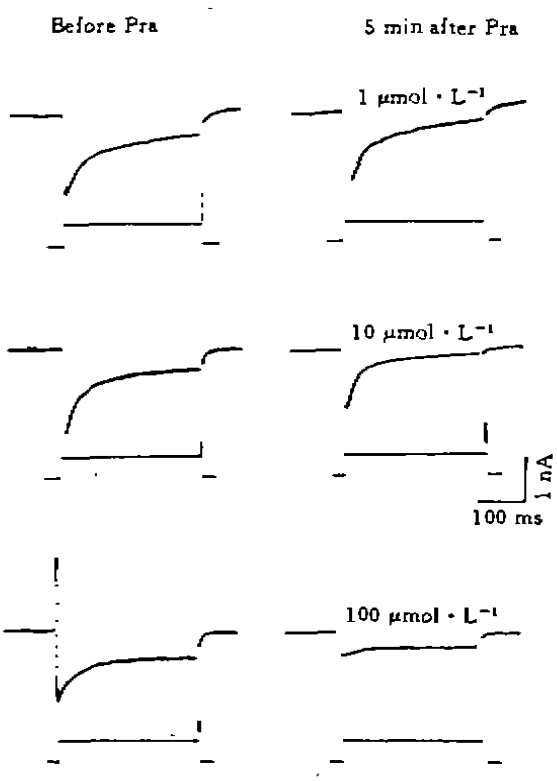

Fig 1. Effect of *dl*-praeruptorin A on I_{Ca} of guinea pig ventricular cells in the presence of CsCl 2 mmol·L^{-1}. Holding potential −40 mV; Command potential 0 mV; Stimulating duration 300 ms.

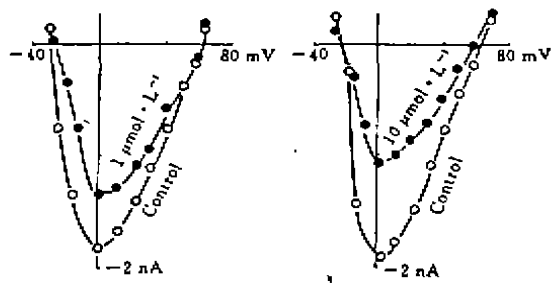

Fig 2. Current-voltage relation of I_{Ca} in *dl*-praeruptorin A. Holding potential −40 mV; Command potentials from −30 to +70 mV in 10 voltage steps; Stimulating duration 300 ms.

DISCUSSION

The present studies showed that Pra decreased the I_{Ca} clearly and dose-dependently

under the conditions where the potassium channels were blocked with CsCl without affecting the apparent reversal potential. These results were only qualitative. In our previous studies, the shortening effects of Pra on APD_{30} and APD_{90} were observed by use of conventional microelectrode technic. It is well known that the action potential duration (APD) at plateau phase is determined by the balance between inward I_{Ca} and outward potassoum current (I_K). The reduction of I_{Ca} increases the net outward currents and thus causes a decrease of APD. Therefore, the reduction of APD_{30} and APD_{90} is thought to be induced by the depression of I_{Ca}. This was further supported by the fact that Pra (Pd-Ia) had a calcium antagonistic action[13]. The precise mechanism for the reduction of I_{Ca} by Pra remains unclear.

To minimize influences of I_K, cesium ions were used in this experiment. The ventricular cells were dealt internally with the Cs-internal pipette solution through the patch electrode and were superfused with Cs-Tyrode solution. Under such a circumstance (Cs/Cs), inward and outward I_K were almost abolished[6]. In addition, when the holding potential was kept at -40 mV, the Na^+ channel basically was inactivated, so inward current recorded was actually a pure I_{Ca}.

The value of a $+60$ mV reversal potential of I_{Ca} obtained in this experiment was correspondent with that reported by Lee and Iijima[8,9]. The results that Pra decreased the peak values of I_{Ca} dose-dependently and did not influence the threshold potential and peak potential suggested that Pra had a Ca^{2+} channel blocking effect.

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白花前胡甲素对豚鼠心肌细胞钙电流的影响

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摘要 利用膜片钳制技术全细胞记录观察了白花前胡根中成分甲素对豚鼠单一心室肌细胞I_{Ca}的作用. 表明：1，10，100 μmol·L^{-1}的甲素使I_{Ca}的峰值变小，具有剂量依赖关系. 给药前后I_{Ca}的翻转电位均为 $+60$ mV，实验结果提示甲素对Ca^{2+}通道有阻滞作用.

关键词 白花前胡甲素；心肌；培养的细胞；钙通道阻滞剂；膜片钳技术