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THE INFLUENCE OF DRUGS ON THE OVERFLOW OF NORADRENALINE AND THE IDENTIFICATION OF RECEPTORS IN GUINEA-PIG ATRIA

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1 Salbutamol (1.0 μM) and isoprenaline (1.2 nM) significantly increased the fractional release of tritiated noradrenaline from driven left atria but phentolamine (10 μM) failed to do so. Butoxamine (4.0 μM) blocked the increase in overflow produced by isoprenaline. Isoprenaline (1.2 nM), phentolamine (10.0 μM) and salbutamol (1.0 μM) failed to increase the overflow of tritiated noradrenaline from spontaneously beating atria.

2 Spontaneously beating atria were therefore used to identify the receptors mediating chronotropism and inotropism.

3 There was no clear relationship between inotropism and chronotropism.

4 The inotropic effects of both dobutamine (0.04–4.0 μM) and isoprenaline (0.11–9.0 nM) were inhibited by practolol (4.0 μM) and by butoxamine (4.0 μM). The chronotropic effects were inhibited only by practolol (4.0 μM).

5 Both inotropic and chronotropic effects of noradrenaline (3.0–200 nM) were antagonized by practolol (4.0 μM), but not by butoxamine (4.0 μM). Thus both functions appeared to be mediated by β_1-adrenoceptors when noradrenaline was the agonist.

6 Inotropic responses to salbutamol (0.45–7.5 μM) were inhibited by both practolol (4.0 μM) and by butoxamine (4.0 μM), but chronotropic responses were anatgonized only by butoxamine (4.0 μM). Thus salbutamol acts on both β_1- and β_2-adrenoceptors to produce an inotropic response but only on β_2-adrenoceptors to produce its chronotropic response.

7 It is concluded that both β_1- and β_2-adrenoceptors can mediate chronotropism and inotropism in guinea-pig isolated atria. Determination of the postsynaptic effects of drugs should be carried out on spontaneously beating rather than driven atria to obviate modification of the responses by noradrenaline released from sympathetic neurones.

Introduction

Lands, Arnold, McAuliff, Luduena & Brown (1967) suggested that the β-adrenoceptors could be divided into two groups because the relative activities of a number of sympathomimetic amines were similar for the production of lipolysis or cardiac stimulation on the one hand, but different from those for the production of bronchodilatation or vasodepressor effects on the other. The receptors producing the former activities were classified as β_1 and the latter as β_2. Nevertheless, they found the cardiac receptors hard to classify with certainty. With the introduction of the β_2-adrenoceptor agonist salbutamol, Cullum, Farmer, Jack & Levy (1969) suggested that the β-adrenoceptor population in guinea-pig atria is not homogeneous. This was supported by Carlsson, Ablad, Brindstrom & Carlsson (1972), who showed that in the isolated heart of the cat both β_1- and β_2-adrenoceptors appear to mediate chronotropic responses. In 1977 Carlsson, Dahloff, Hedberg, Persson & Tangstrand demonstrated that both β_1- and β_2-adrenoceptors were present in the cat atrium, with the β_1-adrenoceptor population predominant overall but with a greater β_2:β_1-adrenoceptor ratio in the sinus node than in the myocardium. By contrast, Kaumann, Birnbaumer & Wittmann (1978) have suggested that salbutamol acts on β_2-adrenoceptors to produce the chronotropic response in the guinea-pig right atrium and that the receptors mediating inotropism in the driven left atrium are a homogeneous population of β_1-adrenoceptors.

Koch-Weser & Blinks (1963) found a relationship between chronotropism and inotropism in isolated spontaneously beating guinea-pig auricles. To avoid this complication, driven left atrial strips have commonly been used to investigate the effects of agonists on inotropism, despite the suggestion of Blinks (1966) that stimulation could cause an increased release of transmitter from the neurones in the prep-

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aration. To complicate the picture even further, we now know that the agonists themselves might act on receptors on the neurones to modify the release of the transmitters. Adler-Graschinsky & Langer (1975) showed that isoprenaline caused an increased fractional release of noradrenaline from isolated driven atria of the guinea-pig preloaded with [^{3}H]-noradrenaline as did phentolamine, whilst propranolol decreased it. They therefore suggested that presynaptic β-adrenoceptors increased the release of noradrenaline and that presynaptic α-adrenoceptors decreased it.

Since noradrenaline preferentially stimulates β_1-adrenoceptors and salbutamol stimulates β_2-adrenoceptors, the use of different β-adrenoceptor agonists to characterize the receptors mediating inotropism and chronotropism could be complicated by the release of noradrenaline, especially in driven auricles. The effects of agonists on the release of noradrenaline in driven and spontaneously beating auricles have therefore been determined and the dose-response curves to various agonists in spontaneously beating auricles considered in the light of the results obtained.

Methods

Measurement of [^{3}H]-noradrenaline overflow from spontaneously beating and from driven atria

Male guinea-pigs (200–650 g) were killed by a blow on the head and exsanguinated. The heart was removed, washed in Krebs solution and then transferred to a Petri dish containing Krebs solution. The ventricles were dissected off the auricles, which latter were then freed from fat, blood vessels and connective tissue. Both atria were used to provide spontaneously beating preparations and the left atria were dissected off when electrically driven preparations were required.

The separated atria were immersed in Krebs solution of the following composition (mM): NaCl 119, KCl 4.9, $CaCl_2$ 2.5, $NaH_2PO_4.2H_20$ 1.2, $NaHCO_3$ 25, $MgSO_4.7H_2O$ 1.2 and glucose 11. Oestradiol (3.3 μM) to block uptake$_2$, ascorbic acid (114 mM) and disodium edetate (EDTA, 27 mM) to preserve noradrenaline were added to the Krebs solution. After preincubation in this solution at 37°C for 10 min each atrium was transferred to a 5 ml conical flask containing 1.8 ml Krebs solution to which had been added 0.2 ml of [^{3}H]-noradrenaline (10 Ci mmol^{-1}) prepared from a stock solution of 1-[7-8-^{3}H]-noradrenaline hydrochloride (40 Ci mmol^{-1}) and sufficient cold noradrenaline to give a final concentration of 1.0 μg ml^{-1}. The tissues were incubated at 37°C for 30 min in this solution. The activity of the incubation medium was 4.8 μCi ml^{-1}.

At the end of the 30 min incubation period, the tissue was washed for 1 min in each of eight beakers containing 50 ml of fresh Krebs solution at room temperature. The atria were then suspended under a tension of 1.0 g in an isolated organ bath containing 4.0 ml (2.5 ml in the case of driven atria, the difference in volume representing the space occupied by the electrode) of Krebs solution bubbled with 95% O_2 and 5% CO_2 which now additionally contained desmethylimipramine (1.0 μM) to prevent uptake$_1$. Desmethylimipramine was used in preference to cocaine as it is less depressant to myocardial activity (Cornish & Miller, 1975).

To determine the time taken for the radioactivity to wash out of the extracellular spaces and for the overflow to stabilize, the organ bath was emptied every 5 min and refilled with fresh Krebs solution. The bath contents after 30, 60, 90, 100, 110, 120, 130, 140, 150, 160, 170 and 180 min were retained and the fractional release of [^{3}H]-noradrenaline estimated as described below. The results were plotted against time and are illustrated in Figure 1. The curve shows that the overflow of [^{3}H]-noradrenaline was stable after 100 min. All preparations were therefore washed for 100 min as described above, the following two 5 min samples were then collected as controls before the bathing solution was changed to one containing phentolamine (10.0 μM) to block pre- and

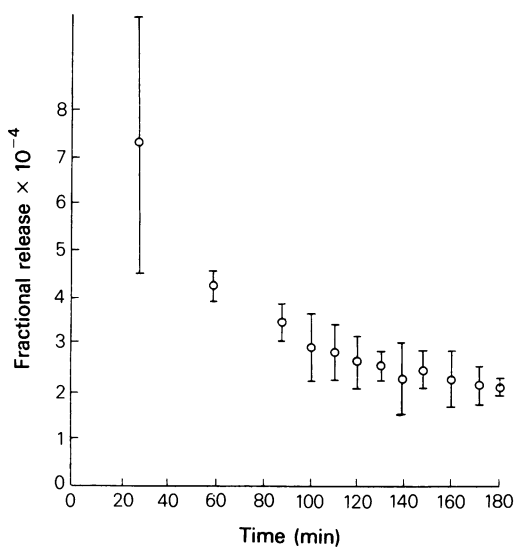

Figure 1 Fractional release of [^{3}H]-noradrenaline from guinea-pig auricles with time showing that release stabilized about 100 min after the start of the washing procedure following the loading of the tissues with tritium. The points shown are the means; vertical lines indicate s.e.means; $n = 6$.

postsynaptic α-adrenoceptors. Two further 5 min samples were collected before adding either isoprenaline or salbutamol in less than 1.5 μl from a micrometer syringe to give final concentrations in the bath of 1.2 nM or 1.0 μM respectively. After the addition of either of these agonists, the bath contents were collected every 5 min for 15 min. At the end of this period the tissue was removed, blotted dry, weighed and homogenized in 1.0 ml of perchloric acid (0.4 N). The homogenate was transferred to a scintillation vial together with a further 1.0 ml of perchloric acid used to rinse out the homogenizer.

One millilitre of sodium acetate buffer (pH 8.6) together wih 0.5 ml of a standard solution containing cold noradrenaline (111 μM), 1-(3,4-dihydroxyphenyl)ethane-1,2-diol (DOPEG, 130 μM) and EDTA (68.5 μM) was added to the 2.0 ml of homogenate and to a 2.0 ml aliquot of each sample of bathing solution. The samples were then stored at −10°C until they could be subjected to the column chromatographic procedure described by Graefe, Stefano & Langer (1973) to separate [^{3}H]-noradrenaline from its metabolites.

Catechols were separated from non-catechols on an alumina column (Anton & Sayre, 1962) pre-equilibrated with 10 ml of sodium acetate (0.2 M, pH 8.6). The noradrenaline and DOPEG were then eluted from the alumina by passing 3.0 ml of acetic acid (0,2 M) followed by 1.0 ml of distilled water through the alumina columns and allowing the eluate to pass through Dowex columns (50W-×4, H^+ form, 200–400 mesh). The Dowex removed the noradrenaline and allowed the DOPEG to pass through. Noradrenaline was eluted from the Dowex with two 2.0 ml volumes of HC1 (2.0 M) followed by 0.5 ml of distilled water. A blank was prepared with 2.0 ml of distilled water. Instagel (2 ml) was added to each tube which was then shaken thoroughly before measuring the radioactivity in a Packard 3220 Tricarb Liquid Scintillation Spectrophotometer. The counts were converted to disintegrations per minute ($dmin^{-1}$) and the released [^{3}H]-noradrenaline expressed as a fraction of that remaining in the tissue at the time of release (Enero, Langer, Rothlin & Stefano 1972).

Determination of dose-response curves to isoprenaline, dobutamine, noradrenaline and salbutamol in spontaneously beating atria

Atria were dissected out as described above and mounted in a 30 ml bath containing Krebs solution at 30°C of the composition already given, but with the addition of desmethylimipramine (1.0 μM), phentolamine (10 μM) and oestradiol (3.3 μM). The solution was gassed with 95% O_2 and 5% CO_2 and a tension of 1.0 g was applied to the tissue. Contractions were recorded on a Grass polygraph via a Grass FT03 force-displacement transducer. Rate changes were recorded by means of a tachograph. Agonists were added to the bath in volumes of 0.8 ml or less at intervals of 20 min. The tissues were washed twice after the responses to agonist drugs had reached a maximum. When antagonists were used they were included in the Krebs solution which was left in contact with the tissue for 45 min before preparing a dose-response curve in their presence.

Inotropic responses were expressed as a percentage of the maximum tension that could be produced by isoprenaline and chronotropic responses as a percentage of the maximum increase in rate that it could produce. This method of expressing the results was adopted because the tension developed or the rate achieved depended on the dose given and bore little or no relationship to the resting tension or rate. Only one agonist and one antagonist was used in each tissue so that control and test curves were obtained in the same tissue.

Statistical analysis

The significance of changes in overflow of tritum produced by various drugs and that of the displacement of dose-response curves for inotropism and chronotropism by various β-adrenoceptor antagonists was determined by Student's *t* test.

Drugs

The following drugs were used: L-ascorbic acid, (BDH); butoxamine hydrochloride (Burroughs Wellcome); desmethylimipramine hydrochloride (Pertofran, Geigy); dobutamine hydrochloride (Eli Lilly & Co.); DOPEG (Sigma); EDTA (Sigma); (−)-isoprenaline sulphate BP (St. Mary's Hospital Pharmacy); 17 oestradiol (Koch-Light Laboratories); phentolamine mesylate (Rogitine, Ciba); practolol (Eraldin ICI); salbutamol (Ventolin, Allen & Hanbury); 1-[7,8,^{3}H]-noradrenaline hydrochloride (specific activity 40 Ci $mmol^{-1}$ diluted in 1% sodium metabisulphite, Amersham).

Drugs were made up freshly in 0.9% w/v NaCl solution (saline) with ascorbic acid (1.0 μM) added to solutions of catecholamines to prevent oxidation.

Results

Table 1 shows that in the presence of phentolamine (10 μM) to block pre- and postsynaptic α-adrenoceptors, both isoprenaline (1.2 nM) and salbutamol (1.0 μM) are capable of releasing noradrenaline from driven left atria but not from spontaneously beating auricles. The release of noradrenaline

Table 1 The effects of drugs on the release of [^{3}H]-noradrenaline in electrically driven left atria and in spontaneously beating guinea-pig auricles

Drug	*Fractional release* $\times 10^{-4}$	n	*Significance*
	Driven left atria		
None	5.79 ± 0.72	16	NS
Phentolamine (10 μM)	7.64 ± 1.20	16	
Phentolamine (10 μM)	5.51 ± 0.60	8	
Phentolamine (10 μM) + isoprenaline (1.2 nM)	9.29 ± 1.40	16	$P<0.01$
Butoxamine (4.0 μM)	4.01 ± 0.62	6	
Phentolamine (10 μM) + butoxamine (4.0 μM)	4.75 ± 0.82	6	NS
Phentolamine (10 μM) + butoxamine (4.0 μM) + isoprenaline (1.2 nM)	5.30 ± 0.75	12	NS
Phentolamine (10 μM)	9.77 ± 1.90	8	
Phentolamine (10 μM) + salbutamol (1.0 μM)	14.75 ± 2.0	16	$P<0.05$
	Spontaneously beating atria		
None	6.53 ± 1.69	14	NS
Phentolamine (10 μM)	6.97 ± 1.49	14	
Phentolamine (10 μM)	6.59 ± 1.39	6	
Phentolamine (10 μM) + isoprenaline (1.2 nM)	9.64 ± 2.03	12	NS
Phentolamine (10 μM)	7.26 ± 2.77	8	
Phentolamine (10 μM) + salbutamol (1.0 μM)	8.62 ± 1.44	16	NS

Mean values ± s.e.mean are given.

provoked by isoprenaline in driven left atria was blocked by butoxamine (4.0 μM), suggesting that the effect was mediated by β_2-adrenoceptors.

Neither phentolamine alone nor isoprenaline or salbutamol in the presence of phentolamine significantly increased the overflow of noradrenaline in spontaneously beating auricles. For this reason the effects of various adrenoceptor agonists on inotropism and chronotropism were examined in spontaneously beating guinea-pig auricles.

Dose-response curves for the inotropic and chronotropic effects of each drug were determined and the shifts in these curves produced by β_1 and β_2-adrenoceptor antagonists (practolol and butoxamine respectively) is represented in Table 2 by the changes in the response to the median effective dose of each agonist. Assuming isoprenaline to be a full agonist for both chronotropism and inotropism, noradrenaline was a full agonist for inotropism but not for chronotropism. Dobutamine and salbutamol were both partial agonists with respect to both force and rate.

In the presence of practolol (4.0 μM) the inotropic and chronotropic dose-response curves for isoprenaline, dobutamine and noradrenaline were all significantly shifted to the right.

All the points on the curves were displaced similarly and significantly to that for the median effective dose given in Table 2. In the case of salbutamol, practolol (4.0 μM) produced a significant rightward shift in the dose-response curve for inotropism, but was without effect on the curve for chronotropism.

Butoxamine (4.0 μM) produced a significant rightward displacement in the inotropic and chronotropic dose-response curves for dobutamine and for salbutamol, but did not significantly affect either of

Table 2 Inotropic and chronotropic responses at the median effective dose of various adrenoceptor agonists in the presence and absence of practolol or butoxamine

Drug	*% of maximal response produced by median effective dose*	n	*Significance*
	Inotropic responses		
Isoprenaline (0.5 nM)	69.0 ± 5.0	14	
Isoprenaline (0.5 nM) + butoxamine (4.0 μM)	37.5 ± 1.5	4	$P < 0.001$
Isoprenaline (0.5 nM) + practolol (4.0 μM)	12.5 ± 1.5	4	$P < 0.001$
Dobutamine (90 nM)	67.0 ± 1.5	8	
Dobutamine (90 nM) + butoxamine (4.0 μM)	47.5 ± 3.5	4	$P < 0.001$
Dobutamine (90 nM) + practolol (4.0 μM)	12.0 ± 1.0	4	$P < 0.001$
Noradrenaline (6.0 nM)	72.5 ± 4.0	4	
Noradrenaline (6.0 nM) + butoxamine (4.0 μM)	64.0 ± 1.5	4	NS
Noradrenaline (6.0 nM) + practolol (4.0 μM)	20.0 ± 3.0	4	$P < 0.001$
Salbutamol (0.75 μM)	70.0 ± 4.0	12	
Salbutamol (0.75 μM) + butoxamine (4.0 μM)	32.0 ± 1.0	4	$P < 0.001$
Salbutamol (0.75 μM) + practolol (4.0 μM)	37.5 ± 7.5	4	$P < 0.001$
	Chronotropic responses		
Isoprenaline (0.8 nM)	43.5 ± 2.5	14	
Isoprenaline (0.8 nM) + butoxamine (4.0 μM)	34.5 ± 4.5	4	NS
Isoprenaline (0.8 nM) + practolol (4.0 μM)	12.5 ± 2.5	4	$P < 0.001$
Dobutamine (135.0 nM)	62.5 ± 4.75	8	
Dobutamine (135.0 nM) + butoxamine (4.0 μM)	45.0 ± 2.5	4	$P < 0.01$
Dobutamine (135.0 nM) + practolol (4.0 μM)	12.5 ± 4.25	4	$P < 0.001$
Noradrenaline (6.0 nM)	66.05 ± 5.5	4	
Noradrenaline (6.0 nM) + butoxamine (4.0 μM)	56.0 ± 6.5	4	NS
Noradrenaline (6.0 nM) + practolol (4.0 μM)	3.0 ± 1.5	4	$P < 0.001$
Salbutamol (0.75 μM)	52.5 ± 4.5	12	
Salbutamol (0.75 μM) + butoxamine (4.0 μM)	13 ± 5.5	4	$P < 0.001$
Salbutamol (0.75 μM) + practolol (4.0 μM)	51.0 ± 12.5	4	NS

these curves in response to noradrenaline. The inotropic response but not the chronotropic response to isoprenaline was also significantly antagonized by butoxamine.

Discussion

With uptake$_1$ and uptake$_2$ blocking agents and an α-adrenoceptor antagonist present in the bathing

solution, isoprenaline and salbutamol have been shown to increase the overflow of noradrenaline from field stimulated guinea-pig left atria, but not from spontaneously beating auricles. Phentolamine, the α-adrenoceptor blocking agent used, did not on its own affect the overflow of noradrenaline in either preparation. This observation, agrees substantially with the report of Adler-Graschinsky & Langer (1975) except that in their experiments phentolamine alone increased the overflow very substantially. However, their method of stimulation was very different as they stimulated the accelerans nerve directly. This may well have resulted in higher concentrations of noradrenaline in the synaptic gap, causing greater feedback inhibition via α-adrenoceptors than occurred in these experiments. Otherwise, in agreement with Adler-Graschinsky & Langer (1975) isoprenaline was found to increase the noradrenaline overflow in driven left atria as did salbutamol. Adler-Graschinsky & Langer (1975) found that the effect could be antagonized with the β_1- and β_2-adrenoceptor blocking agent, propranolol. In our experiments butoxamine was effective which suggests that the adrenoceptors involved were of the β_2 variety.

The finding that isoprenaline and salbutamol could increase the overflow of noradrenaline in driven left atria confirms the suspicions of Blinks (1966) that transmitter may be released in myocardium by the actions of drugs on neurones. Indeed, Furchgott, de Gubareff & Grossman (1959) had shown that increases in responses of driven guinea-pig left atria to increases in stimulus could be blocked with dichloroisoprenaline and prevented by reserpine pretreatment indicating a release of sympathetic transmitter caused by field stimulation and revealing the potential for drug-induced changes.

With the finding that the effect of noradrenaline on inotropism and chronotropism could only be antagonized by the β_1-adrenoceptor blocking agent practolol and not by butoxamine the β_2-adrenoceptor antagonist, doubt is cast upon the purity of effect of sympathetic adrenoceptor agonists examined in driven left atria since they may well enhance the release of noradrenaline with which they would then produce a combined effect on receptors. The results obtained with β-adrenoceptor agonists and antagonists on inotropism and chronotropism clearly indicate the presence of β_1- and β_2-adrenoceptors capable of mediating both effects and are consistent with the suggestion of Carlsson *et al.* (1977) that both β_1- and β_2-adrenoceptors are present with the β_1-receptor population predominant overall but with a greater β_2:β_1 ratio in the sinus node.

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