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Effects of 7-bromoethoxybenzene-tetrahydropalmatine on voltage-dependent currents in guinea pig ventricular myocytes

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KEY WORDS electrophsiology; anti-arrhythmia agents; ion channels; patch-clamp techniques; 7-bromoethoxybenzene-tetrahydropalmatine

AIM: To study the anti-arrhythmic mechanism of 7-bromoethoxybenzene-tetrahydropalmatine (EBP). **METHODS**: Whole-cell current and voltage clamp on isolated guinea pig ventricular cells. **RESULTS**: EBP 30 μmol·L^{-1} prolonged APD_{90} from 430±47 ms to 514±61 ms ($n=5$, $P<0.05$) without effects on the action potential amplitude and resting potential. Delayed outward K^+ current and its tail current were blocked by EBP in a concentration-dependent fashion, while EBP did not change the amplitudes of the sodium current, the L type calcium current, and the inwardly rectifying potassium current. **CONCLUSION**: The mechanism of anti-arrhythmic action of EBP was to prolong the APD through inhibiting the delayed rectified potassium current

7-Bromoethoxybenzene-tetrahydropalmatine (EBP) counteracted experiment arrhythmias[1], the mechanism of which may be due to the blocking actions on ionic currents[2]. To confirm the anti-arrhythmic mechanism, we investigated the effects of EBP on voltage-dependent currents in isolated guinea pig ventricular cells using whole-cell recording techniques[3-4].

MATERIALS AND METHODS

Cell preparation Ventricular myocytes were isolated using a modified procedure[5-6]. Guinea pigs (250 ± s 31 g) hearts were perfused retrogradely at 37 ℃. After washing out of the blood with Ca^{2+} 0.2 mmol·L^{-1} solution for 2 – 3 min, Ca-free Tyrode solution 50 mL was perfused. Then enzyme solution 50 mL was recirculated for 5 min. The enzyme was washed out by "Kraftbrühe" (K – B) solution for 1 – 2 min The ventricles were cut into approximately 3 mm × 3 mm pieces. After the pieces were shaken for 5 min with K – B 10 mL solution, the supernatant was collected. The last procedure was repeated 2 – 4 times. The cells were stored in K – B solutions at 25 ℃ until use within 12 h.

Solutions and drugs Tyrode solution was composed of NaCl 135, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.33, egtazic acid 10 mmol·L^{-1}(pH = 7.2). Enzyme solution was made up by adding collagenase Type I (Sigma) 25 mg and pronase E (Sigma) 5 mg to Ca-free Tyrode solution 50 mL. The standard internal solution contained KCl 120, NaCl 10, Mg-ATP 5, egtazic acid 11, $CaCl_2$ 1, HEPES 10 mmol·L^{-1} (pH = 7.2). Cs internal solution for I_{Na}, I_{Ca} recording contained CsCl 112, $MgSO_4$ 1, egtazic acid 5, Na_2ATP 5 mmol·L^{-1}(pH = 7.2).

EBP (white crystalline, melting point: 191 – 192 ℃, very soluble in acid water) was synthesized by Prof HUANG Chen-Ya (China Pharmaceutical University).

Electrophysiology Current and voltage clamp experiments utilized the whole-cell recording configuration with a PC-I patch clamp amplifier (made in our institute) at 25 ± 1 ℃ except the delayed outward currents were recorded at 28 ± 1 ℃. Each cell was exposed to only 1 or 2 concentrations to minimize the time-dependent changes in K^+ current magnitude not related to the drug. Pipettes had resistances of 2 – 5 MΩ. Series resistances and junction potential were compensated. Membrane currents were filtered at 5 kHz and sampled at 0.5 – 5 ms intervals.

Statistical methods Data were presented as $\bar{x} \pm s$. The t test was used. Curve fitting was performed using the Marquardt least-square method of nonlinear regression analysis.

RESULTS

Action potential Superimposed action potentials recorded from a single guinea pig ventricular cell in the presence or absence of EBP 30 μmol·L^{-1} showed that EBP increased the action potential duration (APD) without modifying the overshot and the resting potential (RP) (Fig 1). Similar results were obtained from four other observations. The

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APD_{90}, RP, and APA in control were 430 ± 47 ms, 69.4 ± 2.7 mV, and 116 ± 8 mV. In the presence of EBP 30 $\mu mol \cdot L^{-1}$ the APD_{90}, RP, and APA were 514 ± 61 ms ($P<0.05$, $n=5$), 68 ± 3 mV ($P>0.05$, $n=5$), and 118 ± 7 mV ($P>0.05$, $n=5$), respectively. Only the rate of phase 2 repolarization was slowed by EBP, indicating that EBP acted on the later part of plateau or repolarizing process.

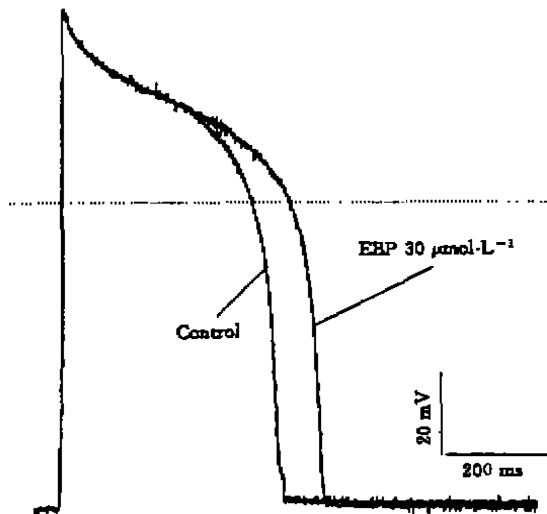

Fig 1. Superimposed action potentials recorded from a single guinea pig ventricular myocyte.

Delayed outward current (I_K) When the cell was bathed in Tyrode solution with the addition of $CdCl_2$ 1 $mmol \cdot L^{-1}$ to block L-type Ca^{2+} currents, only I_K was elicited by depolarizing to 60 mV for 5 s from holding potential of −40 mV at a frequency of 0.1 Hz. EBP 30 $\mu mol \cdot L^{-1}$ strongly decreased both the time dependent K^+ current and the tail current (Fig 2A). While, EBP did not change the magnitude of the time-independent outward currents at holding potential. The concentration-dependent blockage of EBP on I_K was assessed by measuring the decrease in tail currents at −40 mV after 5 s depolarizing pulse to 60 mV. IC_{50} for block of I_K by EBP was estimated to be 17.7 $\mu mol \cdot L^{-1}$ (Fig 2B).

Other membrane currents We elicited the inward rectifier current (I_{K1}) by hyperpolarizing pulse to −40 ~ −180 mV from a holding potential of −40 mV. After exposure to EBP 30 $\mu mol \cdot L^{-1}$, the amplitudes of I_{K1} were not significantly different with that of control (Fig 3).

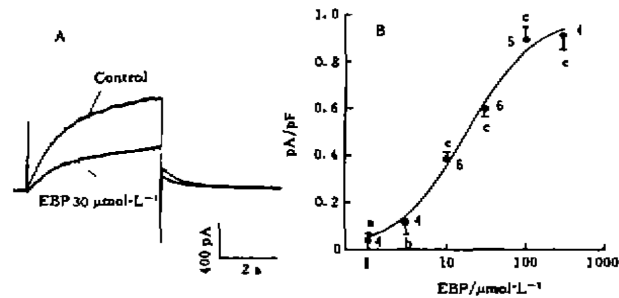

Fig 2. A: Original recording of I_K B: Block of I_K tail currents. Drug sensitive currents expressed relative to cell capacitance. Number of cells was given at each point. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs without EBP. The average data were fitted by the equation: $I = I_{max}/(IC_{50}/[EBP]+1)$, $I_{max} = 0.99$ pA/pF ($R^2 = 0.99$).

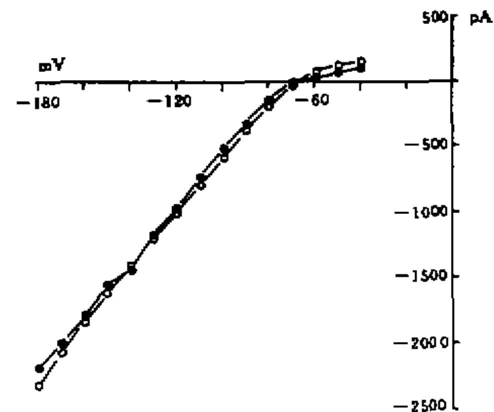

Fig 3. I − V curves of the inward rectifier current in absence (○) and presence (●) of EBP 30 $\mu mol \cdot L^{-1}$.

EBP had no effect on sodium and L-type calcium currents, which were blocked by tetrodotoxin (TTX) 20 $\mu mol \cdot L^{-1}$ and nitrendipine 2 $\mu mol \cdot L^{-1}$, respectively (Fig 4). Similar results were obtained in other observations. In the presence of EBP 100 $\mu mol \cdot L^{-1}$, the relative changes of sodium and L calcium currents were −4.3 % ± 1.4 % ($P>0.05$, $n=3$) and −3.6 % ± 1.1 % ($P>0.05$, $n=4$).

DISCUSSION

The concept of action potential prolongation as a useful anti-arrhythmic mode of action was first advanced over 2 decades ago. The present study of EBP on single cell action potential supported the

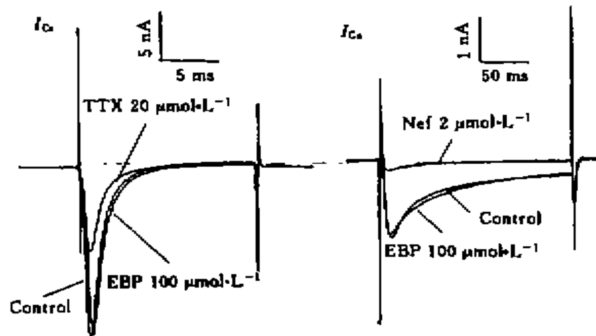

Fig 4. I_{Na}, I_{Ca} were elicited by depolarizing membrane potential from −80, and −40 mV to −30, and 10 mV, respectively.

suggestion that EBP acted as a class Ⅲ agent[1-2].

Moreover, we found that EBP selectively inhibited among voltage-dependent membrane currents in guinea pig ventricular cells. This might be the main mechanism underlying action potential prolongation.

In our whole-cell recordings, EBP had minor influence on I_K. However, published work in canine Purkinje fibers using two microelectrode voltage-clamp techniques provided argument that I_{Ca} was concentration-dependently depressed by EBP. We suggested the disagreement might come from the variation of sample and method: it is possible that EBP might have selective action across cell types and species. In additon, it is well known voltage-clamp on multicellular cardiac preparations often resulted in poor estimation of I_{Ca} properties[7]. The method problem seems to be another strong candidate for explanation.

In conclusion, the results indicated that the mechanism of anti-arrhythmic action of EBP was to prolong the APD through specially inhibiting the delayed rectifier potassium current.

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7-溴化乙氧苯四氢巴马汀对豚鼠心室肌细胞电压依赖性通道电流的影响

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关键词　电生理学；抗心律失常药；离子通道；膜片箝技术；7-溴化乙氧苯四氢巴马汀

目的：研究 7-溴化乙氧苯四氢巴马汀(EBP)对电压敏感通道电流的影响．**方法**：在豚鼠心室肌细胞上进行全细胞电流钳和电压钳记录．**结果**：EBP 30 μmol·L⁻¹可使单细胞 APD_{90}从 430±47 ms 延长至 514±61 ms ($P<0.05$, $n=5$)．电压钳研究表明 EBP 可依剂量地抑制 I_K 及其尾电流，而对 I_{K1}，I_{Ca}和 I_{Na}无明显作用．**结论**：以上结果提示选择性地抑制 I_K，从而延长动作电位时程，可能是 EBP 抗心律失常作用机制之一．