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Possible subdivisions among α-adrenoceptors in various isolated tissues

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The ratio (expressed in $\log_{10}$ units) of the equieffective concentrations of (+)- and (−)-noradrenaline has been measured in a variety of isolated tissues in the presence of cocaine (1×10^{-5}M), tropolone (3×10^{-5}M) and (±)-propranolol (5×10^{-7} to 5×10^{-5}M). The values obtained fall into 3 distinct and statistically different groups. Firstly, a high group comprising (mean ± s.e.) mouse vas deferens (2·78 ± 0·04), rabbit duodenum (2·91 ± 0·07) and ileum (2·86 ± 0·05). Secondly a middle group comprising rabbit vas deferens (2·54 ± 0·04), bladder neck muscle (2·56 ± 0·07) and spleen (2·50 ± 0·02), guinea-pig vas deferens (2·55 ± 0·10) and bladder neck muscle (2·48 ± 0·13) and rat deferens (2·40 ± 0·08) and thirdly, a low group comprising the bladder detrusor muscle from both the rabbit (2·08 ± 0·08) and the guinea-pig (2·07 ± 0·04). Under the same conditions measurement of pA_2 values for phentolamine and piperoxan against noradrenaline gave the following values in rat vas deferens (8·22 ± 0·07 and 6·72 ± 0·03 respectively) and mouse vas deferens (8·31 ± 0·05 and 6·53 ± 0·07 respectively). The results are discussed in relation to other findings concerning the nature of the α-adrenoceptor in these tissues. In spite of the absence of any significant difference between the potency of the α-adrenoceptor blocking agents in the two species it is suggested that α-adrenoceptors may not belong to a single homogeneous population but may vary in their characteristics from tissue to tissue.

It is now widely accepted that adrenoceptors can be classified into α and β groups and that the β-adrenoceptors can be subdivided into β_1 and β_2 types (Lands, Arnold & others 1967; Apperley, Daly & Levy, 1976) although the implied clear-cut division between these two subtypes has been questioned (Furchgott, 1972; see also Daly, Flook & Levy, 1975 for references). Evidence reviewed by Patil, Miller & Trendelenburg (1974) generally indicates however that the α-adrenoceptors form a more homogeneous population although it is possible that, for example, the α-adrenoceptors concerned with thermoregulation in the cat hypothalamus may differ in their characteristics from those encountered elsewhere (Hattan & Wolf, 1974).

A variety of methods have been used to detect differences between different types of receptors; for example, the order of potency of various agonists, comparison of the pA_2 values of antagonists, or determination of the isomeric activity ratio for either agonists, or, less frequently, antagonists. In all these studies unreliable results can be obtained unless complicating factors such as metabolism of the agents or uptake by active processes are controlled (Furchgott, 1972; Patil & others, 1974).

These different methods are by no means of equal value in distinguishing between different receptor types. The use of the order of potency of various agonists or of pA_2 values for antagonists has been shown to be effective and reliable in many cases. The use of isomeric activity ratios is more problematical and it would appear to be inappropriate to conclude that two types of receptor are necessarily identical if identical isomeric activity ratios are obtained. If different isomeric activity ratios are found however a stronger case can be made for a difference in the receptors although this should be supported by comparable results obtained using the other two methods if firm conclusions are to be drawn.

This paper reports an investigation of the characteristics of α-adrenoceptors in several isolated tissues under controlled conditions using mainly the isomeric activity ratio method and employing (−)- and (+)-noradrenaline as agonists. The rationale behind the use of isomeric activity ratios has been reviewed by Patil & others (1974) but briefly, in tissues where stereoselective sites of loss have been eliminated, the ratio of the concentrations of (−)- and (+)-noradrenaline required to produce the same sized response will differ only if the characteristics of the adrenoceptor at which they act differ from tissue to tissue. Differences in penetrability of the tissues by the agonists are unlikely to affect the equieffective concentration *ratio* since the two agonists are stereoisomers and will be equally affected by changes in penetrability. Although the

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presence of β-adrenoceptors in some tissues and not in others may distort the isomeric activity ratio the incorporation of a highly effective concentration of a non-selective β-adrenoceptor blocking agent ensures this factor is controlled.

The tissues examined in this paper were selected because there already exists some evidence in the literature which indicates that there may be differences in the characteristics of the α-adrenoceptors found in certain tissues.

MATERIALS AND METHODS

Mature male animals were killed by a blow on the head. The appropriate tissue was dissected, placed in cold physiological saline, prepared and mounted in an organ bath. Changes in length were recorded isotonically. Details of the experimental conditions for each isolated preparation are given in Table 1 and the positions from which the bladder strips were taken are shown in Fig. 1. All tissues were allowed to equilibrate for 30 min, the maximal response to (−)-noradrenaline was then determined and the amplification of the recording system adjusted appropriately. Constant responses to a fixed submaximal concentration of (−)-noradrenaline were then established and in some cases (especially bladder preparations) this took as long as 90 min.

Table 1. *Details of the experimental conditions used for the various isolated preparations.*

Prep.	°C	Tension (g)	NA dose schedule (min) contact time	cycle time	Approx. NA concn. (M)
Rat vas deferens*	34	0·2	1	5	2×10^{-6}
Guinea-pig vas deferens	34	0·2	1	5	6×10^{-6}
Mouse vas deferens	34	0·15	0·5	5	2×10^{-5}
Guinea pig bladder**	34	0·25	3	15	neck 5×10^{-5} detrusor 5×10^{-5}
Rabbit bladder**	36	1·0	3	15	neck 9×10^{-6} detrusor 5×10^{-5}
Rabbit vas deferens	36	0·2	1	5	4×10^{-6}
Rabbit ileum and duodenum	36	0·5	1·5	7	7×10^{-7}
Rabbit spleen	35	0·5	2	15	8×10^{-8}

The physiological saline in which the tissues were mounted had the following composition: NaCl 130, KCl 2·8, $CaCl_2$ 2·1, $NaHCO_3$ 24·9, NaH_2PO_4 1·2 glucose 11·1, and sucrose 13·1 mM. The solution was aerated with 5% CO_2 in oxygen and also contained (±)-propranolol (5×10^{-7}M), tropolone (3×10^{-5}M) and cocaine (1×10^{-5}M). With some preparations this physiological saline was modified by the inclusion of 0·83 mM $MgSO_4$ (*) or 1 mM $MgSO_4$ together with 5×10^{-5}M (±)-propranolol (**).

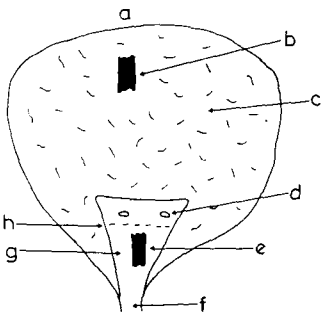

FIG. 1. Diagrammatic view of the interior dorsal aspect of the urinary bladder showing the positions from which the isolated bladder preparations were taken. Strips were approximately 4 mm wide and 10 mm long. a-apex, b-detrusor strip, c-trabeculated detrusor muscle, d-uteric orifice, e-bladder neck strip, f-urethra, g-trigone, h-uteric ridge.

Measurement of isomeric activity ratio for noradrenaline. Equieffective concentrations of (−)- and (+)-noradrenaline were usually determined using a randomized 4 point assay design although occasionally a simpler bracketing assay was used. The mean responses to the high and to the low concentrations of both (−)- and (+)-noradrenaline were calculated and the isomeric activity ratio (expressed in $\log_{10}$ units) was calculated from a $\log_{10}$ concentration-response plot.

Estimation of pA_2 of antagonists. The tissues were exposed repeatedly to two submaximal concentrations of (−)-noradrenaline until reproducible responses were obtained and the physiological saline bathing the tissue was then replaced with physiological saline containing either phentolamine (5×10^{-8}M) or piperoxan ($7{\cdot}7 \times 10^{-7}$M). After 30 min, when the responses to submaximal concentrations of (−)-noradrenaline had become constant and therefore, equilibration between tissue and antagonist had been achieved, two further submaximal concentrations of (−)-noradrenaline were repeatedly tested on the tissue and the responses recorded. Mean responses to each concentration of (−)-noradrenaline (in the presence and in the absence of the antagonist) were calculated, plotted on $\log_{10}$ concentration-response graphs and the pA_2 for the antagonist was calculated from the equation

$pA_2 = \log_{10}((Ar'/Ar)-1)/\text{molar concentration of antagonist}$ where:— Ar′ and Ar represent respectively the concentrations of (−)-noradrenaline

required to produce the same sized response from the tissue in the presence and absence of the appropriate concentration of antagonist.

Statistical procedures. Where appropriate, values are given as means ± standard errors and tests for statistical significance utilized Student's *t*-test.

Drugs used: L-Ascorbic acid (BDH), cocaine hydrochloride (BDH), phentolamine mesylate (Rogitine; Ciba) piperoxan hydrochloride, (±)-propranolol hydrochloride (ICI). (−)-Noradrenaline bitartrate was obtained from Koch-Light Laboratories and the (+)-isomer (m.p. 163°; $[\alpha]^{25}D = +39{\cdot}5$: lit. m.p. 164–165°; $[\alpha]^{25}D = +39{\cdot}9$) was prepared in our own laboratories by the method of Tuller (1948) from (±)-noradrenaline obtained from Fluka AG. Before use, noradrenaline was dissolved in a solution of NaCl (153 mM), ascorbic acid (0·11 mM) and HCl (10 mM). Tropolone (Aldrich Chemicals) was recrystallized to constant melting point (51–52°) from light petroleum (40–60°) and stored at 4° since the original sample showed a brown discolouration.

RESULTS

All but two of the preparations responded to noradrenaline at the concentrations in Table 1 with a contraction; rabbit duodenum and ileum preparations relaxed. As the responses developed (and reversed after washing) at different rates in the different preparations, a suitable contact time for noradrenaline and a suitable time between doses were chosen appropriate to each preparation (Table 1).

Rat vas deferens and all the bladder preparations showed considerable spontaneous activity when bathed in the normal physiological saline and this interfered seriously with the estimates of isomeric activity ratio. However, the addition of a small amount of magnesium sulphate to the physiological saline reduced spontaneous activity to an acceptable level. Higher than normal concentrations of propranolol were incorporated into the physiological saline in the case of the bladder preparations since these tissues are known to contain large numbers of β-adrenoceptors (Awad, Bruce & others, 1974; Downie, Dean & others 1975) and it is essential to eliminate any β-adrenoceptor component of the response if valid isomeric activity ratios are to be obtained.

The values obtained for the isomeric activity ratio of (+)- to (−)-noradrenaline (expressed in $\log_{10}$ units) in the various preparations used are shown in Fig. 2. Clearly, the results fall into three

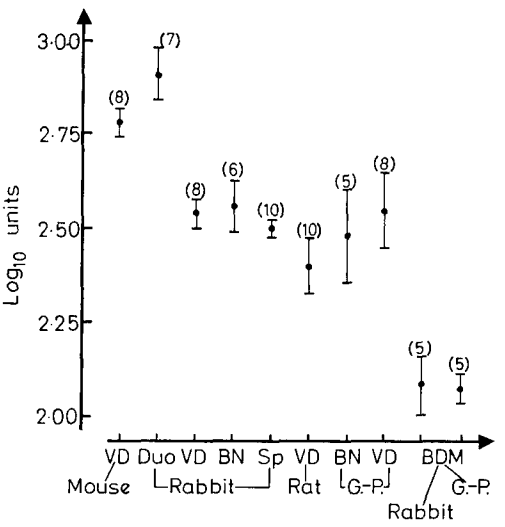

FIG. 2. Mean ± s.e. for the isomeric activity ratio ($\log_{10}$ units) for (+)- and (—)-noradrenaline measured in several tissues in the presence of cocaine (1×10^{-5}M), (±)-propranolol (5×10^{-7}M) and tropolone (3×10^{-5}M). For details of the experimental conditions see Table 1. The figures in parenthesis show the number of observations contributing to each mean and the statistical significance of the differences between the means is given in Table 2. VD-vas deferens, Duo-duodenum, BN-bladder neck, Sp-spleen, BDM-bladder detrusor muscle.

distinct groups. Firstly, a large main group comprising rabbit vas deferens (2·54 ± 0·04), bladder neck (2·56 ± 0·07) and spleen (2·50 ± 0·02), guinea-pig vas deferens (2·55 ± 0·10) and bladder neck (2·48 ± 0·13) and rat vas deferens (2·40 ± 0·08); secondly, a 'high' group comprising mouse vas deferens (2·78 ± 0·04) and rabbit duodenum (2·91 ± 0·07) and thirdly, a 'low' group comprising the bladder detrusor muscle from both the rabbit (2·08 ± 0·08) and the guinea-pig (2·07 ± 0·04). Although the magnitude of the differences between these groups is not large the differences are significant statistically (Table 2) and it must be remembered that these figures are in $\log_{10}$ units. Furthermore, as the size of standard errors show, the isomeric activity ratio is remarkably constant in a given tissue.

With the rabbit duodenum a high value for the isomeric activity ratio might be expected if there were incomplete blockade of the inhibitory β-adrenoceptors known to be present in this tissue. To test this possibility a separate series of experiments was performed in various concentrations of propranolol and the isomeric activity ratio redeter-

Table 2. *The probability (Student's* t*-test) of the difference between the mean isomeric activity ratios in the various tissues being due to chance alone.*

	1	2	3	4	5	6	7	8	9
	Tissue code (see L.H.S. of this figure)								
1 Mouse vas deferens (8)	—								
2 Rat vas deferens (10)	<0·001	—							
3 Rabbit vas deferens (8)	<0·001	>0·1	—						
4 Guinea-pig vas deferens (8)	<0·05	>0·2	>0·9	—					
5 Guinea-pig bladder neck (5)	<0·05	>0·5	>0·6	>0·7	—				
6 Guinea-pig detrusor (5)	<0·001	<0·02	<0·01	<0·02	<0·1	—			
7 Rabbit bladder neck (6)	0·02	>0·1	>0·7	>0·9	>0·5	<0·02	—		
8 Rabbit detrusor (5)	<0·001	<0·05	<0·001	<0·01	<0·05	>0·9	<0·001	—	
9 Rabbit duodenum (7)	>0·1	<0·001	<0·001	<0·02	<0·02	<0·001	<0·01	<0·001	—
10 Rabbit spleen (10)	<0·001	>0·2	>0·2	>0·6	>0·8	<0·001	>0·2	<0·001	<0·001

The figures in parentheses show the number of determinations contributing to each mean and the actual means and standard errors are given in the text.

mined. As can be seen from Table 3, the isomeric activity ratios obtained in this second series of experiments did not differ significantly from those obtained in the first series, neither did increasing the concentration of propranolol 100-fold cause any significant reduction in the ratio obtained. Also presented in this table are data obtained on rabbit ileum preparations and it can be seen that similar value for the isomeric activity ratio was obtained in this tissue as in the duodenum.

The pA_2 values obtained for phentolamine and for piperoxan against (−)-noradrenaline in rat and mouse vas deferens are shown in Table 4. In both species phentolamine was some 40–60 times more potent as an α-adrenoceptor blocking agent than was piperoxan. However, although the isomeric activity ratio for (+)- and (−)-noradrenaline differed in mouse and rat vas deferens, the pA_2 values obtained for phentolamine were not significantly different ($P > 0·3$) in the two tissues. A similar result was also obtained using piperoxan ($P > 0·3$) and it would appear that there is no significant difference between the sensitivity of the rat and the mouse vas deferens to these two α-adrenoceptor blocking agents.

Table 3. *Mean (± standard error) of the isomeric activity ratios obtained on rabbit duodenum or ileum under various experimental conditions.*

Tissue	Propranolol concn. (M)	Isomeric act. ratio (mean ± s.e.)	n	P*
Duodenum	5×10^{-7}	2·93 ± 0·08	3	
Duodenum	5×10^{-6}	2·82 ± 0·05	6	0·2
Duodenum	5×10^{-5}	2·99 & 2·88 (single results)	2	—
Ileum	5×10^{-7}	2·86 ± 0·05	4	0·4

* Testing against value obtained in 5×10^{-7} propranolol for duodenum.
In the previous set of experiments (see Fig. 2) the rabbit duodenum gave an isomeric activity ratio of 2·91 ± 0·07 (n = 7) which is not significantly different from the results obtained under the same conditions and shown above either for duodenum ($P > 0·8$) or ileum ($P > 0·5$).

Table 4. *pA_2 values (mean ± s.e.) for phentolamine and piperoxan on mouse and rat vas deferens using (−)-noradrenaline as the agonist.*

	Phentolamine	Piperoxan
Rat	8·22 ± 0·07 (6)	6·62 ± 0·03 (4)
Mouse	8·31 ± 0·05 (6)	6·53 ± 0·07 (6)
Probability	$P > 0·3$	$P > 0·3$

The experimental conditions under which these determinations were made are given in Table 1. Also included in this table are the number of observations contributing to each mean (figures in parenthesis) and the probability (Student's *t*-test) of the observed differences between the mean values obtained in the two species being due to chance alone.

DISCUSSION

The results show that the isomeric activity ratios obtained fell into three distinct and statistically different groups, the largest group giving isomeric ratios in the range 2·4 to 2·6 $\log_{10}$ units (rat, rabbit and guinea-pig vas deferens, guinea-pig and rabbit bladder neck muscle and rabbit spleen). A second group comprised mouse vas deferens and rabbit duodenum (2·78 and 2·91 respectively) and the third group comprised guinea-pig and rabbit bladder detrusor muscle (2·07 and 2·08 respectively). An initial survey of the results therefore suggests that

the α-adrenoceptors in rat, rabbit and guinea-pig vas deferens, guinea-pig and rabbit bladder neck muscles and rabbit spleen are the same and would appear to be the most abundant form of α-adrenoceptor since other workers have reported values in a similar range for rabbit, frog, rat, guinea-pig and cat aorta, cat spleen, rabbit vena cava and rat seminal vesicle (Patil, Patil & Krell, 1971; Patil, Fudge & Jacobowitz, 1972; Patil & others, 1974).

The results reported here confirm some of the values obtained by these other workers for the isomeric activity ratio of noradrenaline. Thus there is no statistically significant difference between the values reported here and those obtained by Patil & others (1971) for rat vas deferens (2·40 ± 0·08 and 2·51 ± 0·11; $P>0·4$) or for rabbit spleen (2·50 ± 0·02 and 2·43 ± 0·12; $P>0·5$) respectively. With rabbit intestine preparations however a statistically significant difference does exist in that Patil & others (1971) reported a value for the isomeric activity ratio of 2·50 ± 0·11 while our value is 2·91 ± 0·07 ($P>0·01$). Since our values were determined on duodenum while Patil and his colleagues used ileum, the possibility was investigated that the isomeric activity ratio might vary depending on the region of intestine used. In a separate set of experiments however no significant difference was found between values obtained on ileum and duodenum and neither did these values differ from the high value in the first series of experiments.

A second factor which might explain this discrepancy is the possibility that β-adrenoceptor blockade is incomplete in our experiments. The concentration of propranolol was therefore increased (up to 5×10^{-5}M) but high values for the isomeric activity ratio were still obtained which were not statistically different from the values obtained at the low propranolol concentration. The highest concentration of propranolol used is some 4 orders of magnitude above the pA_2 for this agent against (−)-noradrenaline on the β-adrenoceptors of the heart and should be highly effective against the β-adrenoceptors in the intestine and in the bladder where the same high concentration was routinely employed. It was considered unwise to increase the concentration of propranolol further as the pA_2 of this agent against α-adrenoceptors in the rabbit aortic strip is 5·2 (Gulati, Gokhale & others, 1969).

A closer examination of the results obtained by Patil & others (1971) shows that although they did report a value of 2·50 ± 0·11 (n = 10) for the isomeric activity ratio in rabbit ileum they also reported a second value of 2·83 ± 0·14 (n = 8) in a separate set of experiments. This second value is not statistically significantly different ($P>0·6$) from the value reported in this paper (2·91 ± 0·07; n = 7). It is possible therefore that rabbit strains show some variation in the isomeric activity ratio for noradrenaline and that ours belonged consistently to the higher side of the distribution while those used by Patil & others (1971) were more varied.

Although the experimental conditions under which the isomeric activity ratios were determined differed in the several tissues there appears to be no obvious correlation between the conditions used and the values obtained. Furthermore, there appears to be no relation between the value obtained for the isomeric ratio and the concentration of (−)-noradrenaline required to produce a response from the various tissues. It does not appear therefore that the differences observed are artifactual in the sense that they are imposed by variations in the experimental conditions from tissue to tissue. It is possible however that the differences in isomeric activity ratio are artifactual in the sense that they derive from the presence of stereoselective sites of loss still present in some of the tissues. We have no direct evidence that in *each* of the isolated preparations *all* the stereo-selective sites of loss have been *completely* eliminated and it is known that in tissues with stereoselective sites of loss still functioning normally, different isomeric activity ratios can be obtained. These differences were eliminated however in the presence of cocaine, tropolone and sotalol (Patil & others, 1974).

The suggestions that there are indeed differences in the characteristics of the α-adrenoceptors found in some of the tissues investigated in this paper is supported by other published work. A difference in the α-adrenoceptors in rabbit bladder neck and detrusor muscles has also been suggested by Downie & others (1975) who investigated the sensitivity of these preparations to several α-adrenoceptor agonists. In the group of tissues with high isomeric activity ratios, van Rossum (1965) has suggested (based on the potency of various agonists and antagonists) that the α-adrenoceptors in rabbit jejunum differ from those in rat vas deferens and we have reported previously (Hughes, Kneen & Main, 1974) that the pA_2 value of desipramine appears to be different in mouse and guinea-pig vasa deferentia. In none of these reports however, were adequate precautions taken to control all the factors which might complicate interpretation of the results.

In an attempt to confirm the possibility that the α-adrenoceptors in mouse and guinea-pig vas

deferens are different in their characteristics (as suggested by the isomeric activity ratio results), estimates of the pA_2 values of piperoxan and phentolamine were made in these two tissues under controlled conditions. No statistically significant differences were found between the two tissues. Although this does not support the suggestion that the α-adrenoceptors in the two tissues are different, neither does it disprove the possibility. To draw an analogy with the β-adrenoceptor, practolol has been shown to be β_1-selective while propranolol does not show selectivity (Furchgott, 1972). It is possible that a similar situation exists with regard to the α-adrenoceptor blocking agents used above. Although neither may be able to distinguish between the α-adrenoceptor in the two species this does not necessarily imply that the receptors are in fact identical.

Recently, several workers using diverse tissues have suggested that the α-adrenoceptor in particular tissues may differ in its characteristics from those more frequently encountered. Not only may α-adrenoceptors in various tissues of the cod differ in their characteristics (Holmgren & Nilsson, 1975) but also pre- and post-junctional α-adrenoceptors (Starke, Endo & Taube, 1975), rabbit cerebral artery α-adrenoceptors (Duckles & Bevan, 1976) and those α-adrenoceptors in the rat central nervous system which are concerned with the regulation of activity in sympathetic nerves (Struyker Boudier, de Boer & others, 1975) may also show different characteristics from α-adrenoceptors encountered elsewhere. Indeed, it seems likely that the α-adrenoceptor is at least as heterogeneous, if not more so, than is the β-adrenoceptor.

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