Demonstration in *Tupaia* papillary muscle preparations of α -adrenoceptors mediating positive inotropic effects: comparison with guinea-pigs

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1 Positive inotropic responses to α - and β -adrenoceptor agonists of isolated papillary muscles from the tree shrew (*Tupaia*), were compared with those from guinea-pigs.

2 In *Tupaia*, the concentration-response curve for phenylephrine, unlike that for isoprenaline, was not affected by pindolol in a concentration (10^{-8} M) sufficient to block β -adrenoceptor-mediated responses, but it was significantly shifted to the right by phentolamine (10^{-6} M). In guinea-pig papillary muscles, however, the concentration-response curve for phenylephrine, like that for isoprenaline, was shifted to the right by pindolol (10^{-8} M) but was unaltered by phentolamine (10^{-6} M). Furthermore, when the mean concentrations of agonists inducing maximal positive inotropic responses were compared (relative to that of isoprenaline = 1.0), phenylephrine was found to be only slightly less potent (0.84 ± 0.04 ; $n = 5$) in *Tupaia* and much less potent (0.33 ± 0.06 ; $n = 5$) in the guinea-pig.

3 Although in *Tupaia* papillary muscles the increase in developed tension induced by a combination of phenylephrine and isoprenaline did not significantly differ from that by phenylephrine alone, it was approximately 3 times larger than that produced by phenylephrine alone in guinea-pigs.

4 These results indicate that in papillary muscles from *Tupaia*, unlike the guinea-pig, the positive inotropic effects of phenylephrine can be mediated by α -adrenoceptors.

Introduction

Since Wenzel & Su (1966) first reported the presence of α -adrenoceptors in the myocardium of rats, there has been no doubt about the presence of α -adrenoceptors as possible mediators of positive inotropic effects in the heart of common laboratory mammals and also of primates and man (Wagner & Brodde, 1978; Schümann, 1980; Davey, 1986). Current literature indicates that the positive inotropic effects of sympathomimetic agents arise from combined activation of α - and β -adrenoceptor populations, even though the functional significance of the mammalian myocardial α -adrenoceptors remains uncertain (Benfey, 1982; Endoh, 1986).

Tree shrews (e.g. *Tupaia glis*) have some anatomical (Le Gros Clark, 1971) and serological (Goodman, 1966; Moore & Goodman, 1968) analogies to primitive primates and are characterized by rapid reproduction (Schwaier, 1973; 1975). Although

Tupaia may be a promising experimental animal in various research fields (Noyes, 1968; McClure *et al.*, 1972; Sakai *et al.*, 1979), little attention has been paid to their possible usefulness in applied medical research. Therefore, it was of interest to examine whether or not α -adrenoceptors mediating the positive inotropic response to sympathomimetic agents exists in the heart of *Tupaia*.

The effect of α - and β -adrenoceptor agonists on isolated papillary muscles of *Tupaia*, were compared with effects in the guinea-pig.

Methods

Male adult Hartley guinea-pigs and *Tupaia glis* of either sex (bred in the Chugai Farm) were killed by a blow to the back of the neck under light ether anaesthesia. Papillary muscles with a diameter of 1.5 mm or less were excised from the right ventricle of guinea-pigs, or from the left ventricle (the muscle from the right ventricle is too small) of *Tupaia*. The muscles were mounted in a 20 ml bath maintained at 37°C containing a modified Krebs-Henseleit solution

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Table 1 Basal tension, maximal increase in the developed tension (Max ΔT) induced by phenylephrine or isoprenaline, and $-\log EC_{50}$ values for each agonist after repeated determination of the concentration-response curves (CRC) in the papillary muscles of *Tupaia* or guinea-pig

		Phenylephrine			Isoprenaline		
		$-\log EC_{50}$ values	Developed tension (mg)		$-\log EC_{50}$ values	Developed tension (mg)	
			Basal	Max ΔT		Basal	Max ΔT
<i>Tupaia</i>	1st CRC	4.62 $\pm$ 0.06	155 $\pm$ 41	91 $\pm$ 21	7.26 $\pm$ 0.17	207 $\pm$ 47	106 $\pm$ 26
	2nd CRC	4.56 $\pm$ 0.08	134 $\pm$ 31	67 $\pm$ 16	7.10 $\pm$ 0.24	168 $\pm$ 37	88 $\pm$ 14
	3rd CRC	4.69 $\pm$ 0.07	135 $\pm$ 26	57 $\pm$ 14	7.13 $\pm$ 0.20	153 $\pm$ 34*	80 $\pm$ 13
	4th CRC	4.68 $\pm$ 0.09	150 $\pm$ 29	51 $\pm$ 16*	6.95 $\pm$ 0.24	153 $\pm$ 34*	79 $\pm$ 17
Guinea-pig	1st CRC	4.33 $\pm$ 0.11	176 $\pm$ 49	154 $\pm$ 36	7.80 $\pm$ 0.10	179 $\pm$ 64	560 $\pm$ 193
	2nd CRC	4.06 $\pm$ 0.07	155 $\pm$ 35	98 $\pm$ 32	7.80 $\pm$ 0.11	164 $\pm$ 53	529 $\pm$ 162
	3rd CRC	4.21 $\pm$ 0.08	135 $\pm$ 29	89 $\pm$ 18*	7.79 $\pm$ 0.09	145 $\pm$ 44	551 $\pm$ 175
	4th CRC	4.18 $\pm$ 0.10	134 $\pm$ 30	82 $\pm$ 18*	7.81 $\pm$ 0.09	137 $\pm$ 39	557 $\pm$ 174

Given are means $\pm$ s.e. mean of 5 preparations. * $P < 0.05$ compared with the 1st CRC value.

of the following composition (mM): NaCl 119.0, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose, 10.0; the bathing solution was continuously aerated with 95% O_2 and 5% CO_2 . A tension of 0.5 g was applied to the muscles; they were stimulated by two platinum electrodes using field stimulation from a Nihon Kohden electric stimulator (model SEN-7103) (frequency, 0.5 Hz; intensity, 1.2–1.5 times threshold; duration, 5 ms).

The developed tension of the muscle was measured isometrically with a Nihon Kohden force transducer (TB-611T) attached to a Yokogawa pen recorder (Type 3066; frequency response characteristics: 0.7 Hz). The muscle segments at 0.5 Hz stimulation were first equilibrated for at least 30 min, with changes of the bathing medium every 10–15 min before drug challenge. Then, to assess its responsiveness and stability, the preparation was exposed to a test concentration of phenylephrine or isoprenaline likely to be submaximally effective. When these responses had reached a plateau, the drug was washed out and the preparation allowed to recover for at least 60 min. The relationship between concentration of agonists and inotropic responses was subsequently examined by means of cumulative additions of either phenylephrine or isoprenaline, the final bath concentration being increased in three fold increments. Maximum inotropic responses were obtained for each tissue.

Experimental protocols

After equilibration of the preparation, one of 3 different experimental procedures was then started.

(1) Concentration-response curves to phenylephrine or isoprenaline were constructed 4 times in the absence of any antagonists. After completion of the first curve the bathing fluid was renewed every 10–15 min for 60 min until the developed tension of the

papillary muscle returned to control values; the next concentration-response curve was then constructed.

(2) Concentration-response curves to phenylephrine or isoprenaline were constructed in the absence of antagonists. It was confirmed that there were no significant differences between the corresponding values obtained from the second and third curves (Table 1). After the second concentration-response curve was constructed, the tissue was washed out repeatedly in the absence of antagonists and equilibrated for at least 60 min. An antagonist was added to the organ bath and, 30 min later, the cumulative concentration-response curve to an agonist drug was constructed again for each tissue.

(3) Papillary muscles were exposed to a high concentration of phenylephrine, which was incubated with the tissue for 5–10 min before successive applications of isoprenaline.

Drugs

Drugs used were phentolamine mesylate (Ciba-Geigy), (–)-isoprenaline hydrochloride (Nikken Kagaku), (–)-phenylephrine hydrochloride (Sigma), and (±)-pindolol base (Sandoz). All drugs were dissolved in distilled water, and/or diluted with 0.9% saline solution. Drug solutions were usually added to the bath in a volume of 0.1 ml in individual syringes (Terumo Co.). All drug concentrations are expressed as final molar (M) concentrations in the bathing solution.

Statistical analysis

Values in the text are means $\pm$ s.e. mean. The concentrations causing 50% of the maximum response to each agonist (EC_{50} value) for phenylephrine or isoprenaline were determined from each concentration-response curve. The statistical significance of the differences between mean values was

estimated by means of Student's *t* test and expressed as a *P* value; a value of 0.05 or less was considered significant.

Results

Repeated determination of concentration-response curves for phenylephrine or isoprenaline

Summarized data are shown in Table 1. Phenylephrine over the concentration-range 10^{-7} to 10^{-3} M elicited a concentration-dependent increase in developed tension in papillary muscle preparations of *Tupaia* and guinea-pig, and at 3×10^{-4} M the concentration-response curve reached a maximum (Figures 1 and 2). Although the curve for phenylephrine was constructed 4 times in the absence of any antagonists, the basal developed tension remained unchanged by this procedure. Likewise, the negative logarithm of the EC_{50} ($-\log EC_{50}$), values for phenylephrine calculated from four consecutive concentration-response curves, were nearly identical. Even though there were no significant differences between the corresponding values obtained from the second, third and fourth curves, the maximum increases in the developed tension in the three consecutive curves, compared with those in the first curve, were considerably diminished. The degree of attenuation was larger in the guinea-pig than in *Tupaia*.

Figure 1 Effects of pindolol and/or phentolamine on the positive inotropic responses to phenylephrine or isoprenaline in *Tupaia* papillary muscles. The concentration-response curves for phenylephrine (a) or isoprenaline (b) were constructed in the absence or presence of either phentolamine or pindolol. Increases in tension are plotted as a percentage of the maximum response. Each point represents mean of 10 (phenylephrine control) or 5 (the others) experiments; vertical lines show s.e.mean: (○) Control; (●) pindolol 10^{-8} M; (■) phentolamine 10^{-6} M. **P* < 0.05 ****P* < 0.001 compared to the corresponding value from the group not treated with any antagonists (control).

Isoprenaline (10^{-9} to 10^{-6} M) caused a concentration-dependent positive inotropic effect in papillary muscle preparations of *Tupaia* and guinea-pig (Figures 1 and 2). In guinea-pigs, neither the basal developed tension nor the maximum positive inotropic effect of isoprenaline was significantly changed by this procedure. Additionally, the $-\log EC_{50}$ values for isoprenaline calculated from four successive concentration-response curves were also identical, suggesting that no spontaneous decrease in sensitivity to isoprenaline had taken place. A consistent finding in *Tupaia*, was a significant reduction in basal tension following the construction of the first concentration-response curve for isoprenaline; the basal tensions for the third and fourth curves were significantly lower than that for the first curve but there were no significant differences between the relevant values for second, third and fourth curves.

The mean molar concentrations of agonists inducing maximal positive inotropic responses were compared by assigning the value 1.0 to the values of isoprenaline. In *Tupaia* phenylephrine (0.84 ± 0.04 ; $n = 5$) was only slightly less active than isoprenaline, whereas in the guinea-pig, it had only one-third (0.33 ± 0.06 ; $n = 5$) of the potency of isoprenaline.

Effects of β - and α -adrenoceptor antagonists on the positive inotropic response to isoprenaline and phenylephrine

In *Tupaia* papillary muscles, pindolol (10^{-8} M) failed to affect the concentration-response curve for the papillary muscle tension responses to phenylephrine (Figure 1). The $-\log EC_{50}$ value for phenylephrine after blockade of β -adrenoceptors was 4.86 ± 0.15 ($n = 5$) and did not differ significantly from that of the control (4.99 ± 0.09 ; $n = 10$). The concentration of pindolol (10^{-8} M) used was sufficient to block β -adrenoceptors, as shown by a rightward shift of the concentration-response curve for isoprenaline (Figure 1). A significant difference (*P* < 0.05) was observed with the $-\log EC_{50}$ value for isoprenaline between groups treated (6.67 ± 0.18 ; $n = 5$) and untreated (7.16 ± 0.11 ; $n = 5$) with pindolol (10^{-8} M), and the concentration-ratio (CR; EC_{50} value of agonist in the presence of antagonist divided by the control EC_{50} value) was 3.57 ± 1.03 ($n = 5$). On the other hand, the α -adrenoceptor antagonist, phentolamine, significantly blocked the positive inotropic effect of phenylephrine. The concentration-response curve for phenylephrine was shifted to the right in the presence of phentolamine (10^{-6} M) (Figure 1). The $-\log EC_{50}$ value for phenylephrine was significantly (*P* < 0.05) reduced from 4.99 ± 0.09 ($n = 10$) to 4.52 ± 0.16 ($n = 5$) in the presence of phentolamine (CR = 3.47 ± 0.58 ; $n = 5$).

Figure 2 Effects of pindolol and/or phentolamine on the positive inotropic responses to phenylephrine or isoprenaline in guinea-pig papillary muscles. The concentration-response curves for phenylephrine (a) or isoprenaline (b) were constructed in the absence or presence of either phentolamine or pindolol. Increases in tension are plotted as a percentage of the maximum response. Each point represents mean of 10 (phenylephrine control) or 5 (the others) experiments; vertical lines show s.e.mean. (○) Control; (●) pindolol 10^{-8} M; (■) phentolamine 10^{-6} M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the corresponding value from the group not treated with any antagonists (control).

In guinea-pig papillary muscles, pindolol (10^{-8} M) caused a rightward shift of the concentration-response curve for phenylephrine as well as for isoprenaline (Figure 2). The $-\log EC_{50}$ values for phenylephrine and isoprenaline were significantly reduced from 4.56 ± 0.10 ($n = 10$) to 3.99 ± 0.18 ($n = 5$) ($P < 0.05$, CR = 3.18 ± 0.71 ; $n = 5$) and from 7.80 ± 0.10 to 6.92 ± 0.13 ($P < 0.001$, each $n = 5$, CR = 9.86 ± 3.64 ; $n = 5$) in the presence of pindolol (10^{-8} M), respectively. The treatment with phentolamine (10^{-6} M) did not affect the concentration-response curve for phenylephrine. No significant difference was observed in the $-\log EC_{50}$ value for phenylephrine between treated (4.36 ± 0.02 ; $n = 5$) and untreated groups (4.56 ± 0.10 ; $n = 10$) with phentolamine (10^{-6} M).

When compared with the $-\log EC_{50}$ values for phenylephrine and isoprenaline in papillary muscles from *Tupaia* and the guinea-pig, *Tupaia* possessed a significantly higher sensitivity to phenylephrine (*Tupaia*, 4.99 ± 0.09 ; guinea-pig, 4.56 ± 0.10 ; $P < 0.05$, each $n = 10$), whereas the guinea-pig had significantly higher sensitivity to isoprenaline (*Tupaia*, 7.16 ± 0.11 ; guinea-pig, 7.80 ± 0.10 ; $P < 0.05$, each $n = 5$). There were no significant changes in maximum developed tension between treated and untreated groups with each antagonist (not shown).

Positive inotropic responses to simultaneous muscle stimulation by α - and β -adrenoceptor agonists

Papillary muscles from *Tupaia* and guinea-pig were exposed to a maximal concentration of phenylephrine (10^{-3} M). In the presence of phenylephrine, isoprenaline in a concentration (10^{-6} M) sufficient to cause a maximum response was added to the bath. In *Tupaia* papillary muscles, the increase in developed tension induced by a combination of phenylephrine and isoprenaline (95 ± 19 mg; $n = 5$) did not significantly differ from that induced by phenylephrine alone (73 ± 16 mg; $n = 5$). On the other hand, in guinea-pig papillary muscle preparations, the combination of phenylephrine and isoprenaline produced a developed tension (365 ± 70 mg; $n = 5$), which was approximately 3 times higher than that with phenylephrine alone (123 ± 29 mg; $n = 5$).

Discussion

The present experiment revealed that in *Tupaia* papillary muscles the concentration-response curve for phenylephrine was shifted to the right by phentolamine, whereas it was not affected by pindolol in a concentration sufficient to block the β -adrenoceptors. This finding is consistent with results of previous studies on rat cardiac tissues which showed that positive inotropic responses to phenylephrine were mainly due to α -adrenoceptor stimulation (Wagner & Brodde, 1978; Shibata *et al.*, 1980; Williamson & Broadley, 1987). On the other hand, it was noted that in guinea-pig papillary muscles the concentration-response curve for phenylephrine remained unaffected by phentolamine, but like isoprenaline, was significantly shifted to the right by pindolol. Thus, in papillary muscles of the guinea-pig, unlike *Tupaia*, we could not demonstrate the presence of any functionally active α -adrenoceptors under the present conditions.

The question whether phenylephrine causes positive inotropic responses of the myocardium through stimulation of α - and β -adrenoceptors is still under investigation. The α -adrenoceptor-mediated positive inotropic response to phenylephrine appears to be concentration-dependent, species-dependent and varies between different regions of the heart (Schümann *et al.*, 1974; Wagner & Brodde, 1978; Shibata *et al.*, 1980). Nevertheless, there has been a substantial amount of evidence from both functional and binding studies supporting the existence of post-junctional α -adrenoceptors in the mammalian heart, including human heart (Wagner & Brodde, 1978; Benfey, 1982; Endoh, 1986).

In the present experiments, the potency of phenylephrine, relative to that of isoprenaline, probably indicates that in *Tupaia* papillary muscles the posi-

tive inotropic effect of phenylephrine is mediated almost exclusively by α -adrenoceptors, but in guinea-pig predominantly via β -adrenoceptors. Moreover, it should be noted that in *Tupaia* papillary muscles, the increase in developed tension caused by a combination of phenylephrine and isoprenaline did not significantly differ from that caused by phenylephrine alone, whereas in guinea-pig papillary muscles the response to a combined use of phenylephrine and isoprenaline, compared with phenylephrine alone, was approximately three times greater with respect to the developed tension. This finding lends further support to the idea that α -adrenoceptors may play a functionally significant role in the regulation of *Tupaia* myocardial contraction and provides further evidence for the existence of α -adrenoceptors mediating the positive inotropic effect in *Tupaia* papillary muscles.

For the present study, the α -adrenoceptor subtypes in the papillary muscle preparations could not be classified because phentolamine, a non-selective α -adrenoceptor antagonist (Davey, 1986), was used. However, it is known that stimulation of

α_1 -adrenoceptors produces a positive inotropic effect in cardiac tissues from cat, guinea-pig, rabbit, rat and man (Davey, 1986; Ask *et al.*, 1987; Williamson & Broadley, 1987). Taken together, these findings suggest that the α -adrenoceptor subtypes in *Tupaia* papillary muscles also may be of the α_1 -type, but this requires further investigation.

In conclusion, the present study demonstrated the existence of functionally active α -adrenoceptors in *Tupaia* papillary muscles, which clearly mediated the positive inotropic effect in the presence and absence of a β -adrenoceptor antagonist. Although myocardial β -adrenoceptors are well known to mediate effects of sympathetic nerve stimulation and sympathomimetic agents, it is still doubtful whether myocardial α -adrenoceptors have a clinically physiological significance in man. In view of this, the papillary muscle preparation from *Tupaia*, a primitive primate (Schwaier, 1973; 1975) may provide a useful alternative to the other species for studying the physiological role of α -adrenoceptors in the myocardium.

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