Comparative Study of Inotropic Effects of Catecholamines on Blood-perfused Canine Papillary Muscle

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ENDO, M., KIMURA, T. and HASHIMOTO, K. *Comparative Study of Inotropic Effects of Catecholamines on Blood-perfused Canine Papillary Muscle.* Tohoku J. exp. Med., 1972, 106 (2), 165-173 — The relative inotropic potencies of isoproterenol, norepinephrine, epinephrine and phenylephrine were compared in the blood-perfused canine papillary muscle preparation. The muscle was perfused at a constant pressure of 100 mm Hg at 38-39°C with arterial blood from a donor animal. The isometric tension developed by the papillary muscle and the positive inotropic effect of catecholamines were a function of the stimulus frequency. The potencies of the inotropic effect of catecholamines were compared at various stimulus frequencies from 90 to 240/min. Isoproterenol, norepinephrine and epinephrine, 0.001 to 0.1 μ g, caused reproducible positive inotropic effects lasting for from 2 to 5 min, when the perfused blood flow of the muscles was from 3 to 5 ml/min. Phenylephrine, 0.01 to 1 μ g, caused no inotropic effect. The order of potencies of inotropic effect of each amine was isoproterenol > norepinephrine > epinephrine $\gg$ phenylephrine. The dose ratio required to produce 100% increase in isometric tension was IS:NE:EP=1:3:6 at stimulus frequencies of 90 and 120/min. The ratio of the percentage increase produced by 0.03 μ g of each agent was roughly IS:NE:EP=3:2:1 and 5:3:1 at 90 and 120/min, respectively. Norepinephrine produced a distinctly stronger positive inotropic effect than did epinephrine and the differences between these amines were statistically significant. — catecholamines; inotropic effect; papillary muscle

The inotropic potencies of catecholamines, naturally occurring and synthetic, have been determined in various preparations of mammalian ventricular muscle, *i.e.*, isolated papillary muscles (Garb 1950, Lee and Yu 1964), heart-lung preparation (Crismon and Tainter 1938, Fawaz and Tutunji 1960) and hearts *in situ* (Goldberg *et al.* 1953, Cotten and Pincus 1955, Moran 1966). While isoproterenol was found to be the most potent catecholamine (Lands and Howard 1952, Moran 1966), differences in potencies between epinephrine and norepinephrine occur among various species of animals. In the canine heart it is reported that norepinephrine exhibits approximately an equal or slightly stronger inotropic effect than epinephrine. But there are considerable variations when assessed in different preparations, thus quantitative differences are hardly comparable. Difference in the experimental conditions may also influence the results. In the isolated papillary muscle hypoxia may exist because coronary vessels are not perfused (Blinks and Koch-Weser 1963). Such hypoxic conditions are not favorable for the assessment of the inotropic

potency of catecholamines. On the other hand, various factors such as the venuous return, stroke volume, peripheral resistance, and above all heart rate, may modify the precise comparison of the inotropic effect of these drugs *in situ*. Recently we developed the blood-perfused canine papillary muscle preparation by a cross-circulation technique (Endoh and Hashimoto 1970, a, b; Endoh *et al.* 1970). In the present study, these various difficulties were avoided using this preparation for a comparison of the inotropic potencies of norepinephrine, epinephrine, isoproterenol and phenylephrine.

METHODS

Sixteen mongrel dogs, weighing 7 to 10 kg, were anesthetized with ether. After heparinization (1,000 U/kg, *i.v.*), the heart was excised and plunged into cold Tyrode's solution at 3 to 6°C, equilibrated with 95% O₂ and 5% CO₂. The anterior septal artery was dissected and cannulated. All branches except the arteries to the anterior papillary muscle were ligated. Indigo carmine, 0.4% in saline solution, was used to make the papillary muscle arteries visible. The isolated muscle was perfused through the septal artery with arterial blood of a donor dog by the aid of a Sigmamotor pump. A pneumatic resistance was placed in parallel with the perfusion system so that a constant perfusion pressure at 100 mm Hg was maintained. The papillary muscle was maintained at a constant temperature of 38–39°C in a bell shaped glass container which was warmed by circulating warm water pumped from a water bath. The tendinous end of the papillary muscle was connected to a force displacement transducer (Grass FT 03) by a fine silk thread and stretched with a weight of about 1 g. The perfusing blood flow was measured from time to time with a graduated cylinder and ranged from 3 to 5 ml/min. The perfused blood was returned to the donor through the jugular vein. The donor dogs were anesthetized with morphine, 10 mg/kg, *s.c.* and urethan, 1 g/kg, *s.c.*. Sodium heparin, 500 U/kg, was given at the beginning of the experiment and 200 U/kg were added at 1-hour intervals. The muscle was electrically driven by an electronic stimulator (Nihon Kohden MSE-3) through bipolar platinum electrodes with stimulus pulses of 0.5 to 1.5 volts (approximately twice threshold), 5 msec duration and 120/min. It has been ascertained that no electro-release of autonomic transmitters (Blinks 1966) occurred under these conditions (Endoh and Hashimoto 1970, b). Isometric contractions were recorded on an ink-writing oscillograph (Nihon Kohden WI-130) through a force displacement transducer, a carrier preamplifier (Nihon Kohden RP-3) and a main amplifier (Nihon Kohden AD 2-22). The drug solutions, 0.01 to 0.03 ml, were injected at 10-min intervals into the rubber tube close to its connection with the shank of the polyethylene cannula, in a period of 4 seconds by a microinjector (Jintan Terumo Co.). The details of the experimental setup were described in a previous paper (Endoh and Hashimoto 1970, b). The drugs used in these experiments were *l*-norepinephrine (Fluka AG), *l*-epinephrine (Merck AG), *l*-isoproterenol (Kaken Kagaku) and phenylephrine hydrochloride (Kowa). All doses given are expressed in terms of the base. The desired concentration was obtained by dilution of the stock solution with saline. An injection of 0.03 ml of saline in 4 seconds had no effect on the contractile force. Statistical analysis of the results was performed using Student's *t* test and statistical significance of the differences was expressed as *P* values.

RESULTS

1) Frequency-force relationship and positive inotropic effect of sympathomimetic amines

The contractile tension of the blood-perfused canine papillary muscle was markedly influenced by stimulus frequency of the muscle (Fig. 1) as described in

Fig. 1. Effect of changes of stimulus frequency on the isometric contractile tension of the blood-perfused canine papillary muscle.

the previous paper (Endoh and Hashimoto 1970, b). When the stimulus frequency was increased from 60 to 120, 240 and 270/min, keeping the stimulus pulse duration and voltage constant at about twice threshold, isometric contractile tension was augmented. A slight alternation of tension developed at 240/min, which became marked when the frequency was 270/min. The average effect of the stimulus frequency on the contractile tension in 7 papillary muscles is shown in Table 1.

TABLE 1. *Effect of stimulation frequency on the isometric contractile tension of the blood-perfused canine papillary muscle*

		Stimulus frequency (/min)					
		60	90	120	180 ^b	240 ^b	270 ^b
Isometric contractile tension (g)	mean	1.7 ^a	1.9	2.2	2.7	3.1	3.2
	S.E.	0.1	0.1	0.1	0.2	0.3	0.3

^a Isometric contractile tension in gram, each value is mean of 7 muscles (n=7)

^b Values of the isometric tension are expressed as mean of paired large and small contractions of pulsus alternans over the frequencies of 180/min.

This augmentation of contractile tension by high frequency stimulation is not directly related to an electro-release of autonomic transmitters (Endoh and Hashimoto 1970 b) which may occur with strong electrical stimulation as reported in the previous paper (Endoh and Hashimoto 1970, a). As the contractile performance of the ventricular myocardium is a function of the stimulus frequency, it is to be expected that the inotropic effect of catecholamines will be modified by changes in rate. Therefore the inotropic effect of sympathomimetic amines was compared at various frequencies of from 90 to 140/min as shown in Figs. 2 and 3. Fig. 2 illustrates the positive inotropic effect of 0.01 μ g of epinephrine, norepinephrine, and isoproterenol at stimulus frequencies of 120, 150 and 180/min in a typical experiment. Percentage increase of the contractile tension by intra-arterial administration of 0.01 μ g of four sympathomimetic amines at different stimulus frequencies in a typical experiment is shown graphically in Fig. 3. When the stimulus frequency is raised from 90 to 120–150/min, the positive inotropic effect of each amine

Fig. 2. Positive inotropic effects of catecholamines on the blood-perfused canine papillary muscle at stimulus frequencies of 120, 150 and 180/min. EP: epinephrine. NE: norepinephrine. IS: isoproterenol.

Fig. 3. Positive inotropic effects of 0.01 μ g of four sympathomimetic amines at various stimulus frequencies of from 90 to 240/min. $\circ$ IS: isoproterenol. $\bullet$ NE: norepinephrine. $\odot$ EP: epinephrine. $\times$ PH: phenylephrine.

increased gradually in spite of the increased control contractile tension resulting from high frequency stimulation. Further increase of stimulus frequency beyond 150/min reduced the positive inotropic effect of the catecholamines which was most prominent at frequencies of 120 to 150/min.

2) Comparison of the positive inotropic effect of isoproterenol, norepinephrine, epinephrine and phenylephrine at stimulus frequencies of 90 and 120/min

Since the positive inotropic effect of catecholamines was influenced by stimulus frequency as described above, the inotropic effect of these amines was compared at two different stimulus frequencies of 90 and 120/min corresponding approximately to the heart rate in the intact dog, around which the positive inotropic effects were most pronounced. The percentage increase of contractile tension by 0.01 to 0.1 μg of each agent is shown in Fig. 4. Isoproterenol had the greatest positive inotropic effect and the longest duration of action. 0.03 μg of isoproterenol produced a percentage increase of 150 ± 19 (mean $\pm$ S.E.; $n=6$) at 90/min and 233 ± 18 at 120/min ($n=5$). This dose of isoproterenol was found to be the maximum dose permissible for repetitive administration in the present study, because extrasystolic contractions were evoked and the contractile force under the basal condition of stimulation was diminished and not recovered within 30 min. The duration of action of 0.03 μg isoproterenol was 4 to 5 min and the half time of action was 53 ± 5 sec ($n=8$). Isoproterenol sometimes produced extrasystolic contractions, which often disturbed the measurement of the positive inotropic effect. Norepinephrine produced a more prominent positive inotropic effect than

Fig. 4. Positive inotropic effects of sympathomimetic amines on the blood-perfused canine papillary muscle at stimulus frequencies of 90/min (A) and 120/min (B). ● NE: norepinephrine. ○ EP: epinephrine. ○ IS: isoproterenol. × PH: phenylephrine.

epinephrine. Norepinephrine in a dose of $0.1 \mu\text{g}$ caused a percentage increase of 170 ± 23 ($n=6$) at 90/min and 200 ± 19 ($n=6$) at 120/min. The effect of norepinephrine lasted for from 2 to 3 min and the half time of action was 44 ± 6 sec. Epinephrine in $0.1 \mu\text{g}$ doses caused a percentage increase of 128 ± 20 ($n=6$) at 90/min and 161 ± 13 ($n=6$) at 120/min. The duration of action of epinephrine was 1.5 to 2.5 min and the half time of action was 35 ± 6 sec ($n=8$). Phenylephrine, 0.01 to $1 \mu\text{g}$, produced no inotropic effect at any of the stimulus frequencies investigated in the present study.

3) Consideration of the potency of the inotropic effect of catecholamines

The inotropic potencies of catecholamines have been compared by others at various heart rates, controlled or not controlled, in various ways according to the type of preparation used. In the present study the positive inotropic effects of catecholamines were compared in two ways: *a*) the dose ratio required to produce the same percentage increase of contractile tension and *b*) the ratio of the percentage increase of contractile tension produced by equivalent doses of the agents at stimulus frequencies of 90 and 120/min.

TABLE 2. Ratio of percentage increase of contractile tension produced by norepinephrine and isoproterenol to epinephrine control (100)

		Ratio to epinephrine control (100)	
		Stimulus frequency	
		90/min	120/min
Norepinephrine (μg)	0.01	$246 \pm 36(6)$ $p^a < 0.02$	$280 \pm 47(6)$ $p < 0.05$
	0.03	$212 \pm 31(7)$ $p < 0.02$	$254 \pm 55(5)$ $p < 0.05$
	0.1	$178 \pm 1(5)$ $p < 0.01$	$128 \pm 6(5)$ $p < 0.01$
Isoproterenol (μg)	0.01	$506 \pm 102(5)$	$439 \pm 137(6)$
	0.03	$334 \pm 52(6)$	$487 \pm 128(5)$

a Differences between epinephrine and norepinephrine.

a) The dose ratio producing 100% increase of contractile tension was calculated from the dose-response curves shown in Fig. 4, since these are almost parallel for the catecholamines tested. The dose ratios resulting in 100% increase of contractile tension were IS: NE: EP = 1: 3.6: 6.8 at 90/min and 1: 2.3: 5.2 at 120/min, or approximately 1: 3: 6.

b) The increase of the contractile tension resulting from isoproterenol and norepinephrine was expressed as a percentage of the epinephrine control in the same papillary muscle because responses varied considerably from one preparation to another depending on the blood flow. The data are given in Table 2. The ratios of the percentage increase produced by $0.03 \mu\text{g}$ of each agent at which

dose-response curves were relatively parallel were EP: NE: IS=1: 2.1: 3.3 at 90/min and 1: 2.5: 4.9 at 120/min.

Differences in inotropic potency between epinephrine and norepinephrine were compared using Student's *t*-test. *P* values are presented in Table 2, which indicate the statistical significance of the difference between epinephrine and norepinephrine at all doses administered.

DISCUSSION

Blood-perfused canine papillary muscles used in the present study responded to electrical stimulation of 120/min at 38–39°C for over 10 hours without any deterioration of contractile tension, while that of the muscles in physiological solution decreased gradually over many hours under conditions of 38°C and stimulus frequency of 60/min (Garb 1950, Lee and Yu 1964). Thus, the blood-perfused canine papillary muscle is probably free from hypoxia in contrast to the preparation in physiological saline solution. Furthermore, the metabolic state of the blood-perfused papillary muscle should be more closely related to that in the mammalian ventricle *in vivo*. Lee and Yu (1964) suggested that the metabolic state of the muscle should be taken into consideration whenever the influence of sympathomimetic amines on the mechanical efficiency of the heart is investigated.

The contractile tension of the ventricular muscle is a function of heart rate (Fig. 1) and the positive inotropic effect of catecholamines is influenced by stimulus frequency (Fig. 3), therefore the heart rate must be considered when the inotropic effect of catecholamines are compared. Changes in heart rate together with changes in venous return, stroke volume and peripheral resistance might modify the true inotropic effect of catecholamines. Heart rate has not been controlled ordinarily in the *in situ* experiments (Crismon and Tainter 1938, Lands and Howard 1952, Goldberg *et al.* 1953, Cotten and Pincus 1955, Fawaz and Tutunji 1960).

It has been reported that isoproterenol exhibited the most potent inotropic effect among catecholamines in the rabbit (Lands and Howard 1952) and in the dog (Moran 1966). Moran (1966), using anesthetized, vagotomized, open-chest dogs, showed that isoproterenol is 4 to 5 times more potent than epinephrine and norepinephrine. In the present study isoproterenol is 3 to 5 times more potent than norepinephrine and 5 to 7 times more potent than epinephrine based on the dose required to produce a 100% increase in contractile tension. Thus the present data on isoproterenol agree with those of Moran in the canine heart *in vivo*.

It has been reported that norepinephrine and epinephrine exhibit almost equal positive inotropic effects in the cat (Garb 1950, Lee and Yu 1964) and dog (Cotten and Pincus 1955, Moran 1966) heart. Cotten and Pincus (1955) have shown that the positive inotropic effect of *l*-epinephrine and *l*-norepinephrine, as measured directly with a strain gauge in the *in situ* canine heart, to be qualitatively and quantitatively similar over a wide dosage range. But they did not control the heart rate and it is probable that this factor together with others described

previously may modify the true inotropic effect of these amines. Garb (1950), and Lee and Yu (1964), using isolated cat papillary muscle, observed that increases in contractility with norepinephrine were slightly greater than those seen with epinephrine. However, there was considerable variation in the degree of the increase, so that quantitative relationships between drugs could not be established. In the blood-perfused canine papillary muscle norepinephrine had about twice the inotropic potency as did epinephrine, the difference being statistically significant at all doses administered at various stimulus frequencies (Table 2). Crismon and Tainter (1938) reported that norepinephrine exhibited twice the potency of epinephrine in the cat heart-lung preparation. The present results are consistent with those of Crismon and Tainter (1938) and indicate that norepinephrine exhibits a greater inotropic effect than does epinephrine in the controlled and blood-perfused state in cat and canine hearts. The duration of action of these agents was 2 to 5 min and was consistent with the results of Goldbert *et al.* (1953) working with the canine heart *in situ*.

The positive inotropic effect of catecholamines increased gradually as the stimulus frequency was raised from 90 to 150/min and was most prominent at frequencies of from 120 to 150/min. Further increase of frequency reduced the positive inotropic effect. Koch-Weser, Berlin and Blinks (1963) have suggested that the positive inotropic effect of catecholamines was caused by the accumulation of "positive inotropic effect of activation" related to the amount *produced per beat*, and thus the higher the frequency, the stronger is the positive inotropic effect (Koch-Weser and Blinks, 1963). In the present study pulsus alternans always developed at frequencies between 180 and 240/min and the positive inotropic effect of catecholamines decreased remarkably at these frequencies. Pulsus alternans also has been observed at these frequencies in the canine heart *in situ* (Gilbert *et al.* 1965). This suggests that the frequency of 180 to 240/min is the physiological upper limit of the heart rate which the canine ventricular muscle can follow and above which the drug cannot act effectively even in the blood-perfused ventricular muscle. Relative oxygen deficiency may occur at high stimulus frequencies, since the blood supply will be restricted by abbreviation of the diastolic pause, more oxygen will be required than at low frequencies, and further increase of oxygen consumption is caused by catecholamines. For these reasons the papillary muscle response to catecholamines may be limited.

CONCLUSIONS

Inotropic potencies of isoproterenol, norepinephrine, epinephrine and phenylephrine on canine ventricle were compared in the physiological and controlled conditions using the blood-perfused papillary muscle. The order of potencies was isoproterenol > norepinephrine > epinephrine ≫ phenylephrine (5-6:2-3:1:none). Inotropic effects of each amine were stimulus frequency dependent.

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