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Comparison of the β-adrenoceptors in the myocardium and coronary vasculature of the kitten heart

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The relative potencies of (−)-noradrenaline, (−)-adrenaline, (±)-isoprenaline and (±)-salbutamol have been assessed for their positive inotropic and chronotropic actions on kitten isolated atria. These relative potencies have been compared with those obtained for the relative coronary dilator potencies in two preparations. These were intact hearts perfused by Langendorff's method and isolated perfused coronary arteries from the kitten. The relative molar potencies for inotropic effects were noradrenaline 1: adrenaline 1: isoprenaline 7: salbutamol 0·6. The observed ratios for chronotropic effects were not significantly different from those for inotropic effects. The relative potencies of noradrenaline, adrenaline and isoprenaline as myocardial stimulants were similar to their relative potencies as coronary dilators in the intact heart. Similar relative potencies for noradrenaline and isoprenaline were also found in the isolated coronary artery but adrenaline was only one third as active as noradrenaline on this preparation. In both the intact heart and in the isolated coronary artery salbutamol was about one tenth as active as noradrenaline. It was therefore less active relative to noradrenaline as a coronary dilator than as a myocardial stimulant. In spite of these differences in relative potency ratios for myocardial and coronary dilator effects, similar dissociation constants (K_B values) for practolol against isoprenaline were found in driven atria and in isolated coronary arteries. Myocardial and coronary vascular β-adrenoceptors thus can both be placed in the general classification of β_1-adrenoceptors.

Studies using isolated coronary artery preparations indicate that in the dog (Baron, Speden & Bohr, 1972) coronary adrenoceptors resemble myocardial β_1-adrenoceptors. Drew & Levy (1972) and Johansson (1973) reached a similar conclusion from their experiments in the pig whilst Bayer, Mentz & Förster (1972) suggested that pig coronary adrenoceptors were comparable to peripheral vascular β_2-receptors. In guinea-pig perfused hearts Broadley (1970) also found a difference between myocardial and coronary β-adrenoceptors. This author showed that when myocardial β-adrenoceptors were blocked with the β_1-adrenoceptor antagonist practolol, coronary dilator responses to isoprenaline and the β_2-selective compound salbutamol persisted. On the other hand, both myocardial and coronary dilator responses were blocked by propranolol, a non-selective antagonist at both β_1- and β_2-adrenoceptor sites.

In view of the various classifications of coronary β-adrenoceptors outlined above, it was decided to study and compare the types of β-adrenoceptors present in coronary artery and atrial preparations from the kitten.

The coronary arteries of the kitten can be readily dissected free from myocardial tissue and, like those in the intact heart, spontaneously develop and maintain tone during perfusion (Cornish, Miller & Tolmer, 1974). The anterior descending branch of the left coronary artery is a useful preparation for studying β-adrenoceptor-mediated responses since the effects produced are not complicated by concomitant α-adrenoceptor induced constrictor activity (Cornish & others, 1974)

METHODS

Kittens of either sex, 500–850 g, were anaesthetized with ether and the heart removed and placed in cold McEwen solution (1956) gassed with 5% CO_2 in oxygen. The aorta was quickly cannulated and the heart perfused by Langendorff's method. Various isolated preparations from the heart were set up as described below in oxygenated McEwen solution. The solution was maintained at 37° for isolated atria and the Langendorff heart and at 32° for the isolated coronary artery preparation.

Isolated atria

Atrial preparations were placed under a resting tension of 2 g and contractions recorded using a Grass Force-Displacement Transducer (FTO3C) coupled to a Grass Model 79 Polygraph. Chronotropic activity was measured using a Grass tachograph model 7P4C. Spontaneously beating whole atria were used to study the chronotropic effects and atria driven at 4 Hz were used to study the inotropic effects of the compounds.

Langendorff heart

The coronary vessels were perfused by means of a Watson-Marlow H.R. Flow Inducer and resistance of the preparation to perfusion was measured using a mercury manometer with a float writing on a smoked drum. An initial perfusion rate of 10 ml min^{-1} was used and this was gradually increased in 0·5 ml min^{-1} increments over a period of 30 min. During this time the vessels began to develop tone. At the maximal perfusion rates used (13–15 ml min^{-1}), most of the preparations developed a resistance to perfusion of about 75 mm Hg. Once a steady perfusion pressure was obtained single doses of the compounds under study were injected into a rubber tube connecting the aortic cannula to the perfusion apparatus and changes in perfusion pressure monitored. All doses were administered in a volume of 0·1 ml.

Isolated coronary artery

The anterior descending branch of the left coronary artery was dissected free from myocardial tissue and perfused as previously described (Cornish & others, 1974). The arteries spontaneously developed a resistance to perfusion (30–90 mm Hg) and when this became constant single doses of compounds under test were injected in a dose volume of 0·05 ml. Changes in perfusion pressure were measured on a smoked drum using a mercury manometer.

Drugs

(−)-Noradrenaline bitartrate, (−)-adrenaline bitartrate and (−)-phenylephrine hydrochloride (Sigma), (±)-isoprenaline hydrochloride (Winthrop), (±)-salbutamol

sulphate and oxymetazoline hydrochloride (Glaxo-Allenburys), phentolamine mesylate (Ciba), cocaine hydrochloride (May and Baker), practolol and propranolol hydrochloride (Imperial Chemical Industries).

RESULTS

Myocardial stimulation

Inotropic and chronotropic responses in isolated atria. In each experiment two or three cumulative concentration-effect curves to noradrenaline, adrenaline and isoprenaline were first established, then a single concentration-effect curve to salbutamol was obtained. The maximal inotropic and chronotropic responses were similar for salbutamol and the other amines. The molar potency ratios for adrenaline, isoprenaline and salbutamol relative to noradrenaline (= 1) were calculated from the concentrations producing 50% of the maximal increase in rate or force of the atria (Table 1). The four amines gave similar potency ratios for both inotropic and chronotropic effects in atria (comparisons gave P values ranging from 0·2 to 0·6).

Table 1. *Comparison of molar potency ratios for β-adrenoceptor agonists.*

Preparation	Response	(−)NA	(−)ADR	(±)ISO	(±)SAL
Atria	increase in force	1	1·0 ± 0·2	7·0 ± 1·3	0·62 ± 0·10
Atria	increase in rate	1	0·80 ± 0·11	8·3 ± 1·5	0·45 ± 0·09
Langendorff heart	coronary dilatation	1	1·0 ± 0·1	7·4 ± 1·5	0·13† ± 0·03
Isolated coronary artery	dilatation	1	0·29* ± 0·03	8·5 ± 0·7	0·15† ± 0·07

NA = noradrenaline ADR = adrenaline ISO = isoprenaline SAL = salbutamol.
Values shown are means together with their standard errors obtained from 4–8 experiments.
* Significantly different from ratios for adrenaline on other preparations ($P<0·05$).
† Significantly different from ratios for myocardial effects of salbutamol ($P<0·05$).

Coronary dilatation

Langendorff heart and isolated coronary artery. Noradrenaline, adrenaline, isoprenaline and salbutamol produced dose-related decreases in resistance to perfusion in both the intact heart perfused by Langendorff's method and in the isolated coronary artery preparation. In both preparations, the time course of the coronary dilator response was similar as was the threshold dose of noradrenaline (5×10^{-11} mol) required to produce dilatation. Typical responses of an isolated coronary artery to different doses of noradrenaline are shown in Fig. 1.

The dilator responses to noradrenaline, adrenaline, isoprenaline and salbutamol were abolished following addition of propranolol (1×10^{-6}M) to the perfusion fluid. After propranolol no α-mediated constrictor responses were evident. However in the intact heart the more selective α-adrenoceptor agonists phenylephrine (5×10^{-8} mol) and oxymetazoline (1×10^{-7} mol) (Mujic & van Rossum, 1965) produced constriction of the coronary arteries. This constrictor response was most notable in preparations where the perfusion pressure was low (<30 mm Hg). At higher perfusion pressures these drugs produced constriction followed by dilatation or dilatation alone. The constrictor responses were abolished by phentolamine (2×10^{-6}M)

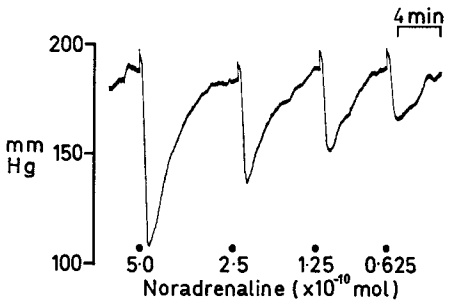

FIG. 1. Responses of an isolated coronary artery from a kitten to single injections of noradrenaline.

suggesting that they were due to stimulation of α-adrenoceptors. In the isolated coronary artery preparation phenylephrine and oxymetazoline produced only dilator responses (Cornish & others, 1974). α-Adrenoceptor-mediated constrictor actions were not observed even at low perfusion pressures.

In initial experiments where dilator responses of the amines were assessed it was noted that reproducible responses to noradrenaline, adrenaline and isoprenaline could be obtained over periods of 3 to 4 h. However, after injections of salbutamol responses to succeeding doses of the catecholamines were slightly depressed. Therefore, in experiments where the coronary dilator responses to the amines were compared, responses to random doses of noradrenaline, adrenaline and isoprenaline were first obtained and then responses to three or four doses of salbutamol were examined. There were no significant differences in the maximal dilator responses to the four amines. Molar potency ratios for the dilator actions of the amines (noradrenaline = 1) were calculated from the concentrations producing 50% of the maximal responses. These ratios are shown in Table 1. The molar potency ratios for isoprenaline and salbutamol relative to noradrenaline in the intact heart were not significantly different from those in the isolated coronary artery preparation. Adrenaline and noradrenaline had similar dilator potencies in the intact heart, but adrenaline was significantly less potent than noradrenaline in the isolated coronary artery preparation ($0{\cdot}01 > P > 0{\cdot}001$).

In view of the weak α-adrenoceptor mediated vasoconstrictor responses which could be elicited by phenylephrine and oxymetazoline in the intact heart, further

Table 2. *Comparison of molar potency ratios for β-adrenoceptor agonists as coronary dilators in the Langendorff heart preparation. Effects of phentolamine and cocaine.*

Treatment	(−)NA	(−)ADR	(±)ISO	(±)SAL
No blockers	1	1·0 ± 0·1	7·4 ± 1·5	0·13 ± 0·03
Phentolamine 2×10^{-6}M	1	0·76 ± 0·08	6·2 ± 1·0	0·053 ± 0·014
Phentolamine 2×10^{-6}M	1	0·41* ± 0·09	4·7 ± 1·1	0·037* ± 0·004
Cocaine 1×10^{-5}M				
Phentolamine 2×10^{-6}M	1	0·39* ± 0·07	3·0* ± 0·06	0·033* ± 0·006
Cocaine 3×10^{-5}M				

Values shown are means together with their standard errors obtained from 4–7 experiments.
* Significantly different from ratios obtained in the absence of phentolamine and cocaine ($P < 0{\cdot}05$).

experiments were performed in this preparation in which the relative potencies of the amines were assessed in the presence of phentolamine (2×10^{-6}M). There were no significant differences in the relative potencies of the amines in the presence and absence of this α-adrenoceptor antagonist (Table 2).

The potencies of adrenaline and isoprenaline relative to noradrenaline in the intact heart, and of isoprenaline relative to noradrenaline in the isolated coronary artery were not significantly different from the relative potencies of these amines in atria (Table 1). In the isolated coronary artery, the potency of adrenaline relative to noradrenaline was 3 times less than in atria. In both the intact heart and the isolated coronary artery the potency of salbutamol relative to noradrenaline was 3–4 times less than in atria.

β_1-Adrenoceptor antagonism

Driven atria and isolated coronary artery. The ability of practolol ($1{\cdot}88 \times 10^{-6}$M) to antagonize isoprenaline-induced inotropic responses in atria and vasodilator responses in coronary artery preparations was assessed. An antagonist contact-time of 30 min was used in these experiments. Dose-ratios were taken at 50% of the maximal responses to isoprenaline and dissociation constants calculated. From 4 experiments in each preparation, mean K_B values ($\pm$ s.e.) of $2{\cdot}8$ ($\pm$ $0{\cdot}1$) $\times 10^{-7}$ and $2{\cdot}6$ ($\pm$ $0{\cdot}4$) $\times 10^{-7}$ were obtained for atria and coronary arteries respectively. These K_B values are not significantly different ($0{\cdot}4 > P > 0{\cdot}3$).

Inhibition of Uptake$_1$

Langendorff heart and isolated coronary artery. The observed differences in the potency of adrenaline relative to noradrenaline in the intact heart and isolated coronary artery might be due to differences in the uptake processes involved in the two preparations. Further experiments were therefore performed in which the relative potencies of the amines were assessed in the presence of cocaine (1 or 3×10^{-5}M), an inhibitor of the Uptake$_1$ process (Iversen, 1965). In these experiments phentolamine (2×10^{-6}M) was also included in the perfusion to preclude possible actions of the drugs at α-adrenoceptors. In the presence of these two compounds the sensitivity of the coronary vessels in the intact heart to noradrenaline was increased approximately twofold. This change was associated with a significant decrease in the potency of the other amines relative to noradrenaline (Table 2). The decrease in the potency of isoprenaline relative to noradrenaline was significant in the presence of 3×10^{-5}M cocaine but was not significant when the lower concentration of the uptake blocker was used.

In the isolated coronary artery perfusion pressure was maintained in the presence of cocaine (1×10^{-5}M) and phentolamine (2×10^{-6}M) but was not maintained with a higher concentration of cocaine (3×10^{-5}M) in the perfusion fluid. In the presence of cocaine (1×10^{-5}M) and phentolamine (2×10^{-6}M) there was a twofold decrease in the potency of isoprenaline relative to both noradrenaline and adrenaline (Table 3). Although an apparent decrease was observed in the potency of salbutamol relative to noradrenaline and adrenaline, this decrease was not statistically significant ($0{\cdot}1 > P > 0{\cdot}05$). The molar potency ratios of the amines found for the isolated coronary artery in the presence of cocaine and phentolamine (Table 3) were not significantly different from those found in the intact heart under the same experimental conditions (Table 2).

Table 3. *Comparison of molar potency ratios for β-adrenoceptor agonists as dilators of the isolated coronary artery. Effects of phentolamine and cocaine.*

Treatment	(−)NA	(−)ADR	(±)ISO	(±)SAL
No blockers	1	0·29 ± 0·03	8·5 ± 0·7	0·15 ± 0·07
Phentolamine 2×10^{-6}M	1	0·39 ± 0·08	4·2* ± 0·08	0·017 ± 0·009
Cocaine 1×10^{-5}M				

Values shown are means together with their standard errors obtained from 4–5 experiments.
* Significantly different from ratio obtained in the absence of phentolamine and cocaine ($P<0·05$).

DISCUSSION

In the intact kitten heart both α-adrenoceptor mediated coronary constrictor responses and β-adrenoceptor mediated coronary dilator responses to sympathomimetic amines could be demonstrated. In contrast, in the isolated coronary artery preparation used in the present study only β-adrenoceptor mediated dilator responses to sympathomimetic amines occurred (Cornish & others, 1974). Bohr (1967) has shown that the large coronary arteries of the dog contain both α- and β-adrenoceptors, whereas the small vessels contain only β-adrenoceptors. It is therefore possible that the α-adrenoceptors found in the intact kitten heart are present in larger coronary arteries than the branch used for the isolated coronary artery preparation. In the intact heart, stimulation of α-adrenoceptors did not appear to be of any significance when assessing the relative dilator potencies of noradrenaline, adrenaline, isoprenaline and salbutamol, since the potencies of noradrenaline and adrenaline relative to the other amines did not increase significantly in the presence of the α-adrenoceptor antagonist phentolamine (Table 2).

There was a marked similarity in the sensitivity and responses of the coronary vasculature in the intact heart and the isolated coronary artery to noradrenaline. This observation, together with comparable potency ratios for isoprenaline and salbutamol relative to noradrenaline in the two preparations (Table 1), suggests that the dilator effects of these amines in the intact heart were due to stimulation of β-adrenoceptors in the coronary vessels. On the other hand, the preparations showed a threefold difference in their sensitivity to adrenaline. The absence of α-adrenoceptors in the isolated coronary artery rules out the possibility that α-mediated constrictor effects were responsible for the lower dilator potency of adrenaline in this preparation. In the intact heart, the potency of adrenaline relative to noradrenaline as a coronary dilator was not significantly different from its potency relative to noradrenaline on the atrial preparations (Table 1). Coronary vessels in the intact heart may thus be affected more by the myocardial effects of adrenaline than by its direct effects on coronary vascular adrenoceptors. If this is the case, then noradrenaline and isoprenaline may also produce coronary dilatation secondary to myocardial stimulation. The marked similarity in the relative potencies of noradrenaline and isoprenaline on all the preparations studied (Table 1) do not, however, allow their effects on myocardial and coronary β-adrenoceptors to be differentiated.

An alternative explanation for the differences in the relative potencies of noradrenaline and adrenaline in the intact heart and isolated coronary artery is that they are due to differences in uptake mechanisms in the two preparations. Certainly in the presence of cocaine, an inhibitor of $Uptake_1$ (Iversen, 1965) no significant

difference was found in the relative potencies of noradrenaline and adrenaline on the two preparations. However, this result is not unequivocal. At the doses tested, cocaine possesses non-specific depressant activity. We found, for example, that in the presence of cocaine (1×10^{-5}M) atria could not be driven at 4 Hz and spontaneously beating atria failed to produce the same maximal increase in rate in response to noradrenaline as untreated atria. In the intact heart cocaine may thus depress myocardial activity allowing the direct effects of noradrenaline and adrenaline on the coronary vessels to dominate. This would account for the close similarity in the dilator potency ratios for noradrenaline, adrenaline, isoprenaline and salbutamol in the intact heart and isolated coronary artery in the presence of cocaine (Tables 2 and 3). It is possible that further experiments with an Uptake$_1$ inhibitor which has less non-specific depressant activity, such as desipramine (Iversen, 1965), would demonstrate which of the two explanations is the correct one.

Salbutamol was significantly less active relative to noradrenaline as a coronary dilator than as a myocardial stimulant. Since its potency relative to noradrenaline was not significantly different in the intact heart and isolated coronary artery, it probably owes its dilator activity in the whole heart to direct stimulation of coronary adrenoceptors.

Despite the fact that the potencies of adrenaline and salbutamol relative to noradrenaline in the isolated artery were 3–4 times less than their relative potencies in driven atria, K_B values for practolol against isoprenaline on the two preparations were very similar. The K_B values for the kitten preparations are of the same order as those reported for guinea-pig and rabbit atria and dog coronary arteries (Table 4). They also resemble the K_B values found in pig coronary arteries by Drew & Levy (1972) but differ by a factor of 1000 from the K_B value found by Bayer & others (1972). The similarity of the β-adrenoceptors present in the coronary vessels of the kitten and dog is evident not only from the similarity in K_B values but also from a comparison of the vasodilator potency ratios. These were noradrenaline 1: adrenaline 0·3: isoprenaline 7 found for the kitten and noradrenaline 1: adrenaline 0·1: isoprenaline 3 found for the dog (Bohr, 1967).

The potency ratios found for the kitten atrial and coronary preparations contrast

Table 4. K_B *values for practolol against isoprenaline on isolated coronary arteries and isolated atria from different species.*

Tissue		Molar concn practolol	K_B	Reference
Coronary artery				
	Kitten	$1{\cdot}88 \times 10^{-6}$	$2{\cdot}6 \times 10^{-7}$	Present study
	Dog	10^{-6} to 10^{-5}	$1{\cdot}5 \times 10^{-7}$	Baron & others, 1972
	Pig	0·37 to $7{\cdot}4 \times 10^{-6}$	$2{\cdot}6 \times 10^{-7}$	Drew & Levy, 1972
	Pig	7×10^{-6}	$2{\cdot}6 \times 10^{-7}$	Bayer, & others, 1972
Atria				
	Kitten	$1{\cdot}88 \times 10^{-6}$	$2{\cdot}8 \times 10^{-7}$	Present study
	Guinea-pig	4×10^{-7}	$2{\cdot}8 \times 10^{-7}$	Bayer & others, 1972
	Rabbit	10^{-7} to 10^{-4}	$1{\cdot}7$ to $2{\cdot}6 \times 10^{-7}$	Bristow & Green, 1970 Wale, 1970

markedly with those found for tissues containing β_2-type adrenoceptors. For example, we obtained ratios of noradrenaline 1: adrenaline 1340: isoprenaline 4340: salbutamol 1740 for relaxation of the kitten uterus (Cornish & Miller, 1973).

It would seem reasonable to conclude that β-adrenoceptors in the coronary arteries are far more like β_1-myocardial receptors than β_2-adrenoceptors but that the receptors in the coronary vasculature and myocardial tissue do not respond identically to adrenergic agonists.

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