

AN ACTION OF 5-HYDROXYTRYPTAMINE ON ADRENALINE RECEPTORS

BY

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Contractions of isolated strips of cat spleen due to 5-hydroxytryptamine, adrenaline, histamine and acetylcholine were antagonized by phenoxybenzamine. Responses to both 5-hydroxytryptamine and adrenaline were not blocked in strips which were protected by a high concentration of either 5-hydroxytryptamine or adrenaline throughout exposure to phenoxybenzamine. The contraction due to a large dose of 5-hydroxytryptamine lasted less than 1 hr even when the drug was still present. Strips thus desensitized to 5-hydroxytryptamine responded normally to acetylcholine and histamine but did not respond to adrenaline. The actions of 5-hydroxytryptamine and adrenaline were blocked by 2-bromolysergic acid diethylamide or by dihydro-ergotamine. These results indicated that 5-hydroxytryptamine and adrenaline act on the same receptors. Cocaine potentiated the action of adrenaline but inhibited the action of 5-hydroxytryptamine. The sensitivity to 5-hydroxytryptamine of spleen strips from cats treated 24 hr earlier with reserpine was only one-fiftieth of that of normal strips. Cocaine potentiated the action of 5-hydroxytryptamine on strips from reserpine-treated cats. A high concentration of 5-hydroxytryptamine in spleen strips from reserpine-treated cats and in cocaine-treated strips prevented phenoxybenzamine from blocking the actions of adrenaline. The effects of tyramine on spleen strips almost exactly paralleled the effects of 5-hydroxytryptamine. Strips showing tachyphylaxis to tyramine did not respond to 5-hydroxytryptamine. It is concluded that 5-hydroxytryptamine has a dual action, viz., a major action due to release of stored noradrenaline and a minor direct action of adrenaline receptors.

Many investigations on isolated and *in vivo* preparations have indicated that smooth muscle has receptors which are specific for 5-hydroxytryptamine. Much of the evidence for the specificity of these receptors comes from the selectivity of a variety of antagonists. Lysergic acid diethylamide, for example, is in many tissues a powerful antagonist to the action of 5-hydroxytryptamine with little effect on the actions of acetylcholine, adrenaline or histamine (Gaddum, Hameed, Hathway & Stephens, 1955; Ginzell & Kottegoda, 1953; Meier, Tripod & Wirz, 1957). On the other hand, the actions of acetylcholine, adrenaline and histamine can be antagonized by atropine, piperoxan and antihistaminic agents respectively without altering the action of 5-hydroxytryptamine (Gaddum & Hameed, 1954; Erspamer, 1953; Battacharya, 1955). In 1954 Furchgott established the specificity of adrenaline, acetylcholine, histamine and 5-hydroxytryptamine receptors of aortic smooth muscle with an elegant *in vitro* technique of selective receptor protection against block by Dibenamine. After demonstrating that Dibenamine blocked the action of all four agonists on isolated strips of rabbit aorta, he exposed the preparation to the blocking

agent only in the presence of a high concentration of one of the agonists. Preparations so treated with 5-hydroxytryptamine subsequently responded to 5-hydroxytryptamine but not to adrenaline, acetylcholine or histamine. Similarly, muscles which had been successfully protected by adrenaline, acetylcholine or histamine failed to react to 5-hydroxytryptamine. It is presumed that the selective protection is due to the agonist in high concentrations occupying a large proportion of the receptors that combine with this agonist, thereby preventing access of the blocking agent to these receptor sites but not to the receptor sites for other agonists.

However, Vane (1960) presented evidence that the 5-hydroxytryptamine receptors in rat stomach were less specific, since sympathomimetic amines of the amphetamine type reacted with the same receptors as 5-hydroxytryptamine. This was confirmed by Innes (1962a) with the technique of selective receptor protection; amphetamine and 5-hydroxytryptamine were shown to act on the same receptors in rat stomach, guinea-pig ileum and cat spleen.

Amphetamine and 5-hydroxytryptamine stimulate rat stomach and guinea-pig ileum whereas adrenaline causes relaxation. All three agonists are excitatory in cat spleen. It therefore appeared to be of some importance to investigate the behaviour of 5-hydroxytryptamine receptors in cat spleen towards sympathomimetic amines other than amphetamine.

Receptor protection experiments, which are reported here, quickly made it clear that 5-hydroxytryptamine and adrenaline act on the same receptors. The effects of drugs which influence the actions of 5-hydroxytryptamine or adrenaline on other tissues were also studied. Such drugs should affect in the same way the actions of 5-hydroxytryptamine and adrenaline if the two agonists act on the same receptors. However, cocaine potentiated the action of adrenaline but inhibited the action of 5-hydroxytryptamine. In this paper a parallel is drawn between the action of 5-hydroxytryptamine and tyramine in an attempt to explain these paradoxical results.

METHODS

For all experiments spleen strips 25 to 30 mm long and 2 to 3 mm wide were prepared from cats (0.3 to 2.8 kg) of either sex anaesthetized by intraperitoneal injection of pentobarbitone sodium 45 mg/kg or killed by a blow on the head. No differences in behaviour were detected between preparations from anaesthetized and unanaesthetized cats. Each strip was suspended in an organ bath containing 10 ml. of Krebs-Henseleit solution, maintained at 38° C and bubbled with 95% oxygen and 5% carbon dioxide. Isotonic contractions at 0.5 g tension were recorded with a kymograph at 5.5 times amplification. Every preparation included a strip of spleen capsule. Preliminary experiments were made with spleen strips from which the capsule had been dissected. Such strips, although they contracted in response to the various agonists, could not be used because of their friability.

Phenoxybenzamine (Dibenzylamine) was used as the blocking agent in the selective receptor protection experiments (Innes, 1962b). Two strips from the same spleen were exposed to the same concentration of phenoxybenzamine, 5×10^{-8} g/ml., but one was protected throughout the entire period of exposure (5 min) by a high concentration of a selected agonist which was added to the organ bath 5 min before the blocking agent. The second strip, which was not protected from block, served as control.

Drugs. The stimulant drugs, (–)-adrenaline bitartrate, acetylcholine chloride, histamine diphosphate, 5-hydroxytryptamine creatinine sulphate and tyramine hydrochloride, were added in amounts to give the indicated final concentrations of free base. The drugs were added at

15 min intervals. Each drug remained in the bath until the full contraction was attained, usually less than 2 min after the drug was added. The bath was then emptied and the fluid replaced. The fluid was exchanged at 5 min intervals between drug additions.

Other drugs used were phenoxybenzamine hydrochloride (Dibenzyline), atropine sulphate, morphine sulphate, cocaine hydrochloride, 2-bromolysergic acid diethylamide (BOL 148), dihydroergotamine methanesulphonate and reserpine phosphate (lyophilized, Serpasil). A 2.5% solution of phenoxybenzamine hydrochloride in acidified propylene glycol was kept as a stock solution and a suitable dilution in 0.9% sodium chloride solution was made freshly on the morning of use. Reserpine (1 mg/kg) was injected intraperitoneally 24 hr before the experiment in cats where noradrenaline stores were to be depleted.

RESULTS

Receptor protection against block by phenoxybenzamine. Responses of the spleen strips to adrenaline, 5-hydroxytryptamine, acetylcholine and histamine were measured before and after exposure to phenoxybenzamine 5×10^{-8} g/ml. in 20 experiments with paired preparations. The effects of phenoxybenzamine were shown by expressing the response to a single concentration of the agonist after phenoxybenzamine as a percentage of the control response. Concentrations of the agonists were used which caused submaximal contractions, generally of 10 to 50 mm, before phenoxybenzamine was added. The concentrations were 5×10^{-7} g/ml. for adrenaline, 10^{-4} g/ml. for 5-hydroxytryptamine, 10^{-6} g/ml. for histamine and

TABLE 1

EFFECT OF PROTECTION OF RECEPTORS AGAINST BLOCK BY PHENOXYBENZAMINE

During exposure to phenoxybenzamine (5×10^{-8} g/ml.) receptors are protected in one of two strips from the same spleen. Each line represents a single experiment with paired strips. Contractions after phenoxybenzamine are expressed as a percentage of the contraction before phenoxybenzamine. Drug concentrations are given in g/ml.

Protecting agent	Residual response to agonist after blockade (% of control)							
	Adrenaline 5×10^{-7}		5-Hydroxy- tryptamine 10^{-4}		Acetylcholine 10^{-6}		Histamine 10^{-6}	
	Unprotected strip	Protected strip	Unprotected strip	Protected strip	Unprotected strip	Protected strip	Unprotected strip	Protected strip
Adrenaline 10^{-4}	0	55	0	83				
	4	35	12	77				
	1	36	0	59				
	0	40	0	80				
	0	44	0	100	0	0	0	0
	0	32	0	71	0	0	0	0
	2	81	0	100	0	0	0	0
	2	30	0	50	0	0	0	0
5-Hydroxytryptamine 5×10^{-8}	0	32	0	81	5	5	0	0
	0	12	0	12	0	0	0	0
	6	37	3	16	0	0	11	13
	0	14	0	12				
Acetylcholine 10^{-4}	0	0	0	0	0	67	0	0
	5	3	0	0	0	85	0	0
	3	3	2	3	20	50	0	0
	0	0	0	0	0	59	0	0
Histamine 10^{-4}	0	0	0	0	0	0	0	21
	4	8	0	0	0	10	0	50
	0	0	0	0	0	0	0	100
	0	0	0	0	0	0	0	30

10^{-5} g/ml. for acetylcholine. Protection during the presence of the blocking agent was given by adrenaline 10^{-4} g/ml. in 8 experiments, by 5-hydroxytryptamine 5×10^{-3} g/ml. in 4 experiments, by histamine 10^{-4} g/ml. in 4 experiments and by acetylcholine 10^{-4} g/ml. in 4 experiments.

The results are presented in Table 1. Phenoxybenzamine abolished or greatly reduced the responses to each of the agonists in all unprotected preparations. In protected strips the effects of phenoxybenzamine on responses to subsequent doses of the protecting agonist were much less. Preparations protected by histamine subsequently responded well to histamine, but gave no significant response to other agonists. Similarly acetylcholine protection was effective for acetylcholine but for none of the other agonists tested.

Strips protected with either 5-hydroxytryptamine or adrenaline responded to both 5-hydroxytryptamine and adrenaline better than the unprotected control strips, but showed no improvement in the responses to acetylcholine or histamine (Fig. 1).

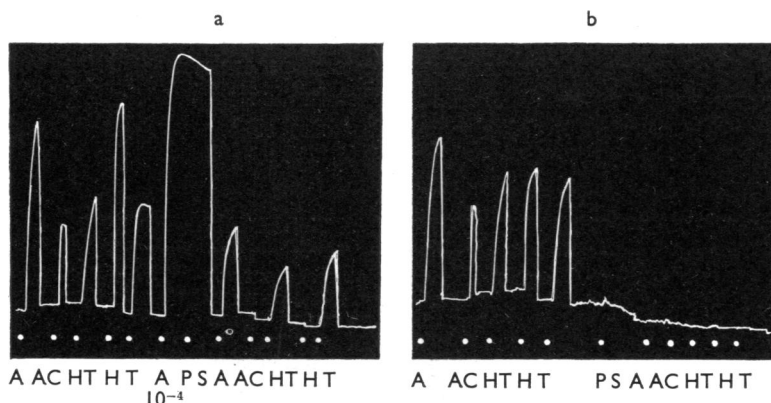


Fig. 1. Effect of phenoxybenzamine on contraction of spleen strips, in *a* with protection by adrenaline throughout exposure to phenoxybenzamine, in *b* without protection. In *a* and *b*, contractions are due to adrenaline 5×10^{-7} g/ml. (A), acetylcholine 10^{-5} g/ml. (AC), 5-hydroxytryptamine 10^{-4} g/ml. (HT), histamine 10^{-6} g/ml. (H) and tyramine 10^{-6} g/ml. (T). Phenoxybenzamine 5×10^{-8} g/ml. was added to the bath for 5 min at P. In *a*, adrenaline 10^{-4} g/ml. was added at A 10^{-4} , 5 min before addition of phenoxybenzamine; adrenaline remained in the bath until phenoxybenzamine was washed out. The drum was stopped for 1 hr at S.

This cross-protection between 5-hydroxytryptamine and adrenaline indicates that both compounds react with the same receptor sites in the muscle.

Effect of desensitization with 5-hydroxytryptamine. Gaddum (1953) observed on isolated guinea-pig ileum that a large dose of 5-hydroxytryptamine caused a contraction which rapidly disappeared in spite of the continued presence of the drug in the organ bath. The preparation was then insensitive to 5-hydroxytryptamine but responded normally to other agonists. If a large dose of 5-hydroxytryptamine similarly desensitized spleen smooth muscle to 5-hydroxytryptamine it might be expected that other agonists acting on the same receptors would also be ineffective. 5-Hydroxytryptamine 10^{-3} g/ml. was therefore tested in 3 experiments where the

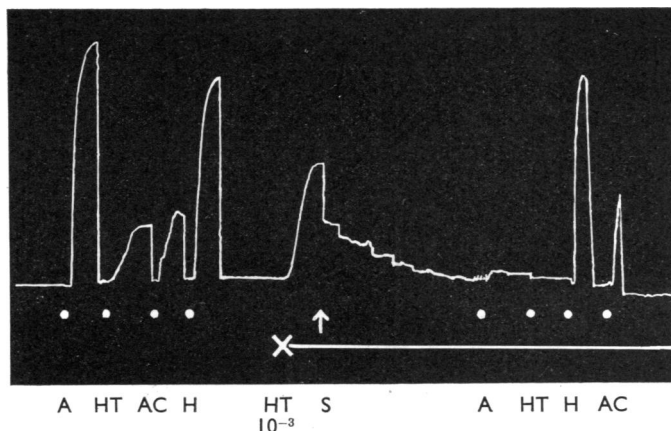


Fig. 2. Effect of desensitization with 5-hydroxytryptamine. Contractions of spleen strip to adrenaline 5×10^{-7} g/ml. (A), 5-hydroxytryptamine 10^{-4} g/ml. (HT), acetylcholine 10^{-5} g/ml. (AC), histamine 10^{-6} g/ml. (H). 5-Hydroxytryptamine 10^{-3} g/ml. was added to the bath at X and replaced whenever the bathing fluid was renewed. The drum was stopped for 45 min at S.

drug remained in the bath for several hours. The contraction due to this high concentration of 5-hydroxytryptamine was not sustained (Fig. 2); in each experiment the muscle returned to its original tone within 60 min. Subsequent responses to acetylcholine and histamine were not greatly changed, but the muscle failed to respond to 5-hydroxytryptamine or adrenaline. The absence of response to adrenaline in muscles desensitized with 5-hydroxytryptamine supports the conclusion that both agonists act on a common receptor site.

Effects of bromolysergic acid diethylamide and dihydroergotamine. Bromolysergic acid diethylamide and dihydroergotamine were applied to spleen strips in concentrations of 10^{-8} to 10^{-6} g/ml. Both drugs reduced the responses to 5-hydroxytryptamine and adrenaline to approximately 20% of the control responses at a concentration of 10^{-8} g/ml. Higher concentrations completely abolished the responses to 5-hydroxytryptamine and adrenaline but had little effect on responses to acetylcholine or histamine. These results again suggested a common receptor site for 5-hydroxytryptamine and adrenaline.

Effects of morphine and atropine. Gaddum & Picarelli (1957) showed that guinea-pig intestine contained two kinds of receptors sensitive to 5-hydroxytryptamine; one type, D receptors, could be blocked by Dibenzylamine, lysergic acid diethylamide or ergot alkaloids, and the second, M receptors, could be blocked by morphine, atropine or cocaine. It was suggested that D receptors were situated in the smooth muscle and M receptors in nervous tissue of the intestine. The complete or almost complete abolition of responses to 5-hydroxytryptamine by phenoxybenzamine described above suggested that there were no M receptors in the spleen strips. To confirm this the effects of morphine and atropine were tested on spleen strips. Neither morphine 10^{-6} g/ml. nor atropine 10^{-6} g/ml. antagonized the actions of 5-hydroxytryptamine or adrenaline.

Effects of cocaine. The potentiating effect of cocaine on the actions of adrenaline has been known since 1910 (Fröhlich & Loewi). Similar potentiation of the action of drugs acting on the same receptors might be expected. The effects of cocaine on adrenaline and 5-hydroxytryptamine actions were therefore tested. Cocaine 10^{-6} g/ml. potentiated the action of adrenaline greatly, but strikingly reduced the responses to 5-hydroxytryptamine in each of 8 experiments. In the experiment shown in Fig. 3, cocaine increased the response to 2.5×10^{-7} g adrenaline/ml. from

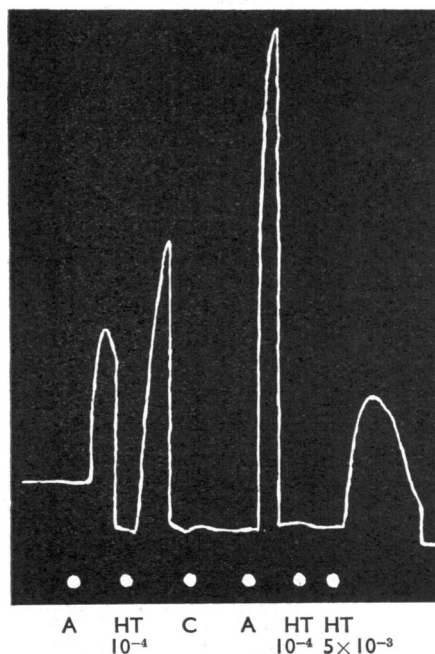


Fig. 3. Effect of cocaine on responses of spleen strip to adrenaline and 5-hydroxytryptamine. Contractions to adrenaline 2.5×10^{-7} g/ml. (A), 5-hydroxytryptamine (HT). Cocaine 10^{-6} g/ml. was added at C and replaced whenever the bathing fluid was renewed.

18 to 60 mm. 5-Hydroxytryptamine 10^{-4} g/ml. caused a 35 mm contraction which was abolished by cocaine. When the concentration of 5-hydroxytryptamine was increased 50 times a contraction of 15 mm occurred.

This dissociation of the actions of adrenaline and 5-hydroxytryptamine was reminiscent of the similar dissociation caused by cocaine of the effects of adrenaline and tyramine which has been known since 1927 (Tainter & Chang). The effects of cocaine on the responses of splenic muscle to tyramine were therefore tested.

For these experiments a concentration of tyramine of 10^{-5} g/ml., causing a contraction of 15 to 40 mm, was chosen. The contraction from a dose of 10^{-3} g/ml. was only slightly greater than from 10^{-5} g/ml., but responses to subsequent doses were markedly reduced. Responses to 10^{-5} g tyramine/ml. were consistently reproducible, provided 45 min elapsed between doses. Tachyphylaxis was often observed even with 10^{-5} g/ml. when doses were given every 15 min. When the

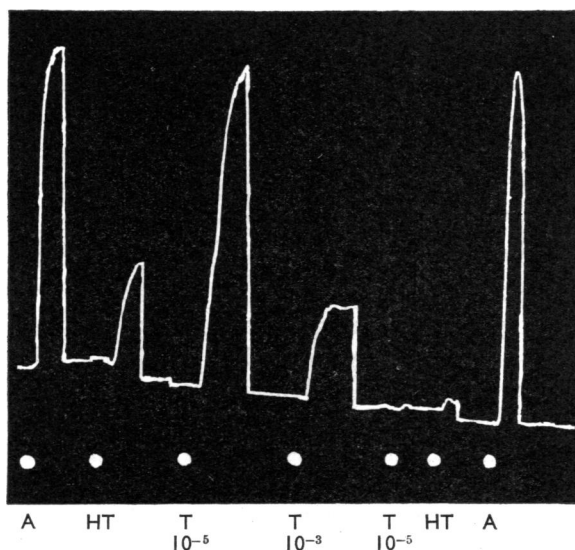


Fig. 4. The effect of tachyphylaxis with tyramine on responses to 5-hydroxytryptamine and adrenaline. Contractions to adrenaline 5×10^{-7} g/ml. (A), 5-hydroxytryptamine 10^{-4} g/ml. (HT) and tyramine (T).

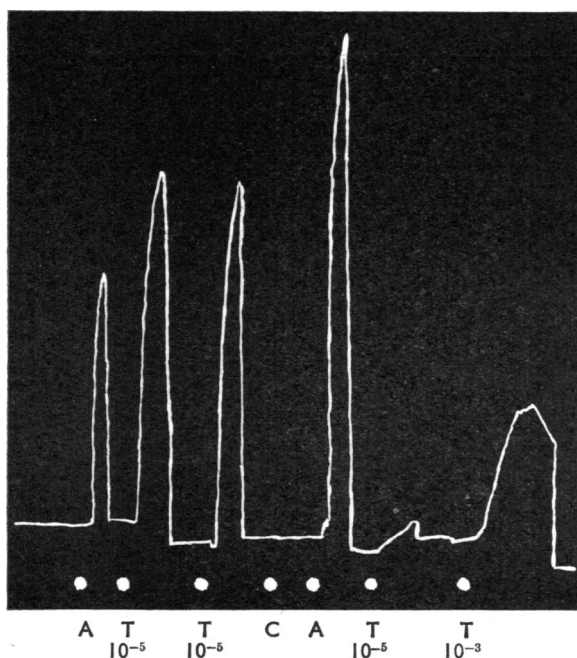


Fig. 5. The effect of cocaine on responses to tyramine. Contractions to adrenaline 2.5×10^{-7} g/ml. (A) and tyramine (T). Cocaine 10^{-6} g/ml. was added at C and replaced whenever the bathing fluid was renewed.

response to tyramine was thus depressed the effect of 5-hydroxytryptamine was also reduced. After a dose of tyramine 10^{-3} g/ml. the muscles contracted poorly in response to 5-hydroxytryptamine and to tyramine for 3 to 4 hr, but responses to adrenaline were not affected (Fig. 4).

The responses to tyramine 10^{-5} g/ml. were abolished or strikingly reduced by the dose of cocaine, 10^{-6} g/ml., which potentiated the action of adrenaline (Fig. 5). After cocaine a 100-fold increase in tyramine concentration failed to cause a contraction equal to the original response.

Receptor protection by tyramine against block by phenoxybenzamine. Tyramine 10^{-3} g/ml. was used to protect spleen strips against phenoxybenzamine 5×10^{-8} g/ml. in 4 experiments. Responses to tyramine 10^{-5} g/ml., as well as to adrenaline, 5-hydroxytryptamine, acetylcholine and histamine, were tested before and after phenoxybenzamine. The test dose of tyramine was given only once before and once after phenoxybenzamine, and enough time was allowed before applying another drug to ensure that the responses would not be depressed. The actions of all the agonists were greatly decreased or abolished by phenoxybenzamine in unprotected strips. Protected strips contracted well in response to adrenaline only; 5-hydroxytryptamine was without effect and responses to tyramine were very small (Table 2).

TABLE 2
RECEPTOR PROTECTION BY TYRAMINE AGAINST BLOCK BY PHENOXYBENZAMINE
Results obtained as in Table 1. Drug concentrations in g/ml.

	Residual response to agonist after blockade (% of control)									
	Adrenaline 5×10^{-7}		5-Hydroxy- tryptamine 10^{-4}		Tyramine 10^{-5}		Acetylcholine 10^{-5}		Histamine 10^{-6}	
	Unpro- tected strip	Pro- tected strip	Unpro- tected strip	Pro- tected strip	Unpro- tected strip	Pro- tected strip	Unpro- tected strip	Pro- tected strip	Unpro- tected strip	Pro- tected strip
Protection	0	60	0	0	0	3	0	0	0	0
with	0	93	0	0	0	6	0	0	0	0
tyramine	10	50	0	0	8	13	7	0	0	0
10^{-3}	2	67	4	5	4	8	4	5	0	0

Responses of spleen strips from cats treated with reserpine. The effects of tyramine have been attributed by Burn & Rand (1958) to an indirect action, namely, by release of stored noradrenaline which then activates the smooth muscle. This view is based on evidence that tyramine has little action on smooth muscle depleted of noradrenaline. The effects of 5-hydroxytryptamine and tyramine were tested on spleen strips taken from cats depleted of noradrenaline by reserpine to find how far the parallelism between 5-hydroxytryptamine and tyramine effects extended.

Doses of 5-hydroxytryptamine and tyramine (10^{-4} g/ml. and 10^{-5} g/ml. respectively) which caused contractions in strips from normal animals had no effect on strips from reserpine-treated animals. The sensitivity of these preparations to 5-hydroxytryptamine and tyramine was similar to the sensitivity of cocaine-treated muscles from normal animals. Contractions due to 5-hydroxytryptamine, 5×10^{-3} g/ml., or tyramine, 10^{-3} g/ml., were generally smaller than contractions due to one-

fiftieth or one-hundredth of these doses in strips from normal cats. Preparations from reserpine-treated cats showed little tachyphylaxis to 5-hydroxytryptamine or tyramine. Contractions from the first dose were somewhat greater than from subsequent doses. After the second dose there was usually no further reduction in the contraction, even when doses were repeated every 15 min.

Receptor protection in spleen strips from cats treated with reserpine. It did not appear likely that effective protection of adrenaline receptors against phenoxybenzamine could be afforded by quantities of noradrenaline such as might be released from tissue stores by 5-hydroxytryptamine, if this proved to be its mode of action. If, however, the protection should be due to released noradrenaline, 5-hydroxytryptamine should fail to protect preparations depleted of noradrenaline. Table 3 shows the results of 4 experiments with protection by 5-hydroxytryptamine. Adrenaline action was effectively protected. The effects of protection on 5-hydroxytryptamine and tyramine responses was not tested in these experiments, since the low sensitivity of the strips would have demanded test doses as great as the protecting dose of the drug. Block of acetylcholine and histamine was not decreased by protection with 5-hydroxytryptamine. Four experiments with protection by tyramine gave similar results (Table 3).

TABLE 3
EFFECT OF PROTECTION OF RECEPTORS AGAINST BLOCK BY PHENOXYBENZAMINE
IN SPLEEN STRIPS FROM CATS TREATED WITH RESERPINE

Results as in Table 1. Drug concentrations in g/ml.

	Residual response to agonist after blockade (% of control)					
	Adrenaline 2.5×10^{-7}		Acetylcholine 10^{-5}		Histamine 10^{-6}	
	Unprotected strip	Protected strip	Unprotected strip	Protected strip	Unprotected strip	Protected strip
Protection with 5-hydroxytryptamine 5×10^{-3}	3	87	0	0	0	0
	8	34	0	0	0	0
	13	33	0	0	0	0
	17	33	0	0	0	0
Protection with tyramine 10^{-3}	8	100			9	22
	20	95			30	29
	2	40	12	8	12	20
	6	48			19	14

Receptor protection in cocaine-treated spleen strips. Three experiments protecting receptors by 5-hydroxytryptamine against block by phenoxybenzamine were done on cocaine-treated strips. Cocaine (10^{-6} g/ml.) was added to both control and experimental strips at the start of the experiment and renewed whenever the fluid was changed. Since usual doses of 5-hydroxytryptamine and tyramine were ineffective, the effects of protection on responses to only adrenaline, acetylcholine and histamine were tested. Protection by 5-hydroxytryptamine decreased the effect of phenoxybenzamine on adrenaline action (Table 4). Responses to acetylcholine and histamine were not increased. The results were the same in 3 similar experiments with protection by tyramine.

TABLE 4
EFFECT OF PROTECTION OF RECEPTORS AGAINST BLOCK BY PHENOXYBENZAMINE
IN SPLEEN STRIPS TREATED WITH COCAINE (10^{-6} g/ml.)

Results as in Table 1. Drug concentrations in g/ml.

	Residual response to agonist after blockade (% of control)					
	Adrenaline 2.5×10^{-7}		Acetylcholine 10^{-5}		Histamine 10^{-6}	
	Unprotected strip	Protected strip	Unprotected strip	Protected strip	Unprotected strip	Protected strip
Protection with 5-hydroxytryptamine 5×10^{-3}	3	37	0	0	0	0
	0	30	0	0	0	0
	22	54	0	0	0	0
Protection with tyramine 10^{-3}	3	13	0	0	0	0
	8	74	0	0	0	0
	0	38				

Effects of cocaine on spleen strips from cats treated with reserpine. Doses of 5-hydroxytryptamine and tyramine were repeated several times in 4 strips from reserpine-treated cats until the response was consistent. Cocaine (10^{-6} g/ml.) then increased the responses to both 5-hydroxytryptamine and tyramine (Fig. 6). Adrenaline was not tested on those strips since the addition of adrenaline is known to

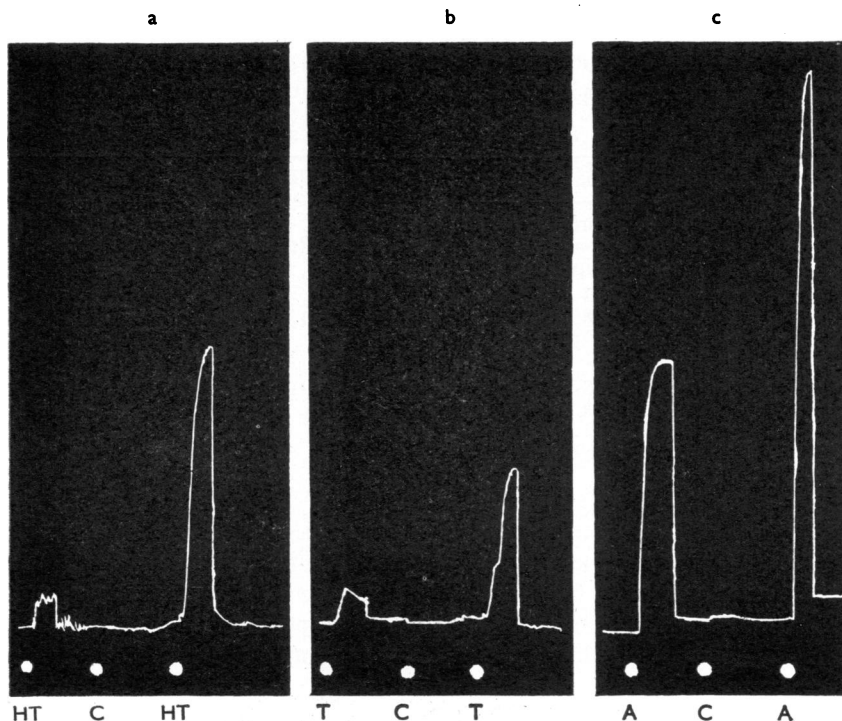


Fig. 6. The effect of cocaine on responses of spleen strips from cats treated with reserpine 1 mg/kg 24 hr previously. In *a*, contractions to 5-hydroxytryptamine 10^{-3} g/ml. (HT); in *b*, contractions to tyramine 10^{-3} g/ml. (T); in *c*, contractions to adrenaline 2.5×10^{-7} g/ml. (A). Cocaine 10^{-6} g/ml. was added at C in *a*, *b* and *c*.

enhance the action of tyramine in other tissues of reserpine-treated animals (Burn & Rand, 1958). In other strips the responses to adrenaline were increased by cocaine.

Restoration of the action of 5-hydroxytryptamine by exposure to noradrenaline. The results obtained from strips from reserpine-treated cats indicated that the action of 5-hydroxytryptamine was largely dependent on noradrenaline stores. Such strips were therefore exposed to a high concentration of noradrenaline 5×10^{-5} g/ml. for 5 min. This procedure greatly increased the responses to 5-hydroxytryptamine; responses to tyramine were also increased. In the experiment shown in Fig. 7 noradrenaline enabled strips to respond to doses of 5-hydroxy-

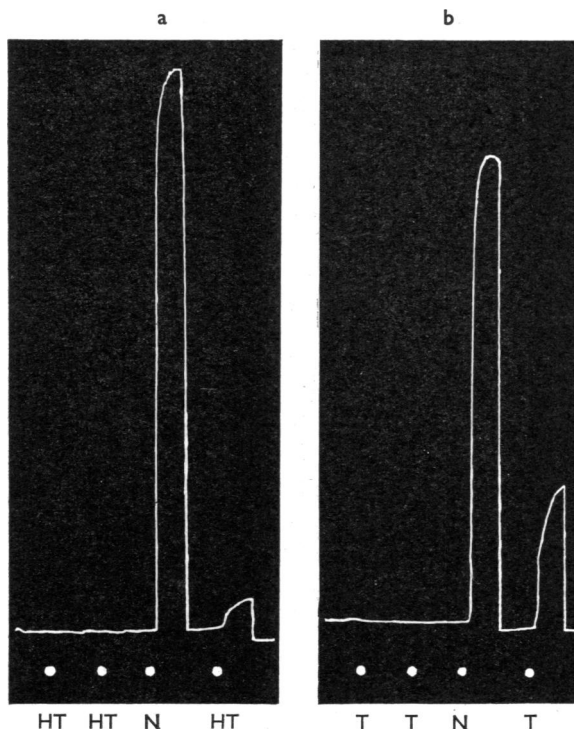


Fig. 7. The effect of a high concentration of noradrenaline on the contractions of spleen strips from cats treated with reserpine 1 mg/kg 24 hr previously. *a* shows responses to 5-hydroxytryptamine 10^{-4} g/ml. (HT); *b* shows responses to tyramine 10^{-5} g/ml. (T). Noradrenaline 10^{-4} g/ml. was added to the bath for 5 min at N in *a* and *b*.

tryptamine and tyramine which were previously ineffective. Exposure to a high concentration of 5-hydroxytryptamine, which is also displaced by reserpine, did not increase the responses.

Strips which had been taken from normal cats were treated with cocaine to reduce the responses to 5-hydroxytryptamine. Exposure to noradrenaline 5×10^{-5} g/ml. for 5 min did not increase subsequent responses to 5-hydroxytryptamine and tyramine.

DISCUSSION

The results of the protection experiments clearly demonstrate that there are three specific types of contraction receptors in the smooth muscle of cat spleen, one for acetylcholine, one for histamine and one for adrenaline and 5-hydroxytryptamine. Specificity of acetylcholine and histamine receptors is indicated by the selectivity of their protective action against phenoxybenzamine. The experiments showing that a high concentration of either 5-hydroxytryptamine or adrenaline decreases the effect of phenoxybenzamine in inhibiting the actions of both drugs provide strong evidence that 5-hydroxytryptamine and adrenaline act on the same receptors. The possibility of a non-specific reduction of block by phenoxybenzamine is ruled out by their failure to protect also acetylcholine and histamine actions in these experiments. The conclusion that 5-hydroxytryptamine and adrenaline act on the same receptor sites is supported by the evidence that adrenaline fails to cause contractions of spleen muscle which has become desensitized to 5-hydroxytryptamine. Since a non-specific depression of smooth muscle has been shown in other preparations after large doses of an agonist (Cantoni & Eastman, 1946), this possibility had to be considered in splenic muscle. However, a non-specific depression by the large desensitizing dose of 5-hydroxytryptamine can be excluded since the effects of acetylcholine and histamine are not depressed. Antagonism to both 5-hydroxytryptamine and adrenaline, but not to acetylcholine and histamine, by bromolysergic acid diethylamide and dihydroergotamine points to the same conclusion, but adds little to the weight of the evidence, since the selectivity of lysergic acid derivatives and ergot alkaloids in antagonizing 5-hydroxytryptamine and adrenaline varies greatly from organ to organ. There are several smooth muscle systems where 5-hydroxytryptamine and adrenaline actions are equally inhibited, as, for example, in inhibition of their pulmonary vasoconstricting actions by ergotamine (Ginzell & Kottegoda, 1953). Such observations, however, suggest that a closer study of the specificity of adrenaline and 5-hydroxytryptamine receptors in those tissues may be warranted.

The difference in effect of cocaine on the actions of 5-hydroxytryptamine and adrenaline can be most readily explained if two mechanisms of action for 5-hydroxytryptamine are proposed, one directly on adrenaline receptors, the second indirectly by release of stored noradrenaline, which in turn acts on adrenaline receptors. This hypothesis can account for the results if it is assumed that the major action of 5-hydroxytryptamine is normally due to the noradrenaline release mechanism, and that the direct action occurs only with large doses and so can be observed alone only when the indirect mechanism has been inactivated by cocaine or previous treatment with reserpine. The behaviour of tyramine on the spleen strip runs parallel to that of 5-hydroxytryptamine in most respects, and the results can be explained on the basis of a similar dual action.

If 5-hydroxytryptamine acts in the way suggested, it has to be assumed also that cocaine has two actions, namely, potentiation of drugs which act on adrenaline receptors and inhibition of release of noradrenaline stores. Thus the major response to 5-hydroxytryptamine and to tyramine would be absent in cocaine-treated strips, as in muscles depleted of noradrenaline stores by reserpine, and only the direct action would remain. This explanation also provides for potentiation of the direct action of

5-hydroxytryptamine and tyramine on adrenaline receptors by cocaine in strips with the indirect action abolished by pretreatment with reserpine.

There is no direct evidence that either 5-hydroxytryptamine or tyramine act by releasing noradrenaline from the spleen. There is, however, a substantial body of indirect evidence that tyramine acts at least in part by this mechanism in some tissues. Tyramine has no pressor effect in animals depleted of noradrenaline by reserpine (Carlsson, Rosengren, Bertler & Nilsson, 1957); an infusion of noradrenaline restores the pressor action of tyramine (Burn & Rand, 1958). The present results show that a large dose of noradrenaline similarly restores the responses to 5-hydroxytryptamine as well as to tyramine in splenic muscle from reserpine-treated cats. Lockett & Eakins (1960) showed that tyramine increased the catechol-amine content of blood in cats, and Hertting, Axelrod & Patrick (1961) showed that tyramine released H^3 -labelled noradrenaline from the rat heart. These investigators disagreed about the effects of cocaine on the releasing action of tyramine. Lockett & Eakins found that cocaine prevented tyramine from increasing the plasma catechol-amine content. On the other hand, Hertting *et al.* found that the reduction in $[H^3]$ -noradrenaline content of the heart due to tyramine was not altered by cocaine. The available evidence does not permit firm conclusions to be drawn about the mode of action of cocaine. The failure of exposure to noradrenaline to restore the responses to 5-hydroxytryptamine and tyramine in cocaine-treated strips could be explained by cocaine either preventing the uptake of noradrenaline by the tissue or inhibiting the release of noradrenaline which has been taken up by the tissue. Whitby, Hertting & Axelrod (1960) have shown that cocaine depresses the uptake of injected noradrenaline by cat spleen, but inhibition of release also is suggested by the present results showing that cocaine depresses the responses to 5-hydroxytryptamine and tyramine in spleen strips which have not been depleted of noradrenaline stores by reserpine pretreatment. It can probably be inferred from work on other tissues that cocaine does not deplete the noradrenaline stores (Muscholl, 1961).

Protection of adrenaline receptors by 5-hydroxytryptamine or tyramine against block by phenoxybenzamine in muscles from cats treated with reserpine indicates that the protection is due to combination of these receptors with the drugs themselves rather than with noradrenaline which they have freed. The results showing protection of the major action of 5-hydroxytryptamine and tyramine by adrenaline may be explained by protection of adrenaline receptors which then respond to normal amounts of noradrenaline released. This interpretation implies either that phenoxybenzamine does not antagonize noradrenaline release by 5-hydroxytryptamine or tyramine, or that adrenaline protection extends to this mechanism as well as to adrenaline receptors. This point cannot be settled by the present experiments.

Although the behaviour of 5-hydroxytryptamine and tyramine was very similar and could be explained by a similar mode of action, differences were observed. Tachyphylaxis occurred more readily with tyramine than 5-hydroxytryptamine. Tyramine tachyphylaxis appeared to be mainly on the noradrenaline release mechanism, since the responses to 5-hydroxytryptamine but not to adrenaline were also inhibited. Moreover, in muscles from reserpine-treated cats tyramine action remained fairly constant after the first dose, which was generally more effective than

subsequent doses. This might be due to the presence of a small amount of noradrenaline in the tissue; after release of a small residual amount of noradrenaline only the direct action on adrenaline receptors would remain. Little tachyphylaxis to 5-hydroxytryptamine was observed, except in experiments where the drug was left in the bath for over an hour. Saturation of adrenaline receptors alone, or of both mechanisms, could account for block of adrenaline and tyramine in these experiments.

The failure of morphine and atropine to antagonize the effects of 5-hydroxytryptamine indicates that the cat spleen has no M receptors. Although phenoxybenzamine, bromolysergic acid diethylamide and dihydroergotamine antagonize 5-hydroxytryptamine effects as they do on the D receptors of guinea-pig ileum, the cat spleen receptors for 5-hydroxytryptamine differ from these D receptors in their specificity towards agonists. The present results strongly suggest that 5-hydroxytryptamine has two actions, neither of which is on receptors that are specific for 5-hydroxytryptamine. One action is directly on receptors for adrenaline, the second releases stored noradrenaline which in turn acts on receptors for adrenaline. Thus both actions are effected finally through the adrenaline receptors.

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