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Tetrandrine inhibits inward rectifying potassium current in cultured bovine aortic endothelial cells

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KEY WORDS tetrandrine; vascular endothelium; potassium channels; patch-clamp techniques

ABSTRACT

AIM: To study the effect of tetrandrine (Tet) on inward rectifying potassium current in cultured bovine aortic endothelial cells. **METHODS**: Inward rectifying potassium current (IRK) was observed by the whole cell patch-clamp technique. **RESULTS**: IRK was inhibited by Tet in a concentration-dependent manner and recovered to normal after wash with drug-free external solution. IRK was reduced from (582 ± 48) pA to (221 ± 40) pA at a holding potential of −70 mV by Tet 30 μmol/L. IC_{50} was 2.8 μmol/L. **CONCLUSION**: Tet inhibited inward rectifying potassium current in cultured bovine aortic endothelial cells.

INTRODUCTION

Tetrandrine (Tet), a vasoactive compound extracted *from the Chinese medicinal herb Radix Stephaniae Tetrandrae*, has been reported to act as a Ca^{2+} channel blocker in various tissues, such as neuroblastoma cells[1], ventricular cells[2], and decrease potassium-induced contraction of smooth muscle cells[3]. Its structure was described by Kawashima[4]. Recently, Tet also served as a specific blocker of the slowly-gating K^+ channel[5].

Endothelium plays an important role in regulating vascular smooth muscle tone[6]. Several agonists can modulate the activity of the endothelium by increasing the intraendothelial calcium concentration caused by release of Ca^{2+} from the intracellular stores and sustained influx of Ca^{2+} from the extracellular space[7]. The Ca^{2+} influx is dependent on the electrochemical gradient for Ca^{2+} and, therefore, on the resting membrane potential of endothelial cells (EC), which is mainly controlled by K^+ and Cl^- [6].

EC are generally regarded as non-excitable cells and in *macrovascular EC inward rectifying* K^+ (IRK) *channels* determine the resting potential[8]. This endothelial inward rectifier current differs from the classical inward rectifier current observed in excitable cells. The most striking difference is that inward rectification seems to be due to an intrinsic gating mechanism rather than a block by Mg^{2+} [11].

Although Tet has been shown to block Ca^{2+} channels in various tissues, its effects on EC have not been reported. In the present report, the effect of Tet on the IRK was *studied in bovine aortic endothelial cells* (BAEC).

MATERIALS AND METHODS

Cell preparation[9] Briefly, aortae were obtained from the Slaughter of Wei Gang Dairy Factory. The culture medium was Dulbecco's modified Eagle's medium (Gibco) supplemented with 10 % heat-inactivated fetal bovine serum (Sigma), streptomycin 50 mg/L, benzylpenicillin 50 mg/L, neomycin 50 mg/L, and glutamine 2 mmol/L. First passage cells were cloned to eliminate contaminated smooth muscle cells. All experimental data were obtained from BAEC in their 3rd to 8th passage. The monolayer was mechanically dispersed with a plastic pipette, and single cells were transported onto glass coverslips in 35-mm diameter plastic culture dishes and kept in a 37 ℃ incubator gassed with 5 % CO_2. Under these conditions, cells were not confluent.

Electrophysiological recordings[10] Conventional whole cell mode was made with an EPC9 amplifier equipped with data acquisition software (Pulse 7.89, HEKA Elektronik, Germany). Currents were recorded in *physiologic extracellular solution containing* (*in mmol/L*) KCl 5, NaCl 145, $CaCl_2$ 2, $MgCl_2$ 1, and HEPES 10.

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The intracellular solution contained (in mmol/L) potassium aspartate 145, NaCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, egtazic acid 2. Total osmolality of solutions was 0.7478 kPa, and pH was adjusted to 7.3. An Ag-AgCl bridge was used for the reference electrode. Compensation for fast and slow capacitive components was obtained with EPC9 software (Pulse 7.89). Pipettes were pulled from glass capillaries (Shanghai Institute of Brain Research, Chinese Academy of Sciences,) by a two-step vertical puller (PP-83, Narishige, Japan) and had resistance between 2 and 5 MΩ after being filled with the standard pipette solution. Whole cell currents were sampled at 20 kHz, filtered at 3 kHz, and stored on hard disk. Drugs were applied to individual cells by self-made perfusion instrument consisting of micropipettes (inner diameter 100 μm). All data were obtained at 20-22 ℃ and analyzed with IGOR software (Applelink Company, USA). Whenever possible, data are presented as $\bar{x} \pm s$, and compared with paired t test.

Chemicals Tet, a gift from Prof HUA Wei-Yi (China Pharmaceutical University), was a white powder, purity > 98 %. It was dissolved in 100 % dimethyl sulphoxide (Me_2SO).

RESULTS

Under resting conditions, an inward current was observed when the membrane potential was hyperpolarized in 20-mV steps from a holding potential of − 70 mV. Outward current was negligible at potentials depolarized from − 70 mV to 40 mV. In these conditions, the main conductance detected in voltage-clamp experiments was 28 pS [(25 ± 3) pS, $n = 8$], IRK was blocked by micromolar concentrations of extracellular Ba^{2+} (100 μmol/L) (Fig 1). This result is in agreement with previously published data on BAEC[8].

The cell membrane potential depolarized from control value of (− 63 ± 3) to (− 42 ± 2) mV after Tet 30 μmol/L was added ($n = 5$) and then slowly recovered after wash and after 2 min, hyperpolarized back to control values. Tet reduced the amplitude of the current compared with control. The suppression of the IRK usually occurred very quickly, approximately 1 − 2 min after the addition of Tet. Since Tet was dissolved in Me_2SO, the effect of Me_2SO was observed and at this concentration Me_2SO did not affect the IRK (data not shown).

Tet (0.03 − 30 μmol/L) inhibited inward rectifying

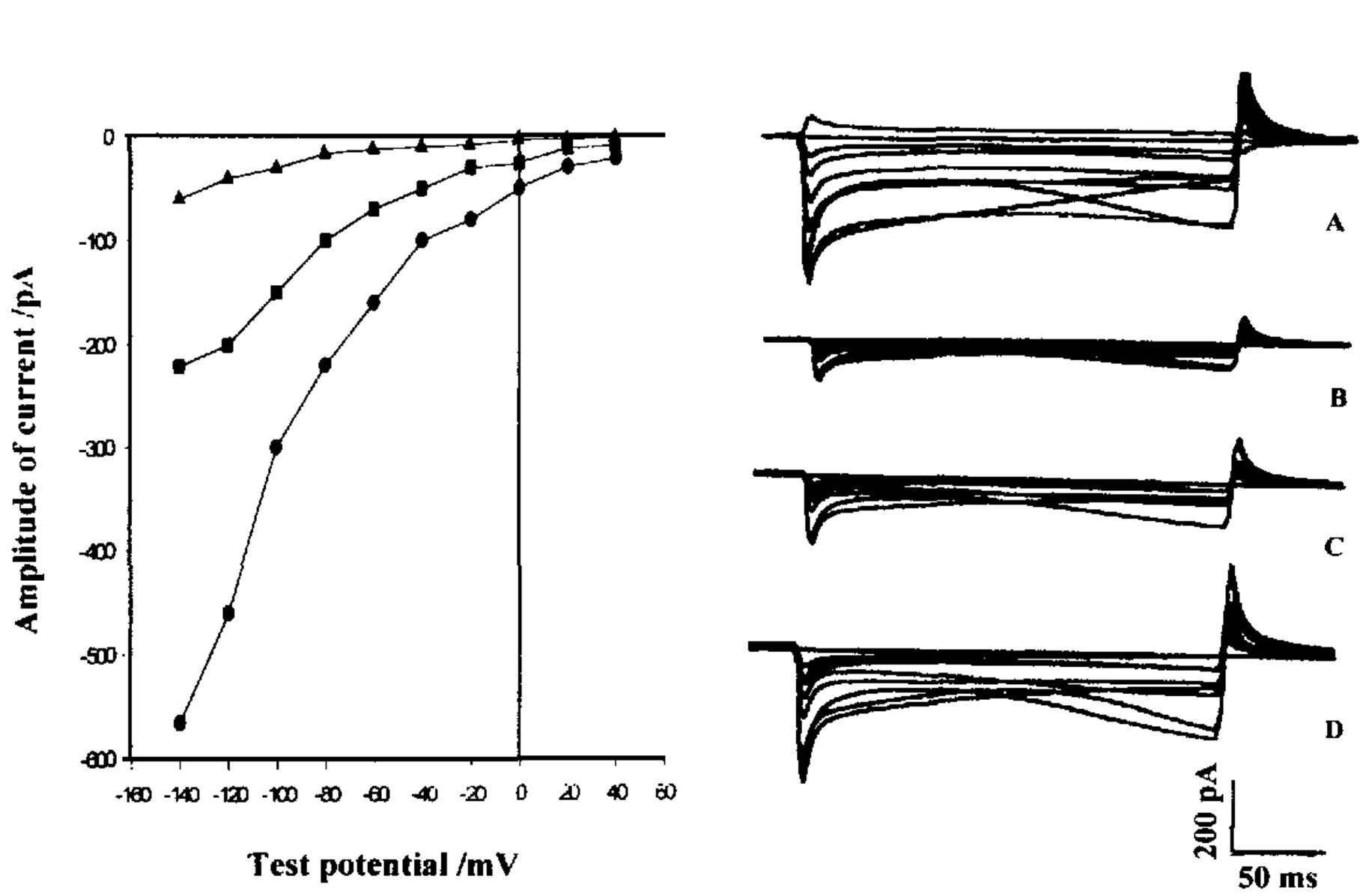

Fig 1. Whole-cell recordings of inward rectifying potassium current before (●) and after (▲) exposure to externally applied 100 μmol/L. (■) Tet 30 μmol/L. (A,B) Normal and suppressed currents on exposure to Ba^{2+}. (C,D) Shows the inhibited currents after application of Tet and the recovered current after wash with drug-free solution. Cells were held at membrane potential of − 70 mV and test potential stepped from − 120 to + 40 mV in 20-mV increment. The test pulses were 300 ms. Fig on the left shows the current-voltage relation.

potassium current in a concentration-dependent manner with IC_{50} 2.8 μmol/L. Compared with control, Tet 3 and 30 μmol/L greatly inhibited the IRK (Fig 2).

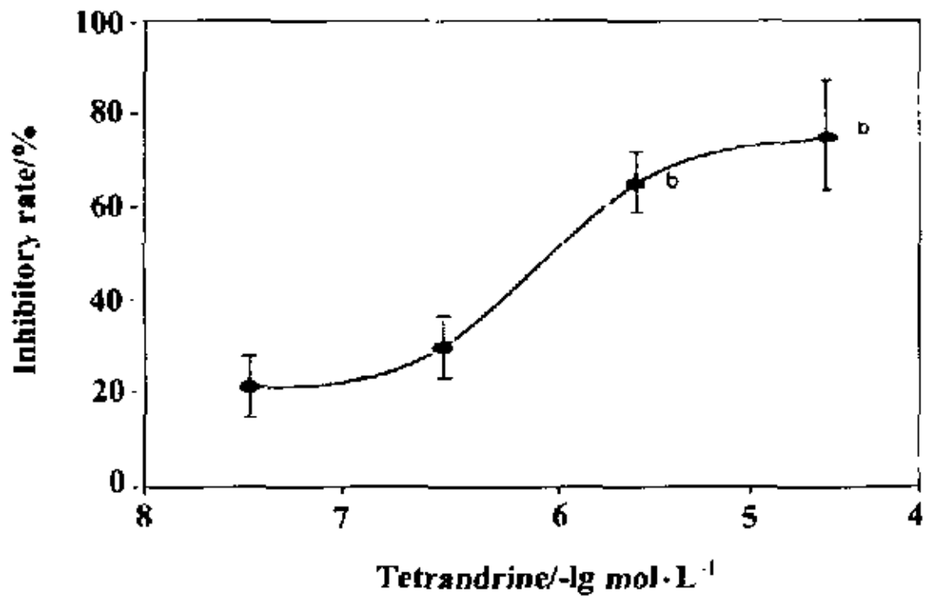

Fig 2. Concentration-dependent inhibition of IRK by Tet. $^bP < 0.05$ *vs* control.

The effect of Tet on the IRK was reversible (Fig 1). Inhibition of the IRK by Tet occurred at 2 min after Tet was added, and at 3 min reached the peak.

DISCUSSION

Activity of K^+ channels may contribute to the shaping of intracellular Ca^{2+} signals by modifying the driving force for Ca^{2+} ions. Modulation of these channels in endothelial cells may therefore be of essential biological relevance. IRK have been described in several types of endothelial cells. Extracellular Ba^{2+}, Cs^+, tetraethylammonium (TEA), and tetrabutylammonium (TBA) block IRK; agonists such as angiotensin II, endothelin 1, and histamine inhibit the IRK[6]. The mechanism of modulation of IRK channels in these EC, as well as their molecular-biological identification, is still to be resolved. So to find a compound with the ability to inhibit IRK will give us a new tool to study the mechanism of IRK.

Traditionally, Tet was used in the treatment of hypertension. Tet inhibited the L-type and T-type Ca^{2+} current in various tissues. The action of Tet as a Ca^{2+} channel antagonist may in part explain its hypotensive effects[2]. Recently, inward rectifying K^+ channels have been described in cultured bovine aortic endothelial cells that are activated by an elevation of $[Ca^{2+}]_i$, and by G-proteins. In addition to the inhibitory effect of Tet on Ca^{2+} influx, Tet also may interfere with Ca^{2+} sequestration. Tet was shown to inhibit cyclopiazonic acid-induced blockage of Ca^{2+} readmission into intracellular storage sites[3]. Tet inhibited Ca^{2+} entry through the sarcolemma by decreasing sarcoplasmic reticular Ca^{2+} uptake[12]. Liu[13] found that Tet inhibited the thapsigargin-induced increase in $[Ca^{2+}]_i$, suggesting an inhibition of Ca^{2+} sequestration. Although endothelial cells differ greatly from smooth cells, Tet may also inhibit IRK in endothelial cells by an elevation of $[Ca^{2+}]_i$. Recently, Tet serves as a specific blocker of the slowly-gating $K^+_{(Ca)}$ channel[5], and its very close analogues dauricine had a blocking effect on IRK in guinea pig ventricular myocytes[14]. In this paper we first report the direct inhibitory effect of Tet on IRK in BAEC. The mechanism is not clear, maybe the specific structure of Tet plays an important role in it[15].

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粉防己碱抑制培养的牛主动脉内皮细胞内向整流钾电流

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关键词 粉防己碱；血管内皮；钾通道；膜片箝技术

目的： 研究粉防己碱(Tet)对培养的牛主动脉内皮细胞的内向整流钾电流(IRK)的作用. **方法：** 在分离培养的牛主动脉内皮细胞上，用全细胞膜片箝技术观察 Tet 对 IRK 的作用. **结果：** Tet 以剂量依赖和可逆的方式抑制 IRK. 用 Tet 30 μmol/L，在控制电压为 −70 mV 时，IRK 从(582 ± 48) pA 降到(221 ± 40) pA. 半数最大抑制浓度为 2.8 μmol/L. **结论：** 粉防己碱抑制培养的牛上动脉内皮细胞内向整流钾电流.

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