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Effects of benzyltetrahydropalmatine on potassium currents in guinea pig and rat ventricular myocytes

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KEY WORDS benzyltetrahydropalmatine; potassium channels; patch-clamp techniques; myocardium

ABSTRACT

AIM: To investigate the effects of benzyltetrahydropalmatine (BTHP) on rapidly activating component (I_{Kr}), slowly activating component (I_{Ks}) of delayed rectifier potassium current, inward rectifier potassium current (I_{K1}), and transient outward potassium current (I_{to}) in single ventricular myocytes. **METHODS**: Whole-cell patch clamp technique was used to record ionic currents. **RESULTS**: (1) BTHP 30 μmol/L reduced I_{Kr} and $I_{Kr,tail}$ by 31 % ± 4 % and 36 % ± 5 % ($n = 6$, $P < 0.01$), respectively and inhibited I_{Ks} and $I_{Ks,tail}$ by 40 % ± 6 % and 45 % ± 5 % ($n = 7$, $P < 0.01$), respectively. I_{Kr} and I_{Ks} were blocked by BTHP 1 - 100 μmol/L in a concentration-dependent fashion (IC_{50} value was 13.5 μmol/L and 95 % confidence limit: 11.2 - 15.8 μmol/L for I_{Kr}, 9.3 μmol/L and 95 % confidence limit: 7.8 - 11.8 μmol/L for I_{Ks}, respectively). (2) BTHP 5 μmol/L inhibited I_{to} by 63 % ± 6 % ($n = 6$, $P < 0.01$). BTHP 1 - 100 μmol/L reduced I_{to} in a concentration-dependent manner (IC_{50} value was 3.6 μmol/L and 95 % confidence limit: 2.9 - 4.3 μmol/L). (3) BTHP 200 μmol/L did not affect I_{K1}. **CONCLUSION**: BTHP inhibited I_{Kr}, I_{Ks}, and I_{to}, but not I_{K1}. The antiarrhythmic effects of BTHP may be mainly due to its blockade on potassium channels.

INTRODUCTION

Benzyltetrahydropalmatine (BTHP) is a derivative of tetrahydropalmatine (THP) which is extracted from *Corydalis ambigua* (Pall) Cham et Schlecht. THP possesses the effect on treating arrhythmias induced by many agents and preventing the myocardium from ischemic and infarcted damage[1]. The mechanism of this effect is prolongation of action potential duration, and inhibition of calcium and potassium currents in myocardium[2]. The previous studies have indicated that BTHP has potent antiarrhythmic effects in several animal models. Electrophysiologic effects of BTHP were related to the prolongation of action potential duration (APD), which is similar to that of class Ⅲ antiarrhythmic agent. BTHP produced concentration-, time-, and frequency-dependent prolongation of action potential duration at 90 % repolarization (APD_{90}) and effective refractory period at 1 - 100 μmol/L[3,4], whereas it had no effect on the resting membrane potential (RP), the action potential amplitude (APA), or V_{max}.

Delayed rectifier potassium current (I_K) including rapidly activating component (I_{Kr}) and slowly activating component (I_{Ks}), inwardly rectifier potassium current (I_{K1}), and transient outward potassium current (I_{to}) are major components in the repolarization process[5,6]. The prolongation of APD by antiarrhythmic drugs is a result of the decrease in outward K^+ currents. To clarify the antiarrhythmic mechanism of BTHP, in the present study, we investigated the effects of BTHP on I_{Kr}, I_{Ks}, and I_{K1} in guinea pig ventricular myocytes and I_{to} in rat ventricular myocytes.

MATERIALS AND METHODS

Agents and animals Benzyltetrahydropalmatine was supplied by China Pharmaceutical University, molecular weight 478.5, mp 204 ℃, a white crystal powder, purity >99.0 %. It was dissolved in distilled water to make a stock solution at 5 mmol/L. Collageoase type Ⅰ, protease E, bovine serum albumin,

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egtazic acid, K_2ATP, $CdCl_2$, HEPES, and K-aspartate were purchased from Sigma Co; dofetilide from Pfizer; tetrodotoxin (TTX) from Hebei Aquatic Product Research Institute; other reagents were of analytical grade. Male or female guinea pigs weighing 250 – 350 g and Wistar rats weighing 150 – 200 g were provided by the Experimental Animal Center of Tongji Medical College of Huazhong University of Science and Technology, Grade Ⅱ, Certificate № 19-025 for guinea pig and Certificate № 19-023 for rat.

Cell preparation Single ventricular myocytes of guinea pigs and rats were isolated using enzymatic dissociation method similar to that previously described[7,8].

Electrophysiologic recording Isolated single cells were transferred to a chamber mounted on the stage of an inverted microscope. To record K^+ currents, the cells were superfused with Ca^{2+}-free Tyrode's solution (mmol/L: NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.33, HEPES 10, glucose 10, pH was adjusted to 7.4 with NaOH) in the presence of TTX 50 μmol/L to block I_{Na} and $CdCl_2$ 100 μmol/L to block $I_{Ca,L}$.

The currents were recorded at 35 ℃ by the whole cell voltage-clamp configuration using a PC-Ⅱ amplifier (Huazhong Science and Technology University, HZUST). Stimuli output or data acquisition and processing were performed by Pclamp software, and an IBM-compatible computer was connected to the amplifier *via* D/A (DAC1210) and A/D (AD1674) converter. Micropipettes were made by using a two-stage puller (pp-83, Narishige) from star-bore capillary tubes (GG-17) and had resistances of 3 – 4 MΩ when filled with the internal solution (mmol/L: K-aspartate 85, KCl 45, Na-pyruvate 5, K_2-ATP 3, $MgCl_2$ 4, egtazic acid 10, HEPES 10, *D*-glucose 11, and pH was adjusted to 7.3 with KOH). The voltage clamp error was usually within 2 mV in the range of the measured currents, otherwise excluded. Voltage signals were low-pass filtered at 1 kHz by a 4 pole Bassel filter before sampling.

Data analysis Data were expressed as $\bar{x} \pm s$ and n represents the number of experiments performed. The Marquardt-Levenberg method of nonlinear regress was used to fit the concentration-response curve and to calculate the IC_{50} value according to the equation $I = I_{max}/[IC_{50}/([C] + 1)]$. Statistical significance was determined by t-test.

RESULTS

Effect of BTHP on I_{Kr} I_{Kr} was elicited by short depolarizing pulses from a holding potential of – 50 mV to 0 mV at the frequency of 1.0 Hz. The step pulse duration was 225 ms. Amplitude of the tail current ($I_{Kr,tail}$) was determined upon repolarization to – 40 mV, which could be blocked almost completely by dofetilide 1 μmol/L. BTHP 30 μmol/L reduced current densities of I_{Kr} and $I_{Kr,tail}$ from (0.7 ± 0.3) pA/pF and (1.4 ± 0.5) pA/pF to (0.5 ± 0.2) pA/pF and (0.9 ± 0.3) pA/pF ($n = 6$ cells from 5 guinea pigs, $P < 0.01$, Fig 1).

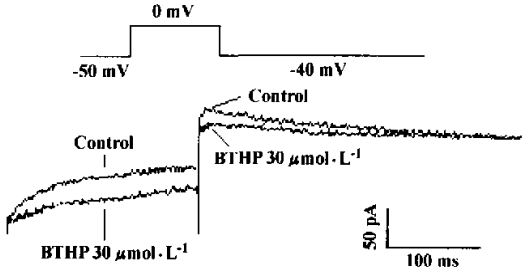

Fig 1. Effect of BTHP on I_{Kr} and $I_{Kr,tail}$ in guinea pig ventricular myocytes.

Effect of BTHP on I_{Ks} Cells were exposed to dofetilide 1 μmol/L to block I_{Kr}. I_{Ks} was recorded by applying various voltage pulses ranging from – 20 to + 50 mV for 7 s from holding potential of – 50 mV, followed by repolarizing to – 30 mV. In the 7 cells examined from 4 guinea pigs, BTHP 30 μmol/L reduced current densities of I_{Ks} and $I_{Ks,tail}$ from (12.1 ± 1.8) pA/pF and (4.6 ± 0.6) pA/pF to (7.3 ± 0.9) pA/pF and (2.6 ± 0.9) pA/pF, respectively ($P < 0.01$, Fig 2).

Concentration-dependent blockade effect of BTHP on I_{Kr} and I_{Ks} BTHP 1 – 100 μmol/L blocked I_{Kr} in a concentration-dependent fashion, with an IC_{50} value of 13.5 μmol/L and 95 % confidence limit: 11.2 – 15.8 μmol/L. BTHP 1 – 100 μmol/L blocked I_{Ks} in a concentration-dependent fashion, with IC_{50} value of 9.3 μmol/L and 95 % confidence limit: 7.8 – 11.8 μmol/L ($n = 6$, Fig 3).

Effect of BTHP on I_{K1} I_{K1} was elicited from holding potential of – 40 mV to a number of test pulses between – 100 mV and + 40 mV during 300 ms with step 10 mV. BTHP 200 μmol/L did not affect this

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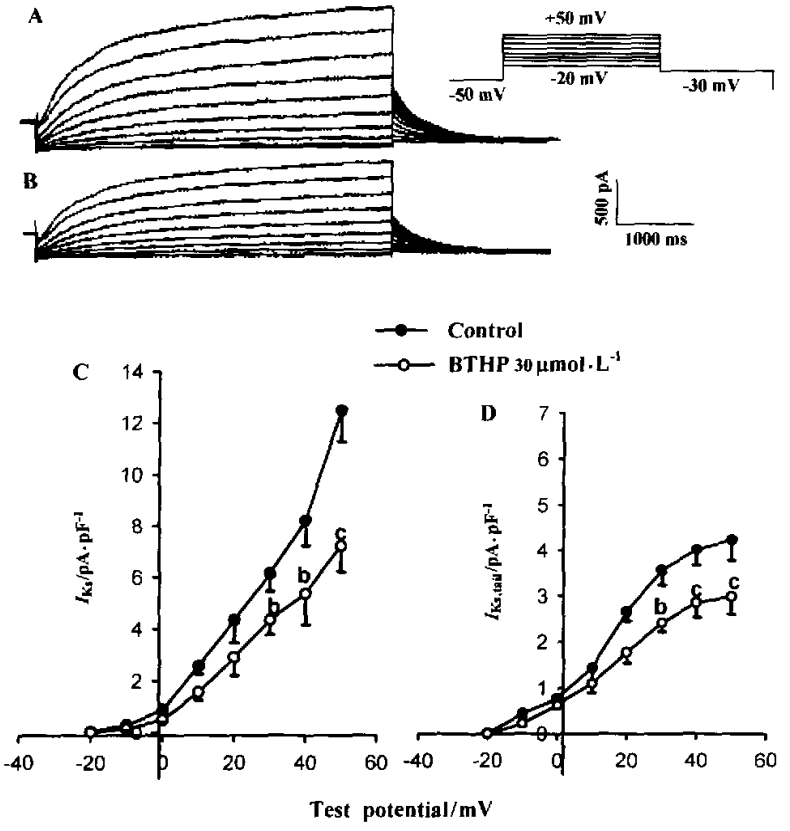

Fig 2. Voltage-dependent blockage on I_{Ks} by BTHP in guinea pig ventricular myocytes. A: Without BTHP; B: BTHP 30 μmol/L. C and D: *I-V* relationships of I_{Ks} and $I_{Ks,tail}$ before and after the exposure to BTHP. $n = 7$ cells from 4 guinea pigs. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

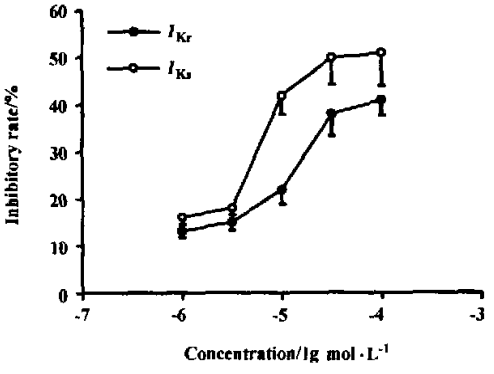

Fig 3. Concentration-dependent block of I_{Kr} and I_{Ks} by BTHP. $n = 6$. $\bar{x} \pm s$.

current (n = 6 cells from 4 guinea pigs, $P > 0.05$, Fig 4).

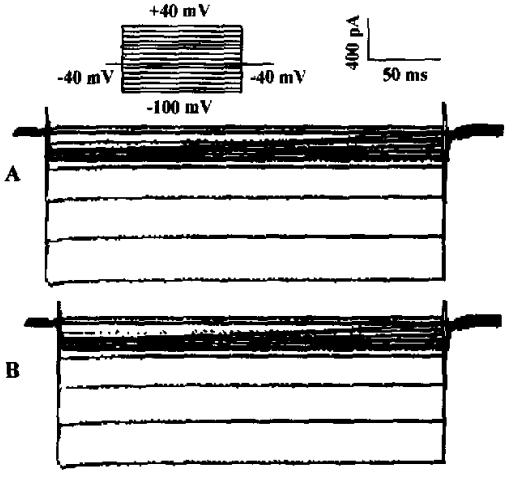

Fig 4. Effect of BTHP on I_{K1} in guinea pig ventricular myocytes. (A) Control, (B) BTHP 200 μmol/L.

Effect of BTHP on I_{to} The cells from rat were held at holding potential of − 80 mV, depolarized to − 40 mV for 25 ms to inactivate sodium current, and then depolarized in 10 mV increment to test potential of + 50 mV during 300 ms. In the 6 cells examined from 3 rats, BTHP 5 μmol/L reduced I_{to} current densities from (13.8 ± 2.2) pA/pF to (5.1 ± 1.4) pA/pF at membrane potential of + 50 mV ($P < 0.01$, Fig 5). BTHP 1 − 100 μmol/L blocked I_{to} in a concentration-dependent fashion, with an IC_{50} value of 3.6 μmol/L (95 % confidence limit: 2.9 − 4.3 μmol/L).

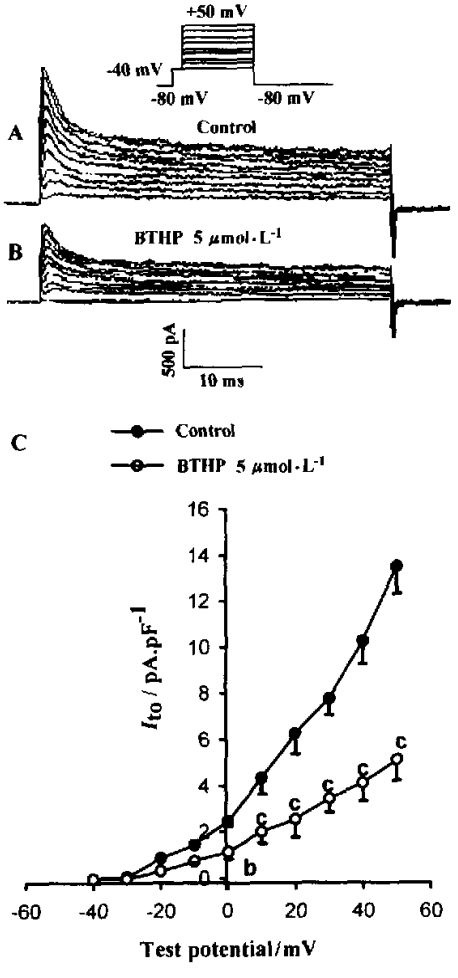

Fig 5. Voltage-dependent block of BTHP on I_{to} of rat ventricular myocytes. The original recording was performed (A: control, B: BTHP 5 μmol/L). C: *I-V* relationship of I_{to} before and after the exposure to BTHP. $n = 6$ cells from 3 rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

DISCUSSION

The results of present study showed that BTHP inhibited I_{Kr}, I_{Ks}, and I_{to}. This is an important characteristic of BTHP that differed from those of dofetilide, E-4031, sotatol, WAY-123, 398 which selectively block I_{Kr}[9,10].

I_{Kr} and I_{Ks} currents are mainly responsible for repolarization. Agents selectively blocking I_{Kr} cause greater prolongation of APD at slow rates of stimulation than rapid rates. This is reverse rate dependence or reverse use-dependence. It has been implicated in the bradycardia-dependent proarrhythmic effects. Reverse rate-dependence has been attributed to the "accumulation" of I_{Ks} during rapid stimulation (results from the incomplete deactivation of this current), which minimizes the effects of I_{Kr} block at faster stimulation rates[11]. Our results showed that the effects of BTHP 30 μmol/L effectively blocked two components of I_k and had no obvious selectivity for guinea pig ventricular myocytes. This could be more advantageous than class Ⅲ antiarrhythimc drugs for selectively blocking I_{Kr}. It is proposed that favorable blockade of I_{Ks} by BTHP results in minimizing proarrhythmic events while still maintaining effective antiarrhythmic property.

I_{to} plays an important role in repolarization plateau height and the early period of the action potential in several mammalian hearts including rat, dog, and human. The decrease in I_{to} could also contribute to the obvious increase in APD. Some antiarrhythmic drugs have been known to affect I_{to} in some animals obviously. The fact that BTHP inhibits I_{to} may be partly responsible for its antiarrhythmic effects.

On the other hand, I_{Ks} and I_{to} play important roles in defining dispersion of repolarization across the ventricle wall because of the distributing heterogeneity of I_{Ks} and I_{to}[12,13]. BTHP significantly inhibits both I_{Ks} and I_{to}, which suggests that BTHP may decrease dispersion of repolarization of transmural ventricle wall.

These characteristics of blocking the multiple ion channels of BTHP can explained well its effects on prolongation of APD_{90} in the rate-dependent fashion[14], which is the basis of its antiarrhythmic effects, minimizing the morbidity of acquired *tosade de points*. Furthermore it can decrease dispersion of repolarization across ventricular wall and reduced the probability of successful transmural reentry.

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苄基四氢巴马汀对豚鼠和大鼠心室肌细胞钾电流的作用

R96 A

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关键词 苄基四氢巴马汀; 钾通道; 膜片箝技术; 心肌

目的: 研究苄基四氢巴马汀(BTHP)对心室肌细胞快激活(I_{Kr})和慢激活(I_{Ks})延迟整流钾电流、内向整流钾电流(I_{K1})和瞬时外向钾电流(I_{to})的作用. **方法**: 采用全细胞膜片箝技术记录豚鼠及大鼠心室肌细胞钾电流. **结果**: BTHP 在 1-100 μmol/L 的范围内以浓度依赖性方式阻滞 I_{Kr}和 I_{Ks}, 其中对 I_{Kr}的 IC_{50}为 13.5 μmol/L (95 %可信限: 11.2-15.8 μmol/L) 而对 I_{Ks}的 IC_{50}则为 9.3 μmol/L (95 %可信限: 7.8-11.8 μmol/L). BTHP 30 μmol/L 时可使 I_{Kr}及 $I_{Kr,tail}$分别降低31 % ± 4 %和36 % ± 5 % (n = 6, P < 0.01); 使 I_{Ks}及 $I_{Ks,tail}$分别降低40 % ± 6 %和 45 % ± 5 % (n = 7, P < 0.01); BTHP 5 μmol/L 可抑制大鼠心室肌细胞 I_{to}电流, 使电流幅值降低63 % ± 6 % (n = 6, P < 0.01), BTHP 1-100 μmol/L 以浓度依赖性方式阻滞 I_{to}, 其 IC_{50}为 3.6 μmol/L (95 %可信限: 2.9-4.3 μmol/L). 但 BTHP 200 μmol/L 对 I_{K1}基本无影响. **结论**: BTHP 对 I_{Kr}、I_{Ks}、I_{to}均有抑制作用, 且其阻滞作用呈现出浓度依赖性特征.

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