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Blocking L-calcium current by *l*-tetrahydropalmatine in single ventricular myocyte of guinea pigs

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KEY WORDS tetrahydropalmatine; patch-clamp techniques; calcium current; myocardium; cultureed cells; membrane potentials

ABSTRACT

AIM: To study the effect of *l*-tetrahydropalmatine (*l*-THP) on L-type calcium channel. **METHODS**: Patch clamp technique (whole cell recording) was used to record L-Ca^{2+} current in single cardiac myocyte. **RESULTS**: 1) *l*-THP 1, 10, and 100 $\mu mol \cdot L^{-1}$ reduced $I_{Ca\text{-}max}$ from (999 ± 93) pA to (700 ± 111) pA, (582 ± 66) pA, and (420 ± 112) pA ($n = 6$, $P < 0.01$), respectively. 2) *l*-THP reduced the voltage at half-maximal inactivation ($V_{1/2}$) of L-Ca^{2+} channel to more negative potentials by 9 mV ($n = 5$, $P < 0.05$). 3) *l*-THP caused both tonic and use-dependent reduction of Ca^{2+} current. Tonic block of *l*-THP on Ca^{2+} current was 46 % ± 8 % ($n = 6$, $P < 0.01$). The degree of use dependent blocking was 13.5 % ± 2.4 % ($n = 6$, $P < 0.05$) at 1 Hz, the degree increased to 44 % ± 5 % ($n = 6$, $P < 0.01$) at 3 Hz. 4) *l*-THP delayed half-recovery time of Ca^{2+} channel recovery from inactivity from (94 ± 39) ms to (170 ± 42) ms ($n = 6$, $P < 0.01$). **CONCLUSION**: *l*-THP has a moderate inhibitory effect on L-Ca^{2+} current.

INTRODUCTION

l-Tetrahydropalmatine (*l*-THP) is an alkaloid extracted from *Corydalis ambigua* Cham. Previous researches had documented its abilities of anti-arrhythmia and L-Ca^{2+} current blocking effects[1,2]. However, the characteristics of its effect on *L*-calcium current is still unknown. Therefore, the present experiments were carried out to reveal them in single guinea pig ventricular myocyte.

MATERIALS AND METHODS

Drugs *l*-THP was made by the Nanning Second Pharmaceutical Factory. *l*-THP stocking solution (10 $mmol \cdot L^{-1}$) was prepared with distilled water and the pH was adjusted to 7.0 with HCl before use. Tyrode's solution contained: NaCl 137, CsCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 1.0, NaH_2PO_4 0.05, glucose 5.55, HEPES 10 $mmol \cdot L^{-1}$, the pH was adjusted to 7.35 with NaOH. Calcium free Tyrode's soluiton was Tyrode's solution without $CaCl_2$. The ventricular myocyte dissociation solution was calcium free Tyrode's solution containing collagenase 0.33 $g \cdot L^{-1}$ (Sigma), BSA 0.25 $g \cdot L^{-1}$ (Sigma), protease E 0.25 $g \cdot L^{-1}$ (Sigma). Intracellular solution consisted of: CsCl 120, NaCl 10, $CaCl_2$ 1.0, Mg-ATP 5.0, egtazic acid 11 (Sigma), HEPES 10 (Sigma) $mmol \cdot L^{-1}$, pH was adjusted to 7.3 with CsOH.

Preparation of single ventricular myocyte Male or female guinea pigs weighing (300 ± 40) g (species: England undercoat guinea pigs; Grade Ⅱ; Certificate number: 19-023; Medical Experimental Animal Center of Tongji Medical University) were used. Single ventricular cells of guinea pig were obtained by an enzymatic dissociation method[3]. First, the heart was perfused with dissociation solution through coronary arteries for 5 min at 37.5 ℃. Subsequently, the dissociation solution was washed out by calcium free Tyrode's solution. Then several pieces of ventricular tissue were dissected and gently agitated

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in Tyrode's solution contained Ca^{2+} 0.2 mmol·L^{-1}. After that the isolated ventricular myocytes were stored *in Tyrode's solution for 2 h before use*.

Electric recordings The isolated cells were set to a 1.0 mL chamber mounted on the stage of an inverted microscope. After being settled to the bottom of the chamber, cells were superfused with Tyrode's solution to wash out the dead cells. The experiments were performed at room temperature (21 ± 2) ℃.

Micropipettes whose tips were 1 – 2 μm were constructed from stardore capillary tubes and were heat-polished to have resistances of 1 – 3.5 MΩ when they were filled with the internal solution and immersed in the Tyrode's solution. Recordings were made in the whole cell voltage-clamp configuration of the patch-clamp technique with a PC-2 patch clamp amplifier. DA-1 data collection and analysis system was used to record and analyse I_{Ca}. Sampled data were stored on the hard disk of computer gor later analysis. To investigate the effect of *l*-THP on the voltage dependent of Ca^{2+} current availability, the twin-pulse inactivation protocol were used to evaluate effects of membrane potential on the peak Ca^{2+} current of a subsequent depolarization (f-curve) with and without *l*-THP. The holding potential was maintained at – 40 mV, and conditioning pulses (4 s) to potentials between – 60 and + 20 mV were applied (Fig 2, inset)[4,5]. Peak Ca^{2+} current was measured during a subsequent test pulse and plotted as a function of the prepulse membrane potential. Prepulses to more positive potentials result in inactivation of Ca^{2+} channel[6,7]. *The data presented were recored after a 10-min drug* exposure. The frequency of stimulation was 0.10 Hz.

In order to observe the use-depentent effect of *l*-THP on Ca^{2+} current, different frequency (1 Hz, 3 Hz) stimulus was used to activate Ca^{2+} current[8]. The membrane potential was held at – 40 mV without pulsing for 5 min before each train. The command potential was + 20 mV followed by a depolarization to – 40 mV with 10 ms. Tonic block was calculated as a diminution in the peak I_{Ca} of the first depolarizing pulse after exposure to *l*-THP compared with control[9]. Twin-pulse was used to study *l*-THP on recovery time from inactivity of Ca^{2+} channel. Holding potential maintained – 80 mV, prepulse and test-pulse both were 10 mV, interval of two pulse was from 5 to 1280 ms (5,10,20,40,80,.....1280).

Statistic analysis Values were expressed as $\bar{x} \pm s$ by *t* test.

RESULTS

Ca^{2+} current When the holding potential was – 40 mV and the cells were voltage-clamped from – 40 mV to different membrane potential (– 30 to + 60 mV) at a frequency of 0.10 Hz, the inward I_{Ca} was invoked. When the command potential was 10 mV, it reached its maximum. After adding *l*-THP 1, 10, and 100 μmol·L^{-1}, respectively, $I_{Ca\text{-}max}$ reduced from (999 ± 93) pA to (700 ± 111) pA, (582 ± 66) pA, and (420 ± 112) pA ($n = 6$, $P < 0.01$), respectively (Fig 1A).

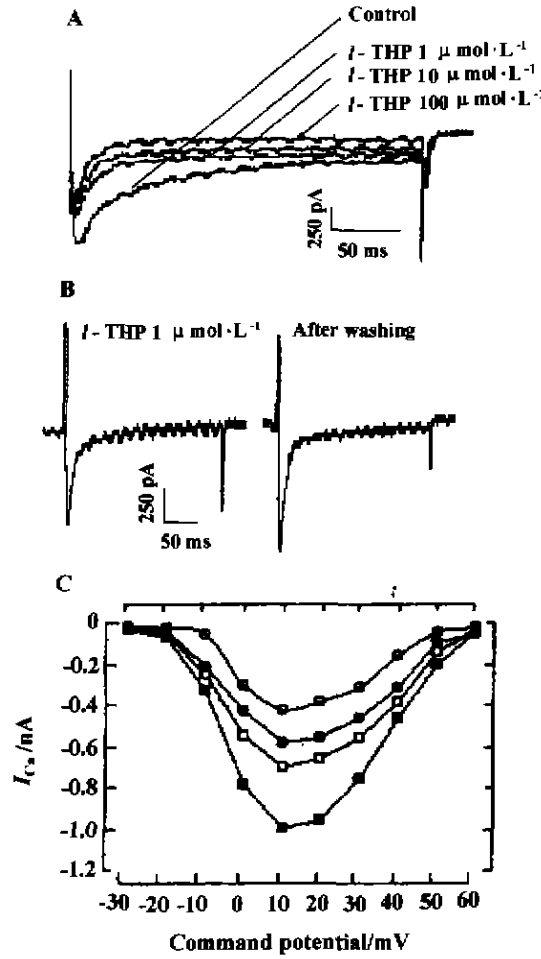

Fig 1. A) *l*-THP on Ca^{2+} current of guinea pig single ventricular cell. B) Example of Ca^{2+} current with (left) or without (right) *l*-THP 1 μmol·L^{-1} in Tyrode's solution. C) $I - V$ relationship curves of L-Ca^{2+}. ■: Control. □: *l*-THP 1 μmol·L^{-1}. ●: 10 μmol·L^{-1}. ○: 100 μmol·L^{-1}.

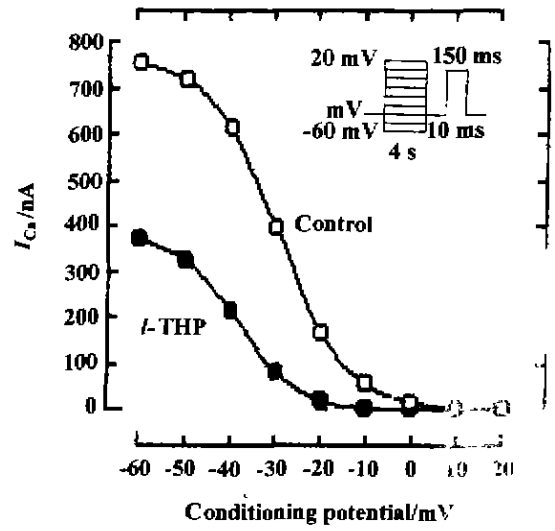

Fig 2. Effect of *l*-THP (10 μmol·L^{-1}) on voltage-dependence of calcium current. $n = 5$ Cells from 5 guinea pigs. $\bar{x} \pm s$.

After *l*-THP was washed out, the Ca^{2+} current reversed (Fig 1B). The data obtained from the experiments fitted with the Boltzmann equation $I = 1/\{1 + EXP[(V_{pre} - V_{1/2})/k]\}$, free parameters, $V_{1/2}$ and k, quantify membrane potential where current was half-maximum and slope of activation curves (d∞). The $V_{1/2}$ value in control and in *l*-THP 10 μmol·L^{-1} were 7.12 mV and 8.33 mV ($P > 0.05$), respectively. The k values in control and in *l*-THP 10 μmol·L^{-1} were −7.11 mV and −6.90 mV ($P > 0.05$), respectively.

Voltage dependent of Ca^{2+} current availability Under control condition, the amplitude of Ca^{2+} current was the largest after a prepulse to potential more negative than −50 mV. *l*-THP decreased Ca^{2+} current at −60 to +20 mV, and the voltage at half-maximal inactivation ($V_{1/2}$) was reduced to more negative potentials by 9 mV [(−29 ± 5) mV vs (−38 ± 7) mV, $n = 5$, $P < 0.05$] (Fig 2).

Use dependent *l*-THP caused a marked tonic block of Ca^{2+} channels. Ca^{2+} current was reduced during the first pulse of the train. The tonic block of *l*-THP 10 μmol·L^{-1} on Ca^{2+} current was 46 % ± 8 % ($n = 6$, $P < 0.01$). After exposure to *l*-THP 10 μmol·L^{-1}, compared with that in control, the degree of use dependent blocking was 13.5 % ± 2.4 % ($n = 6$, $P < 0.05$) at 1 Hz, the degree increased to 44 % ± 5 % ($n = 6$, $P < 0.01$) at 3 Hz. The blocking effect of 3 Hz was stronger than that of 1 Hz stimulus ($n = 6$, $P < 0.01$) (Fig 3).

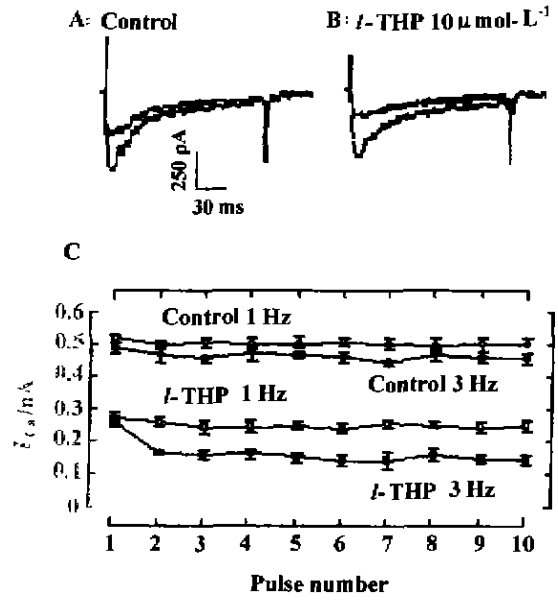

Fig 3. Use-dependent block of Ca^{2+} current by *l*-THP 10 μmol·L^{-1}. Ca^{2+} current at the first stimulus in control (A) and in *l*-tetrahydropalmatine (B) at 3 Hz, frequency-dependent inhibition curve of Ca^{2+} current (C). $n = 6$ cells from 6 guinea pigs. $\bar{x} \pm s$.

Recovery from inactivity of Ca^{2+} channel *l*-THP delayed Ca^{2+} channel half-recovery time from (94 ± 39) to (170 ± 42) ms ($n = 6$, $P < 0.01$) (Fig 4).

DISCUSSION

l-THP can reduce the L-Ca^{2+} current in the ventricular myocytes of guinea pig. This effect seems stronger than that of berberine on L-Ca^{2+} current[10]. Another important result in this experiment was that *l*-THP blocked cardiac L-calcium channel potently at which most channels were inactivated. Just as Fig 2 shown, when the 4-s conditioning pulses were applied to different voltages, the resulting steady-state inactivation curve was shift to the hyperpolarizing direction by 9 mV ($n = 5$, $P < 0.01$), this result showed that its effect on the inactivation of Ca^{2+} channel was the same as that of berberine[10]. The result which is characterised by a number of Ca^{2+} channel ligands

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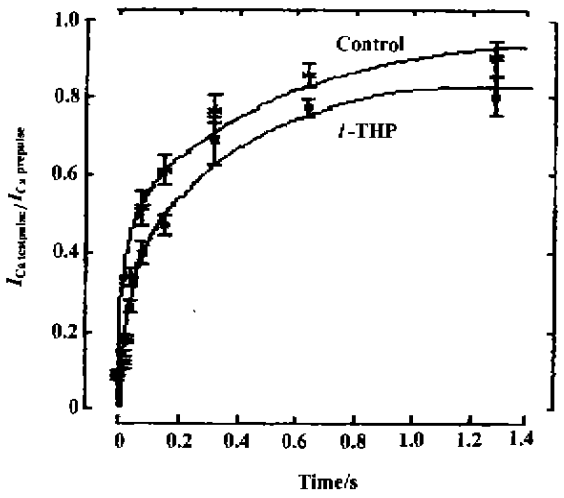

Fig 4. Effect of *l*-THP on recovery time from inactivition of Ca^{2+} channel. n = 6 cells from 6 guinea pigs. $\bar{x} \pm s$.

suggested that *l*-THP should have the affinity to the inactivated state of the Ca^{2+} channel. The modulated receptor model could account for the results described in this paper[11]. The more the channels were bound by *l*-THP, the more the Ca^{2+} current was decreased. This consisted with the concentration-dependent action of *l*-THP on Ca^{2+} channel.

Use dependence and frequency dependence are the common characters of Ca^{2+} channel blockers. *l*-THP had these characters also. Its Ca^{2+} channel blocking effect was significantly stronger at 3 Hz than that at 1 Hz stimulus. But berberine has no effect[10]. This character is very useful in the therapy of tarchycardia when the heart rate is very fast. Another finding in this experiment was that *l*-THP could delay Ca^{2+} channel recovery time from inactivity which means that Ca^{2+} channel would spend much more time to recover. Namely, the duration of inactivation was added. This character should contribute to its blocking effect on Ca^{2+} channel.

We observed a tonic component of block by *l*-THP, which could result from either a specific receptor-mediated inhibition on the channel protein as suggested by the modulated receptor hypothesis for ion channel blockers, or from some indirect or nonspecific action. Our results did not allow us to discriminate between these possibilities.

"Run down" of Ca^{2+} current had ever been reported in whole cell configuration of patch clamp technique. In this experiment, many methods were used to decrease this phenomenon: 1) ATP and egtazic acid were maintained in the pipette solution. 2) The strict selection of cells. After Ca^{2+} current was evoked, it was observed for 10 – 15 min, if the reduction was less than 10 %, then the drug was added, or the cell was given up otherwise. 3) Washing-out, the Ca^{2+} current reduction after *l*-THP was added could recover after drug was washed out by Tyrode's solution. 4) The lessenning of I_{Ca} caused by the run-down in control was less than 10 % throughout the observation period, which has no significant influence on results, so that we could consider that the decrease in I_{Ca} was really caused by *l*-THP, not by the "run down" phenomenon[12].

In summary, the inhibition of Ca^{2+} current caused by *l*-THP was use-dependent and membrane potential-dependent, meanwhile, the time of calcium channel recovery from inactivation was delayed by *l*-THP. These suggest that *l*-THP has a moderate inhibitory effect on L-Ca^{2+} current. On the other hand, these results can be very helpful in the therapy of cardiac arrhythmia with *l*-THP.

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左旋四氢巴马汀对单个豚鼠心室肌细胞钙电流的阻滞作用

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关键词 四氢巴马汀; 膜片箝技术; 钙电流; 心肌; 培养的细胞; 膜电压

目的: 研究左旋四氢巴马汀(*l*-THP)对 L-钙电流的作用. **方法**: 用全细胞膜片箝记录心室肌细胞的 L-钙电流. **结果**: *l*-THP 1 - 100 $\mu mol \cdot L^{-1}$ 将 $I_{Ca^{2+}\text{-max}}$从(999 ± 93) pA 分别减少到(700 ± 111) pA、(582 ± 66) pA 和(420 ± 112) pA ($n = 6$, $P < 0.01$). *l*-THP 对钙通道有紧张性阻滞作用及使用依赖性. *l*-THP 可将钙通道的恢复时间从(94 ± 39) ms 延长到(170 ± 42) ms ($n = 6$, $P < 0.01$). **结论**: *l*-THP 对 L-钙通道有阻滞作用.

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