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粉防己碱对 Bay k 8644 引起兔离体主动脉条收缩的抑制作用

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提要 在有 KCl 19 mmol · L^{-1} 存在的情况下，钙通道激动剂 Bay k 8644 0.47 μmol · L^{-1} 能诱发兔离体主动脉条产生较强的收缩，而粉防己碱则非竞争性地拮抗这种反应，其抑制作用呈浓度依赖性。粉防己碱对卡西霉素诱发兔离体主动脉条收缩也有明显的抑制作用，但其作用机制尚不清楚。

关键词 粉防己碱；二氢吡啶类；卡西霉素；胸主动脉

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Effects of microinjection of picrotoxin into posterior hypothalamus on ventricular electric stability

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ABSTRACT Ventricular fibrillation threshold (VFT), serum potassium, and monophasic action potentials (MAP) have been assessed before and after microinjection of picrotoxin (Pic) into posterior hypothalamus in rabbits. Pic (2 and 3 μg) brought about a biphasic effect on VFT, an initial decrease followed by a notable increase. Pretreatment with spinal cord transection almost abolished the descending phase, whereas pretreatment with vagotomy depressed the ascending phase significantly. Following the microinjection of Pic, serum potassium rose up to 5.0 mmol · L^{-1} at 30 min. The amplitude of MAP (MAPA) and V_{max} were lessened in rabbits, while in vagotomized rabbits, the changes of MAP were aggravated, and the duration of MAP was shortened. Phentolamine counteracted the changes of MAP induced by Pic.

These results suggested that the initial decrease of VFT caused by Pic derived mainly from the direct action and myocardial ischemia of sympathetic activation, while its subsequent elevation was caused by vagal activation and hyperkalemia.

KEY WORDS picrotoxin; hypothalamus; microinjections, ventricular fibrillation, action potentials; potassium; phentolamine

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Although the arrhythmogenic role of higher CNS activity has been established[1], the neurotransmitters involved therein remain speculative. GABA is now thought to modulate autonomic outflow to cardiovascular system; and enhancement or blockade of central GABAergic neurotransmission with central administration of GABA or picrotoxin (Pic) could inhibit or evoke ventricular arrhythmia, respectively[2,3]. This work will focus on defining the effects of blocking the hypothalamic GABAergic inhibition with Pic on the susceptibility to ventricular fibrillation, the basis of

sudden cardiac death. A technique[4] for registering monophasic action potentials of heart (MAP) *in situ* has allowed direct observation of the central modulation of cardiac electric activity.

MATERIALS AND METHODS

Sixty-nine rabbits of either sex, weighing 2.4 ± *s* 0.2 kg, were anesthetized with iv a mixture of urethane (400 mg · kg^{-1}) and α-chloralose (17.5 mg · kg^{-1}). The rabbits were immobilized with gallamine triethiodide under artificial respiration. With rabbit mounted in a stereotaxic instrument, the guide cannula was implanted just above the left posterior hypothalamus (PH) at AP −1.8, L 0.8, H −3.7 referring to the atlas of Sawyer. After the guide cannula was cemented to skull, the rabbit was supinated for determination of ventricular fibrillation threshold (VFT), recording MAP, and sampling blood (1 ml) from the femoral vein. Serum potassium was determined by electrolyte analyzer (E-4A, Beckman). In some rabbits, the spinal cords were transected at about C2 or bilateral vagotomy was performed, then the basic values of VFT were measured before microinjection.

Determination of VFT The chest and pericardium of rabbit were opened, the epicardium at apex and base of left ventricle were clipped with a pair of small electrodes 1 cm apart. ECG and femoral arterial pressure were recorded on a polygraph. A series of rectangular pulses (50 Hz, and 0.5 ms pulse duration) was delivered to the left ventricle with a DCQ-2 stimulator, each stimulation lasted 5-10 s. Ventricular fibrillation was induced by increasing the voltage of pulses[5].

MAP recording A silver electrode was introduced into mediastinum through a stab wound in the left 4th intercostal space. The spherical tip was rendered to be in contact with the pulsation of left ventricle by using a springy buffer apparatus. MAP and its differentiation, arterial pressure, and ECG were recorded synchronously on a polygraph. After all parameters were stabilized for about 10 min, microinjection was carried out.

Picrotoxin (Pic, Fluka AG Chemical Co) was prepared for microinjection[3] with a volume of 1 μl. Injection sites were verified by brain sections. Statistical test employed was paired *t* test.

RESULTS

Changes of VFT and serum potassium Biphasic responses of electrically induced VFT were observed. VFT was decreased significantly at 10-15 min, then increased remarkably 40-45 min after microinjection of Pic into PH. Pretreatment with cervical spinal cord transection almost abolished the descending phase, whereas pretreatment with vagotomy depressed the ascending phase significantly (Tab 1). Serum potassium was increased progressively after similar microinjection of Pic into PH and reached 5.0 mmol · L^{-1} after 30 min (Fig 1).

Tab 1. Effects of microinjection of picrotoxin (Pic) into posterior hypothalamus on ventricular fibrillation threshold (VFT) in rabbits. $n = 6-7$, $\bar{x} \pm s$. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* **0 min (before injection). Vag: Vagotomy. SCT: Spinal cord transection.**

	Dose / μg	Ventricular fibrillation threshold / V 0	10-15 min	40-45 min
Vehicle	0	8.9 ± 1.6	9.1 ± 1.4*	9.4 ± 1.8*
Pic	2	8.6 ± 1.7	6.7 ± 1.3***	10.3 ± 1.9**
Pic	3	9.8 ± 1.5	7.7 ± 1.9***	13.2 ± 1.9**
Vag+Pic	3	8.4 ± 2.2	5.6 ± 2.6***	7.3 ± 2.5**
SCT+Pic	3	10.1 ± 2.9	9.9 ± 2.6*	11.8 ± 2.7**

Changes in MAP In neurally intact rabbits, the amplitude of MAP (MAPA) and maximal velocity of the 0 phase (V_{max}) were decreased after Pic. The action potential duration at 90% repolarization ($MAPD_{90}$) did

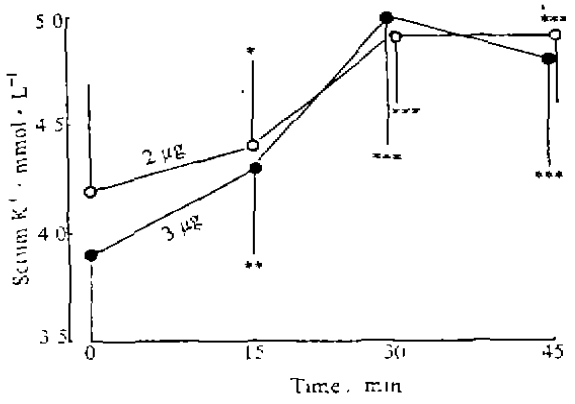

Fig 1. Changes of serum potassium after microinjection of picrotoxin into posterior hypothalamus in rabbits. $n=6$. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **0 min (before injection).**

not show noticeable change. Similar microinjection of vehicle was ineffective. The changes of MAP was counteracted partially by iv phentolamine in small to modest dosage (1, 2 mg · kg^{-1}), but aggravated by 4 mg · kg^{-1}. Atropine (10, 20 mg · kg^{-1}, iv) did not counteract Pic-induced changes of MAP. In vagotomized rabbits, microinjection of Pic (3 μg) induced more significant decreases of MAPA and V_{max}. In addition, $MAPD_{90}$ was shortened. Phentolamine (iv) showed a similar counter action as in the above case (Tab 2).

DISCUSSION

This study showed that microinjection of Pic into PH caused biphasic changes of VFT in neurally intact rabbit. Without vagal protection. Pic could cause ventricular electric instability remarkably. Our results were consistent with the existence of GABAergic inhibition of sympathoexcitatory system in hypothalamus[6]. Electric stimulation of PH could also decrease VFT in the dog, even the heart rate acceleration and pressor response were prevented[7].

A close-chest MAP recording with contact electrode, compared with suction electrode, allowed a longer and more stable recording period, and animals were under a near "physiological" condition. The correspondence between MAP and transmembrane action potentials (TAP) has been confirmed by simultaneous recording[8]. Pic-induced reductions of MAPA, V_{max}, and MAPD were similar to the changes of MAP and TAP during myocardial ischemia[9] and electric stimulation of dorsomedial hypothalamus[10]. The changes of MAP were primarily resulted from α-adrenoceptor mediated coronary spasm, instead of direct activation of α-adrenoceptor in myocardium, since they could be counteracted by phentolamine in small dosage, whereas aggravated in a larger dosage. The reduction of MAPA, V_{max}, and MAPD, in combination with an increase in sympathetic activity to the heart, might result in myocardial electric instability.

The vagal activity of the heart could be elevated by the reflex of pressor response and diffusion of Pic to adjacent nuclei. Blockade of GABAergic neurotransmission in PH also

Tab 2. Effects of microinjection of picrotoxin 3 μg into posterior hypothalamus on monophasic action potentials (MAP) in rabbits. $n=6$. $\bar{x} \pm s$. $^{*}P>0.05$. $^{***}P<0.01$ *vs* **0 min (before injection).**

	Neurally intact rabbits		Vagotomized rabbits	
	0	15 min	0	15 min
MAPA / mV	24.5 ± 2.2	19.0 ± 2.5***	22.7 ± 3.3	14.6 ± 2.6***
V_{max} / V · s^{-1}	1.87 ± 0.29	1.37 ± 0.31***	1.67 ± 0.32	1.03 ± 0.17***
$MAPD_{90}$ / ms	127 ± 10	124 ± 9*	120 ± 9	110 ± 10***
HR / bpm	237 ± 21	230 ± 23*	276 ± 23	293 ± 22***

elicited defense reaction[11] which might increase serum potassium. Vagal activation and hyperkalemia protected the heart against VF primarily by the attendant decrease in heart rate[12] and by inhibiting the automaticity in ectopic pacemaker. Since blood pressure and heart rate were not controlled in our experiments, it was difficult to determine whether the indirect influence of blood pressure and heart rate on VFT contributed to our results.

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下丘脑后区微量注射印防己毒素对心室电稳定性的影响

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提要 兔下丘脑后区注入印防己毒素(Pic，2 及 3 μg)使心室颤动阈先降后升。预先横断颈髓或切断迷走神经分别取消下降相和上升相。注射等量 Pic 使血钾逐渐升高；心肌单相动作电位幅度和 0 相最大上升速率降低。此可被 iv 酚妥拉明部分逆转。提示下丘脑注射 Pic 早期，交感激动及心肌缺血使心室电稳定性下降；随后副交感激动和高血钾则产生保护作用。

关键词 印防己毒素；下丘脑；微量注射；心室颤动；动作电位；钾；酚妥拉明