Effects of tetrandrine on calcium and potassium currents in isolated rat hepatocytes

Hong-Yi Zhou, Fang Wang, Lan Cheng, Li-Ying Fu, Ji Zhou, Wei-Xing Yao

Hong-Yi Zhou, Fang Wang, Lan Cheng, Li-Ying Fu, Ji Zhou, Wei-Xing Yao, Department of Pharmacology, Tongji medical college of Huazhong university of science and technology, Wuhan 430030, Hubei Province, China

Correspondence to: Hong-Yi Zhou, Department of Pharmacology, Tongji medical college of Huazhong university of science and technology, 13 hangkong Road, Wuhan 430030, China. zhouhy518@yahoo.com.cn
Telephone: 027-83644206

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Abstract

AIM: To study the effects of tetrandrine (Tet) on calcium release-activated calcium current (I_{CRAC}), delayed rectifier potassium current (I_K), and inward rectifier potassium currents (I_{K1}) in isolated rat hepatocytes.

METHODS: Hepatocytes of rat were isolated by using perfusion method. Whole cell patch-clamp techniques were used in our experiment.

RESULTS: The peak amplitude of I_{CRAC} was -508 ± 115 pA ($n=15$), its reversal potential of I_{CRAC} was about 0 mV. At the potential of -100 mV, Tet inhibited the peak amplitude of I_{CRAC} from -521 ± 95 pA to -338 ± 85 pA ($P < 0.01$ vs control, $n=5$), with the inhibitory rate of 35 % at 10 μ mol/L and from -504 ± 87 pA to -247 ± 82 pA ($P < 0.01$ vs control, $n=5$), with the inhibitory rate of 49 % at 100 μ mol/L, without affecting its reversal potential. The amplitude of I_{CRAC} was dependent on extracellular Ca^{2+} concentration. The peak amplitude of I_{CRAC} was -205 ± 105 pA ($n=3$) in tyrode's solution with Ca^{2+} 1.8 mmol/L ($P < 0.01$ vs the peak amplitude of I_{CRAC} in external solution with Ca^{2+} 10 mmol/L). Tet at the concentration of 10 and 100 μ mol/L did not markedly change the peak amplitude of delayed rectifier potassium current and inward rectifier potassium current ($P > 0.05$ vs control).

CONCLUSION: Tet protects hepatocytes by inhibiting I_{CRAC} , which is not related to I_K and I_{K1} .

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INTRODUCTION

Tetrandrine (Tet) is a bisbenzylisoquinoline alkaloid from a Chinese medicinal herb (*Stephania tetrandra* S. Moore). In the past decade, lots of studies demonstrated that Tet possessed multiple bioactivities, such as potential immunomodulating, anticarcinoma^[1] and protective effect on CCl₄-injured hepatocytes^[2]. It has also been used in the treatment of ischemic heart diseases^[3] and hypertension^[3,4]. Recently, the antifibrotic effects of Tet have been received considerable attention^[1,5-10]. The former researches of Tet on the liver have probed into cellular and molecular levels^[9,10]. In the present paper, we used

whole-cell patch-clamp technique to observe the effects of Tet on I_{CRAC} , I_K , I_{K1} in normal isolated rat hepatocytes, in order to have a better understanding its hepatoprotective and antifibrotic effects.

MATERIALS AND METHODS

Solutions and drugs

Tet was from Jinhua Pharmaceutical Co. The stock solution (10 mmol/L) was dissolved in distilled water after acidification with 0.1 mol/L HCl and neutralized with 0.1 mol/L NaOH. Ca^{2+} -free Hank's solution was prepared without Ca^{2+} and Mg^{2+} . KB solution contained (mmol/L): glutamic acid 70, taurine 15, KCl 130, KH_2PO_4 10, HEPES 10, glucose 11, egtazic acid 0.5, pH was adjusted to 7.4 with KOH. The external solution used to record I_{crac} contained (mmol/L): NaCl 140, KCl 2.8, $CaCl_2$ 10, $MgCl_2$ 0.5, glucose 11, HEPES 10, pH was adjusted to 7.4 with NaOH. The internal solution used to record I_{CRAC} contained (mmol/L): potassium-glutamate 145, NaCl 8, $MgCl_2$ 1, Mg-ATP 0.5, egtazic acid 10, HEPES 10, pH was adjusted to 7.2 with KOH. The external solution used to record I_K contained (mmol/L): NaCl 144, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, glucose 5.5, HEPES 5, pH was adjusted to 7.4 with NaOH. The internal solution used to record I_K contained (mmol/L): KCl 130, K_2ATP 5, creatine phosphate 5, HEPES 5, pH was adjusted to 7.2 with KOH. The same external and internal solutions used to record I_{K1} contained (mmol/L): KCl 7, $MgCl_2$ 2, egtazic acid 1, potassium-glutamate 130, HEPES 10, pH was adjusted to 7.4 with KOH.

Isolation of single hepatocytes

Hepatocytes were isolated with the modified method reported by Seglen^[11]. Briefly, adult wistar rats of either sex (175 ± 25 g) were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). The portal vein was cannulated and perfused with oxygenated Ca^{2+} -free Hank's solution 30 ml/min at 37 °C for 4-5 min followed by perfusion with Ca^{2+} -free Hank's solution containing collagenase (Type 1, Sigma) (0.3g/L) for 10 min. The liver was chopped in 10 ml Ca^{2+} -free Hank's solution. The cell suspension was filtered through 200 mesh gauze and then centrifuged three times (50 g, 2 min) to separate liver cells. Cells were plated onto the coverslips and incubated in KB medium for 2 hour and preserved in DMEM at 4 °C.

Electrophysiologic recording

Whole-cell recordings were performed using a PC-II patch clamp amplifier (Huazhong university of science and technology). The recording chamber (1.5 ml) was perfused with the corresponding external solution. The pipettes were pulled in two stages from hard glass capillaries using a vertical microelectrode puller (Narishige, Japan). Electrode has a resistance of 2-5 M Ω for whole-cell recording when filled with electrode internal solution. All experiments were conducted at 22 ± 2 °C.

Statistic analysis

The data were expressed as $\bar{x} \pm s$. Statistical significances were analyzed by a unpaired *t*-test. A value of $P < 0.05$ was considered significant.

RESULTS

Effect of Tet on calcium release-activated calcium current (I_{CRAC})

I_{CRAC} was elicited for 200 ms from the holding potential of 0 mV to various potentials ranging from -10 mV to +80 mV with the step of 20 mV every 5 s^[12]. The peak amplitude of I_{CRAC} was -508 ± 115 pA ($n=15$) and the reversal potential of I_{CRAC} was about 0 mV, the current was steady and without run-down in 5 mins. Tet inhibited the peak amplitude of I_{CRAC} from -521 ± 95 pA to -338 ± 85 pA ($P < 0.01$ vs control, $n=5$), with the inhibitory rate of 35 % at 10 $\mu\text{mol/L}$ and from -504 ± 87 pA to -247 ± 82 pA ($P < 0.01$ vs control, $n=5$), with the inhibitory rate of 49 % at 100 $\mu\text{mol/L}$. Tet did not affect the shape of its current voltage curve (Figure 1,2).

Figure 1 Effect of Tet on I_{CRAC} in isolated rat hepatocytes. I_{CRAC} traces before and after Tet 10 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$. I_{CRAC} was elicited for 200 ms from the holding potential of 0 mV to various potentials ranging from -100 mV to +80 mV with step of 20 mV.

Figure 2 Effect of Tet on I-V relationship of I_{CRAC} in isolated rat hepatocytes. Especially at the potential of -100 mV, Tet inhibited the peak amplitude of I_{CRAC} with the inhibitory rate of 35 % at 10 $\mu\text{mol/L}$ and with the inhibitory rate of 49 % at 100 $\mu\text{mol/L}$. ● control; ○ 10 $\mu\text{mol/L}$; □ 100 $\mu\text{mol/L}$.

The amplitude of I_{CRAC} was dependent on extracellular Ca^{2+} concentration. The peak amplitude of I_{CRAC} was -205 ± 105 pA ($n=3$) in tyrode's solution with Ca^{2+} 1.8 mmol/L ($P < 0.01$ vs the peak amplitude of I_{CRAC} in external solution with Ca^{2+} 10 mmol/L). Tet at 10 $\mu\text{mol/L}$ decreased I_{CRAC} from -205 ± 105 pA to -148 ± 96 pA ($n=3$).

Effect of Tet on delayed rectifier potassium current (I_K)

I_K was elicited by 900 ms depolarization steps from +30 mV to +140 mV with step of 10 mV at holding potential of -50 mV^[13]. Tet at 10 and 100 $\mu\text{mol/L}$ did not change the peak amplitude of I_K [from 2014 ± 686 pA to 2030 ± 692 pA and 2047 ± 710 pA respectively, $n=5$, cells from 3 rats, $P > 0.05$]. Tet did not affect the shape of its current voltage curve.

Effect of Tet on inward rectifier potassium current (I_{K1})

I_{K1} was elicited by a number of step pulses (40 ms) from the holding potential (E_h) of 0 mV to test potentials from -200 mV to +175 mV with the step of 10 mV^[14]. Tet at 10 and 100 $\mu\text{mol/L}$ did not change the peak amplitude of I_{K1} [from 2254 ± 718 pA to 2239 ± 700 pA and 2224 ± 658 pA respectively, $n=5$, cells from 3 rats, $P > 0.05$]. Tet did not affect the shape of its current voltage curve.

DISCUSSION

Hepatic fibrosis is a common consequence of chronic liver injury from many causes^[15-19], and the sustained hepatic injury is a primary factor for hepatic fibrogenesis^[20-28]. Preventing hepatocyte from injury is a matter of primary importance in blocking the fibrogenic pathway. Tet has been considered as an effective antifibrotic and hepatoprotective agent^[1,2,5-10], but its protection against toxic cell death has never been well illustrated.

The Ca^{2+} influx is mainly mediated by voltage-operated Ca^{2+} channels and receptor-activated Ca^{2+} channels. Voltage-operated Ca^{2+} channels are not present in hepatocytes^[29]. Calcium influx in isolated hepatocytes mainly depend on receptor-mediated Ca^{2+} entry which has been identified by indirect methods^[30]. Previous studies have shown that one type of receptor-activated Ca^{2+} channels, most likely a store-operated Ca^{2+} channel, in freshly isolated rat hepatocytes is inhibited by high concentrations of L-type voltage-operated Ca^{2+} channels antagonists^[30,31]. Besides, the mRNA encoding isoforms of L-type voltage-operated Ca^{2+} channels has been detected in rat hepatocytes, these observation suggested that receptor-activated Ca^{2+} channels in rat hepatocytes exhibit some characteristics of voltage-operated Ca^{2+} channels^[32]. Cui *et al* reported that I_{CRAC} (an important sub-type of store-operated Ca^{2+} channels) existed in isolated rat hepatocytes and 50 $\mu\text{mol/L}$ of verapamil, diltiazem and nifedipine could decrease the amplitude of I_{CRAC} effectively^[12]. The results in our experiment showed that Tet at the concentration of 10 and 100 $\mu\text{mol/L}$ could decrease the peak amplitude of I_{CRAC} . The concentration that Tet made a half-maximal inhibition of I_{CRAC} was approximately 100 $\mu\text{mol/L}$, which was substantially higher than that needed for the half-maximal inhibition of L-type voltage-operated Ca^{2+} channels by Tet. It suggested that Tet could protect hepatocytes by inhibiting I_{CRAC} and decreasing intracellular Ca^{2+} concentration, in which a higher concentration of Tet was needed.

Nietsch's study demonstrated that the activation of potassium and chloride channels by TNF- α induced apoptosis and death of the HTC rat hepatoma cells, which could be significantly delayed by K^+ channel blockers (Ba^{2+} and quinine)^[33]. Our result showed that Tet had no effect on I_K and I_{K1} , which suggested that the hepatoprotection of Tet might not relate to potassium channels. However, whether calcium activated potassium channels exist in rat hepatocytes or not remains controversial^[34,35]. The effect of Tet on potassium channels requires further investigation.

In conclusion, Tet protects hepatocytes by inhibiting I_{CRAC} , which is not related to I_K and I_{K1} .

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