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Inhibitory effect of tetrahydropalmatine on calcium current in isolated cardiomyocyte of guinea pig

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KEY WORDS *tetrahydropalmatine; myocardium;* calcium channels; patch-clamp techniques; cultured cells

AIM: To study the effect of tetrahydropalmatine (THP) on calcium channels in ventricular single cells of guinea pig heart. **METHODS**: Patch-clamp technique (whole cell recording) was used to observe calcium current in ventricular myocytes. **RESULTS**: THP decreased I_{Ca} in ventricular myocytes with a dose and frequency-dependent manner. THP (0.1, 1, and 10 μmol·L^{-1}) decreased I_{Ca} from 1.15 ± 0.22, 0.91 ± 0.18, and 1.60 ± 0.42 nA (control) to 0.9 ± 0.21 ($P<0.01$), 0.56 ± 0.21 ($P<0.01$), and 0.83 ± 0.21 nA ($P<0.05$), respectively, number of cells is five in each group ($n=5$), and the rates of the depression of I_{Ca} were 22 %, 38 %, and 48 %, respectively. The effect was easily reduced by washing the cell with the Tyrode's solution. The current-voltage relation curve showed that the potential producing peak value of I_{Ca} was 0 mV at which THP had the most markedly inhibited action on I_{Ca}. When the stimulating frequency was changed, I_{Ca} varied in a frequency-dependent manner 5 min after THP was given, and the inhibition of THP was stronger at 2 Hz than that at 0.1 Hz. **CONCLUSION**: THP possessed a Ca^{2+} channel blocking effect.

Tetrahydropalmatine (THP) possessed the effect on preventing and treating arrhythmia induced by many drugs[1], and preventing the myocardium from ischemic and infarcted damage[2,3]. The aim of this study was to explore the influence of THP on I_{Ca} in ventricular cells of guinea pig.

MATERIALS AND METHODS

Preparation of ventricular myocytes Enzymatic dissociation method was used to prepare the single ventricular myocytes[4,5]. Guinea pig weighing 0.45 ± 0.05 kg were anesthetized with ip pentobarbital sodium (90 mg·kg^{-1}). The heart was digested with Ca^{2+}-free Tyrode's solution containing collagenase 0.08 g·L^{-1} (Yakult, Japan) at 36 ℃ for 15 - 20 min. Ventricle was agitated in Krebs buffer (KB) solution and deposited at 4 ℃ for at least 1 h.

Whole cell patch-clamp technique The inside diameter of the electrode tip was 2 - 3 μm and the resistance was 2 - 3 MΩ. When whole cell mode was established and membrane current was observed for 5 min later, the current was recorded. The holding potential was - 40 mV, the stimulating frequency was 0.1 Hz, the duration was 300 ms.

Solutions[6] and THP Tyrode's solution contained (mmol·L^{-1}): NaCl 143, NaOH 24.4, KCl 5.4, $MgCl_2$ 0.5, NaH_2PO_4 0.3, $CaCl_2$ 1.8, glucose 5, HEPES 5. Ca^{2+}-free Tyrode's solution was Tyrode's solution without $CaCl_2$. The KB medium contained (mmol·L^{-1}): *l*-glutamic acid 50, KOH 70, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, GEDTA (glycoether-diaminetetraacetic acid) 0.5, glucose 10, HEPES 10. Intraelectrode solution contained (mmol·L^{-1}): CsCl 130, $MgCl_2$ 1, ATP-2K 5, HEPES 5, GEDTA 1, and creatine phosphate dipotassium salt 5.

THP was synthesized by Harbin 6th Pharmaceutical Factory, purity 99 %.

RESULTS

When the holding potential was - 40 mV and the command potential was 0 mV, the inward I_{Ca} reached its maximum. Tyrode's solution containing THP was perfused 5 min later, I_{Ca} was reduced markedly. THP 0.1, 1, 10 μmol·L^{-1} decreased I_{Ca} from 1.15 ± 0.22, 0.91 ± 0.18, and 1.60 ± 0.42 nA (control) to 0.90 ± 0.21 ($P<0.01$), 0.56 ± 0.21 ($P<0.01$), and 0.83 ± 0.21 ($P<0.05$) nA, respectively ($n=5$). Their inhibitory rates were 22 %, 38 %, and 48 %, respectively. However, the decreased I_{Ca} recovered to its original level when the cell was washed with Tyrode's solution for 10 - 15 min (Fig 1).

The inhibition of THP reached its maximum

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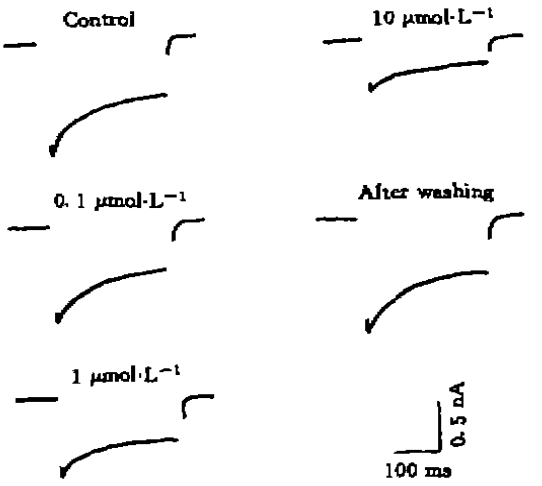

Fig 1. Effect of THP on I_{Ca} of guinea pig ventricular single cell. Holding potential −40 mV; Command potential 0 mV; Stimulating duration 300 ms.

when the command potential was 0 mV at which current reached its peak value (Fig 2).

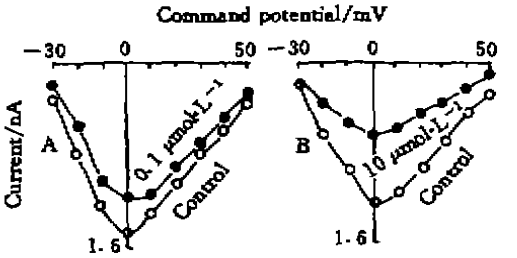

Fig 2. Current-voltage relation of I_{Ca} before and after THP was given. Holding potential −40 mV; Command potential from −30 to +50 mV by 10 mV voltage steps; Stimulating duration 300 ms.

The inhibition of THP on I_{Ca} was enhanced with increasing the stimulating frequency and reached its maximum at 2 Hz (Fig 3).

DISCUSSION

It is well known that the plateau phase of the action potential (AP) is mainly determined by the balance between inward I_{Ca} and outward potassium current (I_k). THP decreased I_{Ca} under the condition where the potassium channels were blocked with CsCl, resulted in the increasing of the

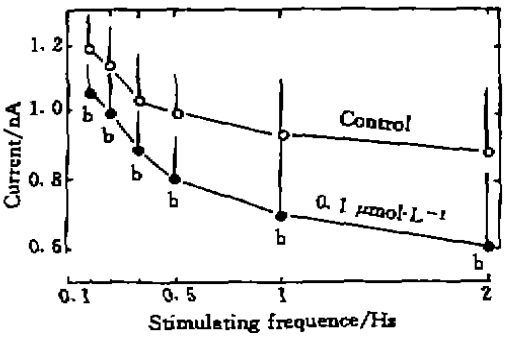

Fig 3. Frequency-dependency of the inhibition of THP on I_{Ca}. Holding potential −40 mV; Command potential 0 mV; Stimulating duration 300 ms; Stimulating frequencies 0.1, 0.2, 0.3, 0.5, 1, 2 Hz; $n = 5$ cells (one cell from each guinea pig). $\bar{x} \pm s$. $^bP < 0.05$ vs control.

net outward current[7], so that the plateau of AP could be influenced, which is perhaps one of mechanisms of antiarrhythmic action. When the stimulating frequency was changed, it was found that the effect of THP on I_{Ca} was stronger at 2 Hz than that at 0.1 Hz, the difference between inhibitory rates of 2 Hz and 0.1 Hz was significant ($P < 0.01$, $n = 5$), so we thought that THP was more beneficial to the patient suffered from rapid arrhythmia. That THP decreased I_{Ca} lowered intracellular Ca^{2+} content which played an important role in processes of excitation-contraction coupling of muscle, therefore, THP decreased myocardial contractility, which was correspondent to the result that THP had a negative inotropic effect in our previous studies, and supported by literature[8].

Run-down of I_{Ca} had ever been reported in whole cell mode, under our experiment conditions which stimulating frequency was 0.1 Hz, the lessen of I_{Ca} caused by the run-down in control was less than 7 % throughout the observation period, which had no significant influence on results, so we considered that the decrease in I_{Ca} was really caused by THP, not by run-down phenomenon.

The result that THP decreased I_{Ca} in dose, frequency-dependent manner indicated that THP had an inhibition to Ca^{2+} channel.

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四氢巴马汀对豚鼠心室肌细胞跨膜内向 Ca^{2+} 电流的抑制作用

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关键词 四氢巴马汀；心肌；钙通道；膜片箝技术；培养的细胞

目的：研究四氢巴马汀（THP）对豚鼠心室肌细胞膜 Ca^{2+} 通道的作用 方法：分离豚鼠心室肌细胞，利用膜片箝技术观察、记录心室肌细胞膜跨膜内向钙电流（I_{Ca}） 结果：THP 灌流 5 min 后明显抑制豚鼠心室肌细胞内向 I_{Ca}，其作用呈现剂量-效应关系，并具有频率依赖性。0.1，1，10 μmol·L^{-1} 的 THP 使 I_{Ca} 从 1.15 ± 0.22，0.91 ± 0.18，1.60 ± 0.42 nA（control，n = 5）分别降低到 0.90 ± 0.21（P < 0.01），0.56 ± 0.21（P < 0.01），0.83 ± 0.21（P < 0.05）nA，抑制率分别为 22 %，38 % 和 48 %，THP 的作用易于洗脱。峰值电压为 0 mV，此时 THP 作用最明显，THP 对较高频率刺激产生的电流抑制作用较强，2 Hz 时的抑制率高于 0.1 Hz 时的抑制率（P < 0.01） 结论：THP 具有 Ca^{2+} 通道阻滞作用。

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Effect of sodium glycyrrhetinate on neonatal rat myocardial cells

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KEY WORDS glycyrrhetinate; myocardium; cultured cells; cyclic AMP; calcium; oxygen

AIM: To investigate the effect of sodium glycyrrhetinate (SG) on neonatal rat myocardial cells. **METHODS**: The neonatal rat myocardial cells were cultured *in vitro*. Radioimmunoassay and fluorimetry were used to determine cAMP and [Ca^{2+}], respectively. **RESULTS**: The beating rate of myocardial cells was depressed by SG 0.4 mmol·L^{-1} at 5, 10, and 15 min, from 73 ± 9 min^{-1} to 62 ± 5, 59 ± 7, and 56 ± 6 min^{-1}, respectively. SG 0.1 and 0.2 mmol·L^{-1} showed above similar results at 15 min and 10, 15 min, respectively. When the myocardial cells were incubated with SG 0.2 and 0.4 mmol·L^{-1} at 37 ℃ for 10 and 15 min, the concentration of cAMP and [Ca^{2+}] were reduced. cAMP contents in SG 0.2 mmol·L^{-1} treated group at 10 and 15 min were lower than

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