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利血平化大、小鼠额叶、纹状体和海马的乙酰胆碱及毒蕈碱受体

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提要 ip 利血平 3 mg · kg⁻¹，24 h 后大、小鼠皮层 ACh 分别增加 155%和 124%，M 受体 B_{max} 数增加，亲和力降低。纹状体 ACh 减少，M 受体 B_{max} 数下降，亲和力不变。在海马，利血平化小鼠的 ACh，M 受体 B_{max} 数及其 K_d 值均减少。ip 利血平后 12 h，小鼠纹状体 ACh 升高 50%，并加强了 Scop 对该部位 ACh 含量降低的作用。

关键词 利血平；东莨菪碱；二苯羟乙酸奎宁酯；乙酰胆碱；毒蕈碱受体；额叶；纹状体；海马

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Protective effect of cycloprotobuxine-A against cardiac arrhythmias induced by ouabain

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ABSTRACT Cycloprotobuxine-A (CPB-A) 1-4 mg · kg⁻¹ iv increased the dose of ouabain required to induce ventricular arrhythmias in guinea pigs. At the equitoxic doses (1/50 LD_{50}), CPB-A was more potent than cyclovirobuxine-D and amiodarone. Pretreatment with reserpine (5 mg · kg⁻¹ ip), vagotomy or pithing spinal cord did not prevent the action of CPB-A, which indicate that the protective effect of CPB-A may be due to its direct action on myocardium without the involvement of nervous system. In isolated guinea pig ventricular muscles, CPB-A 3 μmol · L⁻¹ consistently decreased the amplitude of oscillatory afterpotentials (OAP) and blocked triggered activity elicited by ouabain. At 30 μmol · L⁻¹, CPB-A abolished the appearance of OAP. It seems that one of the mechanisms for the anti-arrhythmic action of CPB-A was a decrease in the amplitude of OAP.

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KEY WORDS arrhythmia; electrophysiology; myocardium; ouabain; cycloprotobuxine-A; amiodarone

Both cycloprotobuxine–A (CPB–A) and cyclovirobuxine–D (CVB–D) are alkaloids extracted from *Buxsus microphylla*[1]. CVB–D exerts anti–arrhythmic effects in both animals and human[2,3]. However, very little has been known on the pharmacology of CPB–A. Recently we have shown CPB–A produces the therapeutic and prophylactic action on experimental arrhythmias. Its therapeutic index (LD_{50} / ED_{50}) is much greater than that of amiodarone (Ami) and CVB–D. The most siginificant effect of CPB–A on the electrophysiology of isolated myocardium is the lengthening of action potential duration and effective refractory period, and the reduction in maximal up– stroke velocity of action potential[4].

The present study was undertaken to observe effect of CPB–A on cardiac arrhythmias and OAP induced by ouabain and to examine whether vagotomy, reserpine or spinal cord pithing prevents the effect of CPB–A in order to provide a further understanding of the mechanisms of its anti–arrhythmic effect.

MATERIALS AND METHODS

Effects on cardiac arrhythmias induced by ouabain in guinea pigs Seventy–two guinea pigs of either sex, weighing 387 ± s 40 g, were anesthetized with urethane 1.8 g · kg^{-1} ip. Since reserpine potentiates the depressing action of anesthetics on the central nervous system[5], the dose of urethane was decreased to 1.2 g · kg^{-1} for all guinea pigs given with reserpine. Lead II electrocardiogram was continuously monitored and recorded every minute to determine heart rate and detect ventricular arrhythmia and cardiac arrest (CA).

Following a bolus of 50 $\mu g \cdot kg^{-1}$, ouabain dissolved in normal saline (NS) was infused at 5 $\mu g \cdot min^{-1}$ by an infusion pump via a steel needle into a cervical vein. The times of the first appearances of ventricular ectopia (VE), ventricular tachycardia (VT), ventricular fibrillation (VF), and CA were recoreded. The amounts of ouabain required to elicit the arrhythmias and CA were calculated.

Guinea pigs were randomly and equally assigned to 12 groups. Two groups were injected ip with reserpine 5 mg · kg^{-1} 24 h prior to ouabain infusion. Another 2 groups went through bilateral cervical vagotomy. Still another 2 groups were pithed and ventilated with room air at a rate of 30 strokes · min^{-1} and a stroke volume of 10 ml · kg^{-1}. The afore–mentioned 6 groups and the other 6 groups without the above treatments were given the drugs tested or the equivalent volume of NS 1.5 ml · kg^{-1} 5 min before ouabain infusion.

Effects on OAP induced by ouabain in isolated guinea pig ventricular myocardium The experimental procedures were the same as mentioned in the previous papers[4,6]. Papillary muscles of right ventricles were obtained from guinea pigs weighing 375 ± 42 g and pinned in a tissue bath that was superfused with Tyrode solution aerated with 95% O_2 + 5% CO_2. The muscles were stimulated with pulses of 3 ms duration, 1.5 times the threshold voltage at I Hz frequency. After an equilibration period of 1 h, OAP was induced by exposing the muscles to ouabain 0.9 $\mu mol \cdot L^{-1}$. The stimulus was switched to 5 Hz frequency and interrupted periodically for 30 s every 3 min to assess the emergence of OAP and triggered activity. Once stable OAP was obtained, effect of CPB–A was observed.

CPB–A and CVB–D were kindly supplied by Mr TANG You–Yuan (Central Laboratory of the 9542 Factory, the General Logistic Department of PLA, China). Ami, reserpine, and ouabain were purchased from Labaz Laboratory (France), Guangzhou Qiaoguang Pharmaceutical Factory (China) and Chemical Sigma Company (Germany).

All results were presented as $\bar{x} \pm s$. Statistically significant differences were

calculated by t test or rank sum test.

RESULTS

Effects on cardiac arrhythmias induced by ouabain in guinea pigs

1 Dose to cardiac arrhythmias induced by ouabain CPB–A 1–4 mg · kg^{-1} iv increased the dose of ouabain required to induce VE, VT, VF, and CA in a dose–dependent manner. It has been demonstrated that ip LD_{50} of CPB–A, CVB–D, and Ami in mice are 98, 55, and 280 mg · kg^{-1}, respectively[4]. At equitoxic dose (1/50 LD_{50}), CPB–A raised the dose of ouabain necessary to evoke VE and VT by 43% and 40%, respectively, yet CVB–D did that by 33% and 23% only. The action of Ami was less potent than that of CPB–A (Tab 1).

Bilateral vagotomy decreased the dose of ouabain needed to elicit cardiac arrhythmias and CA, while reserpine and spinal cord pithing increased the dose of ouabain needed. These results are in accordance with the reported papers[7–9]. Vagotomy, reserpine or spinal cord pithing did not prevent CPB–A from increasing the dose of ouabain required to induce cardiac arrhythmias and CA (Fig 1).

2 Heart rate and blood pressure Compared with NS group, CPB–A exerted no significant effects in heart rate and BP just 1 min before ouabain infusion and the onset of VE and VT in all the groups (Tab 2).

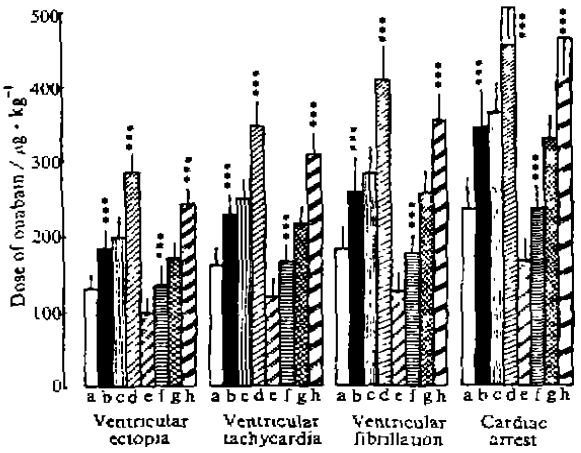

Fig 1. Effect of vagotomy (V), reserpine (R), and pithing (P) on the protective action cycloprotobuxine–A (CPB–A) 2 mg · kg^{-1} iv against ouabain–induced ventricular arrhythmias and cardiac arrest in guinea pigs. $n=6$. a): NS; b): CPB–A; c): R+NS; d): R+CPB–A; e): V+NS; f): V+CPB–A; g): P+NS; h): P+CPB–A. $\bar{x}\pm s$. $^{***}P<0.01$ vs control.

Effects on OAP induced by ouabain in isolated guinea pig ventricular myocardium OAP developed in all the muscles superfused with Tyrode solution containing ouabain 0.9 μmol · L^{-1}. It could reach threshold and thereby lead to triggered activity. Seven preparations were then superfused with a drug–free solution, after OAP was allowed to last at least 30 min. OAP completely dis–

Tab 1. Effects of cycloprotobuxine–A (CPB–A), cyclovirobuxine–D (CVB–D), and amiodarone (Ami) on the dose of ouabain required to induce ventricular arrhythmias and cardiac arrest in guinea pigs. $n=6$. $\bar{x}\pm s$. $^{**}P<0.05$, $^{***}P<0.01$ vs NS. $^{+}P>0.05$, $^{++}P<0.05$ vs CPB–A 2.0 mg · kg^{-1} (by rank sum test).

	Drug / mg · kg^{-1}	Dose of ouabain (μg · kg^{-1}) required to induce VE	VT	VF	CA
NS		130+27	161+29	182+32	233+43
CPB–A	1.0	162+22**	196+25**	238+37**	299+43***
	2.0	186+26***	231+31***	260+49***	341+48***
	4.0	209+24***	247+33***	286+42***	378+56***
CVB–D	1.1	173+29^{**++}	198+28^{**++}	240+33^{**++}	314+54^{**++}
Ami	5.6	176+31^{**++}	211+34^{**++}	251+49^{**+}	329+56^{***+}

Tab 2. Effect of cycloprotobuxine-A (CPB-A), cyclovirobuxine-D (CVB-D), and amiodarone (Ami) on control and ouabain-induced changes in the heart rate (bpm) and blood pressure (kPa) in guinea pigs. $n=6$. $\bar{x} \pm s$. $^{*}P>0.05$ *vs* NS group unpretreated. $^{-}P>0.05$ *vs* NS group pretreated.

	Dose / mg · kg^{-1}	Heart rate 1 min before			Blood pressure 1 min before		
		Ouabain	VE	VT	Ouabain	VE	VT
NS		259 ± 43	226 ± 38	240 ± 39	14.0 ± 2.5	18.0 ± 2.4	16.1 ± 2.4
CPB-A	1.0	245 ± 32*	211 ± 27*	224 ± 37*	14.1 ± 2.7*	18.9 ± 2.9*	17.9 ± 3.2*
	2.0	228 ± 33*	191 ± 33*	212 ± 30*	14.7 ± 3.7*	17.5 ± 2.4*	15.9 ± 3.3*
	4.0	212 ± 41*	186 ± 44*	201 ± 42*	14.2 ± 2.3*	17.1 ± 2.4*	15.2 ± 2.7*
CVB-D	1.1	242 ± 46*	208 ± 47*	225 ± 44*	14.5 ± 2.4*	15.5 ± 2.9*	13.6 ± 2.3*
Ami	5.6	227 ± 55*	204 ± 54*	215 ± 56*	14.4 ± 2.4*	18.8 ± 3.3*	17.1 ± 2.9*
V+NS		282 ± 42	256 ± 41	268 ± 45	14.0 ± 3.1	20.4 ± 4.5	21.7 ± 4.4
V+CPB-A	2.0	246 ± 36^{+}	222 ± 37^{+}	233 ± 34^{-}	16.3 ± 3.5^{+}	19.9 ± 3.1^{-}	21.1 ± 3.7^{+}
R+NS		142 ± 34	124 ± 35^{+}	132 ± 35	6.3 ± 1.9	18.8 ± 2.9	15.6 ± 2.3
R+CPB-A	2.0	125 ± 35^{-}	106 ± 31^{+}	116 ± 36^{-}	7.3 ± 2.0^{-}	16.7 ± 2.4^{+}	17.5 ± 2.7^{+}
P+NS		236 ± 51	215 ± 55	225 ± 56	6.7 ± 2.5	19.2 ± 2.1	17.5 ± 2.7
P+CPB-A	2.0	199 ± 38^{+}	180 ± 32^{+}	189 ± 35^{+}	7.1 ± 1.7^{+}	17.4 ± 2.8^{+}	16.1 ± 2.9^{-}

V: vagotomy; R: reserpine 5 mg · kg^{-1} ip 24 h before ouabain iv; P: pithing.

appeared for about 1 h of washout period. Another 14 preparations were superfused with a solution containing CPB-A (in the presence of ouabain) after stable OAP was obtained. CPB-A 3 μmol · L^{-1} consistently decreased the amplitude of OAP and blocked the triggered activity. At 30 μmol · L^{-1}, CPB-A fully abolished the development of OAP (Fig 2).

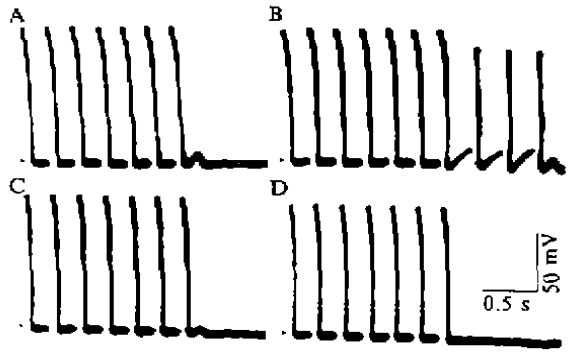

Fig 2. Effect of cycloprotobuxine-A (CPB-A) on OAP and triggered activity elicited by ouabain 0.9 μmol · L^{-1} in isolated guinea pig ventricular muscle. A: OAP; B: Triggered activity; C: Decrease of OAP amplitude caused by CPB-A 3 μmol · L^{-1}; D: Disappearance of OAP casued by CPB-A 30 μmol · L^{-1}.

DISCUSSION

The present study showed that CPB-A 1-4 mg · kg^{-1} iv produced the protective effect against cardiac arrhythmias induced by ouabain in guinea pigs, which appeared to be more potent than that of CVB-D and Ami.

Similar to the reported papers[7-9], we have shown that cardiac arrhythmias elicited by ouabain were affected by the activity of nervous system. Bilateral vagotomy promoted the development of the cardiac arrhythmias. On the contrary, reserpine or spinal cord pithing delayed the appearnace of the cardiac arrhythmias. However, the anti-arrhythmic action of CPB-A was not prevented by vagotomy, reserpine or spinal cord pithing. It seems probable that CPB-A produces the anti-arrhythmic effect by acting directly on the heart without the involvement of nervous system.

Within anti-arrhythmic doses, CPB-A caused no significant changes in heart rate and blood pressure just 1 min before ouabain infusion and the onset of VE and VT compared with the NS group. This appeared to indicate

that anti-arrhythmic action of CPB-A might not be associated with heart rate and blood pressure.

Ouabain has a direct action on cardiac muscles that results in electrophysiologic changes consistent with the appearance of cardiac arrhythmias. There is increasing evidence that OAP is an important manifestation of electrophysiologic changes evoked by ouabain, which can reach threshold voltage and lead to triggered activity[10]. CPB-A depressed and even abolished OAP, which might play a role in the anti-arrhythmic action of CPB-A.

It has been shown that some agents capable of blocking the fast entry of sodium ions into cardiac myocytes, such as TTX, procainamide, quinidine and lidicaine, depress and abolish the OAP[11-13]. At the concentration used in this study, CPB-A reduces the maximal upstroke velocity of action potential in cardiac myocytes, which implies that the drug can block the entry of sodium ions into myocytes. Thus, it is suggested that the depression and abolition of OAP for CPB-A might be dependent in part on a decrease in the fast influx of sodium ions.

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环原黄杨星 A 对抗哇巴因诱发心律失常的作用

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提要 环原黄杨星 A (CPB-A) 1-4 mg · kg^{-1} iv 对抗哇巴因(Oua)诱发豚鼠心律失常. 其作用比 Ami 和 CVB-D 强、且不受利血平、切断迷走神经或毁脊髓的影响. 提示 CPB-A 的抗心律失常作用可能与神经系统无关. CPB-A 3 μmol · L^{-1} 降低 Oua 所致豚鼠离体心肌振荡后电位(OAP)幅度、阻止触发活动产生. 30 μmol · L^{-1} 可消除 OAP. 这可能是 CPB-A 抗心律失常作用的机制之一.

关键词 心律失常; 电生理学; 心肌; 哇巴因; 环原黄杨星 A; 胺碘酮