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RECEPTORS FOR SYMPATHOMIMETIC AMINES IN THE RABBIT AORTA: DIFFERENTIATION BY SPECIFIC ANTAGONISTS

BY

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Some sympathomimetic amines act on 5-hydroxytryptamine receptors in certain preparations of gut—for example, rat stomach (Vane, 1960) and guinea-pig ileum (Innes, 1963; Kohli & Innes, 1965). Using cross-protection against phenoxybenzamine as a means of differentiating drug receptors, Innes (1963) concluded that dexamphetamine acted on 5-hydroxytryptamine receptors in guinea-pig ileum and other smooth muscles including vascular smooth muscle. To extend this conclusion to other sympathomimetic amines, which stimulated guinea-pig ileum and seemed to act on 5-hydroxytryptamine receptors in that tissue, an attempt was made to differentiate receptors for a few sympathomimetic amines in the rabbit aorta by using the cross-protection technique. The results obtained did not enable any clear-cut conclusion to be drawn regarding the type of receptors involved in the action of sympathomimetic amines classed as "inhibitory" and "excitatory" according to their action on guinea-pig ileum (Kohli, 1965).

We have now approached the problem by using specific antagonists as a means of differentiating drug receptors. An antagonist clearly differentiating between 5-hydroxytryptamine and noradrenaline should also differentiate between those sympathomimetic amines which act on 5-hydroxytryptamine receptors and those which act on noradrenaline receptors. The degrees of antagonism by an α-adrenergic blocking agent, phentolamine, and a 5-hydroxytryptamine antagonist, 2-bromolysergic acid diethylamide, against a group of sympathomimetic amines have therefore been compared. pA_{10} values of either of the antagonists against a group of sympathomimetic amines have been determined in order to compare specificity of antagonism. Besides noradrenaline and 5-hydroxytryptamine, three compounds which cause contraction of guinea-pig ileum by acting on 5-hydroxytryptamine receptors (phenylephrine, methoxamine and *d*-amphetamine) and two that cause relaxation (nordefrine and ephedrine) are included in the present study.

METHODS

All experiments were done on spirally cut aortic strips obtained from rabbits (1.5–2.5 kg) of either sex. The strips were suspended in 10–12 ml. baths containing Krebs–Henseleit solution kept at 38° C and bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide. Isotonic contractions

against 1 g tension and magnified 5.5-fold were recorded on a kymograph. Strips were allowed to relax for 75–90 min before testing was begun. The bathing fluid was changed every 15 min during this waiting period. pA_{10} was measured according to the method of Schild (1947).

Four strips from a single aorta were used in each experiment. In all experiments, only one concentration of the antagonist was tested on each strip so that four concentrations of the antagonist were tested on each aorta. Some experiments were done on strips from rabbits treated with reserpine 1 mg/kg intramuscularly 42 and 18 hr before killing.

Drugs and solutions

The standard agonists used were: (−)-noradrenaline bitartrate monohydrate, (−)-adrenaline bitartrate and 5-hydroxytryptamine creatinine sulphate. The test agonists were phenylephrine hydrochloride, methoxamine hydrochloride, *d*-amphetamine sulphate, nordefrine hydrochloride and ephedrine hydrochloride. Stock solutions of these amines were made in acidified 0.9% sodium chloride solution and kept frozen. Concentrations in the text indicate final concentrations of the base in the bath in g/ml.

2-Bromolysergic acid diethylamide bitartrate (BOL) was supplied in 1 ml. ampoules containing 0.5 mg of the salt with 0.25 mg tartaric acid and 8 mg sodium chloride in distilled water. Stock solution of phentolamine hydrochloride 10 mg/ml. was made in saline and stored at 4° C. Dilutions were freshly made when required.

RESULTS

Unit doses of agonists

Dose-response curves of each agonist were studied in three to four strips. Phenylephrine, methoxamine and nordefrine caused maximal contraction of the strips equal to that caused by noradrenaline. Phenylephrine was nearly equipotent, while methoxamine and nordefrine respectively were one-third and one-tenth as potent in comparison with noradrenaline. The highest contractions caused by ephedrine or *d*-amphetamine were only 60–70% of the maximal contraction caused by noradrenaline. Compared at the *ED*50 level, both ephedrine and *d*-amphetamine were about one thousandth as potent as noradrenaline. The unit doses of agonists used to determine pA_{10} values (shown in Table 1) were based on the results of these experiments and were near *ED*50 in each case.

TABLE 1

pA_{10} VALUES OF PHENTOLAMINE AGAINST SOME SYMPATHOMIMETIC AMINES AND 5-HYDROXYTRYPTAMINE ON RABBIT AORTIC STRIPS

Exposure to phentolamine, 3 min. Figures in parentheses are numbers of experiments.

Agonist	Unit dose (g/ml.)	$pA_{10} \pm$ S.E.
Noradrenaline	$1–3 \times 10^{-8}$	6·2±0·04 (6)
Adrenaline	$1–3 \times 10^{-8}$	6·6±0·03 (6)
Phenylephrine	$1–3 \times 10^{-8}$	6·4±0·04 (6)
Methoxamine	$1–5 \times 10^{-7}$	6·5±0·02 (6)
Nordefrine	$3–10 \times 10^{-7}$	6·2±0·05 (6)
Ephedrine	$1–3 \times 10^{-5}$	5·8±0·08 (5)
d-Amphetamine	$3–10 \times 10^{-5}$	5·1±0·1 (5)
5-Hydroxytryptamine	$3–10 \times 10^{-8}$	4·9±0·07 (5)

pA_{10} of phentolamine

When reproducible responses to the unit doses of the test agonist had been obtained, each strip was exposed to a given concentration of phentolamine for 3 min. The concentrations of phentolamine used in final determinations of pA_{10} were based on several

preliminary experiments which indicated the range within which the pA_{10} against each agonist would lie. The relevant parts of the record of a representative experiment are reproduced in Fig. 1. The effect of tenfold dose was measured and calculated as a percentage of the effect of the unit dose before the antagonist. The concentration equivalent to the pA_{10} value was then determined graphically (Hill & Kohli, 1967), from which the pA_{10} was calculated.

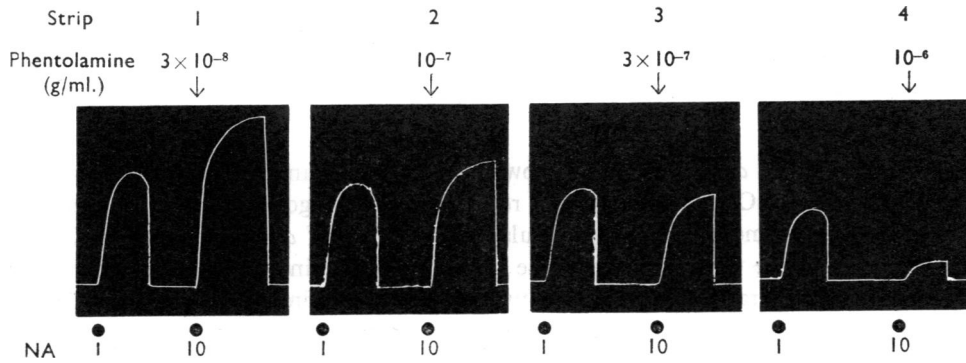

Fig. 1. Determination of pA_{10} of phentolamine against noradrenaline in rabbit aorta. All contractions with noradrenaline (NA) 10^{-8} g/ml. (1) and 10^{-7} g/ml. (10). Indicated concentrations of phentolamine were added at ↓ to each bath 3 min before NA (10).

The pA_{10} values of phentolamine ±S.E. against seven sympathomimetic amines and 5-hydroxytryptamine are shown in Table 1. The values for noradrenaline and the other potent agonists ranged from 6.2 to 6.6. The values for the weaker agonists, ephedrine and *d*-amphetamine, were lower—so much so that the pA_{10} against *d*-amphetamine was only 0.2 log units more than the value for 5-hydroxytryptamine (4.9). The difference between the pA_{10} values against noradrenaline and 5-hydroxytryptamine was 1.3 log units.

pA_2 and pA_{10} of BOL against standard agonists

Kohli & Innes (1964) showed that BOL possessed a marked degree of specificity of antagonism against 5-hydroxytryptamine. pA_2 and pA_{10} values of BOL against 5-hydroxytryptamine and noradrenaline were therefore determined. Tissues were exposed to BOL for 10 min before the effects of a multiple dose in the presence of antagonist was recorded. The results are presented in Table 2. At either level of testing, the difference between the values against 5-hydroxytryptamine and noradrenaline was more than 2.5 log units. In other words BOL was about three hundred times more active against 5-hydroxytryptamine than against noradrenaline.

pA_{10} of BOL against the test amines

Following the procedure used for standard agonists, pA_{10} of BOL against each sympathomimetic amine was determined in six to seven experiments (Table 2). As with phentolamine, the pA_{10} values of BOL against the potent agonists differed by less than 0.5 log units. Values against the weaker agonists, however, showed a greater divergence.

TABLE 2

pA_2 AND pA_{10} VALUES OF 2-BROMOLYSERGIC ACID DIETHYLAMIDE (BOL) AGAINST 5-HYDROXYTRYPTAMINE AND SOME SYMPATHOMIMETIC AMINES ON NORMAL AND RESERPINIZED AORTIC STRIPS

Exposure to BOL 10 min. Figures in parentheses are numbers of experiments.

Agonist	$pA_2 \pm$ S.E. Normal strips	$pA_{10} \pm$ S.E. Normal strips	$pA_{10} \pm$ S.E. Reserpinized strips
5-Hydroxytryptamine	9·0±0·08 (5)	8·3±0·05 (7)	8·6±0·02 (6)
Noradrenaline	6·4±0·04 (8)	5·6±0·08 (6)	5·7±0·09 (5)
Phenylephrine	—	5·8±0·01 (6)	—
Methoxamine	—	5·7±0·04 (6)	—
Nordefrine	—	5·3±0·07 (6)	—
Ephedrine	—	5·0±0·04 (7)	5·0±0·05 (4)
d-Amphetamine	—	4·6±0·06 (6)	4·4±0·08 (5)

Thus ephedrine and *d*-amphetamine showed greater resistance to antagonism by both phentolamine and BOL. Because this resistance to antagonism might be related to indirect sympathomimetic activity, particularly in the case of *d*-amphetamine, pA_{10} values of BOL against these two amines and the two standard amines were also determined in strips obtained from rabbits treated with two doses of reserpine 1 mg/kg given 42 and 18 hr before killing. This dose of reserpine was considered adequate to cause depletion of the endogenous catecholamines (Carlsson, Rosengren, Bertler & Nilsson, 1957; Muscholl & Vogt, 1958). The pA_{10} values of BOL from these strips were nearly identical with those from normal strips (Table 2).

DISCUSSION

The main object of the present study was to determine whether the sympathomimetic amines, which caused contraction of the guinea-pig ileum by acting on 5-hydroxytryptamine receptors, had any part of their excitatory activity on the rabbit aorta through 5-hydroxytryptamine receptors. With this objecive, the pA_{10} values of the two antagonists against the various amines were compared. In Fig. 2 the pA_{10} values of phentolamine and BOL against the various amines have been plotted on two parallel lines and the pA_{10} values against each agonist have been joined. The slopes of these lines bring out a clear distinction between 5-hydroxytryptamine and the sympathomimetic amines; all sympathomimetic amines were more sensitive to blocking by phentolamine than they were to BOL, while 5-hydroxytryptamine showed markedly greater sensitivity to BOL and least sensitivity to phentolamine. Thus the slope of the line joining the pA_{10} values of the two antagonists against 5-hydroxytryptamine is in the opposite direction from the slopes of the lines for all the sympathomimetic amines. This fact places sympathomimetic amines, including *d*-amphetamine, in a different class from 5-hydroxytryptamine.

According to the results obtained with cross-protection against phenoxybenzamine, *d*-amphetamine seemed to act on 5-hydroxytryptamine receptors in the rabbit aorta (Innes, 1963; Kohli, 1965). It is therefore significant that the difference between the pA_{10} values of BOL against 5-hydroxytryptamine and any sympathomimetic amine is highest in the case of *d*-amphetamine (3.7 log units). According to the pA_{10} values of BOL the "excitatory" amines (phenylephrine, methoxamine and *d*-amphetamine) are indistinguishable from the "inhibitory" amines (noradrenaline, nordefrine and ephedrine). It therefore seems reasonable to conclude that none of the amines studied has any part of its action on the rabbit aorta through 5-hydroxytryptamine receptors.

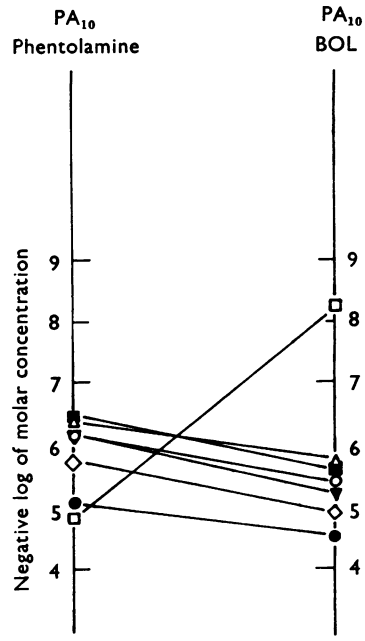

Fig. 2. pA_{10} values of phentolamine and 2-bromolysergic acid diethylamide (BOL) against 5-hydroxytryptamine and some sympathomimetic amines. 5-Hydroxytryptamine, □——□; noradrenaline, ○——○; phenylephrine, Δ——Δ; methoxamine, ■——■; nordefrine, ▼——▼; ephedrine, ◇——◇; *d*-amphetamine, ●——●.

The difference between the conclusions drawn from protection experiments and the present study, specifically with regard to *d*-amphetamine, deserves special comment. Based on theoretical considerations, Waud (1962) criticized the validity of the cross-protection technique in identifying drug receptors. Furchgott (1964), however, considered the cross-protection method useful in differentiating receptors as long as it was used with an understanding of its possible pitfalls. The results of the present study point to one such pitfall. In cross-protection experiments (see Innes, 1962) large concentrations of agonists are used for protection against block by phenoxybenzamine. It is conceivable that, in such large concentrations, agonists can protect receptors other than the specific receptors, so that the ability to protect responses is merely a measure of the affinity of an agonist for different receptors. It would therefore seem that, whereas *d*-amphetamine may have marked affinity for 5-hydroxytryptamine receptors, it does not produce its effect in conventional doses through these receptors, this effect being remarkably resistant to block by BOL in contrast to 5-hydroxytryptamine.

A general parallelism among the lines joining the pA_{10} values of the two antagonists against the various sympathomimetic amines (Fig. 2) would seem to support the idea of a single receptor system being concerned in the action of all these agonists. A variation of 0.4–1.2 log units among the pA_{10} values against various sympathomimetics has, however, been observed for each of the antagonists. The concept that different agonists acting on the same receptor would give the same pA_x values applies only if such agonists are acting directly on the receptor (Arunlakshana & Schild, 1959) and if

interference through action on other receptors can be excluded. The possibility of interference through the indirect action of some of the amines was considered, but the pA_{10} values of BOL from strips obtained from rabbits depleted of noradrenaline by reserpine were nearly identical with the values obtained from the normal strips (Table 2).

The possibility of interference through an action on the β-inhibitory receptors cannot be excluded by the present study, but this factor does not seem likely on account of the closeness of pA_{10} values of either antagonist against such amines as adrenaline, noradrenaline, phenylephrine and methoxamine. On the other hand the divergence from the standard amine, noradrenaline, seemed to be roughly related to the potency of the agonists. Whether or not this means that partial agonists may not show the same pA_x values as the full agonists, even if both act through the same receptor system, requires further investigation. The present work has, however, demonstrated that more than one antagonist should be used for the purpose of differentiation of receptors for a group of agonists. If phentolamine alone had been used in the present investigation, the similar pA_{10} values against 5-hydroxytryptamine and *d*-amphetamine would have supported the cross-protection study which indicated that *d*-amphetamine acted on 5-hydroxytryptamine receptors. Only when BOL was used was the marked difference between 5-hydroxytryptamine and *d*-amphetamine brought out, the difference between the pA_{10} values against the two agonists being 3.7 log units.

SUMMARY

1. pA_{10} of phentolamine and pA_2 and pA_{10} of 2-bromolysergic acid diethylamide (BOL) against noradrenaline and 5-hydroxytryptamine were determined in rabbit aortic strips.

2. BOL showed a greater specificity against 5-hydroxytryptamine than did phentolamine against noradrenaline ; the difference between the pA_{10} values of BOL against the two agonists was more than 2.5 log units as compared with a difference of 1.3 log units in the case of phentolamine.

3. pA_{10} values of phentolamine and BOL against phenylephrine, methoxamine and *d*-amphetamine which contract guinea-pig ileum (excitatory amines) and nordefrine and ephedrine which cause relaxation (inhibitory amines) were also determined.

4. The pA_{10} values of BOL and phentolamine did not distinguish between the " excitatory " and the " inhibitory " sympathomimetic amines in the rabbit aorta. Moreover, the slopes of the lines joining the pA_{10} values of the antagonists against various sympathomimetic amines were similar to the slope for noradrenaline and opposite to the slope for 5-hydroxytryptamine. These findings suggest that all the sympathomimetic amines studied act through noradrenaline receptors.

5. The difference between the pA_{10} values of BOL against 5-hydroxytryptamine and *d*-amphetamine is 3.7 log units. This seemed to rule out the possibility that 5-hydroxytryptamine receptors are concerned in the action of *d*-amphetamine in the rabbit aorta. These results thus point to a pitfall in the use of cross-protection technique as a means of differentiating drug receptors.

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