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Dual effects of tetrandrine on calcium-activated potassium channels in pulmonary artery smooth muscle cells

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KEY WORDS tetrandrine; ion channels; potassium channels; calcium; patch-clamp techniques; pulmonary artery

ABSTRACT

AIM: To study the effects of tetrandrine (Tet) on calcium-activated potassium channels. **METHODS**: Inside-out configuration of patch-clamp single channel recording techniques. **RESULTS**: Tet 7.5 and 15 $\mu mol \cdot L^{-1}$ increased the open probability from control value 0.251 ± 0.012 to 0.340 ± 0.013 and 0.415 ± 0.011, respectively ($P < 0.01$), decreased the close time from (61 ± 15) ms to (33 ± 10) and (28 ± 11) ms, respectively ($P < 0.01$). But Tet 30 $\mu mol \cdot L^{-1}$ decreased the open probability and open time to (0.114 ± 0.008) and (1.47 ± 0.09) ms, respectively [$P < 0.01$ *vs* control (0.251 ± 0.012) and (20 ± 8) ms]. **CONCLUSION**: Tet has concentration-dependent dual effects on K_{Ca} channels in isolated rat pulmonary smooth muscle cells.

INTRODUCTION

Tetrandrine (Tet) has been clinically used as an anti-arrhythmic and antihypertensive agent. It blocked the Ca^{2+} channels, as well as calcium-activated potassium (K_{Ca}) channels in nerve terminals, and produced a relaxation of smooth muscle[1-4]. Because potassium channel activation, especially the K_{Ca} and ATP-sensitive potassium channel, reduced the pulmonary vasoconstriction, this study investigated the effects of Tet on K_{Ca} of rat pulmonary smooth muscle cells.

MATERIALS AND METHODS

Drugs and reagent Collagenase (type XI), papain, bovine serum albumin, tetraethylammonium bromide, dithiothreitol, and poly-*L*-lysine were purchased from Sigma Chemical Co. Tet was a product of Jinhua Pharmaceutical Factory (Jinhua, Zhejiang Province, China), with a purity of 98 %.

Isolation of rat pulmonary artery smooth muscle cells[5,6] Wistar ♂ rats ($n = 18$, 200 - 250 g, Certificate № 24301050), supplyed by Experimental Animal Center of Third Military Medical University, were anethetized after 1 % sodium barbiturate ip 33 $mg \cdot kg^{-1}$. The main left and right branches of pulmonary artery were placed in cold medium containing: NaCl 130, KCl 5, $MgCl_2$ 1.2, $CaCl_2$ 1.5, HEPES 10, and glucose 10 $mmol \cdot L^{-1}$, pH 7.3 adjusted with NaOH, arteries were cleaned and cut into pieces (1 mm^2), and allowed to recover for 20 min in low-Ca^{2+} (20 $\mu mol \cdot L^{-1}$) isolation medium at 37 ℃. Tissue was transferred to 1 mL low-Ca^{2+} medium, collagenase 1 mg, papain 1 mg, dithiothreitol 1 $mmol \cdot L^{-1}$, bovine serum albumin 2 mg were added to digest at 37 ℃ for 40 min, and then centrifuged at 1000 × *g* for 5 min. In 2 mL enzyme-free Ca^{2+}-free isolation

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medium, tissue was triturated with a fire-polished Pasteur pipette to disperse cells. Loose cells were placed on poly-*L*-lysine coated glass coverslips and stored at 4 ℃ for 8 h.

Single-channel recording Single-channel recording was performed at room temperature (23 ± 2) ℃ using the inside-out patch configuration of the patch-clamp technique[7]. Single-channel currents were detected using a patch-clamp amplifier (CEZ-2200, Nihon Kohden, Japan) and gap-free recorded using Fetchex program in the pClamp 6.02 software (Axon Instrument, USA) at a frequency of 10 kHz. The filter frequency of amplifier was set at 1 kHz.

After the control channel currents were recorded, Tet was added cumulatively into solution at intervals of 15 min. Then the currents in the same patch were recorded. The concentrations of Tet were 7.5, 15, and 30 $\mu mol \cdot L^{-1}$.

Solutions The patch electrode was filled with: KCl 140, $MgCl_2$ 1, Na_2ATP 0.5, egtazic acid 6, $CaCl_2$ 5.4, and HEPES 10 $mmol \cdot L^{-1}$, pH 7.4 adjusted with KOH. Bath solution was the same as the patch pipette solution.

Data analysis The open and close times were fitted exponential fitting. Current amplitudes were fitted by Gaussian distributions. Open probability was calculated from the sum of open time for each sweep divided by sweep duration. The conductance of K_{Ca} (G_k) was calculated by

$$E_k = (RT/ZF)\ln([K^+]_o/[K^+]_i) \text{ and}$$

$$G_k = \triangle I/(V_m - E_k)$$

The data were represented as $\bar{x} \pm s$ and treated with ANOVA.

RESULTS

General properties of K_{Ca} in rat pulmonary artery smooth muscle cells On inside-out membrane patches from pulmonary artery smooth muscle cells, about 90 % of patches contained K_{Ca} (58/65). These channels were highly sensitive to changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration. When the intracellular membrane surface was bathed with a range of Ca^{2+} concentrations at a membrane holding potential of −60 mV in symmetrical KCl 140 $mmol \cdot L^{-1}$, the open probability was increased as $[Ca^{2+}]_i$ concentration increased. When $[Ca^{2+}]_i$ was 0.1, 0.2, 0.3, 0.5, and 0.8 $\mu mol \cdot L^{-1}$, the open probability values were 0.010 ± 0.001, 0.083 ± 0.005, 0.213 ± 0.010, 0.428 ± 0.014, and 0.754 ± 0.012, respectively (Fig 1).

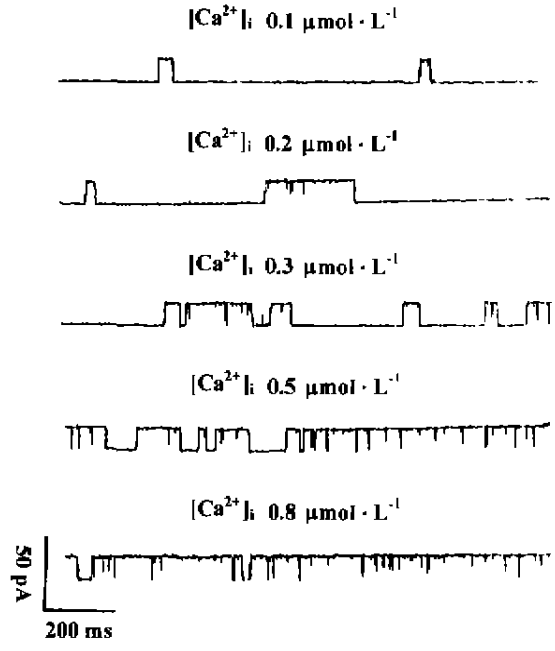

Fig 1. Single Ca^{2+}-activated K^+ channel currents recorded from an inside-out membrane patch held at a membrane potential of −60 mV in rat pulmonary artery smooth muscle cells. The upward deflections indicated outward currents. The concentration of Ca^{2+} bathing the intracellular membrane surface indicated to the top of each current record. This is one recording of 10 cell patches on 10 rats.

The single channel conductance was (245 ± 13) pS. The channel currents were inhibited by

adding tetraethylammonium 10 mmol·L^{-1} to the bath solution (n = 10 cell patches).

Effects of Tet on K_{Ca} in rat pulmonary artery smooth muscle cells The open time and the close time of K_{Ca} were (20 ± 8) ms and (62 ± 15) ms with a Ca^{2+} concentration of bathing solution 0.3 μmol·L^{-1} at a membrane potential of −60 mV. The open probability was 0.251 ± 0.012 (n = 8).

Tet 7.5 and 15 μmol·L^{-1} increased the open probability from control values to 0.340 ± 0.013 and 0.415 ± 0.011, respectively (P < 0.01), and the close times were decreased to (33 ± 10) and (28 ± 11) ms (P < 0.01). But when Tet concentration was increased to 30 μmol·L^{-1}, the open probability decreased to (0.114 ± 0.008), and the open time also decreased to (1.47 ± 0.09) ms (P < 0.01 *vs* control, n = 8). (Fig 2, 3)

DISCUSSION

Our results demonstrated that the open probability of K_{Ca} in rat pulmonary artery smooth muscle cells increased as the Ca^{2+} concentrations in bathing solution were increased. The conductance of K_{Ca} in symmetical K^+ 140 mmol·L^{-1} is 245 pS. The results are similar to that reported by others[5,8].

Tet at low concentration opened the K_{Ca} in rat pulmonary artery smooth muscle cells, the open probability was increased. But when Tet concentration increased to 30 μmol·L^{-1}, it inhibited the open of K_{Ca}, the open time and open probability were decreased. The dual effects of Tet on K_{Ca} indicated that in appropriate concentration, Tet activated the K_{Ca} in pulmonary artery smooth muscle cells, which resulted in K^+ outflow increasing, together with its Ca^{2+} channels inhibiting effects[4], reduced the pulmonary vasoconstriction. If high concentration was used, Tet would inhibit the K_{Ca}, which may be the toxic effects. These indicated

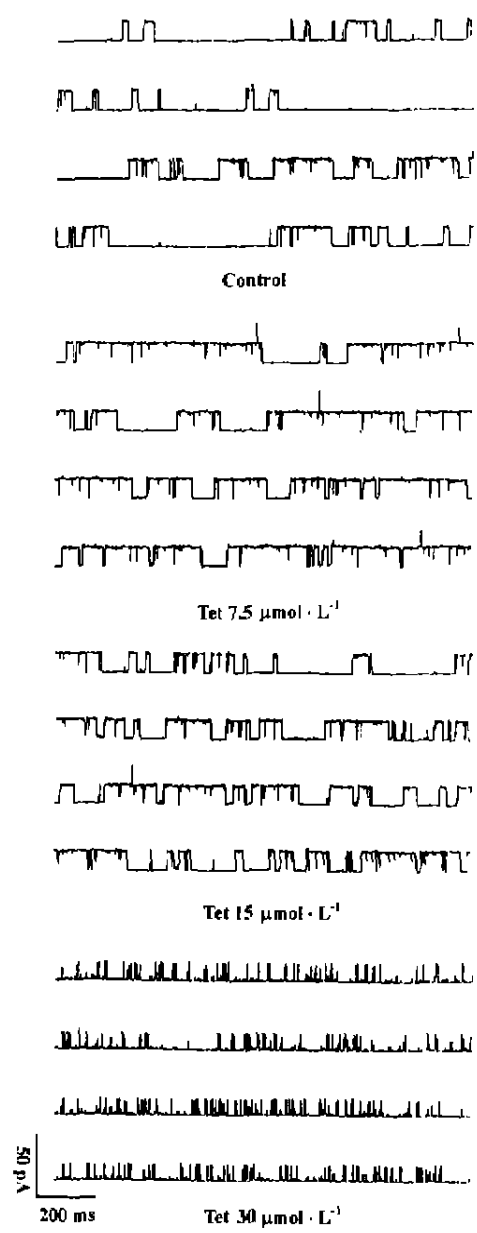

Fig 2. Effects of tetrandrine (Tet) on Ca^{2+}-activated K^+ channels in isolated rat pulmonary artery smooth muscle cells at a membrane potential of −60 mV from an inside-out membrane patch exposed to symmetrical KCl 140 mmol·L^{-1} at Ca^{2+} 0.3 μmol·L^{-1} applied to its intracellular membrane surface. The upward deflections indicated outward currents. Tet 7.5, 15, and 30 μmol·L^{-1} were added cumulatively to bath at intervals of 15 min. This is one of 8 cell patches on 8 rats. The 4 tracings in each group were gap-free recordings.

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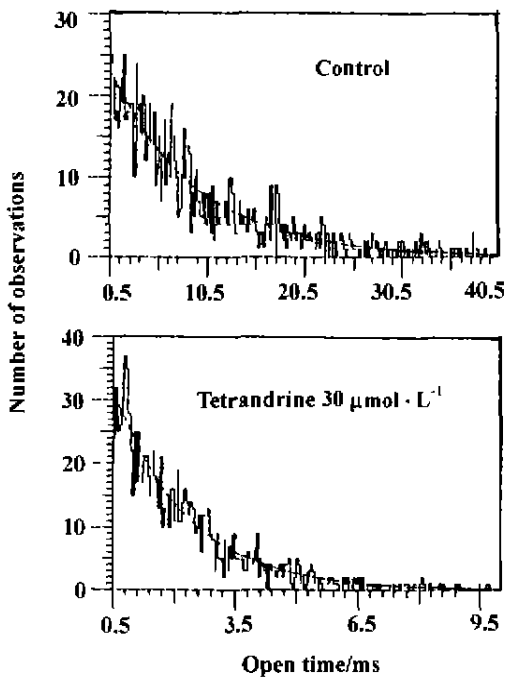

Fig 3. Example of open time exponential fitting curve from one of 8 cell patches on 8 rats.

that there would be different K_{Ca} subunits in different organs, which are identical to that pulmonary vasoconstriction is a unique process in response to hypoxia that distinguishes the pulmonary from the systemic circulation[2,3,8].

REFERENCES

1 Liu QY, Karpinski E, Rao MR, Pang PKT. Tetrandrine, a novel calcium channel antagonist inhibits type L calcium channels in neuroblastoma cells. Neuropharmacology 1991; 30: 1325 - 31.

2 Wang G, Lemos JR. Tetrandrine blocks a slow, large-conductance, Ca^{2+}-activated potassium channel besides inhibiting a non-inactivating Ca^{2+} current in isolated nerve terminals of the rat neurohypophysis. Pflügers Arch 1992; 421: 558 - 65.

3 Wang G, Jiang MX, Coyne MD, Lemos JR. Comparison of effects of tetrandrine on ionic channels of isolated rat neurohypophysial terminals and Y1 mouse adrenocortical tumor cells. Acta Pharmacol Sin 1993; 14: 101 - 6.

4 Yang XH, Yao WX, Xia GJ, Guo LJ, Jiang MX. Effects of tetrandrine on canine various vascular smooth muscles. Chin J Pharmacol Toxicol 1995; 9: 116 - 8.

5 Albarwani S, Robertson BE, Nye PCG, Kozlowski RZ. Biophysical properties of Ca^{2+}- and Mg-ATP-activated K^+ channels in pulmonary arterial smooth muscle cells isolated from the rat. Pflügers Arch 1994; 428: 446 - 54.

6 Smirnov SV, Robertson TP, Ward JPT, Aaronson PI. Chronic hypoxia is associated with reduced delayed rectifier K^+ current in rat pulmonary artery muscle cells. Am J Physiol 1994; 266: H365 - 70.

7 Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 1981; 391: 85 - 100.

8 Post JM, Hume JR, Archer SL, Weir EK. Direct role for potassium channel inhibition in hypoxic pulmonary vasoconstriction. Am J Physiol 1992; 262: C882 - 90.

353-356

粉防己碱对肺动脉平滑肌细胞钙激活钾通道的双重作用

R972

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关键词 粉防己碱；离子通道；钾通道；钙；膜片箝技术；肺动脉

目的：研究粉防己碱（Tet）对大鼠肺动脉平滑肌细胞钙激活钾（K_{Ca}）通道的影响。**方法**：内面朝外膜片箝单通道记录法。**结果**：Tet 7.5 和 15 μmol·L^{-1} 使 K_{Ca} 的开放概率由 0.251 ± 0.012 增加到 0.340 ± 0.013 和 0.415 ± 0.011（$P < 0.01$）。关闭时间由（61 ± 15）ms 缩短到（33 ± 10）和（28 ± 11）ms（$P < 0.01$）。Tet 30 μmol·L^{-1} 使开放概率和开放时间分别降低到（0.114 ± 0.008）和（1.47 ± 0.09）ms（$P < 0.01$）。**结论**：Tet 对大鼠肺动脉平滑肌细胞 K_{Ca} 通道有双重作用。

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