

## Positive inotropic effect of apomorphine on guinea pig myocardium is mediated by dopamine DA<sub>1</sub> receptors

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**ABSTRACT** Dopaminergic agonist apomorphine (Apo, 1-100  $\mu\text{mol} \cdot \text{L}^{-1}$ ) had a concentration-dependent positive inotropic effect on guinea pig left atria. This effect was not changed obviously when the influences of sympathetic and parasympathetic nerves were eliminated by reserpine and atropine, respectively. Apo had little inotropic action on guinea pig papillary muscles. The time course of positive inotropic effect of Apo was different from that of isoprenaline and phenylephrine. Apo influenced the isometric contraction curves in a different way from isoprenaline. Dopaminergic antagonist haloperidol antagonized the positive inotropic effect of Apo. This effect was also competitively antagonized by dopamine DA<sub>1</sub> antagonist SCH 23390. While dopamine DA<sub>2</sub> antagonist domperidone,  $\beta$ -adrenergic antagonist propranolol and  $\alpha$ -adrenergic antagonist phentolamine did not obviously influence the effect of Apo. We concluded that the positive inotropic effect of Apo was mediated by postsynaptic dopamine DA<sub>1</sub> receptors in guinea pig left atria.

**KEY WORDS** apomorphine; haloperidol; SCH 23390; domperidone; myocardial contraction

Dopamine has a positive inotropic effect on myocardium. But it is an argument whether dopamine receptors mediate this effect. Many experiments show that dopamine receptors play no role in the positive inotropic effect of dopamine<sup>(1-3)</sup>, although it has been challenged by evidence<sup>(4)</sup>. So we used dopaminergic agonist apomorphine and some specific receptor blockers to detect if dopamine receptors play a role in the positive inotropic action.

## MATERIALS AND METHODS

Apomorphine hydrochloride (Apo, Hoffmann-La Roche). Haloperidol (Hal, Haipu Pharmaceutic Factory, Shanghai). Domperidone (Dom, Jassen Pharmaceutica, Belgium), dissolved in acetic acid, the highest concentration of acetic acid in bath being 0.008%. SCH 23390 malcate (a gift from Dr Ennio ONGINI, Essex Italy), dissolved in methanol, the highest concentration of methanol in bath being 0.01%. Propranolol hydrochloride (Pro, Beijing Pharmaceutic Factory, Beijing). Phentolamine methane sulphonate (Phc, Ciba-Geigy). Reserpine (Res, Red Flag Pharmaceutic Factory of Shanghai Medical University, Shanghai). Atropine sulphate (Atr, Beijing Pharmaceutic Factory, Beijing). Isoprenaline hydrochloride (Iso, Tianfeng Pharmaceutic Factory, Shanghai). Phenylephrine hydrochloride (Pheny, Shanghai Tenth Pharmaceutic Factory, Shanghai). All other chemicals were of AR.

Guinea pigs of either sex, weighing  $357 \pm \text{SD } 59$  g, supplied by Experimental Animal Center of Fourth Military Medical University, were stunned by a blow on the head. In oxygenated saline at 26°C, the left atria and right ventricular papillary muscles were dissected free and mounted in a lightproof organ bath of 25 ml saline containing NaCl 140, CaCl<sub>2</sub> 2.5, KCl 5.0, MgSO<sub>4</sub> · 7H<sub>2</sub>O 1.2, dextrose 10, HEPES 3.0 mmol · L<sup>-1</sup>, aerated with O<sub>2</sub> and kept at  $35.5 \pm 0.5^\circ\text{C}$ , pH 7.4.

The preparations were electrically driven with field stimulation (1 Hz frequency, 1 ms

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duration and an intensity of twice the threshold voltage), which was delivered by an electronic stimulator (DCQ-2 physiological electronic stimulator, Shanghai). The isometric tension was recorded with a transducer (YL-1 mechanic-electronic transducer, Changsha) on a pen-recorder (XWT-264 autonomic balanced recorder, Shanghai). The rest tension was adjusted to 0.3 g for papillary muscles and 0.5 g for left atria. All preparations were equilibrated for 1 h before addition of any drug. Dose-effect relationships of Apo were obtained cumulatively.

In some experiments, catecholamine-depleted atria were used. Res (5 mg · kg<sup>-1</sup>, ip) was given to the animals 18-24 h prior to the experiment; some reserpinized atria were further treated with Atr (3 μmol · L<sup>-1</sup> added in the bath).

When the antagonistic effects were tested, each left atrium was cut into two halves of atrial strips longitudinally which were mounted in two organ baths. An antagonist was added to one of them 15 min before administration of Apo, while the vehicle of this antagonist was added to another as control.

The dose-effect curves were fitted with logistic model on computer<sup>(5)</sup>. The mean curves were obtained by calculating the geometric means of those concentrations which caused responses of 10%, 30%, 50%, 70%, and 90% of the maximal responses<sup>(6)</sup> and the parameters were calculated.

Statistical significances of differences were determined by ANOVA.

## RESULTS

**Inotropic effects of Apo on left atria and right ventricular papillary muscles** Apo produced an increase in contractile force of left atria, while its vehicle ascorbic acid (AA) did not influence the contractile force (Tab 1). By ANOVA of split-out experiment, the effect of Apo differed significantly from that of AA ( $P < 0.01$ ). Apo produced concentration-dependent increase in contractile force ( $P < 0.01$  in ANOVA of regressions) while AA produced little change in contractile force of left atria ( $P > 0.05$ ). Accumulated increase of concentrations of neither Apo nor AA produced changes in contractile force of papillary muscles.

**Influences of Res and Atr on the inotropic effect of Apo on left atria** Normal left atria, reserpinized left atria, and (Res + Atr)-treated left atria responded to Apo in the same manner (Tab 2). In ANOVA of split-out experiment, there were no significant differences among them ( $P > 0.05$ ). Apo caused concentration-dependent increases in contractile force in all preparations. The  $pD_2$  values were  $5.36 \pm 0.14$  (control),  $5.18 \pm 0.21$  (Res) and  $5.25 \pm 0.22$  (Res + Atr), which showed little difference in ANOVA of completely randomized experiment ( $P > 0.05$ ).

### Time course of positive inotropic effect of

Tab 1. Contractile force (mg) of guinea pig hearts under cumulative concentrations of apomorphine or ascorbic acid as control  $\bar{x} \pm SD$ .

	Drugs	n	Final concentration (μmol · L <sup>-1</sup> )			
			0	3.16	10	31.6
Left atria	Apomorphine	6	186 ± 41	194 ± 49	206 ± 48	221 ± 44
	Control	6	182 ± 28	194 ± 34	178 ± 35	178 ± 39
Papillary muscles	Apomorphine	7	26 ± 6	26 ± 6	27 ± 6	26 ± 7
	Control	7	35 ± 20	35 ± 20	37 ± 22	36 ± 22

**Tab 2. Effect of apomorphine (cumulative concentrations) on contractile force (mg) of guinea pig left atria treated with reserpine (Res. ip, mg · kg<sup>-1</sup>) or atropine (Atr. in bath, μmol · L<sup>-1</sup>). n=5,  $\bar{x} \pm SD$ .**

Apomorphine (μmol · L <sup>-1</sup> )	Res Atr	0	5	5
		0	0	3
0		174 ± 49	212 ± 42	178 ± 53
1.0		180 ± 53	213 ± 45	180 ± 59
1.78		186 ± 57	217 ± 52	183 ± 61
3.16		196 ± 59	222 ± 55	189 ± 59
5.63		210 ± 63	233 ± 60	198 ± 61
10.0		227 ± 68	250 ± 60	211 ± 64
17.8		236 ± 68	259 ± 59	224 ± 75
31.6		235 ± 66	260 ± 52	224 ± 70

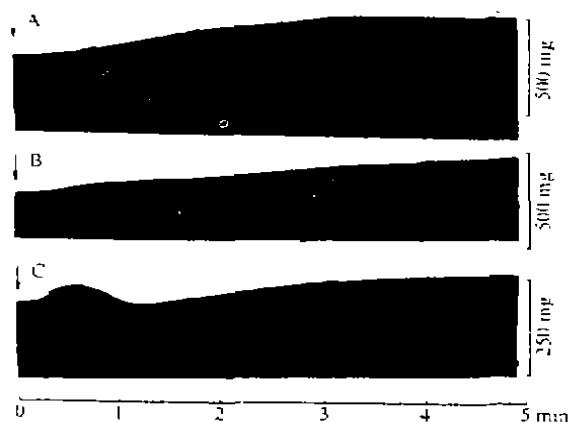
Apo Apo 1–100 μmol · L<sup>-1</sup> produced a positive inotropic action while > 100 μmol · L<sup>-1</sup> exerted a depressant effect on left atria. Apo 30 μmol · L<sup>-1</sup> produced monophasic time course which reached its steady state at 5 min; while Iso 30 nmol · L<sup>-1</sup> produced monophasic time course which reached its steady state at 5 min, and Pheny 500 μmol · L<sup>-1</sup> in the presence of Pro 1 μmol · L<sup>-1</sup> caused a typical triphasic time course of inotropic changes (Fig 1).

**Effect of Apo on isometric contraction**

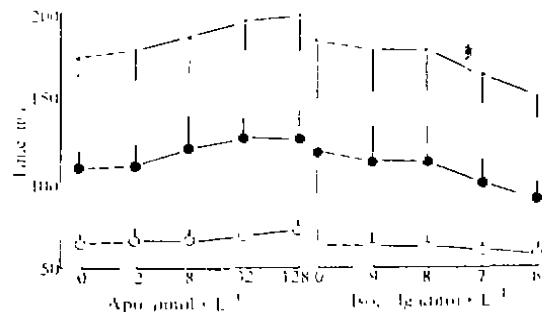
During isometric contractions, Apo prolonged the time to peak force, relaxation time, and total contraction time (*P* < 0.01), while Iso shortened these durations (*P* < 0.01) (Fig 2).

**Influences of some specific receptor blockaders on the action of Apo** Since AA may influence the binding of dopamine receptors with its ligands<sup>(7)</sup>, we used water-dissolved Apo and investigated the antagonistic effects of some specific receptor blockaders on parallel halves of left atrial strips.

Dopaminergic antagonist Hal antagonized the effect of Apo on contractile force in a concentration-dependent way (Fig 3). In the Schild regression for Hal, the regression line was  $\hat{Y} = 3.975 - 0.642 X$ , *pA*<sub>2</sub> = 6.190 ±



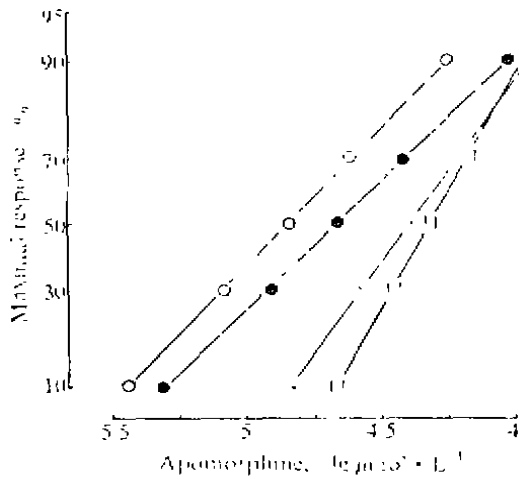
**Fig 1. Positive inotropic effect of apomorphine on guinea pig left atrium, compared with isoprenaline and phenylephrine. Arrows indicate administration of A: isoprenaline 30 nmol · L<sup>-1</sup>, B: apomorphine 30 μmol · L<sup>-1</sup>, C: phenylephrine 500 μmol · L<sup>-1</sup> in the presence of propranolol 1 μmol · L<sup>-1</sup>.**



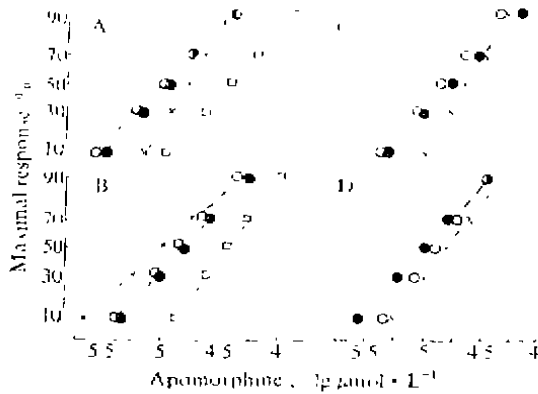
**Fig 2. Influences of apomorphine (Apo, n=6) and isoprenaline (Iso, n=5) on the time to peak force (○), relaxation time (●) and total contraction time (×) of isometric contraction in guinea pig left atria.  $\bar{x} \pm SD$ . All *P* < 0.01 in ANOVA of linear regressions.**

0.180. But its linear correlation was not good (*r* = 0.927, *P* > 0.05) (Fig 3).

DA<sub>1</sub> antagonist SCH 23390 also antagonized the effect of Apo concentration-dependently (Fig 4, A). The Schild regression line was  $\hat{Y} = 9.208 - 1.586 X$ , and its linear correlation was significant



**Fig 3.** Effects of apomorphine on contractile force of guinea pig left atrial strips in the absence (O,  $n=17$ ) and presence of haloperidol (●,  $0.3 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=6$ ; ×,  $1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=5$ , □,  $3 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=6$ ).



**Fig 4.** Effects of apomorphine on contractile force of guinea pig left atrial strips. A: in the absence (O,  $n=12$ ) and presence of SCH 23390 (●,  $0.3 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ ; ×,  $1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ , □,  $3 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ ). B: in the absence (O,  $n=12$ ) and presence of domperidone (●,  $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=3$ ; ×,  $1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=5$ , □,  $10 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ ). C: in the absence (O,  $n=8$ ) and presence of propranolol (●,  $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ ; ×,  $1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ ). D: in the absence (O,  $n=8$ ) and presence of phentolamine (●,  $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ ; ×,  $1 \mu\text{mol} \cdot \text{L}^{-1}$ ,  $n=4$ ).

( $r=0.9995$ ,  $P<0.05$ ). Slope of the line (95% confidential limits being  $-1.586 \pm 0.671$ ) was similar to unity ( $P>0.05$ ),  $pA_2=5.807 \pm 0.014$ .

DA<sub>2</sub> antagonist Dom did not antagonize the effect of Apo concentration-dependently (Fig 4, B). Pro  $0.1$  and  $1 \mu\text{mol} \cdot \text{L}^{-1}$  (Fig 4, C), Phe  $0.1$  and  $1 \mu\text{mol} \cdot \text{L}^{-1}$  (Fig 4, D) also had little effect on inotropic action of Apo.

**DISCUSSION**

Hal, a less selective dopamine receptor antagonist<sup>(6)</sup>, antagonized the effect of Apo, although it could not be confirmed definitely that this antagonism is competitive. Highly selective DA<sub>1</sub> antagonist SCH 23390<sup>(8)</sup> competitively antagonized the positive inotropic effect of Apo, according to the criteria for competitive antagonism<sup>(9)</sup>. DA<sub>2</sub> antagonist Dom showed no competitive antagonism to Apo, and the randomized shifts of dose-effect curves of Apo in this test may be due to experimental errors.

Pro and Phe had little influences on the effect of Apo, but at the same concentrations they respectively antagonized the effect of Iso<sup>(10)</sup> and sympathomimetics<sup>(11)</sup>, apparently. It indicated that the positive inotropic effect of Apo was irrelevant to  $\beta$ - or  $\alpha$ -adrenergic receptors. The observations on the time course of inotropic changes and isometric contraction curve changes of Apo, which showed different results from that of Pheny or Iso, conform to this conclusion.

We demonstrated that Apo produced the positive inotropic action on guinea pig left atria by activating post-synaptic DA<sub>1</sub> receptors. But it had no inotropic action on guinea pig ventricular muscles, which was in consistent with the finding<sup>(12)</sup> that there were only DA<sub>2</sub> receptors which might locate on pre-synaptic sites in guinea pig ventricular muscles.

REFERENCES

- 1 Motomura S, Brodde OE, Schumann HJ. No evidence for involvement of dopaminergic receptors in the positive inotropic action of dopamine on the isolated rabbit papillary muscle. *Jpn J Pharmacol* 1978; 28: 145
  - 2 Brodde OE, Schumann HJ, Inui J. The  $\alpha$ - and  $\beta$ -adrenoceptors but not dopaminergic receptors are involved in the positive inotropic action of dopamine in rabbit heart. *Adv Biosci* 1979; 20: 123
  - 3 Brodde OE, Inui J, Motomura S, Schumann HJ. The mode of direct action of dopamine on the rabbit heart. *J Cardiovasc Pharmacol* 1980; 2: 567
  - 4 Brown L, Lorenz B, Erdmann E. The inotropic effects of dopamine and its precursor levodopa on isolated human ventricular myocardium. *Klin Wochenschr* 1985; 63: 1117
  - 5 Wang N, Zhao DH, Sheng BH. A program for analysis of dose-response relationship with logistic model. *Acta Pharmacol Sin* 1990; 11: 187
  - 6 Patil PN, Ruffolo RR Jr. Evaluation of adrenergic  $\alpha$ - and  $\beta$ -receptor activators and adrenergic  $\alpha$ - and  $\beta$ -blocking agents. In: Szekeres L, ed. *Adrenergic activators and inhibitors*. Part I. Berlin: Springer, 1980: 89-134 (Born GVR, Farah A, Herken H, Welch AD, eds. *Handbook of experimental pharmacology*; vol 54/I)
  - 7 Heikkila RE, Manzino L. Ascorbic acid, redox cycling, lipid peroxidation, and the binding of dopamine receptor antagonists. *Ann NY Acad Sci* 1987; 498: 63
  - 8 Hilditch A, Drew GM, Naylor RJ. SCH 23390 is a very potent and selective antagonist at vascular dopamine receptors. *Eur J Pharmacol* 1984; 97: 333
  - 9 Kenakin TP. The classification of drugs and drug receptors in isolated tissues. *Pharmacol Rev* 1984; 36: 165
  - 10 Blinks JR. Evaluation of the cardiac effects of several beta-adrenergic blocking agents. *Ann NY Acad Sci* 1967; 139: 673
  - 11 Endoh M, Schumann HJ, Krappitz N, Hillel B. Alpha-adrenoceptors mediating positive inotropic effects on the ventricular myocardium: some aspects of structure-activity relationship of sympathomimetic amines. *Jpn J Pharmacol* 1976; 26: 179
  - 12 Sandrini M, Benelli A, Baraldi M. Evidence that dopamine receptors identified by [<sup>3</sup>H]dopamine in the ventricles of guinea-pig heart are of DA<sub>2</sub> type. *Pharmacol Res Commun* 1986; 18: 1151
- 阿扑吗啡对豚鼠心肌的正性变力作用是由多巴胺 DA<sub>1</sub> 受体介导的
- 王 楠 (东方医院老年病科, 福州 350001, 中国)  
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- 提要 为探讨多巴胺受体是否参与心脏变力效应, 观察了阿扑吗啡(Apo)对豚鼠心肌收缩力的影响。Apo对左房肌有正性变力作用, 且不受利血平及阿托品的影响。该作用与异丙肾上腺素及苯肾上腺素特征不同。氟哌啶醇和 SCH 23390 均可拮抗该作用, 而双咪哌酮、普萘洛尔及酚妥拉明则无明显影响。表明 Apo 的正性变力作用是由豚鼠左房突触后多巴胺 DA<sub>1</sub> 受体介导的。
- 关键词 阿扑吗啡; 氟哌啶醇; SCH 23390; 双咪哌酮; 心肌收缩

Instructions to authors

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