arachidonic cascade.

It is interesting to note that lens protein injected intracamerally induced PG syntheses mainly in the anterior part of the eyes (iris and ciliary body) but not in the back of the eyes (retina). Further, leukotriene produced in this lens protein-induced inflammation model was too little to be detected with RIA. Therefore, leukocyte chemotaxis has to be used to represent the late phase of inflammation.

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Positive inotropic and toxic action of direct lytic factor on isolated working guinea pig hearts[1]

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ABSTRACT The positive inotropic and the toxic effects of direct lytic factor (DLF) on the isolated working guinea pig hearts were studied. As compared with baseline values, DLF 1–10 $\mu g \cdot ml^{-1}$ increased aortic flow up to 138% cardiac output 116%, left ventricular pressure volume work 136%, left ventricular pressure 114%, dP/dt_{max} 130%, V_{max} 128%, and mean aortic systolic pressure 114%, but coronary flow was decreased by 16% on an average ($n=6$). However, heart rate remained constant, myocardial oxygen consumption and efficiency were changed little. The cardiotonic effect of DLF was also observed by recording the isometric contractions of the isolated guinea pig papillary muscles and by determining the left ventricular pressure and dP/dt_{max} in anesthetized dogs. Neither spontaneously beating rate of right atrium nor the excitability of left atrium *in vitro* was affected by DLF. The results show that DLF is one of the cardiotonic agents without chronotropic effect and its coronary vasoconstriction effect plays an important part in heart failure.

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KEY WORDS cobra venoms; direct lytic

factors; myocardial contraction; heart function tests

Direct lytic factor (DLF, cobra cardiotoxin), a pharmacologically active polypeptide other than neurotoxins in the cobra venoms, is also called membrane active polypeptide[1] as its depolarization and disorganization effects on cell membranes involve almost all the tissues including skeletal and smooth muscles, erythrocytes, nerves, etc. However, its cardiac toxicity is believed to be the main lethal cause[2]. It has been reported that DLF augments the heart beats until the contracture appears in the isolated, perfused heart preparations[3] and lowers the cardiac output (CO) *in vivo*[4]. But these results did not exclude the influence of the vasoconstriction induced by DLF on cardiac performance. In order to lessen the influence of changed preload and afterload, we chose the isolated working guinea pig heart preparations to analyse its inotropic action.

MATERIALS AND METHODS

DLF Lyophilized venom from *Naja naja atra* was fractionated by CM sephadex C25 column chromatography[5] as shown in Fig 1. The shadowed fraction was rechromatographed by SP sephadex C50 colume. The main peak was identified as hemogeneous by both immunoelectrophoresis and polyacrylamide gel electrophoresis. The iv LD_{50} was 2.0 (95% confidence limits 1.9–2.4) mg · kg^{-1} in mice according to the method of weighted probit analysis.

Isolated working guinea pig hearts Hearts isolated from guinea pigs of either sex weighing 0.31 ± SD 0.06 kg were perfused according to Flynn's method[6]. The composition of modified Krebs–Henseleit (K–H) solution is as follows: in mmol · L^{-1}, KCl 5.7; $MgCl_2 \cdot 6H_2O$ 1.2; $CaCl_2 \cdot 2H_2O$ 2.5; NaCl 112; $NaHCO_3$ 26.2; Na_2EDTA 0.5; glucose 11; and Na pyruvate 2.0. pCO_2 and

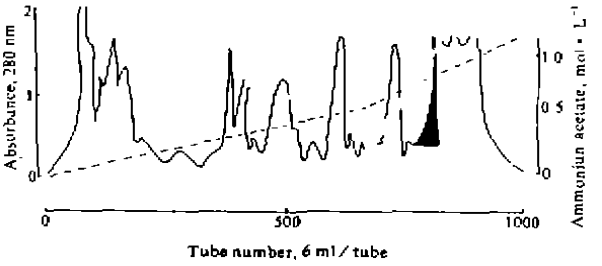

Fig 1. Cation exchange chromatography on CM sephadex C25 of cobra venom 10 g. Eluting buffer is NH_4Ac from 0.05 mol · L^{-1} (pH 5.5) to 1.1 mol · L^{-1} (pH 7.0); flow rate 36 ml · h^{-1} and column dimension 4.5 × 80 cm. The dotted line indicates the concentration of NH_4Ac. The shadowed fraction is available for this experiment after further purification with SP sephadex C50 column rechromatography.

pO_2 of K–H solution at 37℃ were controlled at 4.7–7.3 and 67 kPa or more respectively by adjusting the flow of oxygen and of carbon dioxide before infusion. the coronary flow (CF) was measured manually and then discarded. The aortic flow (AF) was measured by an electromagnetic flowmeter and returned to the perfusion solution reservoir. Left atrial filling pressure was kept constant at 1.17 kPa (12 cm H_2O) and the height of aortic column at 5.87 kPa (60 cm H_2O). Left ventricular pressure (LVP) was measured with a small plastic apical cannula connected to a pressure transducer. LVP signal was differentiated using a differentiator with time constant of 2.5 ms. LVP and dP/dt were recorded in a physiograph (LMS–2A, China). Left ventricular end diastolic pressure (LVEDP) was read from a memory oscilloscope VC–10 (Nihon Kohden, Japan). Maximal velocity of contractile element shortening (V_{max}) was calculated according to the LVP–dP/dt vector loops, which were plotted with a X–Y recorder (Nihon Kohden, Japan) from the LVP and dP/dt signals. pO_2 in K–H solution before and after perfusion was alternately shown in a mass spectrometer Medspect II, Chemitron, USA).

CO was expressed as the sum of AF and CF, which were all corrected for dry heart weight; mean aortic systolic pressure (MASP) was estimated from the area under the ejection phase of LVP tracing run at 10 cm · s^{-1}; external left ventricular pressure volume work (LVPW) was calculated from the formula by Neely[7]; myocardial oxygen consumption (MVO_2) was calculated according to CF and difference between arterial and venous pO_2[8]; efficiency was calculated as the ratio of LVPW / energy equivalent of MVO_2[9].

DLF was administered into the perfusion solution by cumulative method. Then, the hemodynamic parameters were recorded every 2 min. The mean of successive five measured reading was compared with control values.

Isolated papillary muscles The papillary muscle, isolated from right ventricle of guinea pig weighing 216± SD 25 g, was mounted in 20 ml K–H solution at 35℃ aerated with 95% O_2+ 5% CO_2. The composition of K–H solution is free from Na pyruvate, the remainders are the same as above. The resting tension was fixed at 5 mN. Contractions were induced by field stimulation set at a frequency of 2 Hz, duration of 0.5 ms and voltage of 10 V. Tension developed (T) and its differential (dT/dt) were recorded by an isometric transducer and a physiograph (LMS–2A, China). T, $+dT/dt_{max}$, $-dT/dt_{max}$, time to peak tension (t_1), relaxation time (t_2) and contration duration (t_3) were measured. DLF was given as soon as contraction became stable.

Isolated atria The atrium, isolated from above guinea pig hearts, was mounted in 10 ml K–H solution at 35℃ aerated with 95% O_2 + 5% CO_2. Spontaneously developing tension of right atrium was recorded with a force–displacement transducer and a physiograph (SJ–42, China). The threshold voltages exciting left atrium were determined with a pair of stainless steel electrodes and a stimulator set at frequency of 5 Hz and duration from 0.5–12 ms.

Anesthetized dogs Two ♂ dogs (11 and 12 kg), anesthetized with iv sodium pentobarbital 30 mg · kg^{-1}, were used to investigate the cardiac effect of DLF. Respiration was recorded with a pneumotachograph (Nihon Kohden, Japan). LVP was measured through a catheter inserted in the left ventricular cavity. Two hypodermic needle electrodes were used for lead II ECG. Above tracings were recorded in a polygraph, RM 6000 (Nihon Kohden, Japan). DLF 0.5 mg · kg^{-1} every 15 min was given iv.

Statistics Data were expessed as $\bar{x}$ ± SD. Differences of mean values before and after giving DLF were assessed by a pair–t test.

RESULTS

Effect on isolated working hearts In 4 preparations for the stability tests, all the cardiac function parameters, namely LVP, dP/dt_{max}, CO, CF, AF, HR, MASP, LVPW, MVO_2 and efficiency, remained relatively stable at the end of the second hour. As the left atrial filling pressure was set at 0.39, 0.59, 0.78, 0.98, 1.17 and 1.47 kPa respectively, AF was changed correspondingly to 32.3± 3.5, 56± 12, 88± 21, 120± 22, 139± 35 and 140± 36 ml · min^{-1} · g^{-1} (n=7), indicating that the cardiac performance reached the plateau of the Starling curve at the preload of 1.17 kPa (120 mm H_2O). The control values obtained from 6 preparations before administration of DLF were as follows: HR 236± 12 bpm; CO 166± 39 ml · min^{-1} · g^{-1}; CF 61± 19 ml · min^{-1} · g^{-1}; AF 105± 29 ml · min^{-1} · g^{-1}; LVP 8.4± 0.6 kPa; dP/dt_{max} 264± 18 kPa · s^{-1}; MASP 6.7± 0.5 kPa; LVEDP 0.6± 0.4 kPa; MVO_2 28± 7 μmol · min^{-1} · g^{-1}; LVPW 1.15 ± 0.32 J · min^{-1} · g^{-1} and efficiency 9.9 ± 2.6% (n=6). As compared with these control values, the mean changes following DLF 1–10 μg · ml^{-1} were shown in Fig 2.

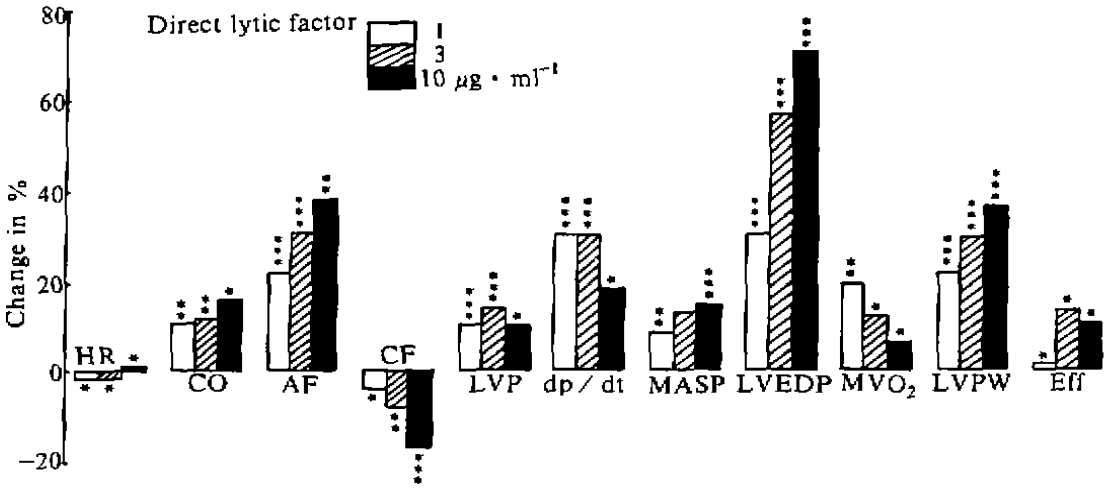

Fig 2. Effect of direct lytic factor, 1, 3, and 10 $\mu g \cdot ml^{-1}$, on isolated working guinea pig hearts. Bars show the mean percentages of changes in parameters from control values seen in text. HR, heart rate; CO, cardiac output; CF, coronary flow; AF, aortic flow; LVP, left ventricular pressure; dP/dt, the maximal rate of rise of left ventricular pressure; MASP, mean aortic systolic pressure; LVEDP, left ventricular end diastolic pressure; MVO_2, myocardial oxygen consumption; LVPW, left ventricular pressure volume work; Eff, efficiency. $n=6$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control values.

The inotropic effects of DLF were manifested as follows:

1 AF and LVPW were increased in a dose dependent manner and the increases in amplitude were more prominent than those of LVP and MASP, but effeciency had no significant improvement.

2 CF was decreased by 16%, which might be responsible for the fact that MVO_2 did not increase as LVPW did.

3 HR remained unchanged throughout the course of giving DLF although AF had been increased significantly.

4 LVEDP was increased, which might be induced by augmentation of atrial contractions.

5 MVO_2 was increased and efficiency was unchanged at DLF 1 $\mu g \cdot ml^{-1}$. However, when the level of DLF reached 3 or 10 $\mu g \cdot ml^{-1}$, MVO_2 turned to decline and efficiency seemed to be increased, but these changes were not significant ($P>0.05$).

6 Both the slope of the isovolumic phase of contraction and the area of LVP–dP/dt vector loops were increased. V_{max} was increased from control values of $39 \pm 5\ s^{-1}$ to $50 \pm 9\ s^{-1}$ ($n=6$, $P<0.05$) after the DLF level reached 10 $\mu g \cdot ml^{-1}$.

Effect on isolated guinea pig papillary muscles T, $+dT/dt$ max and $-dT/dt_{max}$ were 2.24 ± 0.18 mN, 15.7 ± 3.4 mN · s^{-1} and 22.4 ± 3.7 mN respectively ($n=4$) before DLF was given. DLF 1.25–10 $\mu g \cdot ml^{-1}$ increased T, $+dT/dt_{max}$ and $-dT/dt_{max}$ in a dose–dependent manner as shown in Fig 3.

Although T had reached the plateau at DLF 10 $\mu g \cdot ml^{-1}$, t_2 remained unchanged, but t_1 and t_3 were increased from 72.5 ± 17 and 210 ± 12 ms to 85 ± 31 and 224 ± 16 ms respectively ($n=4$, $P>0.05$).

Arrhythmias had never been found in all preparations. However, if the concentration of Ca^{2+} in K–H solution was increased to 7 mmol · L^{-1}, DLF 10 $\mu g \cdot ml^{-1}$ induced premature contractions, which was easily reversed by washing with K–H solution.

Effect on isolated guinea pig atria Before giving DLF, the spontaneously beating

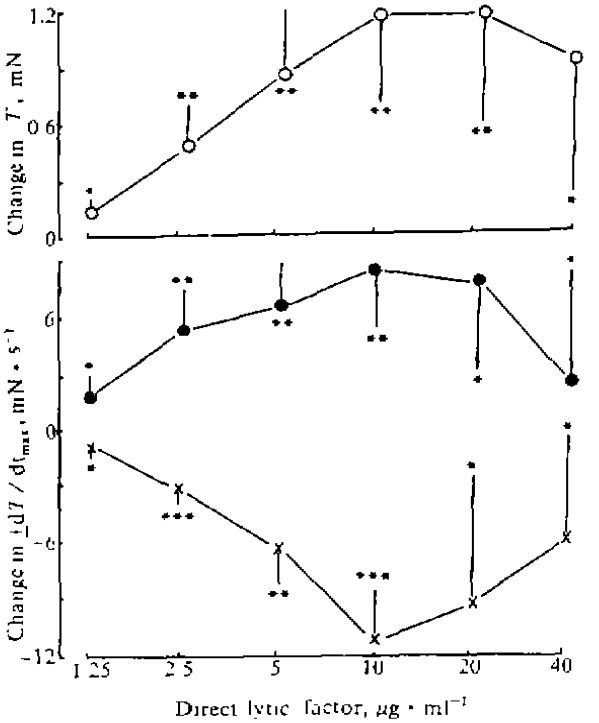

Fig 3. Effects of direct lytic factor on isolated guinea pig papillary muscles. Tension developed (T) and dT/dt were recorded under the following condition: resting force, 5 mN; rectangular wave pulse frequency, 2 Hz; voltage, 10 V; width, 0.5 ms. Points show the absolute changes in parameters from control values. (○) T; (●) $+dT/dt_{max}$. (×) $-dP/dt_{max}$. $n=4$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ vs control values.

tension of right atria was 0.47 ± 0.09 mN and the atrial rate, 276 ± 14 bpm ($n=6$). T was augmented by DLF 1–4 μg · ml^{-1} or more induced contracture, but the spontaneously beating rate remained constant continually, as shown in Fig 4. Moreover, stimulating threshold voltages determined in left atria was not shifted by DLF 3 or 6 μg · ml^{-1}.

Effect on anesthetized dogs DLF increased LVP and dP/dt_{max} in a dose dependent manner. In one anesthetized dog, when DLF 0.5 mg · kg^{-1} was given iv, LVP and dP/dt_{max} were increased from the control value of 16.7 kPa (125 mm Hg) and 267 kPa · s^{-1} (2000 mm Hg · s^{-1}) to the maximal value of 20.0 kPa (150 mm Hg) and 373 kPa · s^{-1} (2800 mm Hg · s^{-1}). HR, however, remained stable and regular. After cumulative DLF dose reached 6 mg · kg^{-1}, the QRS waves became widened and LVEDP began to rise graduately. The chest was opened immediately after the idioventricular rhythm appeared, the rhythmical atrial beats were seen continually even after the ventricular standstill. The same intoxication course was also seen in another anesthetized dog.

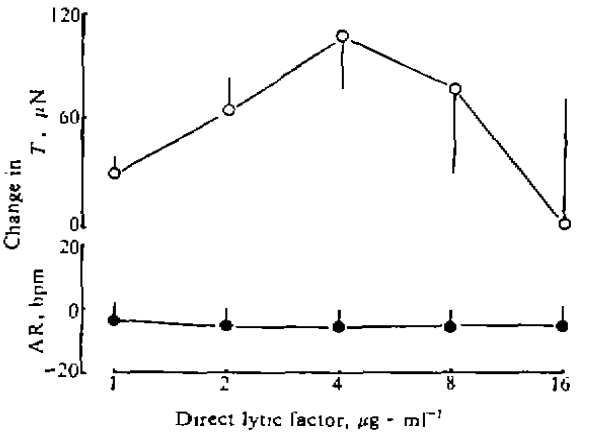

Fig 4. Effects of direct lytic factor on isolated guinea pig right atria. Spontaneously developing tension (T) was recorded with a force–displacement transducer. Points show the absolute changes in parameters from control values. (○) T; (●) atrial rate (AR). $n=6$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ vs control values.

DISCUSSION

The significant increase in CO, AF and LVPW, found in the isolated working guinea pig hearts, indicated that DLF increased the cardiac contractility independent of preload and afterload. The increases in LVP dP/dt_{max} and V_{max} supported above statement. The cardiotonic effect of DLF was also observed by recording the isometric contractions of isolated guinea pig papillary muscles and by determining LVP and dP/dt_{max}

in anesthetized dogs. DLF increased LVEDP in the isolated working guinea pig hearts, which might be the result of strengthened atrial contractions. The raised LVEDP increased the developing tension, however, this was not the primary cause of its positive inotropic effect because the left atrial filling pressure had been fixed at 1.17 kPa at which the cardiac performance had reached the plateau of starling curve. According to the three-component model, the pressure rise velocity is related to the pressure developed[10]. DLF increased V_{max} and the slope of the isovolumic phase of contraction in the LVP–dP/dt vector loops. Therefore, the augmentation of contractility plays a main part in the rise in cardiac performance.

Either *in vitro* or *vivo*, DLF had a rather narrow dose range to strengthen the heart contraction. If DLF exceeded this range, contracture would appear. In isolated working guinea pig hearts given DLF, LVP ran a downhill course along with decreasing CF and MVO_2, and MVO_2 varied directly with CF but not with LVPW. Therefore, coronary vasoconstriction caused by DLF was a limiting factor to its positive inotropic action and was harmful to the working heart. The short lived cardiotonic effect *in vivo* is obviously related to its coronary vasoconstriction effect. The atrial thinness allows a good supply of oxygen and substrates, which may be the reason why atria can continue beating for a certain period following ventricular arrest. The fact that DLF neither induced changed in rate and rhythm of the hearts both *in vitro* and *in vivo*, nor disturbed excitability of the isolated left atrium indicated that the inotropic function of the heart was dissociable from its chronotropic function and thus DLF might be used as a research tool for myocardial contractility.

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直接溶解因子对离体豚鼠工作心脏的正性肌力作用及毒性

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提要 直接溶解因子 (DLF) 1–10 μg·ml^{-1} 增加离体豚鼠工作心脏的 AF, CO, LVPW, LVP, dP/dt$_{max}$, V_{max}, MASP, 并呈剂量依赖性; 但 HR

不改变；CF 下降；MVO_2 及效率改变不明显. DLF 也能增强离体豚鼠乳头肌等长收缩张力，增加麻醉狗的 LVP 及 dP/dt_{max}，但离体右心房自发搏动频率及左心房兴奋阈不受影响. DLF 引起的心脏衰竭和它的冠脉收缩作用有关.

关键词 眼镜蛇毒；直接溶解因子；心肌收缩；心脏功能试验

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Effects of phencyclidine on contractile forces of isolated rabbit papillary muscles[1]

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ABSTRACT Phencyclidine (PCP) (0.01–50 μmol · L^{-1}) and its analogue, TCP (0.01–50 μmol · L^{-1}) exhibited positive inotropic effects on electrically stimulated rabbit papillary muscle preparations. Dextrorphan (5 or 10 μmol · L^{-1}) antagonized the actions of PCP in non-competitive manner ($pD_2' = 5.25$). This demonstrated the involvement of PCP receptors in the positive inotropic effects of PCP. By using high performance liquid chromatography with electrochemical detector (HPLC-ECD), an increase of DOPAC content was found in bath medium after PCP addition. Each of the dopamine receptor antagonists SCH23390, haloperidol and sulpiride (1 μmol · L^{-1}) attenuated the maximal inotropic effects of PCP. These results suggest that PCP induces positive inotropic effects by increasing the release and / or blocking the uptake of dopamine.

KEY WORDS papillary muscles; phencyclidine; high pressure liquid chromatography; dextrorphan; dopamine

Phencyclidine (PCP) has well-described effects on the cardiovascular system[1,2]. There were contradictory reports of positive[3] and negative[4] inotropic effects of PCP on ventricular muscle preparations. Radioligand binding assay demonstrated the existence of specific PCP receptors in guinea pig and rat hearts[2]. Activation of PCP receptors increased the release and blocked the uptake of norepinepherine (NE)[5], dopamine (DA)[6], etc. The purpose of the present study is to investigate the involvement of PCP receptors in inotropic effects of PCP on papillary muscles, and to examine the relationship between monoamine transmitters and the inotropic actions, to explore the mechanism of effects of PCP on ventricular muscles.

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MATERIALS AND METHODS

PCP was synthesized by Shanghai Medical University. Dextrorphan, SCH23390 and *N*-(1-[2-thienyl]cyclohexyl)3,4-piperidine (TCP) were kindly donated by Prof Avram Goldstein (Addiction Research Foundation, USA), Prof Reizo Inoki (Osaka University, Japan) and Beijing Military Medical Institute, respectively. Haloperidol and sulpiride were purchased from Shanghai Tianfeng Pharmaceutical Factory.

Rabbits of either sex, weighing 2.61 ± SD 0.23 kg, were stunned by a blow to the head, and the right heart papillary muscles were dissected free, suspended vertically under 1.0 g of tension, and incubated in a bath containing 4 ml of Tyrode's solution (pH = 7.4). The soluion was gassed with 95% O_2 +