中国药理学报 *Acta Pharmacologica Sinica* 1989 Nov; 10 (6) : 516-519

Positive inotropic effect of cycloprotobuxine-A on isolated guinea pig myocardium

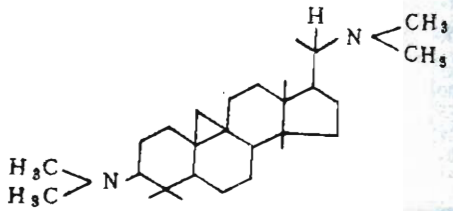
WANG Yong-Xiao, LIU Jin-Wen[1], TAN Yue-Hua, SHENG Bao-Heng
(Department of Pharmacology, Fourth Military Medical University, Xi-an 710033, China)

ABSTRACT Cycloprotobuxine-A (CPB-A) 0.1-100 μmol/L produced positive inotropic effects in left and right atria in a concentration-dependent manner. CPB-A 30 μmol/L enhanced post-rest contraction, augmented the response of left atrium to increase in stimulating frequency, and increased the developing tension evoked by paired pulse stimulation. By taking simultaneous recordings of action potentials and contractile force of papillary muscles, it was found that CPB-A 30 μmol/L increased the contractile force and prolonged the action potential duration at 50% of depolarization. It is concluded that the positive inotropic effect of CPB-A on myocardium may be associated with an increase in transsarcolemmal influx of calcium as well as an augmentation of the amount of calcium released from intracellular stores.

KEY WORDS cycloprotobuxine-A; myocardial contraction; action potentials; heart atrium; papillary muscles

Cycloprotobuxine-A (CPB-A) is an alkaloid extracted from *Buxsus microphylla*[1]. Very little has been studied on the pharmacology of this compound. We have demonstrated that CPB-A produced anti-arrhythmic action in animal models and that the prominent effect of CPB-A on the electrophysiology of myocardium was the prolongation of action potential duration and effective refractory period (to be published).

It has long been recognized that the patterns of stimulation, such as post-rest contraction, positive staircase effect and paired pulse stimulation, have profound effects on the force developed by cardiac muscle. These effects are thought to be attributed to an increase in the concentration of activator calcium ion by using intracellular or extracellular sources of calcium[2]. The present study is intended to observe the inotropic effect of CPB-A on isolated guinea pig myocardium and to examine whether the inotropic effect of CPB-A is associated with intracellular and/or extracellular sources of calcium by taking the patterns of stimulation described above as well as simultaneous recordings of contractile force and action potentials of myocardium.



Cycloprotobuxine-A

METHODS

Guinea pigs of either sex weighing 348±SD 51 g were stunned by a blow to the head, the papillary muscles of the right ventricle and/or the left and right atria were removed and placed in a tissue bath containing Tyrode's solution (mmol/L: NaCl 137.0, $MgCl_2$ 0.5, $NaHCO_3$ 12.0, NaH_2PO_4 1.8, KCl 4.0, $CaCl_2$ 2.7, glucose 5.5). The solution was continuously aerated with 95% O_2 and 5% CO_2 and warmed

Received 1988 Dec 13 Accepted 1989 May 4

[1] Now in *Department of Pharmacology, Yan-an Medical College, Yan-an 716000, China*

at 33℃ for left atria and papillary muscles and at 37℃ for right atria with pH 7.35±0.3. The preparation was connected to a force displacement transducer with a resting tension of 0.5 g for left and 0.3 g for right atria and papillary muscles. Right atria with intact sinus node produced spontaneous beat. Left atria and papillary muscle were stimulated by a stimulator (model SEN-7103, Nihon Kohden, Japan) through a pair of teflon-coated silver wire electrodes. The parameters of stimulation were 3 ms pulse duration and 1.5 Hz frequency and 1.2-1.5 times threshold voltage. The developed force signal was recorded on a recorder (model XWT-264, Da-Hua, China) after an equilibration period for about 1 h.

All data are expressed as mean±SD values. Statistical difference were determined using *t* test for paired or grouped data.

RESULTS

Positive inotropic effects The concentration of CPB-A in the bath was increased cumulatively at 5 min intervals by a factor of 1/2 log mol/L. The measured contractile force was recorded 4 min after medication. As shown in Fig 1, CPB-A 0.1-100 μmol/L exerted positive inotropic effects concentration-dependently in the left and right atria.

Post-rest contraction Left atria were stimulated at 0.5 Hz until steady-state contractile force attained, then stimulation was interrupted for 3, 5, 10 and 20 s respectively and resumed at 0.5 Hz subsequently. The first contraction occurred after the brief rest was potentiated markedly, which was described as the post-rest contraction. Fig 2 illustrates the effects of CPB-A 30 μmol/L on the post-rest contraction. In the presence of CPB-A, the post-rest contraction was augmented significantly.

Positive staircase effect When the stimulating frequency was switched from 0.5 to 2 Hz through 1.0 and 1.5 Hz each for 3 min intervals, the contractile force of left atrium was increased gradually. This phenomenon is referred to as the positive staircase effect. As can be seen in Fig 3, CPB-A 30 μmol/L enhanced the contractile force at each stimulating frequency during the positive staircase effect.

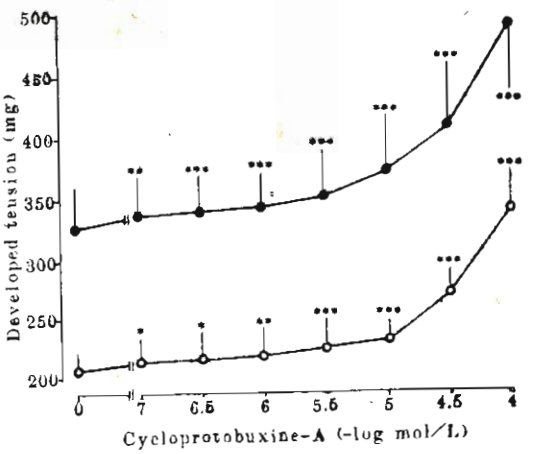


Fig 1. **Effects of cycloprotobuxine-A (CPB-A) on contraction of left atrium(●) and right atrium (○). $n=6$, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.**

Paired pulse stimulation After 60 min equilibration under 1.5 Hz stimulation, left atrial preparations were stimulated with

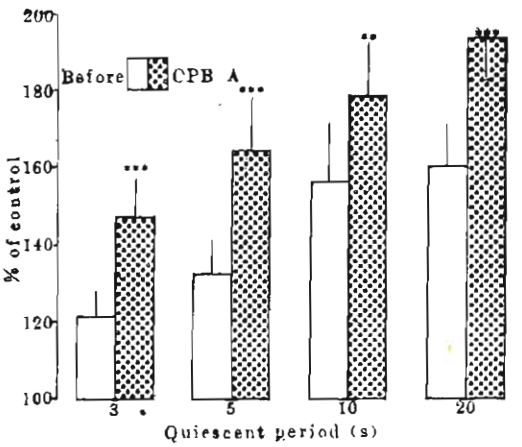


Fig 2. **Effect of CPB-A 30 μmol/L on post-rest contraction in left atrium. $n=6$, $\bar{x}\pm SD$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* values before medication.**

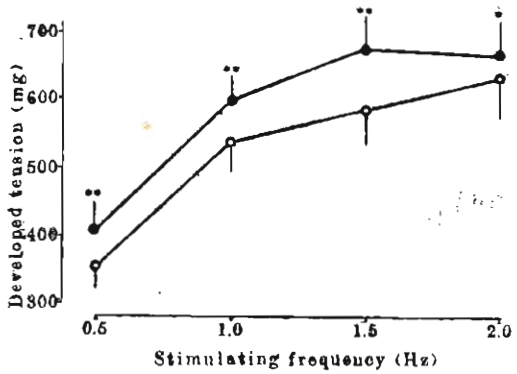


Fig 3. Influence of CPB-A 30 μmol/L (●) on positive staircase effect in left atrium. $n=6$, $\bar{x}\pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$ *vs* control (○).

paired pulse for a 3 min period. In this procedure, the first pulse with 1.5 Hz was followed 150 ms latter by the second of equal intensity. The preparations respond to the paired pulse stimulus with two action potentials and a single, augmented contraction. When left atrial muscles were exposed to 30 μmol/L CPB-A, the contraction was markedly increased from 670 ± 56 to 746 ± 52 mg ($n=6$, $P<0.01$).

Simultaneous recordings of action potentials and contractile force of myocardium Papillary muscles of the right ventricle from guinea pigs were placed in 1 ml chamber and superfused with Tyrode's solution at 5 ml/min. One end of the preparation was pinned with a fine stainless steel needle, the other end was connected to a force transducer. Signals of action potentials and contraction of myocardium were displayed on an oscilloscope and photographed. The apparatus and general experimental procedure have been thoroughly described in the preceding paper[3]. The effects of CPB-A were observed for 1 h. They became apparent within 5 min and reached to the peak at 30 min. The action potential duration at 50% of repolarization was lengthened from 123 ± 23 to 146 ± 27 ms and the developed tension was enhanced from 241 ± 51 to 314 ± 42 mg ($n=6$, $P<0.01$) after 30 min exposured to CPB-A 30 μmol/L. CPB-A also prolonged action potential duration at 90% of repolarization, and slightly reduced maximal rate of the upstroke, amplitude of the action potentials and the resting potentials (Fig 4).

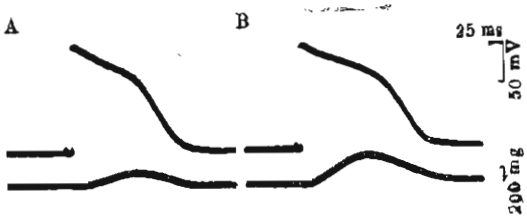


Fig 4. Effect of CPB-A 30 μmol/L on contractile force and action potential of papillary muscle of right ventricle from guinea pig. A) Control, B) 30 min after cycloprotobuxine-A.

DISCUSSION

In the left atrium and right atrium, CPB-A 0.1-100 μmol/L produced a positive inotropic action in a concentration-dependent manner. We have demonstrated that CPB-A displayed anti-arrhythmic effects in another study. The anti-arrhythmic effects of CPB-A with a positive inotropic property might be a very helpful therapy to some cardiac diseases.

The positive staircase effect is supposed to be due to an increase of calcium ion influx through the cell membrane of heart[4,5]. The plateau of the action potentials of myocardium is mainly dependent on slow inward current, which is carried by calcium ion[6]. Thus, the plateau is shortened by a decrease of the slow inward current, while it is prolonged by an increase of the slow inward current[7,8]. CPB-A enhanced the positive staircase effect and lengthened the plateau of the action potentials. These results suggest that the positive inotropic action of CPB-A might be related to an increase in transsarcolemmal influx of calcium ion.

The post-rest contraction and the paired pulse stimulation might be attributed to

an augmentation in the amount of calcium ion released from intracellular stores[9,10]. CPB-A potentiated both of them. Therefore, the increased contraction caused by CPB-A may also be associated with an enhancement in the concentration of activator calcium ion via its release from intracellular sources.

ACKNOWLEDGMENT TANG You-Yuan for his generous supply of cycloprotobuxine-A.

REFERENCES

1 Liang BW, Deng CA, Wang XB, *et al*. Isolation and structural elucidation of *Buxus* alkaloids I, II, III, IV. *Chin Pharm Bull* 1981; 16 : 195
2 Siegl PKS. Overview of cardiac inotropic mechanisms. *J Cardiovasc Pharmacol* 1986; 8 (Suppl 9): S 1
3 Wang YX, Tang YH. Effects of berberine on action potential of guinea pig papillary muscle and contractility in rat left ventricle. *J Northwestern Pharm* [Chin] 1987; 2 : 155
4 Teiger DG, Farah A. The effect of calcium and sodium ion on the frequency-force relationship in cardiac muscle. *J Pharmacol Exp Ther* 1968; 164 : 1
5 Henderson AH, Brutsaert DL, Parmley WW, Sonnenblick EH. Myocardial mechanics in papillary muscles of the rat and cat. *Am J Physiol* 1969; 217 : 1273
6 Beeler GW, Reuter H. Membrane calcium current in ventricular myocardial fibres. *J Physiol* (Lond) 1970; 207 : 19
7 McAllister RE, Noble D, Tsien RW. Reconstruction of the electrical activity of cardiac Purkinje fibres. *Ibid* 1975; 251 : 1
8 Reuter H. Properties of two inward membrane currents in the heart. *Annu Rev Physiol* 1979; 41 : 413
9 Haacke H, Lüllmann H, van Zwieten PA. Calcium metabolism in atrial tissue during frequency potentiation and paired stimulation. *Pflügers Arch* 1970; 314 : 113
10 Temma K, Akera T, Brody TM. Inotropic effects of digitoxin in isolated guinea-pig heart under conditions which alter contraction. *Eur J Pharmacol* 1981; 76 : 361

环原黄杨星 A 对豚鼠离体心肌的正性变力作用

汪永孝、刘锦文、谭月华、盛宝恒 （第四军医大学药理教研室，西安 710033，中国）

提要 环原黄杨星 A (CPB-A) 0.1-100 μmol/L 对左、右心房肌都是呈现浓度依赖性正性变力作用. CPB-A 30 μmol/L 增加左心房肌静息后收缩以及正性阶梯和成对刺激效应. 心肌动作电位和收缩同步记录显示, CPB-A 30 μmol/L 加强心肌收缩力, 延长动作电位复极 50% 的时程. 因此, CPB-A 的正性变力作用可能是由于促进心肌细胞外 Ca^{2+} 跨膜内流和增加细胞内 Ca^{2+} 释放所致.

关键词 环原黄杨星 A; 心肌收缩; 动作电位; 心房; 乳头状肌