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Anti-arrhythmic action of cycloprotobuxine-A

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ABSTRACT Cycloprotobuxine-A (CPB-A) 1-4 mg/kg (1/100-1/25 LD_{50}) produced therapeutic and prophylactic effects which were found to be dose-dependent on experimental arrhythmias induced by $BaCl_2$, aconitine and chloroform. Given at equitoxic doses, the anti-arrhythmic action of CPB-A was as potent as cyclovirobuxine-D (CVB-D) and amiodarone (Amio). However, its therapeutic index (LD_{50}/ED_{50}) was 1.8 times that of CVB-D and 1.2 times that of Amio. The most pronounced effects of CPB-A (0.3-30 μmol/L) on the electrophysiology of ventricular muscle of guinea pig were the lengthening of APD_{50}, APD_{90} and ERP. This may contribute to its anti-arrhythmic action and suggests that CPB-A most likely belongs to class III anti-arrhythmic drugs (prolongation of APD). Perfused with the same concentration (3 μmol/L), CPB-A brought about more significant increases in APD_{50}, APD_{90} and ERP than CVB-D and Amio did.

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KEY WORDS cycloprotobuxine-A; cyclovirobuxine-D; amiodarone; anti-arrhythmia agents; action potentials

Both cycloprotobuxine-A (CPB-A) and cyclovirobuxine-D (CVB-D) are alkaloids extracted from *Buxus microphylla* Sieb. *et* Zucc. var. *sinica* Rehd. *et* Wils[1]. It has been shown that CVB-D has anti-arrhythmic effects in both animals and human[2,3]. Electrophysiologic studies indicate that the most noteworthy effect of CVB-D on cardiac muscle is the lengthening of action potential durations and effective refractory period[4].

Very little has been undertaken concerning the pharmacology of CPB-A. In this study we carried out an investigation of the effects of CPB-A on experimental arrhythmias and transmembrane action potential of myocardium, compared with CVB-D and amiodarone (Amio).

METHODS

Acute toxicity One hundred and fifty ♀ and ♂ albino mice weighing 21 ± SD 2 g were randomly and equally divided into 15 groups. CPB-A, CVB-D and Amio were administered ip. The ip LD_{50} and LD_1 values were then calculated by log-dose probit-response analysis.

Therapeutic action on $BaCl_2$-induced arrhythmias Sprague-Dawley rats of either sex weighing 181 ± 17 g were anesthetized with chloral hydrate (300 mg/kg, ip). The electrocardiogram (ECG) (Standard lead II) was recorded. Two minutes after the appearance of arrhythmias induced by iv $BaCl_2$ 4 mg/kg, the rats were iv with either a drug or an equivalent volume of normal saline (NS, 1 ml/kg). The number of rats which recovered from established arrhythmias was determined and the time of restoration of normal sinus rhythm (NSR) and the time of maintenance were observed and recorded.

Protection against development of ventricular fibrillation caused by chloroform Male albino mice weighing 20 ± 2 g were immobilized on a specially designed iron wire rack. Lead II ECG was recorded through subcutaneously placed needle electrodes. The mice inhaled chloroform 5 min after the drugs or an equivalent volume of NS (10 ml/kg) was given iv. The incidences of ventricular fibrillation (VF) were observed and recorded. Anti-arrhythmic ED_{50} and ED_{99} values were calculated by linear regression.

Antagonism of aconitine-evoked ventricular arrhythmias Fifty mice weighing 31 ± 3 g were assigned to 5 groups. The animals were anesthetized with ip sodium pentobarbital 60 mg/kg. The ECG (Standard lead II) was recorded intermittently. Aconitine (10 μg/kg) was infused by a pump at a rate of 1 μg/min via a steel needle inserted into a tail vein. Drugs or NS were iv 5 min before aconitine infusion. The time of appearance of the first ventricular ectopic (VE), VF and cardiac arrest (CA) were recorded and the amount of aconitine (μg/kg) required to elicit the arrhythmias was calculated. All drugs and NS were given at a volume of 5 ml/kg.

Electrophysiologic effects on ventricular muscle Papillary muscles obtained from the right ventricles of guinea pigs of either sex weighing 307 ± 71 g and pinned in a tissue bath that was constantly perfused with Tyrode's solution (4 ml/min) aerated with 95% O_2 and 5% CO_2 (35 ± 0.5℃, pH 7.35 ± 0.3). The composition of the Tyrode's solution in mmol/L was: NaCl 137.0, $NaHCO_3$ 12.0, NaH_2PO_4 1.8, $MgCl_2$ 0.5, KCl 4.0, $CaCl_2$ 2.7, glucose 5.5. The muscles were stimulated with pulses of 3 ms duration and 1.2 times threshold voltage at 1.25 Hz frequency through a pair of tefloncoated silver wire electrodes. Transmembrane action potentials were recorded through KCl 3 mol/L-filled glass microelectrodes (resistance 10-30 MΩ). The maximal upstroke velocity of the action potential was obtained from a differentiator. The output signal, together with that from the microelectrode amplifier, was displayed on a dual beam oscilloscope and photographed with an camera. A computer program[5] was used to analyse the data. Control records were taken after an initial equilibration period of 1 h. A single impalement was maintained throughout the control and experimental periods.

RESULTS

Acute toxicity Tab 1 lists the LD_{50} and LD_1 values calculated from the log-dose probit-response lines to CPB-A, CVB-D and Amio. The order of the LD_{50} values is as follows: CVB-D < CPB-A < Amio.

Therapeutic action on $BaCl_2$-induced arrhythmias As shown in Tab 2, CPB-A 1 and 2 mg/kg reestablished normal sinus

Tab 1. LD_{50}, LD_1 and anti-arrhythmic ED_{50}, ED_{99} calculated from log-dose probit-response lines to cycloprotobuxine-A (CPB-A), cyclovirobuxine-D (CVB-D) and amiodarone (Amio) in mice.

	n	CPB-A	CVB-D	Amio
LD_{50}(mg/kg)	50	98.4	55.0	279.7
LD_1(mg/kg)	50	48.7	18.4	82.3
ED_{50}(mg/kg)	30	1.0	1.0	3.5
ED_{99}(mg/kg)	30	12.9	21.3	28.7
LD_{50}/ED_{50}		98.4	55.0	79.9
LD_1/ED_{99}		3.8	0.9	2.9

rhythm (NSR) in 7/10 and 9/10 rats, respectively. At a dose of LD_{50}, the therapeutic actions of CVB-D and Amio were less potent than that of CPB-A, although there was no statistical difference. In the control group, no rat had reestablished NSR within 30 min.

Tab 2. Effects of CPB-A, CVB-D and Amio on recovery of normal sinus rhythm from arrhythmias induced by $BaCl_2$ in rats. $n=10$, $\bar{x}\pm SD$. $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Drug	Dose (mg/kg)	Number of recovery	T_R (min)	T_M (min)
NS		0		
CPB-A	1.0	7***	2±3	22±6
	2.0	9***	1±2	25±4
CVB-D	0.55	4*	10±7	20±7
Amio	2.8	5**	10±10	15±10

T_R: time of recovery. T_M: time of maintenance.

Protection against development of ventricular fibrillation caused by chloroform Pretreatment of the mice with 1/100, 1/50 or 1/25 LD_{50} doses of CPB-A, CVB-D or Amio reduced the incidence of VF. All of the effects were dose-dependent.

Anti-arrhythmic ED_{50} and ED_{99} values were calculated from the log-dose probit-response lines to CPB-A, CVB-D or Amio. The therapeutic index (LD_{50}/ED_{50}) and the ratio between LD_1 and ED_{99} are presented in Tab 1. It may be noted that the therapeutic index and LD_1/ED_{99} of CPB-A are 1.8 and 4.2 times as those of CVB-D, and 1.2 and 1.3 times as those of Amio, respectively.

Antagonism of aconitine-evoked ventricular arrhythmias The doses of aconitine (μg/kg) required to bring about ventricular arrhythmias and cardiac arrest for the control and drug-treated groups of animals were depicted in Fig 1. All drugs tested were effective at antagonizing elicited by aconitine.

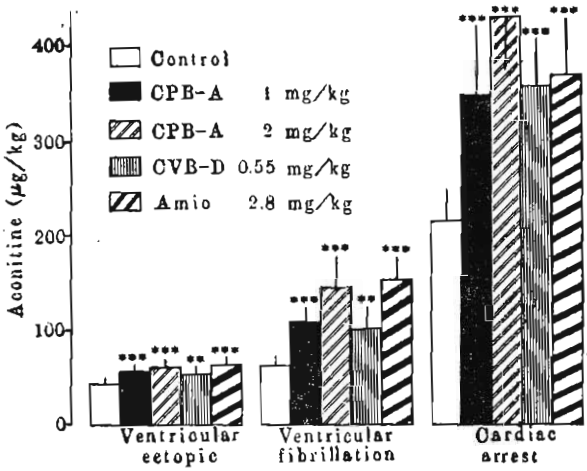

Fig 1. Effects of iv CPB-A, CVB-D and Amio on the doses of aconitine required to elicit ventricular arrhythmias and cardiac arrest in mice. $n=10$, $\bar{x}\pm SD$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Electrophysiologic effects on ventricular muscle The effects of CPB-A were observed for 1 h. They became apparent within 5 min and reached a steady state within 30 min. The features of the transmembrane action potentials after 30 min exposure to 0.3-30 μmol/L CPB-A were shown in Tab 3. CPB-A 0.3 μmol/L lengthened action potential durations at both 50% (APD_{50}) and 90% (APD_{90}) repolarization, however, no changes were observed in the maximal rate of the upstroke ($\dot{V}_{max}$), amplitude of the action potential (APA) or the resting potential (RP). At higher concentration, CPB-A produced more marked prolongation of APD_{50} and APD_{90}, and caused reductions in $\dot{V}_{max}$, APA and RP. CPB-A 0.3-30 μmol/L also gave rise to a progressive lengthening of the effective refractory period (ERP) ($n=6$, $P<0.01$).

Tab 3. Effects of cycloprotobuxine-A on transmembrane action potentials of guinea pig papillary muscles. $n=6$, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.01$ $^{***}P<0.01$ *vs* before.

Concn (μmol/L)		0.3	3	30
RP	Before	−86±3	−88±3	−88±2
(mV)	After	−85±5*	−86±2*	−83±4**
	Washout	−86±2*	−87±2*	−87±2*
APA	Before	122±3	125±3	123±4
(mV)	After	121±3*	120±4**	115±5***
	Washout	122±4*	122±3*	123±2*
APD_{50}	Before	119±7	110±18	102±11
(ms)	After	125±9***	126±28***	129±21***
	Washout	123±11*	111±21*	107±13*
APD_{90}	Before	177±13	175±16	178±15
(ms)	After	189±16***	210±18***	284±24***
	Washout	179±13*	175±10*	204±31*
V_{max}	Before	289±17	287±65	290±64
(V/s)	After	284±29*	265±71**	262±59**
	Washout	287±25*	287±66*	271±73*
ERP	Before	155±16	152±17	156±15
(ms)	After	167±20***	185±15***	216±9***

All of the effects described above were abolished after a 30–60 min washout period with drug-free Tyrode's solution.

The effects of CPB-A, CVB-D and Amio at 3 μmol/L were compared in Fig 2. CPB-A caused more obvious prolongation of APD_{90} and ERP and also produced a decrease in $\dot{V}_{max}$. Both CPB-A and CVB-D extended APD_{50}, while Amio did not.

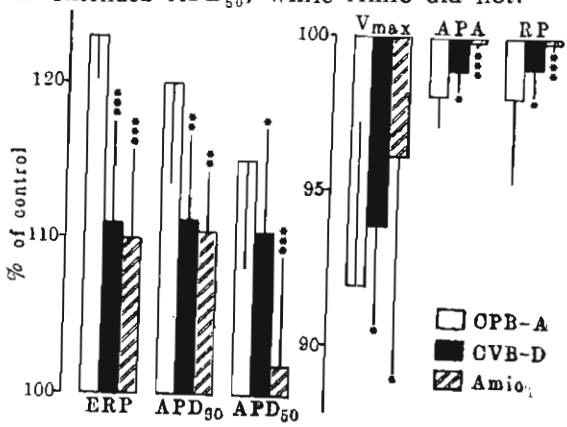

Fig 2. Influence of CPB-A, CVB-D and Amio at 3 μmol/L on transmembrane action potential of papillary muscles. $n=6$, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* CPB-A group.

DISCUSSION

CPB-A 1 and 2 mg/kg given to rats suffering from $BaCl_2$-induced arrhythmias brought about a restoration of normal sinus rhythm as well as increasing the doses of aconitine required to elicit ventricular ectopic, ventricular fibrillation and cardiac arrest. CPB-A 1–4 mg/kg reduced the incidences of ventricular fibrillation evoked by chloroform in a dose-dependent manner. It is suggested that CPB-A possesses a definite anti-arrhythmic action.

With equitoxic doses, the anti-arrhythmic effects of CPB-A were as potent as those of CVB-D and Amio. However, the therapeutic index and LD_1/ED_{99} values were 1.8 and 4.2 times as those of CVB-D and greater than those of Amio.

Similar to CVB-D and Amio, the prominent electrophysiologic effects of CPB-A on ventricular muscle were the prolongation of APD and ERP. This may play an important role in its effectiveness on arrhythmias and endow CPB-A with class III drug characteristics according to Vaughan Williams's classification[6]. Higher concentrations of CPB-A could reduce the $\dot{V}_{max}$ of the action potential, which may contribute to its anti-arrhythmic action.

When perfused at the same concentration (3 μmol/L), CPB-A produced greater lengthening of APD_{90} and ERP, and decreases in V_{max} and APA. Both CPB-A and CVB-D also caused prolongation of APD_{50}, while Amio did not. These results suggest that CPB-A perhaps possesses more potent effects on the myocardium than CVB-D dose.

The phase 2 of repolarization is dependent on the slow inward current (I_{si})[7,8]. An increase in I_{si} may enhance the contraction force of myocardium. Prolongation of APD_{50} with CPB-A may result from increase in I_{si}. In other studies, we observed that CPB-A produced a concentration-

dependent positive inotropic effect in isolated cardiac muscles (to be published). Thus, this action seems to be attributed to the augmentation of I_{si} to a certain extent. The combination of the anti-arrhythmic effects and its positive inotropic property may be therapeutically very useful for arrhythmias patients with heart failure.

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环原黄杨星 A 的抗心律失常作用

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提要 环原黄杨星 A (CPB-A)1-4 mg/kg (1/100-1/25 LD_{50})能防治 $BaCl_2$、乌头碱和氯仿所致的心律失常. 这种作用呈明显的量-效关系.在等毒性剂量下，CPB-A的抗心律失常作用 与 CVB-D 及 胺碘酮相近. 但 CPB-A 的治疗指数(LD_{50}/ED_{50})是 CVB-D 的 1.8 倍，胺碘酮的 1.2 倍. CPB-A 0.3-30 μmol/L 对心室肌电生理最显著的影响是延长 APD_{50}, APD_{90} 和 ERP，这可能是其抗心律失常作用的重要机理， 并提示它属于Ⅲ类抗心律失常药. 灌流相同浓度(3 μmol/L)时，CPB-A 延长 APD_{50}, APD_{90} 和 ERP 的作用比 CVB-D 和胺碘酮均强.

关键词 环原黄杨星 A； 环维黄杨星 D； 胺碘酮； 抗心律失常药物； 动作电位

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