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Possible differences in α-adrenoceptors in rabbit ileum and spleen

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In isolated tissues from reserpinized rabbits (5 mg kg^{-1}, i.m. 20 h before experiment) and in the presence of cocaine (3×10^{-5} M), corticosterone ($2{\cdot}8 \times 10^{-5}$ M), tropolone (3×10^{-5} M), propranolol (4×10^{-6} M) and disodium EDTA (3×10^{-5} M), the potency ratios (relative to (−)-noradrenaline) of (−)adrenaline, (−)-phenylephrine and (±)-methoxamine were (m ± s.e.) 2·03 ± 0·13, 0·045 ± 0·003 and 0·0062 ± 0·0018 respectively in splenic strips and 1·77 ± 0·41, 0·093 ± 0·018 and 0·029 ± 0·004 respectively in isolated ileum. Although the pA_2 values for phentolamine and thymoxamine against (−)-noradrenaline in the two tissues were very similar there was a statistically significant difference when using yohimbine as the α-adrenoceptor blocking agent (pA_2 = 6·80 ± 0·30 in spleen; 5·60 ± 0·12 in ileum). These differences suggest that the α-adrenoceptor in the two tissues is not identical. The pA_2 value of phentolamine in rabbit ileum was not significantly different whether (−)-noradrenaline or (±)-methoxamine was used as agonist (7·91 ± 0·07 and 7·97 ± 0·06 respectively) while that of yohimbine was 5·56 ± 0·10 using (−)-noradrenaline and 6·19 ± 0·12 using (±)-methoxamine. In the light of this latter result and, considering the scatter of the experimentally determined values, there may be two α-adrenoceptors in rabbit ileum and either or both may not be identical in all respects to the α-adrenoceptor found in rabbit spleen.

The division of adrenoceptors into α- and β-types is now well established and it seems probable that some subdivision of β-adrenoceptors may be valid (Lands, Arnold & others, 1967; Furchgott, 1972; Apperley, Daly & Levy, 1976). Evidence has recently accumulated that a subdivision of α-adrenoceptors may also be appropriate although this evidence is strong only for a distinction between the pre- and post-synaptic α-adrenoceptors (Starke, 1977). Differences have, however, been suggested to occur elsewhere at a variety of sites and in a variety of species (Downie, Dean & others, 1975; Holmgren & Nilsson, 1975; Struyker Boudier, de Boer & others, 1975; Duckles & Bevan, 1976).

The classification and subclassification of receptors in functioning isolated tissues is based mainly on the application of one or (preferably) more of three basically different techniques. Firstly, the use of relative potency measurements on a series of agonists at the two sites under investigation. Secondly, the use of a variety of antagonists in an attempt to show a different affinity or dissociation constant (usually expressed as a pA_2) at one site as opposed to another and thirdly, the use of the isomeric activity ratio of agonists or, less frequently, antagonists. The first two of these techniques are well established and have proved their utility in a number of situations (the β-adrenoceptor and histamine receptors for example) while the third technique, the use of isomeric activity ratios, is a relatively new method of, at best, unproven utility.

We have previously used this latter method to investigate the characteristics of the α-adrenoceptor and found that small differences did exist in the potency ratio between (−)- and (+)-noradrenaline in a variety of isolated tissues implying that the α-adrenoceptors in these tissues may not be identical (Barker, Harper & Hughes, 1977). If this is correct it should be possible to obtain compatible results using the alternative and better established methods of receptor characterization and such an investigation is the subject of this paper. We have chosen to investigate the α-adrenoceptor in rabbit ileum (which has a high isometric activity ratio) in comparison with that in spleen (which has an isomeric activity ratio similar to that in a variety of other tissues). The suggestion that the α-adrenoceptor in rabbit intestine is not identical to that found at other sites does find support elsewhere in that van Rossum (1965) found differences between the α-adrenoceptor in rabbit intestine and that in rat vas deferens though in the light of modern understanding the conditions under which the experiments were performed were not wholly adequate for firm conclusions to be drawn.

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MATERIALS AND METHODS

Rabbits of either sex (New Zealand White strain; 1·8–3·5 kg) were given reserpine (5 mg kg^{-1}, i.m.) 20 h before death by a blow on the head. The spleen and a length of ileum (taken from approximately 8 cm proximal to the ileo-caecal junction) were removed and cleared of mesentery in cold physiological saline. Pieces of ileum (approximately 1·5 cm long) and splenic strips (approximately 2 mm wide and 25 mm long) were mounted in organ baths in physiological saline (NaCl 118, KCl 4·8, $CaCl_2$ 2·5, $MgSO_4$ 0·6, $NaHCO_3$ 25, KH_2PO_4 1·0 and glucose 11·1 mM; aerated with 5% carbon dioxide in oxygen). Incorporated in the physiological saline were cocaine (3×10^{-5} M to block uptake$_1$), corticosterone ($2{\cdot}8 \times 10^{-5}$ M to block uptake$_2$), tropolone (3×10^{-5} M to block catechol-*O*-Me-transferase), propranolol (4×10^{-6} M to block β-adrenoceptors) and disodium ethylenediaminetetra-acetic acid (3×10^{-5} M to prevent oxidative degradation of catecholamines). The temperature of the organ baths was maintained at 36·5° and changes in length of the tissues were recorded isotonically with a load of 0·5 g for spleen and 1·0 g for ileum.

Dose response curves to various agonists (contact time ~2 min) were determined using cumulative techniques in the presence and absence of various concentrations of antagonists which were contained in the bulk of the bathing fluid and were allowed to equilibrate with the tissues for 30 min before responses were recorded.

Spleen strips responded to the agonists with a well maintained contraction and all four of the agonists used were full agonists. The ileum preparations showed a reduction in net tone and a reduction in the size and rate of the spontaneous movements when exposed to the agonists and the response measured was the reduction in the size of the spontaneous movements. This reduction was expressed as a percentage of the size of the spontaneous movements in a control period immediately before addition of the agonists. All four agents were capable of completely abolishing the spontaneous movements.

Values for the pA_2 of the antagonist were calculated from plots of $\log_{10}$(dose ratio -1) against $\log_{10}$ molar concentration of antagonist present in the bathing fluid (Arunlakshana & Schild, 1959) or directly from the equation.

$pA_2 = \log_{10}((Ar'/Ar)-1)$/molar concentration of antagonist where:— Ar′ and Ar represent respectively the concentrations of the agonist required to produce the same sized response from the tissue in the presence and absence of the appropriate concentration of the antagonist. Agonist potency ratios and ED50 values were determined using a 4 point assay design and data were only considered acceptable if the slopes of the $\log_{10}$ dose—response relations did not differ in a statistically significant manner from that obtained with (−)-noradrenaline.

Statistical methods. Tests for deviations from parallelism of $\log_{10}$ dose—response curves were carried out according to the British Pharmacopoeia (1958), regression lines were calculated according to Snedecor & Cochran (1967) and tests for statistical significance utilised Student's *t*-test. Where appropriate, results are given as mean ± standard error (m ± s.e.).

Drugs used: (−)-adrenaline hydrogen tartrate (Sigma), cocaine hydrochloride (B.P.), corticosterone (Sigma), disodium ethylene diamine tetra-acetic acid (BDH), (±)-methoxamine hydrochloride (Wellcome), (−)-noradrenaline bitartrate (Sigma), phentolamine mesylate (Ciba), (−)-phenylephrine hydrochloride (Sigma), (±)-propranolol hydrochloride (ICI), reserpine (Sigma) and thymoxamine hydrochloride (Warner). Tropolone (Aldrich) was recrystallized to constant melting point (51–52°) from light petroleum (40°–60°) since the original sample showed a brown discolourization. Reserpine was dissolved in 20% ascorbic acid (BDH) immediately before use. Corticosterone was dissolved in ethanol (~1 mg ml^{-1}) and an appropriate volume of the ethanolic solution was added to the physiological saline.

RESULTS

Values for the EC50 of each of the agonists tested in both spleen and ileum are shown in Table 1. In both tissues adrenaline is the most potent agonist and the order of potency of the agonists is the same, namely adrenaline > noradrenaline > phenylephrine > methoxamine. With regard to the actual magnitude of the EC50 values, although there is no statistically significant difference between the EC50 for adrenaline in the two tissues or between the EC50 for noradrenaline in the two tissues, the EC50 values for phenylephrine and for methoxamine in spleen are significantly different from the corresponding values in ileum. A more satisfactory method of examining these data is to calculate potency ratios relative to noradrenaline in each individual experiment since this procedure removes the variability associated with overall sensitivity differences between experimental animals. The potency

Table 1. *Concentrations* (M; *m* ± *s.e.*) *of the various agonists producing 50% maximal response* (*EC50*) *in rabbit ileum and spleen.* The individual EC50 values obtained in the experiments were converted to $\log_{10}$ values before calculation of means and standard errors which have then been converted back to concentrations (M).

Agonist	Tissue (no. of exps in parentheses) Spleen	Ileum	*P*
(−)-Adrenaline	$7{\cdot}06 + 1{\cdot}81 \times 10^{-8}$ $- 1{\cdot}45$ (8)	$4{\cdot}81 + 2{\cdot}12 \times 10^{-8}$ $- 1{\cdot}47$ (5)	>0·3
(−)-Noradrenaline	$1{\cdot}42 + 0{\cdot}42 \times 10^{-7}$ $- 0{\cdot}31$ (8)	$1{\cdot}03 + 0{\cdot}25 \times 10^{-7}$ $- 0{\cdot}21$ (9)	>0·3
(−)-Phenylephrine	$3{\cdot}20 + 0{\cdot}84 \times 10^{-6}$ $- 0{\cdot}68$ (8)	$1{\cdot}17 + 0{\cdot}33 \times 10^{-6}$ $- 0{\cdot}25$ (6)	<0·02
(±)-Methoxamine	$3{\cdot}13 + 0{\cdot}84 \times 10^{-5}$ $- 0{\cdot}66$ (8)	$0{\cdot}57 + 0{\cdot}18 \times 10^{-5}$ $- 0{\cdot}14$ (6)	<0·001

ratios of the agonists relative to noradrenaline are shown in Table 2 and it is apparent that adrenaline has the same relative potency in spleen as in ileum. This is not so with either phenylephrine or methoxamine where statistically significant differences in potency ratio relative to noradrenaline do exist between the two tissues. A similar picture emerges if the ratios of the mean EC50's given in Table 1 are compared (see values in parentheses in Table 2) though the differences are marginally less marked. This suggests that the receptors on which the agonists act might not be identical in the two tissues.

In an attempt to confirm such a difference the pA_2 values for various antagonists against noradrenaline have been measured in the two tissues by calculation from the effect of a single concentration of the antagonist on the agonist dose ratio and application of the equation given in the methods section (which cannot of course give any indication as to the competitiveness of the interaction). Using this method the mean pA_2 values for phentolamine in spleen (7·71; n = 2) and in ileum (7·65; n = 2) were not markedly different and the comparable values for thymoxamine in spleen (6·92 ± 0·14; n = 5) and in ileum (6·58 ± 0·06; n = 4) showed no statistically significant difference ($P > 0{\cdot}5$).

Table 2. *Potency ratios relative to* (−)-*noradrenaline* (*taken as unity*) *for various agonists in rabbit ileum and spleen.* The results shown are the mean (± s.e.) of the values obtained in individual experiments. The results in parentheses were calculated from the mean EC50 values given in Table 1. * n = 5 in this group.

Agonist	Tissue Spleen (n = 8)	Ileum (n = 6)	*P*
(−)-Adrenaline	2·03 ± 0·13 (2·01)	1·77 ± 0·41* (2·14)	>0·4
(−)-Phenylephrine	0·045 ± 0·003 (0·044)	0·093 ± 0·018 (0·088)	<0·01
(±)-Methoxamine	0·0062 ± 0·0018 (0·0045)	0·029 ± 0·004 (0·018)	<0·001

Initial experiments with yohimbine suggested that there might be a difference between the pA_2 values for this antagonist in the two tissues and therefore a more rigorous estimation of the pA_2 value was carried out using plots of $\log_{10}$ (dose ratio −1) against $\log_{10}$ antagonist concentration (Arunlakshana-Schild plots) which are shown in Fig. 1. Calculated regression lines for the data gives slopes of 1·04 ± 0·25 (n = 11) in spleen and 0·95 ± 0·18 (n = 13) in ileum neither of which values differ statistically ($P > 0{\cdot}8$) from a value of unity which is the theoretical value expected if the antagonism is competitive. The pA_2 values for yohimbine were 6·80 ± 0·30 (n = 11) in spleen and 5·60 ± 0·12 (n = 13) in ileum which are significantly different ($P < 0{\cdot}01$) suggesting again that the receptors in the two tissues might not be identical.

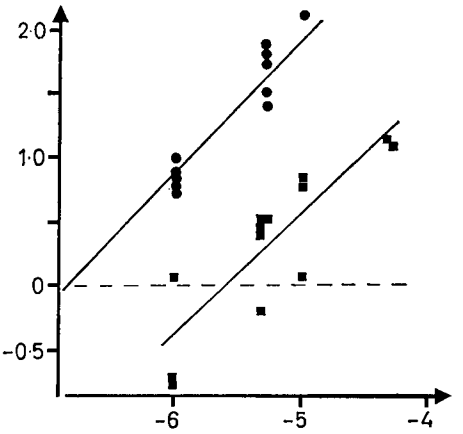

FIG. 1. Arunlakshana & Schild plots used to determine the pA_2 values of yohimbine in ● spleen (11 experimental points derived from 7 animals) and ■ ileum (13 experimental points derived from 8 animals). For clarity, where points should be superimposed a marginal displacement in the position of the point on the abscissa has been made. Ordinate: Log_{10} (DR-1). Abscissa: Log_{10} concentration of antagonist (M).

To further clarify the situation, in separate experiments, the pA_2 values for phentolamine have been determined in ileum employing the Arunlakshana-Schild method and utilizing both noradrenaline and methoxamine as agonists. Similar measurements have been made with yohimbine as the antagonist. The results are shown in Table 3 and the Arunlakshana-Schild plots from which these results have

Table 3. *Showing the pA_2 values for yohimbine and phentolamine against noradrenaline and methoxamine in ileum.*

Agonist	Antagonist	Slope (m ± s.e.)	No. of points	n	pA_2 (m ± s.e.)	P
(−)-Noradrenaline	Phentolamine	0·92 ±0·08	21	10	7·91 ±0·07	>0·05
(±)-Methoxamine	Phentolamine	1·05 ±0·08	24	11	7·97 ±0·06	
(−)-Noradrenaline	Yohimbine	0·92 ±0·17	22	10	5·56 ±0·10	<0·001
(±)-Methoxamine	Yohimbine	0·93 ±0·12	30	13	6·19 ±0·12	

been derived are shown in Fig. 2. In no case is the slope of the calculated regression line significantly different from the theoretical value of unity which is to be expected if the antagonism is competitive. For phentolamine the pA_2 values against noradrenaline and against methoxamine are not significantly different but with yohimbine a statistically significantly difference is apparent in that the pA_2 value using noradrenaline as agonist is some 0·63 $\log_{10}$ units lower than that obtained with methoxamine. This difference is not large but it should be noted that the pA_2 value for yohimbine against noradrenaline in ileum (5·56 ± 0·10) is very close to the value obtained in the previous and separate set of experiments (5·60 ± 0·12) indicating the excellent reproducibility of the method.

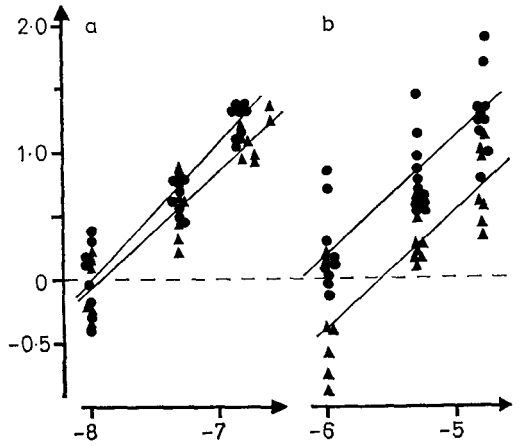

FIG. 2. Arunlakshana-Schild plots used to determine the pA_2 values of a–phentolamine and b–yohimbine against either noradrenaline (▲) or methoxamine (●) in rabbit ileum. For clarity, where points should be superimposed a marginal displacement in the position of the point on the abscissa has been made. Ordinate: Log_{10} (DR-1). Abscissa: Log_{10} concentration of antagonist (M).

DISCUSSION

The investigation of the characteristics of receptors in isolated tissues can only be carried out satisfactorily if precautions are taken to eliminate various complicating factors which can distort the magnitude of the characterizing parameters usually measured (Furchgott, 1972; Patil, Miller & Trendelenberg, 1974). For this reason a variety of materials were incorporated into the physiological saline (see methods) and under these conditions quantification of the actions of α-adrenoceptor blocking agents on α-adrenoceptors is uncomplicated by other properties they may possess (for example uptake blockade). Furthermore, the relative potency of α-adrenoceptor agonists is not distorted by differences in the contribution from the various sites of loss in different tissues or by differences in the susceptibility of α-adrenoceptor antagonists to these various sites of loss.

The order of potency of the four α-adrenoceptor agonists investigated was found to be the same in both tissues indicating a basic similarity between the α-adrenoceptors in spleen and ileum. A more detailed examination of the relative potency ratios reveals some differences in that phenylephrine was approximately twice as potent and methoxamine four to four and a half times more potent in ileum than in spleen. This difference is not large but is significant statistically and suggests that the α-adrenoceptors at the two sites may not be identical.

If this is correct it might be expected that α-adrenoceptor antagonists might be able to distinguish between these two closely related α-adrenoceptors and show some degree of selectivity in their ability to block the effects of α-adrenoceptor agonists in the two tissues. This is not so, however, with phentolamine or with thymoxamine which showed similar pA_2 values in spleen and ileum. With the α-adrenoceptor blocking agent yohimbine an apparent selectivity was found as this agent was some 15

times more potent in spleen than in ileum and this observation supports the suggestion that the α-adrenoceptors in the two tissues are not identical. Closer examination of the Arunlakshana-Schild plots from which the pA_2 values were obtained shows that, whilst the experimental points determined on spleen cluster fairly closely to the fitted line, there is a greater scatter with ileum. This variability is disturbing as it seems possible that either the fine structure of the receptor may differ from animal to animal or that there may be a variable mixture of two (or more) differing populations of α-adrenoceptors in the ileum, both of which contribute to the observed response. This latter possibility complicates the situation since a general assumption in all receptor characterization measurements is that a single type of receptor is under investigation. The use of the pA_2 to characterize receptors becomes debatable when two receptor sites are involved in the interaction since the implicit assumption is that this empirically determined value represents the affinity or dissociation constant of the antagonist at *the* receptor at which the antagonist acts.

If a single receptor type is present which has variable characteristics from animal to animal and which can be activated by both noradrenaline and methoxamine then the pA_2 value of an antagonist acting at that receptor should be independent of whether noradrenaline or methoxamine is used as agonist. Variability in the pA_2 value will occur between animals as the fine structure of the receptor varies but, in a given animal, the pA_2 values for the antagonist should be independent of the agonist used. Unfortunately, in our hands it has proved impossible to measure the pA_2 of an antagonist against both noradrenaline and methoxamine on the same piece of tissue without involving an excessively long experimental procedure with all the attendant hazards of changing sensitivity and decreasing viability. Over a large number of animals however, providing the variation in the fine structure of the receptor is randomly distributed throughout the animal sample, the *mean* pA_2 value obtained from a *set* of determinations should still be independent of the agonist used since the variability in the estimations is due to variations between animals and should affect both sets of determinations equally. We have therefore measured the pA_2 of two antagonists (phentolamine and yohimbine) against noradrenaline and methoxamine. With phentolamine the mean pA_2 values obtained with the two agonists did not differ significantly and this would suggest that there is a single type of receptor present characteristics of which vary from animal to animal. However, the experimental points on the Arunlakshana-Schild plots show no evidence of an unreasonable scatter about the fitted line indicating that any variability in the characteristics of the receptor must be small. Alternatively, phentolamine may not be able to distinguish between the two closely related, but different, receptors.

Experiments with yohimbine did show a difference between the pA_2 value using methoxamine as agonist ($6{\cdot}19 \pm 0{\cdot}12$) and that obtained using noradrenaline as agonist ($5{\cdot}56 \pm 0{\cdot}10$). This difference is not large but is statistically significant and therefore tends to support the possibility that two types of closely related α-adrenoceptor might exist in the ileum.

This is compatible with the degree of scatter of the experimental points used to determine the pA_2 values in the ileum. With phentolamine as antagonist the points are reasonably closely distributed along the fitted line while with yohimbine as antagonist, a much wider scatter is observed. It seems possible therefore that phentolamine fails to distinguish between closely related receptors whose varying proportions do not affect the resultant dose ratio values. Yohimbine is able to distinguish the two receptors and variations in their proportions therefore induces considerable variation in the results. The presence of two α-adrenoceptors in the ileum may also account for the variability of the isomeric activity ratio recorded by Patil, Patil & Krell (1971) and our inability to reproduce their lower value (Barker & others, 1977).

One feature of our results which is perhaps not fully compatible with the suggestion that two slightly different α-adrenoceptors are involved in the ileum is the values obtained for the slopes of the Arunlakshana-Schild plots. While this should be equal to unity in the case of a competitive antagonist acting at a single receptor this may not be the case when two receptors are involved. It might be that some value less than unity could have been expected if two α-adrenoceptors were involved in the interaction but although the values for the slopes obtained with yohimbine as antagonist were less than unity this deviation was not staistically significant.

The possibility that a division of adrenoceptors simply into α-, β_1- and β_2 types may be unsatisfactory in the intestinal tract has been suggested by Gillespie & Khoyi (1977) who investigated the response of rabbit colon to a variety of tonic and inhibitory influences. They found that not all the β-adrenoceptors present had the properties associated with

classical β-adrenoceptors and we have now demonstrated that all the α-adrenoceptors in ileum do not have identical characteristics. Although Wikberg (1977) has suggested that the α-adrenoceptors of the rabbit jejunum may be located on smooth muscle cells as well as on neuronal elements, the design of his experiments does not allow any conclusion about the possbility of this anatomical difference in location being accompanied by a difference in receptor characteristics corresponding to the differences we have reported above.

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