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Characterization of β-adrenoceptors mediating relaxation of the guinea-pig ileum

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Relaxation responses to sympathomimetic amines were recorded in potassium-contracted segments of guinea-pig ileum. Experiments were performed in the presence of phentolamine (5×10^{-6} M) to eliminate β-adrenoceptor-mediated effects. Metanephrine (10^{-5} M) and desmethylimipramine (5×10^{-7} M) were also present to prevent extraneuronal and neuronal uptake respectively. A potency order (−)-isoprenaline > (−)-noradrenaline > (−)-adrenaline was established, indicating a β_1-adrenoceptor involvement for this relaxation. The potency of salbutamol (β_2-selective) relative to isoprenaline in the ileum compared closely with its relative potency in isolated cardiac tissues (β_1) but differed significantly from the value in lung parenchymal strips and vas deferens (β_2). The pA_2 values for antagonism of selective agonists (−)-noradrenaline (β_1-selective) and fenoterol (β_2-selective) by practolol (β_1-selective) were identical, indicating a single β_1-adrenoceptor population. The pA_2 values for antagonism of these agonists by ICI 118,551 (β_2-selective) were also identical and compatible with a β_1-adrenoceptor population. Relaxation of the guinea-pig ileum is therefore mediated via a homogeneous population of β_1-adrenoceptors.

In subdividing β-adrenoceptors into the β_1- and β_2-types, Lands et al (1967) assigned the adrenoceptors mediating relaxation of rabbit jejunum to the β_1-category. Since then it has been widely assumed that guinea-pig intestinal β-adrenoceptors are also of the β_1-subtype (O'Donnell & Wanstall 1975; Levy & Apperley 1978; Williams & Broadley 1982). However, this assumption is based upon very sparse evidence (Furchgott 1967) and no systematic classification of these β-adrenoceptors appears to have been performed. Furthermore, it now appears that tissues may contain a mixture of β_1- and β_2-adrenoceptors, both mediating the same response. For example, heterogeneous populations have been identified pharmacologically in the heart (Carlsson et al 1972; O'Donnell & Wanstall 1979a) and respiratory system (Furchgott et al 1975; Zaagsma et al 1979).

Because the guinea-pig ileum is a widely used, simple and inexpensive tissue, and may represent a useful alternative to the heart for examining β_1-adrenoceptors, this study was undertaken to characterize the β-adrenoceptors mediating the relaxation by the use of three different methods. Firstly, the order of potency of the classic agonists isoprenaline, noradrenaline and adrenaline was established; secondly the potency of the β_2-adrenoceptor-selective agonist salbutamol (Farmer et al 1970), relative to isoprenaline, was determined and compared with values obtained in other tissues having either β_1- or β_2-adrenoceptors; and thirdly, the potencies of the selective antagonists practolol (β_1-selective) and ICI 118,551 (β_2-selective) were determined as their pA_2 values. The last of these methods would also indicate the presence of homogeneous or heterogeneous populations when β_1- or β_2-selective agonists are used.

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MATERIALS AND METHODS

Isolated tissues

Guinea-pigs of either sex (>300 g) were killed by a blow to the head and exsanguinated. Ileum, left and right atria, lung parenchymal strips and vas deferens were removed and set up in organ baths containing Krebs bicarbonate solution (composition, mM in distilled water; NaCl 118·4, KCl 4·7, $CaCl_2.2H_2O$ 1·9, $NaHCO_3$ 25, $MgSO_4.7H_2O$ 1·2, glucose 11·7, $KH_2PO_4.2H_2O$ 1·2) gassed with 5% CO_2 in oxygen and maintained at 38 °C.

Segments (2 cm) of mid-region ileum were mounted on tissue holders for recording longitudinal contractions under a resting tension of 0·5 g. Relaxation responses were always obtained after contraction of the tissue by the introduction of additional potassium to the bath (100 mM), as potassium chloride.

Left and right atria were suspended under a resting tension of 0·6 to 0·8 g. The left atria were secured to

bipolar electrodes and paced at 2 Hz with threshold voltage (+50%) and 5 ms pulse width using an SRI stimulator (Type 6053), for measurement of tension responses. Right atrial rate was recorded by means of a ratemeter (Devices, Lectromed, Type 2751) triggered by the tension signal.

Lung parenchymal strips (1–2 cm long) were set up by the method of Lulich et al (1976) under a resting tension of 1·5 g. Following a 1 h equilibration with changes of bathing medium every 15 min, the tissues were contracted with carbachol (10^{-5} M) before recording relaxation responses to sympathomimetic amines.

Vasa deferentia were set up at a resting tension of 1 g and contraction induced by field stimulation via two ring electrodes. Stimulation parameters were 10 Hz for 3 s at 1 ms pulse width and supramaximal voltage. Relaxation responses to sympathomimetic amines were then recorded.

All tissues were attached by cotton threads to strain gauges (Devices UFI, 57 g sensitivity range) and isometric tension recorded on a Devices M19 Polygraph (Lectromed).

Drug administration

All tissues were allowed to equilibrate for at least 30 min, changing the bathing medium every 10 or 15 min before drug challenge. They were incubated throughout with metanephrine (10^{-5} M) to inhibit extraneuronal uptake, and phentolamine (5×10^{-6} M) to inhibit α-adrenoceptors. In addition, the ileum was incubated with desmethylimipramine (5×10^{-7} M) in all experiments where adrenaline or noradrenaline were used. Sympathomimetic amines were added cumulatively by half log increments in concentration until the maximum response was obtained. The increases or decreases in tension in individual experiments were plotted as the percentage of the maximum response and the molar EC50 value determined. Geometric mean EC50 values were determined in tissues from at least four animals and the significance level of any difference was calculated by Student's *t*-test.

Agonist studies

For comparison of isoprenaline, noradrenaline and adrenaline in guinea-pig ileum, cumulative concentration-response curves were obtained in the same segment. After exposure of the K^+-contracted ileum to the first agonist, the tissue was washed before recontracting with K^+ ready for a second curve to the next agonist. This was then repeated for the third agonist. The order of adding the three agonists was varied to include all six possible permutations (each with duplicate animals), so that no control experiments were necessary to allow for the effects of time or repeated exposure.

The potency of salbutamol relative to isoprenaline was determined in all tissues by constructing a concentration-response curve to isoprenaline followed, after washout, by one to salbutamol. Control experiments were performed in which the tissues were exposed to two consecutive isoprenaline curves. Changes in EC50 value occurring between first and second curves were expressed as a correction concentration-ratio, and the mean value (n = 4) was applied to the isoprenaline EC50 value of test experiments. Although salbutamol was a partial agonist in certain tissues (e.g. left and right atria), the EC50 value was also calculated by expressing uncorrected changes in tension as a percentage of its own maximum. The EC50 ratio of isoprenaline to salbutamol was obtained in individual experiments and the potency of salbutamol expressed relative to a value of 100 for isoprenaline.

Antagonist studies

pA_2 values for antagonists were determined by constructing cumulative concentration-response curves to either fenoterol or noradrenaline before and after practolol (5×10^{-6}, 10^{-5} or 5×10^{-5} M) or ICI 118,551 (5×10^{-7}, 10^{-6}, 5×10^{-6} or 10^{-5} M). After the initial concentration-response curve to the sympathomimetic amine in the K^+-contracted ileum, the tissue was washed and incubated with one concentration of antagonist for 15 min before contracting the tissue again with K^+. The agonist administration was then repeated after a further 15 min in the presence of the antagonist. One concentration of antagonist only was used in each preparation. Concentration-ratios (CR) were determined by dividing the EC50 value obtained in the presence of antagonist by the pre-antagonist value. These ratios were divided by the mean correction values obtained from control experiments, which were performed identically except that the tissues were not exposed to antagonist. Mean corrected concentration-ratios were determined for each concentration of antagonist and log (CR-1) plotted against log molar concentration (M). The slopes of the calculated regression lines were calculated and the pA_2 values determined for each concentration of antagonist by use of the equation: $pA_2 = \log (CR-1) - \log M$ (Mackay 1978; O'Donnell & Wanstall 1979b). This provided mean values ±s.e.m. and

permitted statistical comparisons between values for the two agonists.

The drugs used were (−)-adrenaline (Hopkin & Williams), desmethylimipramine hydrochloride (Sigma), (±)-fenoterol hydrobromide (Boehringer Ingelheim), ICI 118,551 (erythro-DL-1(7-methylindan-4-yloxy)3-isopropyl-aminobutan-2-ol) (ICI), (−)-isoprenaline bitartrate dihydrate (Ward-Blenkinsop), (±)-metanephrine hydrochloride (Sigma), (−)-noradrenaline bitartrate (Sigma), phentolamine mesylate (Ciba-Geigy), practolol (ICI) and salbutamol sulphate (Glaxo).

RESULTS

Agonist potency order

(−)-Isoprenaline, (−)-noradrenaline and (−)-adrenaline produced concentration-dependent relaxations of the K^+-contracted guinea-pig ileum. The mean concentration-response curves (n = 12) are shown in Fig. 1. The geometric mean EC50 values for (−)-noradrenaline and (−)-adrenaline were significantly ($P < 0.05$) different from the (−)-isoprenaline value and from each other (Table 1). The potency order was (−)-isoprenaline > (−)-noradrenaline > (−)-adrenaline.

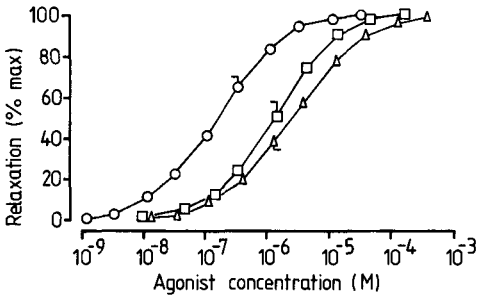

FIG. 1. Mean concentration-response curves for the relaxation of K^+-contracted guinea-pig ileum by (−)-isoprenaline (○), (−)-noradrenaline (□) and (−)-adrenaline (△). Each tissue was exposed to the three agonists and they were incubated throughout with metanephrine (10^{-5} M), phentolamine (5×10^{-6} M) and desmethylimipramine (5×10^{-7} M). Each point is the mean from 12 tissues with the s.e.m. shown at a mid-curve point.

Potency of salbutamol relative to isoprenaline

(−)-Isoprenaline and salbutamol were compared in the K^+-contracted ileum, left and right atria, lung strip and vas deferens of the guinea-pig. The mean potency values of salbutamol (n > 4) measured relative to the value of 100 assigned to (−)-isoprenaline are shown in Table 2. In all tissues the potency of salbutamol was less than (−)-isoprenaline, but the relative potencies in left (0·63 ± 0·40) and right atria (0·31 ± 0·20) were significantly ($P < 0.05$) less than in the vas deferens (26·9 ± 10·0) and lung strip (8·2 ± 3·0). The relative potency of salbutamol in the ileum (0·44 ± 0·20) did not differ significantly ($P > 0.05$) from the values in the two cardiac preparations, but it was significantly different ($P < 0.05$) from the vas deferens and lung strip values.

Table 1. Mean EC50 values and relative potencies of (−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline for relaxation of K^+-contracted guinea-pig ileum. Values in parentheses are the 95% confidence limits for the geometric mean EC50 value. Levels of significance for differences between EC50 values, as determined by Student's *t*-test, are represented by $*P < 0.05$ and $**P < 0.001$. Tissues were incubated throughout with phentolamine (5×10^{-6} M) metanephrine (10^{-5} M) and desmethylimipramine (5×10^{-7} M). n = Number of animals from which ileum was used.

Agonist	EC50 (μM)		Potency	n
(−)-Isoprenaline	0·18 (0·10–0·29)	**	1·0	12
(−)-Noradrenaline	1·39 (1·11–1·74)	** *	0·13	12
(−)-Adrenaline	2·61 (1·36–5·02)	*	0·06	12

Antagonist pA_2 values

The relaxation responses of the K^+-contracted ileum to (−)-noradrenaline (β_1-selective agonist) or fenoterol (β_2-selective agonist) were antagonized by either practolol (β_1-selective antagonist) or ICI 118,551 (β_2-selective antagonist). Neither of these antagonists caused a significant ($P > 0.05$) alteration of the K^+-induced contraction of the ileum, the mean contraction heights obtained before and after practolol (5×10^{-5} M) were 1·73 ± 0·14 and 1·60 ± 0·10 g and for ICI 118,551 (10^{-5} M) the values were 1·46 ± 0·25 and 1·15 ± 0·09 g respectively. The Schild plots for antagonism by practolol of (−)-noradrenaline and fenoterol were virtually superimposed, as were those for the antagonism of these agonists by ICI 118,551 (Fig. 2). The mean pA_2 values of ICI 118,551, calculated by the method of Mackay (1978), were not significantly ($P > 0.05$) different for the antagonism of (−)-noradrenaline or fenoterol. Furthermore, the mean pA_2 values for practolol were not significantly ($P > 0.05$) different when (−)-noradrenaline or fenoterol were the agonists (Table 3). A relatively low slope of the Schild plot for antagonism by practolol of fenoterol was obtained, the pA_2 values of practolol were therefore additionally calculated from extrapolation of the mean calculated Schild regression to the concentra-

tion axis. These values were 5·37 for antagonism of (−)-noradrenaline and 5·68 for antagonism of fenoterol.

DISCUSSION

This study has attempted to classify the β-adrenoceptors mediating relaxation of the guinea-pig ileum. This tissue normally exhibits little spontaneous activity and tone and therefore to examine relaxation responses contractile tone had to be induced. We chose to contract the tissue by addition of potassium which produced a sustained level of contraction from which dose-related relaxations could be induced by the sympathomimetic amines. This method of examining intestinal β-adrenoceptors is superior to using transmural electrical stimulation to induce contractions by the Paton technique (1957), where the relaxation is primarily by stimulation of pre-synaptic α-adrenoceptors on intramural cholinergic plexuses

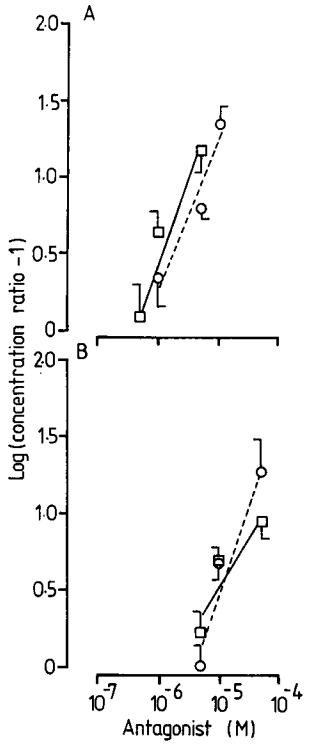

FIG. 2. Schild plots for antagonism by: A, ICI 118,551 and B, practolol of the relaxation responses of K^+-contracted guinea-pig ileum to (−)-noradrenaline (○---○) and fenoterol (□——□). The calculated regression lines of mean log (corrected concentration ratio −1) values (±s.e.m.) against log concentration of agonist (M) are shown. Tissues were incubated throughout with metanephrine (10^{-5} M), phentolamine (5×10^{-6} M) and desmethylimipramine (5×10^{-7} M).

Table 2. Potency of salbutamol relative to (−)-isoprenaline for the β-adrenoceptor-mediated responses of various guinea-pig isolated tissues. Mean (±s.e.m.) potency of salbutamol is expressed relative to a value of 100 for isoprenaline. Levels of significance for differences between the ileum and the other tissues, as determined by Student's *t*-test, are represented by $^*P < 0.05$ and NS, not significant. Tissues were incubated throughout with phentolamine (5×10^{-6} M) and metanephrine (10^{-5} M). n = Number of animals supplying each tissue.

Tissue	Receptor type	Salbutamol potency	n
Left atria	β_1	0·63 ± 0·40^{NS}	5
Right atria	β_1	0·31 ± 0·20^{NS}	4
Vas deferens	β_2	26·9 ± 10·0*	4
Lung strip	β_2	8·2 ± 3·0*	4
Ileum	—	0·44 ± 0·20	4

Table 3. Mean pA_2 values for antagonism of (−)-noradrenaline- and fenoterol-induced relaxation of guinea-pig ileum by ICI 118,551 and practolol. Mean pA_2 values (±s.e.m.) were determined from individual concentration-ratios (CR) as $pA_2 = \log (CR-1) - \log M$. Slope values are from calculated regression lines of the Schild plots. Levels of significance for comparisons between agonists, as determined by Student's *t*-test, are represented by NS, not significant ($P < 0.05$). n = Number of animals from which ileum was used.

	Antagonist					
	ICI 118,551			Practolol		
Agonist	pA_2	slope	n	pA_2	slope	n
(−)-Noradrenaline	6·31 ±0·09	0·96	12	5·41 ±0·10	1·20	12
Fenoterol	6·43^{NS} ±0·09	1·14	12	5·45^{NS} ±0·08	0·64	12

(Kosterlitz et al 1970; Drew 1978). Although α-adrenoceptors are present on the smooth muscle, the relaxation of K^+-contracted ileum is weak compared with the β-adrenoceptor-mediated effect (unpublished data) but in the present study this is prevented by the presence of phentolamine throughout.

A relative potency order of (−)-isoprenaline > (−)-noradrenaline > (−)-adrenaline was established for the relaxation responses to these agonists. This was obtained in the presence of desmethylimipramine; the potency order of these catecholamines was not therefore influenced by differences in their neuronal uptake. Omission of a neuronal uptake antagonist such as desmethylimipramine can seriously alter potency orders, for example, in cardiac tissue (Furchgott 1967, 1972). A potency order of these three agonists appears to have been determined previously in guinea-pig ileum (Furchgott 1967), at a time when the $\beta_1 : \beta_2$ subclassification had

yet to be established, but the order was as found here. This potency order compares favourably with that obtained in guinea-pig right atria which are generally thought to contain a homogeneous β-adrenoceptor population (Broadley 1982), although a recent study indicates that β_2-adrenoceptors may be present (Johansson & Persson 1983). The relative potency obtained by Buckner & Patil (1971) in the presence of an uptake blocker was 1·00 : 0·07 : 0·03 for (−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline, which is similar to the values obtained here.

In contrast, the guinea-pig uterus has a homogeneous β_2-adrenoceptor population (O'Donnell et al 1978; Krstew et al 1982). The relaxation of the rat uterus is also mediated via β_2-adrenoceptors, and the potency order is (−)-isoprenaline > (−)-adrenaline > (−)-noradrenaline (1·0 : 0·21 : 0·002) (Arnold 1972). The relaxation of the guinea-pig trachea is mediated by a mixed population of β_1- and β_2-adrenoceptors (Furchgott et al 1975; O'Donnell & Wanstall 1979b; Zaagsma et al 1979), and in this case the potency order is (−)-isoprenaline > (−)-adrenaline > (−)-noradrenaline (1·0 : 0·11 : 0·01) (Buckner & Patil 1971; Grana et al 1974), indicative of a β_2-adrenoceptor system. However, noradrenaline which is relatively selective for β_1-adrenoceptors is closer to adrenaline in potency and is 50 times more potent than on the rat uterus. Therefore, from the relative potency order of these three agonists, the relaxation of the guinea-pig ileum would appear to be closer to β_1-adrenoceptor-mediated effects in tissues such as the heart, than to tissues such as the uterus and respiratory system where β_2-adrenoceptors predominate.

The second approach to classifying the β-adrenoceptors of the guinea-pig ileum was to compare the potency of salbutamol, a selective β_2-adrenoceptor agonist, with isoprenaline. This could then be compared with values obtained in other tissues where the β-adrenoceptors are better understood. Since such relative potencies were not readily available in the literature, and because they should be determined under identical conditions such as temperature (Broadley 1980) and the presence of antagonists of receptors not under study (Furchgott 1972), they were determined in the present study for a range of tissues. The relative potency for the relaxation of K^+-contracted ileum was compared with the values for the positive inotropic and chronotropic responses of left and right atria which are mediated via a homogeneous β_1-adrenoceptor population (Broadley 1982); the relaxation of lung parenchymal strips which is mediated via a homogeneous β_2-adrenoceptor population (Zaagsma et al 1979); and relaxation of the vas deferens which is also mediated via predominantly β_2-adrenoceptors (Von Euler & Hedqvist 1975; Gerthoffer & Westfall 1976). As with agonist potency order, the relative potency of salbutamol was comparable with the values for the two cardiac preparations having β_1-adrenoceptors, rather than those for the lung strip or vas deferens which differed significantly from the ileal value. Thus, from the combined evidence of the potency order of isoprenaline, noradrenaline and adrenaline and the relative potency of salbutamol, the β-adrenoceptor mediating relaxation of the guinea-pig ileum appears to be of the β_1-subtype. However, this alone does not indicate whether it is a homogeneous or heterogeneous population of predominantly β_1-adrenoceptors.

To answer this question, pA_2 values of the selective antagonists practolol (β_1-selective) and ICI 118,551 (β_2-selective) were examined for antagonism of the selective agonists (−)-noradrenaline (β_1-selective) and fenoterol (β_2-selective). If the guinea-pig ileum contains only β_1-adrenoceptors (i.e. a homogeneous population), Schild plots would be superimposed and the pA_2 values of antagonists would be identical irrespective of the agonist used (O'Donnell & Wanstall 1981). This indeed was the case, since the pA_2 values for practolol did not differ significantly whether (−)-noradrenaline or fenoterol was used and similarly the pA_2 values of ICI 118,551 were not significantly different for these two agonists. The use of the equation of Mackay (1978) to calculate pA_2 values was justified by the unity slopes of the Schild plots in all but the antagonism of fenoterol by practolol. In this case, however, the pA_2 values were of the same order when calculated by extrapolation of the Schild plot. Thus from pA_2 values it can be concluded that a homogeneous population of β_1-adrenoceptors appears to be responsible for the relaxation responses of guinea-pig ileum.

This tissue therefore compares with the left and right atria of this species in which pA_2 values of practolol and ICI 118,551 have been shown in these laboratories to be the same whether noradrenaline or fenoterol are the agonists (Broadley & Hawthorn 1983). Virtually identical pA_2 values of ICI 118,551 were obtained in the ileum (noradrenaline, 6·31 and fenoterol, 6·43) and in the left (noradrenaline, 6·3 and fenoterol 6·64) and right atria (noradrenaline, 6·51 and fenoterol, 6·43) of the guinea-pig. These are

relatively low compared with values obtained for a β_2-adrenoceptor system (O'Donnell & Wanstall 1980) and indicate the presence of β_1-adrenoceptors. However, the values for practolol obtained in the ileum were lower than those for the left and right atria (range 6·25–6·59), which at first sight may suggest a difference in the adrenoceptor type. In the case of fenoterol as the agonist, the low slope of the Schild plot may partly explain the low pA_2 values. However, this cannot apply to (−)-noradrenaline which yielded slopes of approximately unity. It is possible that equilibrium may not have been achieved, resulting in a slight reduction of pA_2 values, but a 30 min contact time is usually considered sufficient. Nevertheless, there are other examples of unusually low pA_2 values for the antagonism by practolol of the β_1-adrenoceptors of the rabbit intestine (Farmer & Levy 1970; Wagner et al 1972). Indeed, there are instances of discrepancies when agonists and antagonists are used for receptor characterization, antagonists probably being of more value to corroborate the finding obtained with agonists (Arnold 1972).

In conclusion, this study has shown that the relaxation of guinea-pig ileum is mediated via a homogeneous population of β_1-adrenoceptors. This observation establishes the hitherto anecdotal assumptions which appear to be based upon extensions of data obtained with the rabbit ileum (Lands et al 1967).

Acknowledgements

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