

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

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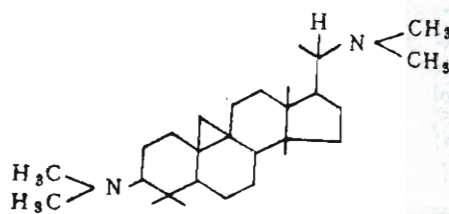
**ABSTRACT** Cycloprotobuxine-A (CPB-A) 0.1-100  $\mu\text{mol/L}$  produced positive inotropic effects in left and right atria in a concentration-dependent manner. CPB-A 30  $\mu\text{mol/L}$  enhanced post-rest contraction, augmented the response of left atrium to increase in stimulating frequency, 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  $\mu\text{mol/L}$  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 *Buxus microphylla*<sup>(1)</sup>. 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<sup>(2)</sup>. 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  $348 \pm \text{SD } 51 \text{ g}$  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,  $\text{MgCl}_2$  0.5,  $\text{NaHCO}_3$  12.0,  $\text{NaH}_2\text{PO}_4$  1.8, KCl 4.0,  $\text{CaCl}_2$  2.7, glucose 5.5). The solution was continuously aerated with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  and warmed

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at 33°C for left atria and papillary muscles and at 37°C for right atria with pH  $7.35 \pm 0.3$ . 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  $\pm$  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  $1/2 \log \text{ mol/L}$ . The measured contractile force was recorded 4 min after medication. As shown in Fig 1, CPB-A 0.1-100  $\mu\text{mol/L}$  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  $\mu\text{mol/L}$  on the post-rest contraction. In the presence of CPB-A, the post-rest contraction was augmented significantly.

**Positive staircase effect** When the stim-

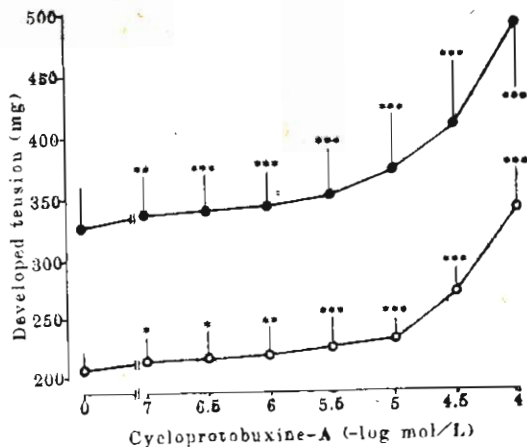


Fig 1. Effects of cycloprotobuxine-A (CPB-A) on contraction of left atrium (●) and right atrium (○).  $n=6$ ,  $\bar{x} \pm \text{SD}$ . \* $P > 0.05$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$  vs control.

ulating 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  $\mu\text{mol/L}$  enhanced the contractile force at each stimulating frequency during the positive staircase effect.

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

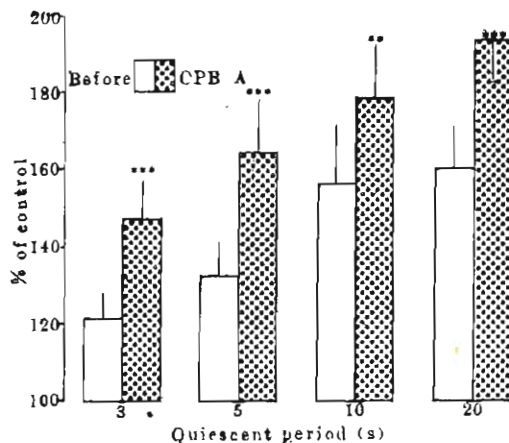


Fig 2. Effect of CPB-A 30  $\mu\text{mol/L}$  on post-rest contraction in left atrium.  $n=6$ ,  $\bar{x} \pm \text{SD}$ . \*\* $P < 0.05$ , \*\*\* $P < 0.01$  vs values before medication.

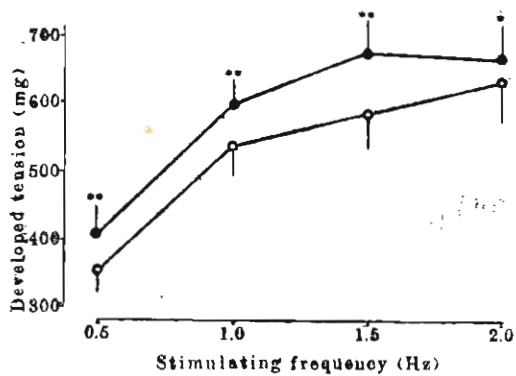


Fig 3. Influence of CPB-A 30  $\mu\text{mol/L}$  (●) on positive staircase effect in left atrium.  $n=6$ ,  $\bar{x} \pm \text{SD}$ . \* $P > 0.05$ , \*\* $P < 0.05$  vs control (○).

paired pulse for a 3 min period. In this procedure, the first pulse with 1.5 Hz was followed 150 ms later 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  $\mu\text{mol/L}$  CPB-A, the contraction was markedly increased from  $670 \pm 56$  to  $746 \pm 52$  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<sup>(3)</sup>. 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  $123 \pm 23$  to  $146 \pm 27$  ms and the developed tension was enhanced from  $241 \pm 51$  to  $314 \pm 42$  mg ( $n=6$ ,  $P < 0.01$ )

after 30 min exposed to CPB-A 30  $\mu\text{mol/L}$ . 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).

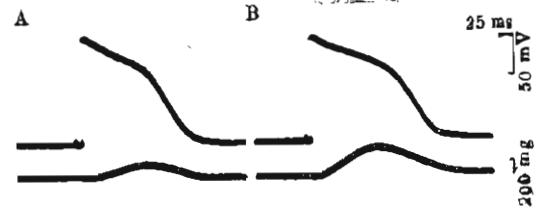


Fig 4. Effect of CPB-A 30  $\mu\text{mol/L}$  on contractile force and action potential of papillary muscle of right ventricle from guinea pig. A) Control, B) 30 min after cycloprotopobuxine-A.

## DISCUSSION

In the left atrium and right atrium, CPB-A 0.1–100  $\mu\text{mol/L}$  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<sup>(4,5)</sup>. The plateau of the action potentials of myocardium is mainly dependent on slow inward current, which is carried by calcium ion<sup>(6)</sup>. 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<sup>(7,8)</sup>. 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<sup>(9,10)</sup>. 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.

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## 环原黄杨星 A 对豚鼠离体心肌的正性变力作用

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**摘要** 环原黄杨星 A (CPB-A) 0.1-100  $\mu\text{mol/L}$  对左、右心房肌都是呈现浓度依赖性正性变力作用。CPB-A 30  $\mu\text{mol/L}$  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  $\mu\text{mol/L}$  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  $\text{Ca}^{2+}$  跨膜内流和增加细胞内  $\text{Ca}^{2+}$  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

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