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Excitatory action of sympathomimetic amines on 5-hydroxytryptamine receptors of gut

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1. Twenty-two sympathomimetic amines were tested for excitatory activity on isolated guinea-pig ileum, rabbit jejunum, and rat stomach.

2. Eight amines contracted all or almost all guinea-pig ileum preparations; five amines contracted half the preparations and relaxed the others. Nine amines were consistently relaxant. Structural requirements for excitatory activity were not clear.

3. Six amines contracted rat stomach and only four contracted rabbit jejunum. Only β-phenylethylamine and (−)-amphetamine contracted all three preparations.

4. The excitatory effects of the sympathomimetic amines on guinea-pig ileum seem to be due to an action on 5-hydroxytryptamine receptors. This conclusion is based on evidence that their excitatory action is antagonized by 2-bromolysergic acid diethylamide, by morphine and by desensitization with 5-hydroxytryptamine, and that there is cross-protection between 5-hydroxytryptamine and the excitatory sympathomimetic amines against block by phenoxybenzamine.

In general, sympathomimetic amines relax the gastrointestinal tract and inhibit its motility. This is the customary response to catecholamines, but some other sympathomimetic amines—for example, amphetamine (Detrick, Millikan, Modern & Thienes, 1937) or β-phenylethylamine (Hoyt, Patek & Thienes, 1934)—cause most preparations of the gut to contract. We have studied the effects of various sympathomimetic amines on rat stomach, rabbit jejunum and guinea-pig ileum to try to relate the chemical structure with this gastrointestinal excitatory activity.

According to current concepts both α and β-adrenoceptive receptors are involved in inhibition of the gut (Ahlquist & Levy, 1959; Furchgott, 1960; Kosterlitz & Watt, 1964). Vane (1960) concluded that sympathomimetics causing contraction of rat stomach act on 5-hydroxytryptamine receptors, and Innes (1963) showed that dexamphetamine contracts guinea-pig ileum by acting on 5-hydroxytryptamine receptors. We have used guinea-pig nonterminal ileum, which is relaxed by

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catecholamines, to study the excitatory receptors for sympathomimetic amines which consistently cause contraction. A preliminary report on this work was made to the 23rd International Congress of Physiological Sciences (Kohli & Innes, 1965).

Methods

Three preparations of gastrointestinal muscle were used: jejunum from rabbits (1.8–3 kg) of either sex ; nonterminal ileum from guinea-pigs (300–700 g) of either sex, taken at least 10 cm away from the ileocaecal junction in order to avoid ileum which may contract in response to adrenaline (Munro, 1952) ; stomach strips prepared as described by Vane (1957) from rats (150–300 g) of either sex. Each preparation was suspended in a 10–12 ml. bath in Krebs–Henseleit solution, bubbled with a mixture of oxygen and carbon dioxide (95%–5%) and kept at 38° C. Isotonic contractions against 1 g tension and amplified 5.5-fold were recorded on a kymograph. After the tissues were suspended, at least 1 hr was allowed before drugs were tested. Unless otherwise specified, tests were made at 10 min intervals. The agonist was added at zero time and washed out after any contraction produced had reached its maximum ; 5 min after zero time the wash was repeated, and 5 min later the next test was made.

Desensitization to 5-hydroxytryptamine

In each experiment of this type we obtained control responses of guinea-pig ileum to one or two doses of 5-hydroxytryptamine, to 3–4 graded doses of the excitatory sympathomimetic being tested, and to 3–4 graded doses of either histamine or acetylcholine. The preparation was then desensitized to 5-hydroxytryptamine by a concentration of 5-hydroxytryptamine of $2–2.5 \times 10^{-6}$ g/ml. kept in the bath for the rest of the experiment. Every 15 min the bathing fluid was changed and the drug immediately added again. The contraction due to 5-hydroxytryptamine was brief ; the muscle rapidly relaxed completely in spite of the 5-hydroxytryptamine in the bath. Desensitized preparations no longer responded to additional doses of 5-hydroxytryptamine after 45 min exposure to the drug. For the rest of the experiment agonists were tested every 15 min in the continued presence of 5-hydroxytryptamine. The responses are expressed as a percentage of the control responses.

Selective receptor protection

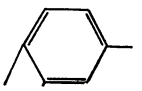
Receptor protection experiments were done as described earlier (Innes, 1962, 1963). Control responses were obtained in four strips prepared from adjacent segments of gut from the same animal. All strips were then exposed to the same concentration of phenoxybenzamine, 10^{-5} g/ml., but three strips were protected throughout the entire period of exposure (5 min) by a high concentration of an agonist added to the bath 5 min before the blocking agent. One strip was protected by 5-hydroxytryptamine (10^{-4} g/ml.), another by an excitatory sympathomimetic amine (10^{-4} g/ml.) and the third by acetylcholine (10^{-4} g/ml.) in seven experiments or by histamine (10^{-4} g/ml) in ten experiments. The fourth strip, which was not protected against the blocking agent, served as control. Protecting and blocking agents were washed out at the same time and washes were repeated every 10–15 min for 60–90 min. Responses were than tested again and the results expressed as a percentage of the control response.

Antagonism by morphine or 2-bromolysergic acid diethylamide

After control responses had been obtained, the agonists were tested in the presence of these antagonists. Morphine was kept constantly in the bath by addition of the drug immediately after each wash. When 2-bromolysergic acid diethylamide was kept constantly in the bath, however, it often caused the gut to contract and usually delayed relaxation of the gut from contractions due to the agonists. 2-Bromolysergic acid diethylamide was therefore added 5 min after the second wash following each test so that the preparation was exposed to the antagonist for only 5 min before each test dose of agonist. With this procedure 2-bromolysergic acid diethylamide rarely caused contractions and did not delay relaxation after the test agonists. The degree of antagonism remained constant with the exposure of 5 min before each test.

Inhibition is indicated quantitatively either by the dose-ratio, the factor by which a dose must be multiplied to produce the same response in the presence of a particular amount of antagonist, or by the residual response expressed as a percentage of the control response to the same dose of agonist. The effect of a dose of agonist

TABLE 1. *Chemical structures of sympathomimetic amines studied*

Drug	Substituent						Source
				H H H | | | C — C — N | | |			
	4	3	other	α	β	R	
(−)-Noradrenaline bitartrate monohydrate	OH	OH		OH	H	H	Calbiochem
(—)-Adrenaline bitartrate	OH	OH		OH	H	CH_3	*Sterling Winthrop
Nordefrine hydrochloride	OH	OH		OH	CH_3	H	*Sterling Winthrop
Dopamine hydrochloride	OH	OH		H	H	H	Sigma Chemical Co.
Epinine hydrochloride	OH	OH		H	H	CH_3	*Burroughs Wellcome
Oxedrine tartrate	OH	H		OH	H	CH_3	*Sterling Winthrop
Octopamine hydrochloride	OH	H		OH	H	H	*Sterling Winthrop
Hydroxyamphetamine hydrobromide	OH	H		H	CH_3	H	*Smith Kline & French
Phenylephrine hydrochloride	H	OH		OH	H	CH_3	*Sterling Winthrop
Norphenylephrine bitartrate	H	OH		OH	H	H	*Sterling Winthrop
Metaraminol bitartrate	H	OH		OH	CH_3	H	*Merck Sharp & Dohme
β-Phenylethylamine	H	H		H	H	H	Matheson Coleman & Bell
(−)-Amphetamine sulphate	H	H		H	CH_3	H	*Smith Kline & French
Methamphetamine hydrochloride	H	H		H	CH_3	CH_3	*Burroughs Wellcome
Phenylpropanolamine hydrochloride	H	H		OH	CH_3	H	*Merck Sharp & Dohme
Ephedrine sulphate	H	H		OH	CH_3	CH_3	Nutritional Biochemicals Corp.
Metanephrine hydrochloride	OH	OCH_3		OH	H	CH_3	Calbiochem
Normetanephrine hydrochloride	OH	OCH_3		OH	H	H	Calbiochem
3-methoxy-4-hydroxyphenylethylamine	OH	OCH_3		H	H	H	Calbiochem
Methoxamine hydrochloride	H	H	2-OCH_3 5-OCH_3	OH	CH_3	H	*Burroughs Wellcome
Methoxyphenamine hydrochloride	H	H	2-OCH_3	H	CH_3	CH_3	*Upjohn Company
Diethylpropion hydrochloride	H	H		†	CH_3	†	*W. S. Merrell Co.

† $\overset{O}{\overset{\|}{}}$ on β carbon and $(C_2H_5)_2$ on the nitrogen. * Indicates organizations which generously donated the drugs, and we gratefully acknowledge these gifts.

was determined, then responses were obtained to 2, 3, 5, and 10-fold concentrations of the agonist in the presence of the antagonist. The exact dose-ratios were not determined but are presented as the range within which the equieffective dose lies.

Drugs

Agonists. The sympathomimetic amines used, their structures and sources of supply are given in Table 1. Other agonists were acetylcholine chloride, histamine acid phosphate and 5-hydroxytryptamine creatine sulphate. Solutions of all agonists were made in 0.9% sodium chloride solution acidified with HCl.

Antagonists. 2-Bromolysergic acid diethylamide was supplied in 1 ml. ampoules containing 0.5 mg of the bitartrate with 0.25 mg tartaric acid and 8 mg NaCl in distilled water. A stock solution of phenoxybenzamine hydrochloride containing 25 mg/ml. was made in acidified propylene glycol (0.1 M HCl). A stock solution of morphine sulphate contained 1 mg/ml. distilled water. Stock solutions were kept at 4° C and suitable dilutions in 0.9% NaCl solution were made freshly for each experiment.

All doses of agonists and antagonists indicate the final concentration of the free base in the bath.

Results

Table 2 shows the effects of twenty-two sympathomimetic amines on guinea-pig ileum, rabbit jejunum, and rat stomach. Each preparation was tested first for responses to acetylcholine and 5-hydroxytryptamine, invariably excitatory, and to adrenaline or noradrenaline, invariably inhibitory. Only one other sympathomimetic amine was then tested on each preparation, so that each compound was tested on muscle which had not been exposed to other sympathomimetic amines except adrenaline or noradrenaline.

The compounds are arranged in Table 2 into three groups according to their effects on guinea-pig ileum.

Group 1: Inhibitory amines. All effective concentrations caused relaxation; these varied from 10^{-8} g/ml. to the highest tested, 10^{-5} g/ml.

Group 2: Amines with variable effects. High concentrations (10^{-6}–10^{-5} g/ml.) inhibited some preparations and stimulated others. The compounds fell into two subgroups based on the effects of smaller doses. Dopamine and nordefrine (10^{-8}–10^{-7} g/ml.) were inhibitory, while similar doses of metaraminol, methamphetamine and 3-methoxy-4-hydroxyphenylethylamine had no effect.

Group 3: Excitatory amines. Concentrations of 10^{-6} g/ml. or higher caused contraction. Lower doses had no effect.

This grouping did not apply precisely in rabbit jejunum and rat stomach. With one exception, diethylpropion, drugs which inhibited guinea-pig ileum also relaxed the other two preparations. The amines which stimulated guinea-pig ileum, however, were not necessarily excitatory in rabbit jejunum and rat stomach. Of the eight compounds in Group 3, only two, β-phenylethylamine and (−)-amphetamine, stimulated all three preparations. In addition normetanephrine stimulated rabbit jejunum and methoxamine stimulated rat stomach.

TABLE 2. *Effects of sympathomimetic amines on guinea-pig ileum, rabbit jejunum and rat stomach*

Sympathomimetic amine	Guinea-pig ileum (Nonterminal) Number of preparations		Rabbit jejunum Number of preparations		Rat stomach Number of preparations	
	Contracted	Relaxed	Contracted	Relaxed	Contracted	Relaxed
Group 1						
Noradrenaline	0	15	0	4	0	4
Adrenaline	0	12	0	6	0	4
Diethylpropion	0	4	2	0	4	0
Ephedrine	0	6	0	3	0	2
Epinine	0	4	0	3	0	3
Methoxyphenamine	0	4	0	2	0	2
Octopamine	0	4	0	3	0	4
Oxedrine	0	4	0	3	0	4
Phenylpropanolamine	1	4	0	2	0	2
Group 2						
Dopamine	2	3	0	2	0	2
Metaraminol	2	3	0	2	0	2
Methamphetamine	4	3			2	0
3-methoxy-4-hydroxy-phenylethylamine	3	3	0	2	2	0
Nordefrine	4	3	0	2	0	2
Group 3						
Amphetamine	12	0	4	0	3	0
β-Phenylethylamine	8	0	3	1	4	0
Hydroxyamphetamine	4	0			0	3
Metanephrine	7	0	0	2	0	2
Methoxamine	10	0	0	4	4	0
Normetanephrine	7	1	2	0	0	2
Norphenylephrine	7	0	0	3		
Phenylephrine	8	1	0	4	2	2

The preparations are divided according to the response of contraction or relaxation.

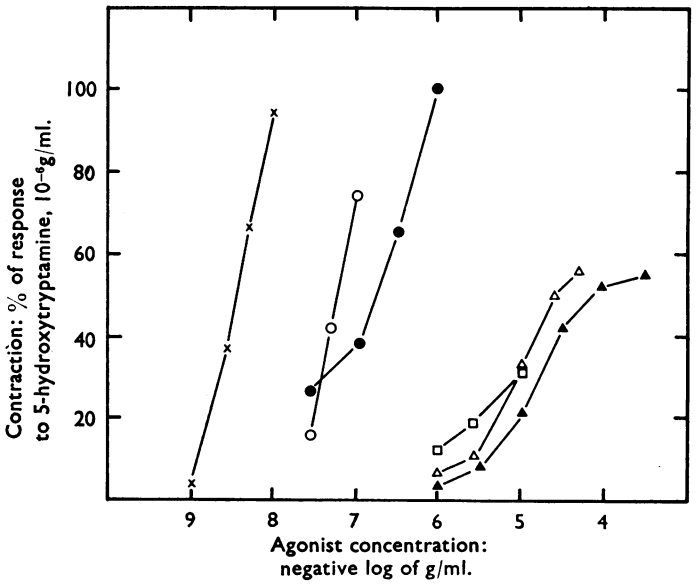


FIG. 1. Log dose-response curves in guinea-pig ileum for acetylcholine (7), × ; histamine (6), ○ ; 5-hydroxytryptamine (10), ● ; (−)-amphetamine (5), □ ; β-phenylethylamine (5), △ ; phenylephrine (4), ▲. All contractions are calculated as a percentage of the contraction due to the largest dose of 5-hydroxytryptamine (10^{-6} g/ml.) in the same segment of ileum. Each point represents the mean of the percentages for the number of experiments indicated in parentheses.

Comparative excitatory activities of sympathomimetics and other agonists on guinea-pig ileum

Dose-response curves for acetylcholine, histamine and 5-hydroxytryptamine were roughly parallel. The curves for the sympathomimetic amines differed from these both in the maximum attained and in slope (Fig. 1). We did not always try to obtain maximum responses to the sympathomimetic amines, but where we did the biggest responses were only about half of the response to 5-hydroxytryptamine, 10^{-6} g/ml., the largest dose used. The sympathomimetic amines were much less potent than 5-hydroxytryptamine and usually the slope of the curve was flatter. For this reason we could not make a meaningful numerical comparison of potency valid throughout the whole dose range. To give a rough idea of the relative potencies of

TABLE 3. *Excitatory activity of sympathomimetic amines on guinea-pig ileum*

Agonist	Concentration (g/ml.)	Number of Tests	Percentage of response to 5-hydroxytryptamine Mean	Range
5-Hydroxytryptamine	10^{-7}	42	100	—
Hydroxyamphetamine	10^{-5}	5	105	53–130
Amphetamine	10^{-5}	6	87	33–171
β-Phenylethylamine	10^{-5}	6	76	55–100
Phenylephrine	10^{-5}	6	54	38–66
Methoxamine	10^{-5}	7	53	25–118
Norphenylephrine	10^{-5}	7	39	15–62
Metanephrine	10^{-5}	5	17	5–43

Contraction due to the sympathomimetic is expressed as a percentage of the contraction due to 5-hydroxytryptamine, 10^{-7} g/ml., in the same preparation.

TABLE 4. *Responses of guinea-pig ileum to sympathomimetic amines after desensitization with 5-hydroxytryptamine* 2–2·5 × 10^{-6} g/ml.

Agonists	Concentration (g/ml.) 10^{-9}	3×10^{-9}	10^{-8}	3×10^{-8}	10^{-7}
Acetylcholine	84±5·1 (9)	90±3·0 (13)	86±3·3 (8)	—	—
Histamine	—	—	61±6·2 (5)	77±5·7 (11)	71±7·5 (4)
5-Hydroxytryptamine	—	—	—	<3	0 (33)

Sympathomimetic amines	10^{-6}	3×10^{-6}	10^{-5}	3×10^{-5}	5×10^{-5}
(−)-Amphetamine	28 (2)	26±9·7 (4)	26±3.4 (6)	—	—
β-Phenylethylamine	23±2·8 (3)	27±5·9 (4)	45±4·7 (5)	—	—
Phenylephrine	61±18·7 (4)	62±16·2 (5)	48±4·6 (3)	—	—
Methoxamine	—	0 (6)	0* (5)	—	—
Hydroxyamphetamine	—	—	52±10·3 (6)	—	—
Norphenylephrine	—	—	15±5·8 (9)	—	17 (2)
Normetanephrine	—	—	—	22±10·7 (5)	27 (2)

Contractions of desensitized ileum are expressed as a percentage ± S.E. of the previous control response in the same preparation. Figures in brackets indicate the number of tests. * Indicates relaxation of the desensitized preparation instead of contraction.

the drugs we have therefore arbitrarily compared the potencies of various sympathomimetics at the same concentration of each (10^{-5} g/ml.), and expressed the responses to this dose as a percentage of the response to a standard dose of 5-hydroxytryptamine (10^{-7} g/ml.) in the same segment of ileum (Table 3).

Effects of sympathomimetic amines on guinea-pig ileum desensitized by 5-hydroxytryptamine

Gaddum (1953) observed that with guinea-pig ileum a large dose of 5-hydroxytryptamine caused a contraction which rapidly disappeared although the drug remained in the bath. The preparation was then insensitive to 5-hydroxytryptamine but responded well to other types of agonist. If the excitatory sympathomimetic amines act on 5-hydroxytryptamine receptors, this desensitizing procedure should block their action as well as that of 5-hydroxytryptamine. Thirty-three preparations were desensitized by 5-hydroxytryptamine ($2–2.5 \times 10^{-6}$ g/ml.) to test this point. Table 4 presents the response at each dose level in the desensitized tissue as a percentage of the corresponding control response.

Although responses to 5-hydroxytryptamine were virtually abolished, responses to acetylcholine were only slightly reduced and response to histamine reduced by only 23 to 39%. Residual responses to the stimulant sympathomimetic amines varied widely. The response to methoxamine was abolished or became relaxation instead of contraction. Responses to (−)-amphetamine, β-phenylethylamine, norphenylephrine and normetanephrine were greatly reduced, but responses to hydroxyamphetamine and phenylephrine were affected little more than were responses to histamine.

Selective receptor protection against block by phenoxybenzamine in guinea-pig ileum

The effects of receptor protection against block by phenoxybenzamine (10^{-5} g/ml.) was tested in seventeen experiments on guinea-pig ileum. Four preparations

TABLE 5 *Effect of protection by acetylcholine, histamine, 5-hydroxytryptamine and excitatory sympathomimetic amines against block by phenoxybenzamine in guinea-pig ileum*

	Residual response (% of control) Protecting agents							
Test Agonist	Acetylcholine 10^{-4}	Histamine 10^{-4}	5-Hydroxytryptamine 10^{-4}	Hydroxyamphetamine 10^{-4}	Methoxamine 10^{-4}	β-Phenylethylamine 10^{-4}	Phenylephrine 10^{-4}	Unprotected
Acetylcholine (7) 10^{-9}	37		3	3	0	1	2	2
Histamine (10) 10^{-8}		42	0	0	0	0	0	0
5-Hydroxytryptamine (17) 10^{-7}	1	1	35	34	33	39	43	0
Hydroxyamphetamine (4) 10^{-5}	0	0	25	36				0
Methoxamine (4) 10^{-5}	0	2	33		51			1
β-Phenylethylamine (4) 10^{-5}	0	0	18			28		3
Phenylephrine (5) 10^{-5}	0	0	59				63	0

Contractions after phenoxybenzamine (10^{-5} g/ml.) are expressed as a percentage of the contraction before phenoxybenzamine. Each number represents the mean of these percentages; the number of experiments with each agonist is given in brackets. Drug concentrations are in g/ml.

were used in each experiment: one protected by 5-hydroxytryptamine, one by an excitatory sympathomimetic drug, one by either acetylcholine or histamine, and one unprotected. The results of four experiments each with hydroxyamphetamine, methoxamine, and β-phenylethylamine and five with phenylephrine are combined in Table 5. The effects of all the agonists were almost completely blocked in unprotected preparations. Acetylcholine and histamine provided self-protection but no cross-protection; that is, the preparations subsequently responded only to the agonist used to protect. Self-protection and cross-protection occurred between 5-hydroxytryptamine and each of the four sympathomimetics.

Antagonism by 2-bromolysergic acid diethylamide

2-Bromolysergic acid diethylamide, 5×10^{-7} g/ml., rarely caused rapid contractions or increased tone of guinea-pig ileum exposed to the drug for only 5 min. This dose did not inhibit acetylcholine but reduced responses to 5-hydroxytryptamine by about 25%. Its inhibitory effects on the excitatory sympathomimetic amines are given in Table 6. The dose-ratios varied widely. Hydroxyamphetamine, (−)-amphetamine and β-phenylethylamine were inhibited more than was 5-hydroxytryptamine. Dose-ratios could not be established for phenylephrine, norphenylephrine and methoxamine, however, because contractions as large as the control responses could not be induced. Large doses of these amines caused relaxation instead of contraction in the presence of the antagonist. The relaxation was more obvious in the few preparations where the antagonist increased basal tone. 2-Bromolysergic acid diethylamide slowed the rate of contraction due to the sympathomimetics besides reducing the height. The peak was reached in 3–4 min in contrast to 60–90 sec without antagonist. This slowing was least striking with

TABLE 6. *Inhibition by 2-Bromolysergic acid diethylamine and morphine in guinea-pig ileum*

		Antagonist		
		2-Bromolysergic acid diethylamide 5×10^{-7}		Morphine 10^{-6}
Agonist	Concentration	Residual response % of control	Dose-ratio	Residual response % of control
Acetylcholine	10^{-8}	100 (6)	1	
Histamine	10^{-8}			86±1·6 (5)
5-Hydroxytryptamine	$1–5 \times 10^{-7}$	75±4·7 (12)	<2	12±1·4 (5)
(−)-Amphetamine	$1–3 \times 10^{-5}$	49±10·8 (4)	>3, <5	87±5·7 (4)
Hydroxyamphetamine	10^{-5}	63±14·4 (4)	<3	83±4·5 (3)
β-Phenylethylamine	10^{-6}	62±9·4 (4)	>5, <10	69±2·6 (5)
Methoxamine	$1–3 \times 10^{-5}$	0 (5)	*	23±2·4 (5)
Norphenylephrine	10^{-5}	0 (5)	>10*	0 (5)
Phenylephrine	10^{-5}	0 (5)	>10*	15·2±6·2 (5)

Contractions in the presence of antagonist are expressed as a percentage of the control response. Each number represents the mean±S.E. of these percentages from the number of experiments indicated in brackets. Drug concentrations are in g/ml. * Dose ratios could not be determined because large doses caused relaxation (see text).

(−)-amphetamine and β-phenylethylamine and did not occur with 5-hydroxytryptamine or acetylcholine.

Antagonism by morphine

Morphine (10^{-6} g/ml.) inhibited the sympathomimetics less than it inhibited 5-hydroxytryptamine (Table 6). Hydroxyamphetamine and (−)-amphetamine were affected no more than was histamine, suggesting an unspecific type of antagonism. In contrast, responses to norphenylephrine, phenylephrine and methoxamine were abolished or greatly reduced.

Discussion

The structure-activity relationships for the excitatory action of sympathomimetic amines differed in the three preparations of gut tested. More compounds stimulated the guinea-pig ileum than stimulated the rabbit jejunum or rat stomach. Several amines excited the guinea-pig ileum but relaxed the other preparations while only one compound, diethylpropion, excited rabbit jejunum without a similar action on guinea-pig ileum. Catecholamines were generally inhibitory, but dopamine and nordefrine had an excitatory component in several guinea-pig ileum preparations. As in earlier studies phenylethylamines were predominantly excitatory (Vane, 1960; Gunn, Gurd & Sachs, 1939; Lands, 1950; McDougal & West, 1954). A methoxy group on the benzene ring favoured contraction, especially in guinea-pig ileum. Substitution of a single hydroxyl group into the benzene ring had varying effects. Oxedrine and octopamine (4-OH) relaxed guinea-pig ileum but hydroxyamphetamine (4-OH), phenylephrine and norphenylephrine (3-OH) caused contraction. We have therefore been unable to define the structural requirements for excitatory activity.

The experiments on guinea-pig ileum in the presence of 2-bromolysergic acid diethylamide, after desensitization by 5-hydroxytryptamine, and with selective receptor protection support the idea that 5-hydroxytryptamine receptors are involved in the excitatory action of sympathomimetic amines. The cross-protection between 5-hydroxytryptamine and the sympathomimetic amines against block by phenoxybenzamine suggests a common receptor. 2-Bromolysergic acid diethylamide, a fairly specific antagonist to 5-hydroxytryptamine, was more effective against the excitatory sympathomimetics than against 5-hydroxytryptamine, even to the extent, with methoxamine, of unmasking a relaxant component in the action. The dose-ratios with 2-bromolysergic acid diethylamide for the various sympathomimetic amines were different, and were all greater than the dose-ratio for 5-hydroxytryptamine. Fairly close values for all the dose-ratios might be expected if the drugs acted on the same receptors. The variation between dose-ratios, however, does not indicate that the excitatory action is not on 5-hydroxytryptamine receptors, for two factors seen in some of the results might account for the differences. First, a response to the sympathomimetic amines may be the resultant of two actions, an excitatory action on 5-hydroxytryptamine receptors and a variable amount of a relaxant action such as we found with methoxamine. For any given degree of block of 5-hydroxytryptamine receptors, the dose-ratio would therefore vary with the relaxant potency of the drugs. Further studies, on tissues depleted of noradrenaline stores or with α- and β-receptor blocking agents, will be required to exclude the complication of such an indirect or direct relaxant action. Second, drugs which are partial agonists may not, in the presence of the antagonists, be able to occupy enough receptors to

equal the control response with acceptable concentrations of the drug. This difficulty would apply to phenylephrine the maximum effect of which was much less than that of 5-hydroxytryptamine, and which is therefore a partial agonist if it acts on 5-hydroxytryptamine receptors. An additional complication is the possibility that the sympathomimetic amines may act on both M and D types of 5-hydroxytryptamine receptor, discussed below, and the proportion of action on each type of receptor may differ with each drug.

The results of desensitization with 5-hydroxytryptamine, although they too suggest an action on 5-hydroxytryptamine receptors, are also difficult to explain. This procedure, which is generally taken to be a very selective test for 5-hydroxytryptamine receptors, abolished the effect of 5-hydroxytryptamine but did no more than reduce the excitatory actions of most of the sympathomimetics. This discrepancy may become explainable when the phenomenon of desensitization is more thoroughly understood.

Gaddum & Picarelli (1957) believed that the guinea-pig ileum had two kinds of 5-hydroxytryptamine receptor, which they named M and D receptors from the drugs which block them selectively. M receptors, blocked by morphine, atropine or cocaine, were thought to be in intramural neurons and the response to their stimulation to be due to released acetylcholine. D receptors, blocked by Dibenamine and ergot alkaloids, were in the smooth muscle. This concept is now controversial (Brownlee & Johnson, 1963 ; Day & Vane, 1963 ; Harry, 1963 ; Kosterlitz & Wallis, 1964), and Day & Vane (1963) suggested that both types of receptor are in the nervous elements. Our results with protection against block by phenoxybenzamine show that 5-hydroxytryptamine provides self-protection but does not protect for acetylcholine (Table 5), indicating that some part of the action of 5-hydroxytryptamine is not mediated by released acetylcholine and is therefore probably on muscle cells. On the other hand morphine was very effective in blocking 5-hydroxytryptamine in our experiments. It is not clear whether this block is wholly due to inhibition of the release of acetylcholine from intramural plexuses as shown by Paton (1957) or by some other blocking action more specific for 5-hydroxytryptamine The dose of morphine which markedly inhibited responses to 5-hydroxytryptamine, however, had a similar effect on methoxamine, phenylephrine and norphenylephrine but had little effect on other excitatory sympathomimetic amines tested. It therefore seems premature to try to draw conclusions regarding the types of 5-hydroxytryptamine receptors on which these act.

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