Br. J. Pharmac. (1978), **64**, 53–61.

CHARACTERIZATION OF ADRENOCEPTORS MEDIATING POSITIVE INOTROPIC RESPONSES IN THE VENTRICULAR MYOCARDIUM OF THE DOG

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1 The pharmacological characteristics of adrenoceptors mediating the positive inotropic action in the dog heart were assessed by the use of blood-perfused papillary muscles and isolated strips of ventricular myocardium.

2 On the blood-perfused papillary muscle driven at 2 Hz and in sinus node preparations, phenylephrine induced positive inotropic and chronotropic responses in the same dose range and was much less potent than isoprenaline. The dose-response curve for the chronotropic action of phenylephrine was parallel to that of isoprenaline, whilst the dose-response curve for the inotropic action of phenylephrine was less steep than that of isoprenaline.

3 The infusion of pindolol, a β-adrenoceptor blocking agent, at a rate of 1 μg/min, shifted the isoprenaline dose-response curves to the right, and to the same extent, in both papillary muscle and sinus node preparations. In contrast to isoprenaline, the antagonism of phenylephrine by pindolol was noncompetitive. Phentolamine did not affect the positive inotropic and chronotropic actions of phenylephrine.

4 On isolated ventricular strips α-adrenoceptor blockade by 10^{-6} M phentolamine did not affect dose-response curves to phenylephrine or dopamine. Pindolol shifted the dopamine dose-response curves to the right in a competitive manner and those of phenylephrine in a noncompetitive manner.

5 On ventricular strips from reserpine-pretreated dogs phenylephrine and tyramine dose-response curves were shifted markedly to the right and downwards. Desipramine (10^{-5} M) which enhanced the action of noradrenaline considerably reduced the myocardial responses of phenylephrine.

6 Papaverine (10^{-5} M) decreased the threshold concentration of phenylephrine required to stimulate the myocardium and shifted phenylephrine dose-response curves to the left.

7 Raising the temperature from 32°C to 37°C shifted phenylephrine dose-response curves to the right; when the temperature was raised from 37°C to 42°C the affinity of the drug was not changed.

8 Other α-adrenoceptor stimulants, methoxamine and clonidine, decreased the active tension of ventricular strips. The responses to noradrenaline and adrenaline (in the presence of pindolol; 3×10^{-8} M) were not affected by phentolamine (10^{-6} M).

9 The results indicate that adrenoceptors mediating positive inotropic responses in the dog ventricle are of the β-type and that post-synaptic α-adrenoceptors are not involved. Phenylephrine acts mainly by releasing noradrenaline from adrenergic nerve endings and partly by a weak direct action on β-adrenoceptors.

Introduction

It has been a matter of discussion whether or not α-adrenoceptors are involved in positive inotropic responses in the mammalian heart. Govier (1967; 1968) has reported that α-adrenoceptors are partly involved in the effects of phenylephrine, adrenaline and noradrenaline on guinea-pig left atria. His observations have been confirmed in rat (Nakashima, Tsuru & Shigei, 1973) and rabbit (Parr & Urquilla, 1972; Benfey, 1973) atria, in rabbit papillary muscle (Schümann, Endoh & Wagner, 1974) and in cat papillary muscle (Rabinowitz, Chuck, Kligerman & Parmley, 1975). However, no relevant detailed studies have been performed on the dog heart although Kabela, Jalife, Peon, Cros & Mendez (1969) noted that phenylephrine responses were inhibited by propranolol. In the present experiments the isolated blood-perfused

papillary muscle (Endoh, 1975) and isolated ventricular strips from the dog were used in order to clarify this problem. The influence of temperature, stimulation frequency, phosphodiesterase inhibition and of α- and β-adrenoceptor blocking agents on phenylephrine-induced myocardial stimulation were investigated. These interventions have been shown to be useful in differentiating the role of α- and β-adrenoceptors in the rabbit and guinea-pig heart (Schümann, *et al.*, 1974; Endoh & Schümann, 1975a; Endoh, Wagner & Schümann, 1975; Ledda, Marchetti & Mugelli, 1975; Mugelli, Ledda & Mantelli, 1976).

Methods

Mongrel dogs of either sex and weighing 7 to 13 kg were anaesthetized with intravenous sodium pentobarbitone (30 mg/kg) and were given heparin (500 units/kg intravenously). The heart was removed after exsanguination and immediately plunged into cold (3 to 6°C) Tyrode solution equilibrated with 95% O_2 and 5% CO_2.

Blood-perfused papillary muscle and sinus node preparations

Details of the experimental procedures for preparing the blood-perfused preparations have been given previously (Endoh, 1975). The anterior papillary muscle and the sinus node region were perfused by means of a peristaltic pump (separately, through the cannulated anterior septal artery and the sinus node artery, respectively) with arterial blood from a donor dog at a constant perfusion pressure of 100 mmHg. The temperature of the preparations was maintained at 37 to 38°C. The venous blood from the isolated tissues was collected in a reservoir and returned to the donor dog via the jugular vein. The donor dog was also anaesthetized with sodium pentobarbitone (30 mg/kg i.v.) and heparin (500 units/kg) was given intravenously immediately before the beginning of the perfusion and then (in doses of 200 units/kg) at 1 h intervals. The papillary muscle was electrically driven at a rate of 2 Hz with square wave pulses of 5 ms duration and a voltage of 20% above the threshold. The developed tension of the muscle was measured isometrically with a force displacement transducer (Grass FTO3B) and recorded on an ink-writing oscillograph (San-ei Instrument). The rate of the isolated sinus node preparation was determined from the electrocardiogram. Drug solutions in volumes of 0.01 to 0.03 ml were injected into the rubber tube at the base of the Y piece connecting the outflow from the donor dog to the arterial cannulae of the isolated preparations. They were given over a period of 4 s with a microsyringe (Jintan Terumo Co.). Injections were made at a point sufficiently distant from the Y piece so that both the papillary muscle and sinus node preparations were exposed to the same concentration of the drug after a single injection. Infusion of the β-adrenoceptor blocking agent, pindolol, at a rate of 1 μg/min was made at this same point.

Isolated ventricular strips

Trabeculae carneae were excised from the right ventricle and fixed in a 20 ml organ bath containing Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at a temperature of 37°C. The muscle was stretched with a tension of about 0.5 g and driven at a rate of 0.5 Hz. The developed tension was recorded isometrically. Four muscles were isolated from one heart and run in parallel. After an equilibration period of 1 h, the cumulative drug dose-response curves were determined in the presence or absence of adrenoceptor blocking agents. The volume of injectate was kept constant (0.1 ml). Since phenylephrine dose-response curves were not repeatable in this preparation, only one was determined in each preparation. One or two muscles excised from the same heart were always used to provide the control responses. The maximal contraction was determined in each preparation by administering calcium either at the beginning or at the end of the experiment and, unless otherwise described, the positive inotropic effect of the agonists was expressed as a percentage of this maximal contraction. Phentolamine (10^{-6} M), pindolol (3×10^{-9} to 10^{-7} M), papaverine (10^{-5} M) were allowed to act for 1 h before agonist dose-response curves were determined. Desipramine (10^{-5} M) was allowed to act for 40 min.

The affinity of agonists for the receptors was expressed as the pD_2-value (negative logarithm of ED_{50}) and the intrinsic activity of agonists was determined as compared with the maximal response induced by calcium in each preparation (Ariëns, Simonis & Van Rossum, 1964). In some experiments reserpine was given twice in a dose of 0.1 mg/kg subcutaneously 48 and 24 h beforehand.

The drugs used were: (−)-phenylephrine hydrochloride (Kowa, Nagoya), (−)-isoprenaline hydrochloride (Nikken Kagaku, Nagoya), dopamine hydrochloride (Kyowa Hakko, Tokyo), (−)-noradrenaline base (Fluka, Bucks), (−)-adrenaline base (Merck, Darmstadt), tyramine hydrochloride (Wako, Osaka), (±)-pindolol base (Sandoz, Basel), phentolamine methanesulphonate (Ciba, Basel), reserpine (Daiichi Seiyaku, Tokyo), desipramine hydrochloride (Ciba, Basel), clonidine hydrochloride (Boehringer, Ingelheim) and methoxamine hydrochloride (Nihon Shinyaku, Kyoto). All doses refer to the base. Experimental values are given as means with s.e. mean. Statistical comparisons were performed by means of

Student's t test. A P value of less than 0.05 was considered to be significant.

Results

Blood-perfused papillary muscle and sinus node preparations

The basal active tension of the papillary muscle and basal rate of the sinus node preparation were 4.0 ± 0.32 g ($n = 10$) and 96.6 ± 3.4 beats/min ($n = 10$), respectively. Isoprenaline (Iso) and phenylephrine (PE) administered into the papillary muscle and sinus node preparations by single injections caused dose-related positive inotropic and chronotropic actions as shown in Figure 1. PE was about 3.5 log units less potent than Iso both in inotropic and chronotropic actions when compared on a molar basis. The Iso and PE dose-response curves (positive chronotropic action) were parallel. The slope of the dose-response curve for the positive inotropic action of PE was less steep than that of Iso and the maximal increase in active tension produced by PE was less than half of that induced by Iso.

During infusion of the β-adrenoceptor blocking agent, pindolol, at a rate of 1 µg/min, the dose-response curves for the positive inotropic and chronotropic actions of Iso were shifted to the right in a parallel fashion by 2 and 1.5 log units, respectively. Pindolol alone did not change the basal developed tension during the infusion. Since the perfusion blood flow rate of the preparations was between 6 and 8 ml/min, the blood concentration of pindolol was roughly 5.1 to 6.7×10^{-7} M. During infusion of pindolol PE dose-response curves (positive inotropism and chronotropism) were displaced to the right in a noncompetitive fashion. The degree of depression of the maximal responses to PE was the same in both preparations.

The influence of the α-adrenoceptor blocking agent, phentolamine, on the positive inotropic and chronotropic actions of PE was examined in four preparations in which β-adrenoceptors were blocked by the infusion of pindolol (1 µg/min). Phentolamine administered intra-arterially did not affect the actions of PE: increases in active tension ($25.6 \pm 2.9\%$; $n = 4$) and sinus rate ($18.9 \pm 4.4\%$; $n = 4$) induced by 1.5×10^{-6} mol of PE after the administration of 100 µg of phentolamine were not significantly different from the control increases of $20.5 \pm 1.7\%$ and $13.8 \pm 3.8\%$ ($n = 4$ each), respectively.

Isolated ventricular strips

The basal and maximal contractions of ventricular strips electrically driven at a rate of 0.5 Hz or 0.2 Hz are given in Table 1. The values in each group do not differ significantly from each other.

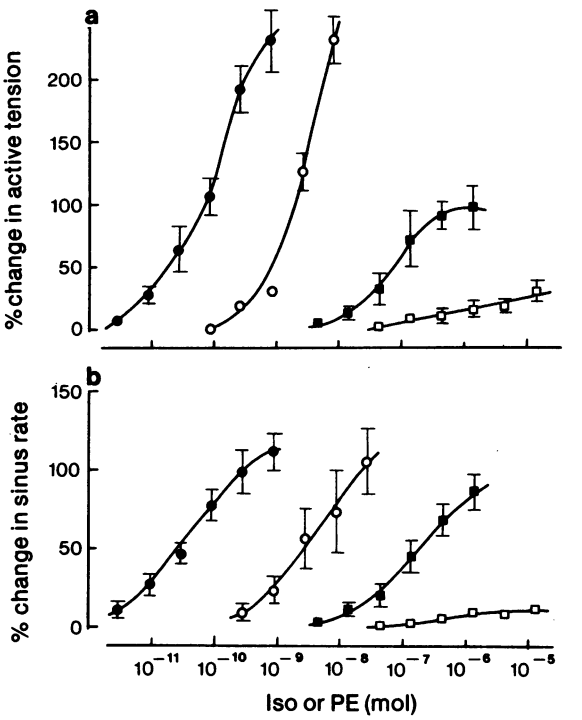

Figure 1 The influence of pindolol (1 µg/min) on the positive inotropic and chronotropic actions of isoprenaline and phenylephrine in isolated blood-perfused preparations. Ordinates: percentage changes in the developed tension (a) and in the sinus rate (b); abscissae: doses of isoprenaline (Iso) and phenylephrine (PE) in moles. (●): Isoprenaline-control ($n = 8$ in a and 7 in b); (○): isoprenaline-pindolol ($n = 4$ in a and b); (■): phenylephrine-control ($n = 6$ in a and 5 in b); (□): phenylephrine-pindolol ($n = 6$ in a and b). Vertical bars show s.e. means.

Influence of α-adrenoceptor blockade

PE, in concentrations higher than 3×10^{-6} M, caused a positive inotropic effect (Figure 2). The intrinsic activity of PE was 0.6, while that of dopamine was 1.0 (Figure 2). PE and dopamine responses were not affected by phentolamine (10^{-6} M; Fig 2). The pD_2-values for PE (4.81 ± 0.07; $n = 7$) and for dopamine (5.42 ± 0.17; $n = 4$) in the presence of 10^{-6} M phentolamine were not significantly different from those (4.96 ± 0.08; $n = 5$ and 5.42 ± 0.12; $n = 4$, respectively) in the control experiments. The maximal responses to PE and dopamine were also not influenced by phentolamine.

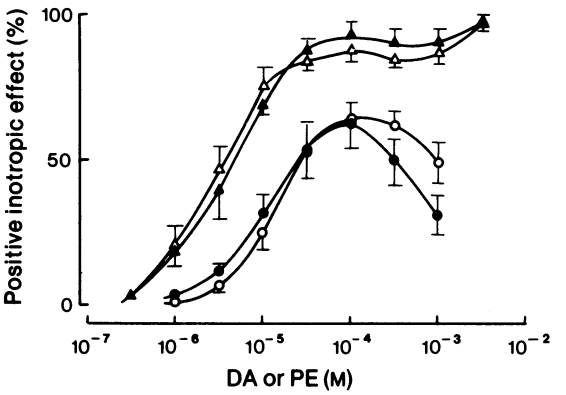

Figure 2 Influence of phentolamine (10^{-6} M) on the positive inotropic actions of dopamine (DA) and phenylephrine (PE) in dog isolated ventricular strips driven at 0.5 Hz. Ordinate scale: the positive inotropic actions expressed as a percentage of the maximal contraction; abscissa scale: molar concentration on logarithmic scale. (▲): Dopamine-control (n = 4); (△): dopamine-phentolamine (n = 4); (●): phenylephrine-control (n = 5); (○): phenylephrine-phentolamine (n = 7). Vertical bars show s.e. means.

Table 1 The basal and maximal contractions of isolated ventricular strips of the dog, electrically driven at a rate of 0.5 or 0.2 Hz at 37°C

Groups	n	*Active tension (mg)* *Basal*	*Maximal**
(A) 0.5 Hz			
Phenylephrine			
Control	5	654 ± 105	2720 ± 335
Phentolamine	7	540 ± 89	2008 ± 440
Control	6	503 ± 130	2733 ± 631
Pindolol	14	594 ± 56	2211 ± 177
Control	4	723 ± 169	3309 ± 484
Desipramine	4	809 ± 91	2660 ± 655
Control	7	549 ± 55	2451 ± 400
Papaverine	5	668 ± 78	1810 ± 268
Dopamine			
Control	4	670 ± 70	1940 ± 185
Phentolamine	4	657 ± 108	1696 ± 206
Control	6	453 ± 109	2170 ± 316
Pindolol	16	526 ± 48	2231 ± 257
Clonidine	7	851 ± 109	1991 ± 256
(B) 0.2 Hz			
Methoxamine	4	515 ± 71	—
Tyramine	4	303 + 46	2900 ± 205
Phenylephrine	7	618 ± 50	2535 + 208
Reserpine-treated	16	522 ± 58	2345 ± 113

* The maximal contractions were induced by calcium in each preparation. Values are mean ± s.e.

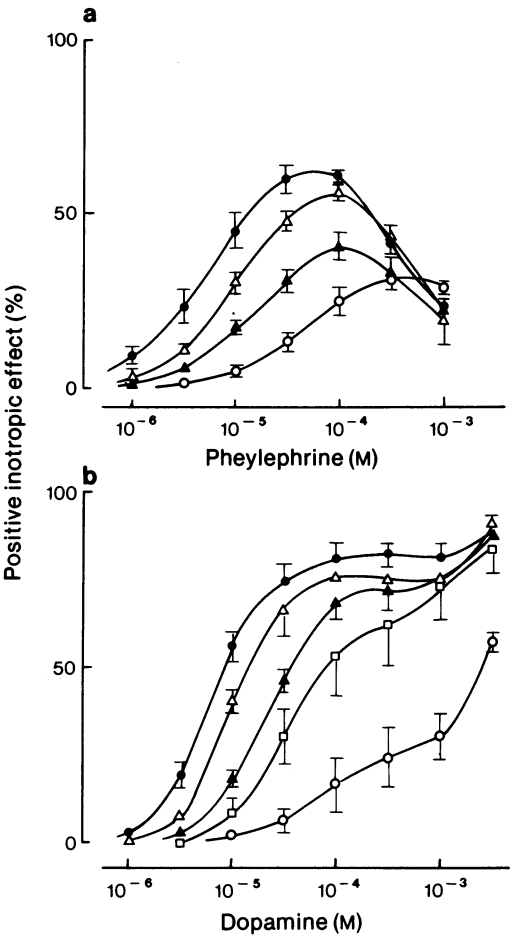

Figure 3 Influence of pindolol on the positive inotropic actions of phenylephrine (a) and of dopamine (b) in dog isolated ventricular strips driven at 0.5 Hz. Ordinates: the positive inotropic actions expressed as a percentage of the maximal contraction; abscissae: molar concentration on a logarithmic scale. (a) Control responses to phenylephrine (●; n = 6) and those in the presence of 3×10^{-9} M (△; n = 6), 10^{-8} M (▲) and 3×10^{-8} M (○) pindolol (n = 4 each) are shown. (b) Control responses to dopamine (●; n = 6) and those in the presence of 3×10^{-9} M (△), 10^{-8} M (▲), 3×10^{-8} M (□) and 10^{-7} M (○) pindolol (n = 4 each). Vertical bars show s.e. means.

Influence of β-adrenoceptor blockade

The positive inotropic actions of PE and dopamine were inhibited by pindolol. In the presence of pindolol (3×10^{-9} M) the maximal response to PE was not significantly reduced but the pD_2-value for PE

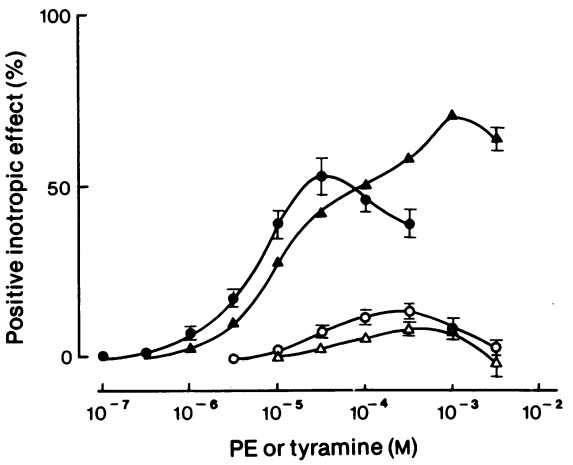

Figure 4 Influence of pretreatment with reserpine on the positive inotropic actions of phenylephrine (PE) and tyramine in dog isolated ventricular strips driven at 0.2 Hz. Ordinate scale: the positive inotropic effect expressed as a percentage of the maximal contraction; abscissa scale: molar concentration on a logarithmic scale. (●): Phenylephrine-control (n = 7); (○): phenylephrine-reserpine (n = 8); (▲): tyramine-control (n = 4); (△); tyramine-reserpine (n = 8). Vertical bars show s.e. means.

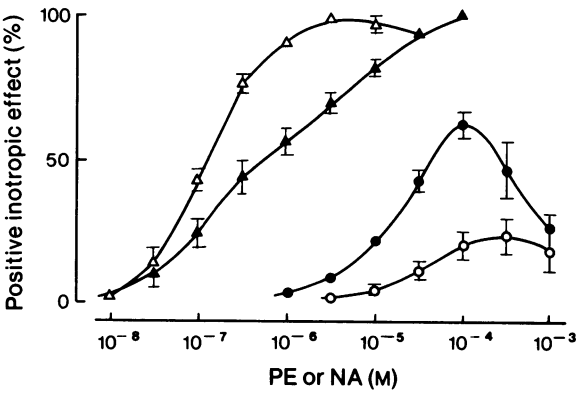

Figure 5 Influence of 10^{-5} M desipramine on the positive inotropic actions of phenylephrine (PE) and noradrenaline (NA) in dog isolated ventricular strips driven at 0.5 Hz. Ordinate scale: the positive inotropic effect expressed as a percentage of the maximal contraction. (▲): Noradrenaline-control (n = 6); (△): noradrenaline-desipramine (n = 4); (●): phenylephrine-control (n = 4); (○): phenylephrine-desipramine (n = 4). Vertical bars show s.e. means.

was significantly decreased to 4.98 ± 0.06 ($P < 0.01$; $n = 6$) from the control value of 5.31 ± 0.07 ($n = 6$). In the presence of higher concentrations of pindolol (10^{-8} M and 3×10^{-8} M) the maximal response to PE was significantly depressed (Figure 3a). On the other hand dopamine responses were antagonized by pindolol in a competitive manner: the maximal response was not affected and the pD_2-value was decreased to 4.95 ± 0.05 ($P < 0.05$ vs control value of 5.31 ± 0.07; $n = 6$), 4.45 ± 0.05 and to 3.86 ± 0.19 in the presence of 3×10^{-9} M, 10^{-8} M and 3×10^{-8} M of pindolol, respectively (Figure 3b). The antagonism between dopamine and pindolol was shown to be competitive by the use of the method of Arunlakshana & Schild (1959): the slope of the regression line for the plot of log $(x - 1)$ against negative log B, where x is the dopamine dose-ratio in the presence of a B molar concentration of pindolol, was −1.07; the curve intersected the abscissa scale at the pA_2-value of 8.5, which is compatible with the value against isoprenaline as agonist (8.9, calculated from 7 muscles) in this preparation. In the presence of pindolol (10^{-7} M) the maximal response to dopamine was also decreased.

Influence of reserpine

Tyramine caused a concentration-dependent positive inotropic effect in ventricular strips not treated with reserpine: the pD_2-value was 4.77 ± 0.04 ($n = 4$) and the intrinsic activity, 0.71 ± 0.01 ($n = 4$) (Figure 4). In muscles excised from animals previously treated with reserpine both the tyramine and PE responses were markedly reduced (Figure 4) although the intrinsic activity of PE (0.14 ± 0.03; $n = 8$) in these muscles was still higher than that of tyramine (0.08 ± 0.03; $n = 8$; $P < 0.05$).

Influence of desipramine

Desipramine (10^{-5} M) decreased the basal developed tension by 32.4 ± 7.1% ($n = 8$) after an equilibration time of 40 min. The positive inotropic effect of noradrenaline was enhanced in the presence of desipramine (10^{-5} M) (Figure 5) and the pD_2-value significantly increased to 6.90 ± 0.06 ($n = 4$; $P < 0.05$) from the control value of 6.27 ± 0.18 ($n = 6$). The threshold concentration of noradrenaline was not affected. On the other hand, PE responses were significantly reduced by desipramine; the intrinsic activity of 0.62 ± 0.05 ($n = 4$) in the control experiments was decreased to 0.23 ± 0.06 ($n = 4$; $P < 0.01$; Figure 5).

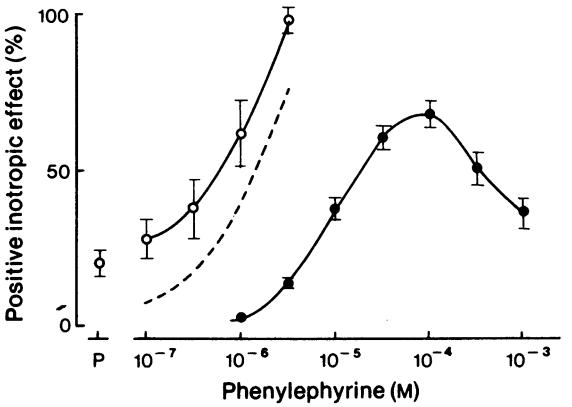

Figure 6 Influence of 10^{-5} M papaverine on the positive inotropic action of phenylephrine in dog isolated ventricular strips driven at 0.5 Hz. Ordinate scale: positive inotropic effect expressed as a percentage of the maximal contraction. Dotted line: the dose-response curve of phenylephrine in the presence of papaverine obtained by subtracting the positive inotropic action of papaverine. (●): Phenylephrine-control ($n = 7$); (○): phenylephrine-papaverine ($n = 5$). Vertical bars show s.e. means. P: positive inotropic action of papaverine.

Influence of papaverine

Papaverine, 10^{-5} M, itself increased developed tension by 20.4 ± 4.4% ($n = 5$) of the maximal contraction and enhanced PE responses (Figure 6). Thus the threshold concentration of PE was decreased to 10^{-7} M (% increase in tension = 7.26 ± 2.33; $n = 5$; $P < 0.05$) in the presence of 10^{-5} M papaverine from 10^{-6} M (% increase = 2.96 ± 0.78; $n = 7$; $P < 0.01$) in the control experiments (Figure 6). It is noteworthy that arrhythmias were elicited in 2 out of 5 muscles by 3×10^{-6} M PE and in all muscles by 10^{-5} M PE in the presence of papaverine.

Influence of temperature

Since α-adrenoceptor-mediated positive inotropic responses have been shown to be affected by change in temperature, or stimulation frequency, in a different manner from that of β-adrenoceptor-mediated responses (Endoh *et al.*, 1975; Endoh & Schümann, 1975a) in isolated rabbit papillary muscle, the influence of temperature on the myocardial responses to PE at different stimulation frequencies was investigated in isolated ventricular strips. The results are presented in Table 2. PE dose-response curves determined at 37°C lay to the right of those at 32°C and the pD_2-value for PE at 37°C (5.24) was significantly smaller than that at 32°C (5.62). However, PE dose-response curves and pD_2-values at 42°C were not different from those at 37°C. The basal tension and the maximal contractions were significantly lower at 42°C than at either 32°C or 37°C at the same stimulation frequency.

Table 2 Influence of temperature on pD_2-values for the positive inotropic effect of phenylephrine (A), and on the basal and maximal contractions induced by calcium (B) at different stimulation frequencies in the isolated ventricular strips of the dog

(A) *pD_2-values*

	32°C	*37°C*	*42°C*
0.2 Hz	5.08 ± 0.12 (9)	5.20 ± 0.06 (18)	—
0.5 Hz	5.29 ± 0.11 (5)	5.10 ± 0.06 (4)	5.05 ± 0.05 (4)
1.0 Hz	5.62 ± 0.08 (6)**	5.24 ± 0.14 (4)*	5.03 ± 0.07 (4)

*$P < 0.05$ compared with the value at 32°C, 1.0 Hz; **$P < 0.05$ compared with the value at 32°C, 0.5 Hz.

(B) *Basal and maximal contractions (mg)*

		32°C		*37°C*		*42°C*	
0.2 Hz	Basal	644 ± 47	(9)	444 ± 52	(18)	—	
	Maximal	2257 ± 347		2754 ± 320			
0.5 Hz	Basal	948 ± 106	(5)	990 ± 87	(4)	168 ± 60*	(4)
	Maximal	1720 ± 155		2580 ± 322		580 ± 91*	
1.0 Hz	Basal	1040 ± 119	(6)	420 ± 31	(4)	143 ± 33*	(4)
	Maximal	2000 ± 177		2128 ± 229		620 ± 176*	

*$P < 0.01$ vs the corresponding values at lower temperatures.
Values in (A) and (B) are mean ± s.e. Number of experiments in parentheses.

Inotropic action of methoxamine and clonidine

Methoxamine and clonidine, which act on rabbit papillary muscle as partial agonists (Schümann & Endoh, 1976), caused only negative inotropic actions on dog ventricular muscle stimulated at low frequencies of 0.2 Hz and 0.5 Hz, respectively, in the same concentration range above 3×10^{-6} M. The active tension was decreased in a concentration-dependent manner and was reduced by $48.7 \pm 5.4\%$ ($n = 4$) and by $47.3 \pm 6.7\%$ ($n = 7$) of the basal value with 10^{-4} M of methoxamine and clonidine, respectively. These negative inotropic actions of methoxamine and clonidine were not affected by phentolamine (10^{-6} M).

Inotropic actions of noradrenaline and adrenaline

The α-adrenoceptor blockade induced by 10^{-6} M phentolamine did not affect the positive inotropic responses of noradrenaline and adrenaline in the presence of 3×10^{-8} M pindolol (Table 3). The pD_2-values for noradrenaline and adrenaline in the presence of 10^{-6} M phentolamine were 5.67 and 5.12 respectively which were not significantly different from those in the absence of phentolamine (5.51 and 5.28, respectively).

Discussion

Blood-perfused preparations

Since it has been shown that the positive inotropic action elicited by stimulation of α-adrenoceptors in the mammalian heart depends very much on the experimental conditions (Nakashima *et al.*, 1973; Endoh & Schümann, 1975a; Endoh, *et al.*, 1975; Ledda *et al.*, 1975; Mugelli *et al.*, 1976; Kunos, 1977; Kunos & Nickerson, 1977), in this study the ventricular myocardium and sinus node of the dog were maintained under near physiological conditions (Endoh, 1975) by perfusing with arterial blood from a donor dog. The actions of PE were then compared with those of Iso. On the heart of other mammalian species PE is the most potent agent in inducing a positive inotropic action through stimulation of α-adrenoceptors (Govier, 1968; Benfey, 1973; Schümann & Endoh, 1976; Endoh, Schümann, Krappitz & Hillen, 1976). Both in inotropic and chronotropic actions PE was about 3.5 log units less potent than Iso. Hence a similar type of adrenoceptor may be responsible for inducing chronotropic as well as inotropic actions in the dog heart under these physiological conditions. The dose-response curve for the positive inotropic action of PE was less steep than that of Iso, while those for the chronotropic action of PE and Iso were parallel to each other; the intrinsic activity of PE was higher for the chronotropic action than for the inotropic action. A depressant action of high concentrations ($> 3 \times 10^{-4}$ M) of PE was consistently observed in canine isolated ventricular strips in the present study and may be a reason for the differences in responsiveness between the papillary muscle and the sinus node. During the infusion of pindolol (approx. 5×10^{-7} M in the perfusion blood) the dose-response curves for both inotropic and chronotropic actions of Iso were shifted to the right in a parallel manner and to the same extent. Since pindolol in this concentration did not itself affect basal developed tension and sinus rate, it may have occupied β-adrenoceptors almost com-

Table 3 Influence of phentolamine (10^{-6} M) on the positive inotropic actions of noradrenaline and adrenaline in dog ventricular strips stimulated at 0.5 Hz in the presence of pindolol (3×10^{-8} M)

		Pindolol	*Pindolol plus phentolamine*
Noradrenaline	n	6	7
	pD_2-value	5.51 ± 0.08	5.67 ± 0.13
	Basal tension (mg) developed	650 ± 80	581 ± 65
	Maximal tension (mg)	1599 ± 108	1810 ± 142
	Calcium response (mg)	1637 ± 151	1828 ± 126
Adrenaline	n	5	5
	pD_2-value	5.28 ± 0.12	5.12 ± 0.05
	Basal tension (mg) developed	584 ± 77	644 ± 63
	Maximal tension (mg)	1800 ± 273	1799 ± 289
	Calcium response (mg)	1952 ± 313	1847 ± 257

Values are mean ± s.e.
The calcium response was the maximum developed tension (in mg) obtainable with this agonist.

pletely without any 'direct' cardiodepressant action. The dose-response curves for the positive inotropic as well as chronotropic actions of PE were shifted markedly to the right in a noncompetitive manner during infusion of this concentration of pindolol. This shows that the positive inotropic and chronotropic actions of PE in the blood-perfused preparations of the dog are mainly caused by stimulation of β-adrenoceptors. This view is supported by the observations that the α-adrenoceptor blocking agent, phentolamine, administered intra-arterially during the pindolol infusion, did not affect the positive inotropic response to large doses of PE.

Isolated ventricular strips

The preparation was driven at low rates (0.2 or 0.5 Hz) since it has been shown, in the rabbit and guinea-pig heart, that the positive inotropic action elicited through stimulation of α-adrenoceptors is more pronounced at lower stimulation frequencies (Endoh & Schümann, 1975a; Mugelli *et al.*, 1976). The intrinsic activity of PE in inducing a positive inotropic effect in dog ventricular muscle equalled that found in the rabbit heart (McNeill, Davis & Muschek, 1972; Schümann, *et al.*, 1974) and amounted to 0.6 in both species. However, the affinity of the drug in the dog ventricle (pD_2-value = 4.96 to 5.13) was lower than that in isolated rabbit papillary muscle determined under similar experimental conditions (pD_2-value = 6.28 to 6.34; Schümann & Endoh, 1976). This suggests that in dog ventricular muscle α-adrenoceptors are not involved in increasing the contractile force even at a low stimulation frequency. In other mammalian species, PE induces positive inotropic effects through stimulation of α-adrenoceptors at low concentrations and through stimulation of β-adrenoceptors at higher concentrations (Govier, 1968; Parr & Urquilla, 1972; Benfey, 1973; Schümann *et al.*, 1974). The present finding that α-adrenoceptor blockade by phentolamine did not affect the positive inotropic action of PE, but that β-adrenoceptor blockade by pindolol inhibited the action of PE in a noncompetitive manner, indicates that the type of adrenoceptors mediating the inotropic effect in the dog ventricle are different from those of other mammalian species (see Introduction) i.e. α-adrenoceptors are not involved and only β-adrenoceptors are responsible for mediating the positive inotropic action in the dog heart. Furthermore, the positive inotropic actions of noradrenaline and adrenaline in the presence of adequate β-adrenoceptor blockade were not influenced by α-adrenoceptor blockade with phentolamine. The observations that the positive inotropic action of dopamine (which was not affected by pindolol because of masking by the α-adrenoceptor-mediated action in the rabbit heart; Endoh *et al.*, 1976) was antagonized in a competitive manner by pindolol in the dog ventricle, and that the other α-adrenoceptor stimulating agents, methoxamine and clonidine which have an α-adrenoceptor-mediated positive inotropic action in other species (Nakashima *et al.*, 1973; Endoh & Schümann, 1975a; Schümann & Endoh, 1976; Rabinowitz *et al.*, 1975), had only a negative inotropic effect in the dog support this view. The results obtained with papaverine, a phosphodiesterase inhibitor and with changes in experimental temperature are also consistent with the view that the adrenoceptors mediating the positive inotropic action in the dog ventricle are only of the β-type. In the rabbit heart phosphodiesterase inhibition by papaverine did not affect positive inotropic responses mediated through α-adrenoceptors, but enhanced those mediated through β-adrenoceptors. Furthermore, the threshold concentration of PE was not changed and the shift to the left of the dose-response curve of PE by papaverine was not parallel; the shift became parallel only when α-adrenoceptors had been blocked beforehand (Schümann, Endoh & Wagner, 1974). In the dog ventricle papaverine decreased the threshold concentration of PE for inducing a positive inotropic action and shifted the dose-response curve for PE to the left. This is similar to the way papaverine modifies the action of Iso in rabbit papillary muscle (Endoh & Schümann, 1975b). The change of temperature affected the positive inotropic action of PE in the dog ventricle in a way which also suggests that the action is elicited through β-adrenoceptors. Raising the temperature from 37°C to 42°C did not affect the dose-response curve of PE in the dog ventricle whereas in the rabbit heart the α-adrenoceptor-mediated inotropic action is greatly reduced by this change of the temperature (Endoh *et al.*, 1975).

In the rabbit and guinea-pig heart the positive inotropic action of PE is not influenced by pretreatment of the animals with reserpine (Schümann *et al.*, 1974; Verma & McNeill, 1976). On the contrary, pretreatment of the dog with reserpine decreased the positive inotropic action of PE; this reduction of the action of PE, however, was slightly less marked than that seen with tyramine. Furthermore, desipramine which enhanced the action of noradrenaline also greatly reduced the effect of PE. It is considered from these observations that in the dog ventricle, PE is mainly taken up into adrenergic nerve endings, then releases noradrenaline and thereby induces the positive inotropic action since it has only a weak effect in the β-adrenoceptors themselves. Thus PE acts on the dog heart to induce the positive inotropic action through a completely different mechanism from that in the other mammalian species. Although other functions of the dog heart may be controlled by stimulation of α-adrenoceptors (as has been recently reported in

Purkinje fibres; Rosen, Hordof, Ilvento & Danilo, 1977), the present experiments clearly show that postsynaptic α-adrenoceptors are not involved in mediating the positive inotropic action in the dog heart.

The authors wish to thank Professor H.J. Schümann and Professor N. Taira for useful comments on the manuscript. This work was partly supported by the Japanese Heart Foundation Research Grant for 1976.

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(Received January 5, 1978
Revised March 23, 1978)