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ACTIONS OF SOME ANALOGUES OF 5-HYDROXYTRYPTAMINE ON THE ISOLATED RAT UTERUS AND THE RAT FUNDUS STRIP PREPARATIONS

BY

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The search for substances which antagonize 5-hydroxytryptamine, and for those which act like it, has been extended to cover 5-substituted indoles which are analogues of 5-hydroxytryptamine, rather than analogues of tryptamine like the compounds previously studied by us. The isolated rat uterus and rat fundus strip preparations have been used to determine activity. The relationships between structure and activity of the compounds studied were not the same on the two preparations nor were they the same from one homologous series to another. These differences may be partly explained by the presence of amine oxidase in the rat fundus, as Vane (1959) has suggested, and by supposing that it is absent from the rat uterus. None of the compounds had marked antagonist activity. The most active antagonist on the rat uterus was 3-(2-aminopropyl)-5-benzyloxyindole, but this was less potent than 5-benzyloxygramine. On the rat fundus strip, however, the only antagonist, 5-benzyloxy-3-(2-dimethylaminoethyl)indole, was more active than 5-benzyloxygramine. On both preparations the most active stimulant was 3-(2-aminopropyl)-5-hydroxyindole, which was about half as potent as 5-hydroxytryptamine. The next most active were 3-(2-aminopropyl)-5-methoxyindole and bufotenine.

A previous paper (Barlow and Khan, 1959) described our search among analogues of tryptamine for substances which act like tryptamine and/or 5-hydroxytryptamine and those which antagonize tryptamine and/or 5-hydroxytryptamine. We found that substitution of alkyl groups in the amino group of the side-chain of tryptamine produced an increase in tryptamine-like and 5-hydroxytryptamine-like activity.

A series of derivatives of 5-hydroxytryptamine which have alkyl substituents in the side-chain amino group was prepared, and the study also included: (1) the corresponding 5-benzyloxy compounds, which, from previous work (see, for example, Gaddum, Hameed, Hathway, and Stephens, 1955), might be expected to be antagonists of 5-hydroxytryptamine, (2) the effects both on stimulant and antagonistic activity of substituting a methyl group in the side-chain in the α position relative to the amino group, this substitution making the compound resistant to attack by amine oxidase, and (3) the actions of 5-methyl and/or 5-methoxy compounds. These, being non-phenolic, might indicate how much the phenolic nature of the 5-hydroxyl group determined the potency of 5-hydroxytryptamine.

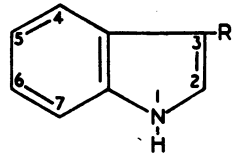
We have continued to use the isolated rat uterus and rat fundus strip preparations for measuring both agonistic and antagonistic potencies because they are convenient preparations which respond to tryptamine and 5-hydroxytryptamine. The compounds tested are shown in Table I. Although they are referred to as bases, a suitable salt was always used.

METHODS

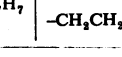
Compounds.—Analogues of 5-hydroxytryptamine have already been prepared by Speeter and Anthony (1954), Stoll, Troxler, Peyer and Hofmann (1955), Speeter (1955), and Young (1958). Samples of some of these have kindly been made available to us. The other compounds were prepared by the method of Speeter and Anthony (1954) starting from 5-benzyloxyindole. The intermediate 5-benzyloxy-3-(2-dialkylaminoethyl)indoles were de-benzylated by hydrogen and palladium-charcoal (10%) at atmospheric pressure. Methanol was used as solvent and the mixture warmed, if necessary. The quaternary salts of 5-benzyloxy-3-(2-dimethylaminoethyl)indole could not be de-benzylated catalytically:

TABLE I

STIMULANT ACTIVITY OF THE COMPOUNDS STUDIED



Microanalyses are by Dr. J. W. Minnis (Department of Biochemistry); melting points were taken on a Kofler hot stage. An asterisk refers to remarks in the section on drugs in the text. EtOH=ethanol, MeCOMe=acetone, EMK=ethyl methyl ketone, and Et_2O=ether. † Indicates that the dose/response curve was not parallel to that of 5-hydroxytryptamine. ‡ Indicates that these compounds have been prepared previously by other workers: No. 2 by Wieland, Konz, and Mittasch (1934), who recorded m.p. 210°: No. 7 by Speeter (1955), who recorded m.p. 213 to 214°. Compounds 4 and 11 have been prepared as bases and oxalates by Stoll, Troxler, Peyer, and Hofmann (1955). The explanation of (S), (C), (Cu), and (V) is given in the acknowledgments section and refers to the source of these compounds. Number of experiments are shown in parentheses after equipotent molar ratios. (I) Indicates that results with these compounds were recorded by Barlow and Khan (1959) and are included for purposes of comparison.

Comp. No.	Ring Sub-stituent	Side-chain (R) (Trivial Name)	Salt	Crystallized from	m.p. (°C.)	Found		Theory		Stimulant Activity: Equipotent Molar Ratios: Mean±S.E.	
						C	H	C	H	Rat Uterus 30°	Rat Fundus Strip 37°
1	5-OH	$-CH_2CH_2NMe_2$ (Bufotenine)	Base Fumarate	MeCOMe/Et_2O EtOH	125–6°* 228–30° (dec)	70·9 64·1	7·77 6·79	70·6 64·2	7·92 6·91	2·7±1·6 (3)	3·1±1·8 (3)
2	5-OH	$-CH_2-CH_2-N(CH_3)_2$	Methiodide	EtOH	210–11°‡					>20,000 (2)	>10,000 (2)
3	5-OH	$-CH_2-CH_2-N(CH_3)_2$	Ethiodide	EtOH	192–3°	46·6	5·61	46·6	5·89	>20,000 (2)	>10,000 (2)
4	5-OH	$-CH_2-CH_2-N(C_2H_5)_2$ (*N'N'*-Diethyl-5-hydroxytryptamine)	HCl	EtOH/EMK	169·5–170°	62·9	7·55	62·5	7·90	9·0±2·6 (3)	18±9·5 (3)
5	5-OH	$-CH_2CH_2N(C_3H_7)_2$ (*N'N'*-Dipropyl-5-hydroxytryptamine)	HCl	EtOH/EMK	204–5°	64·7	8·33	64·8	8·52	39±1·5 (3)	16±4·0 (6)
6	5-OH	$-CH_2-CH_2-N(isoC_3H_7)_2$	HCl	EtOH/EMK/Et_2O	109–11°	64·5	8·59	64·8	8·52	16±0 (3)	4·5±1·5 (3)
7	5-OH	$-CH_2-CH_2-N(C_4H_9)_2$	HCl	EtOH/EMK	212–15°‡	66·9	8·97	66·5	9·02	1,111† (2)	151±40 (3)
8	5-OC_7H_7	$-CH_2-CH_2-N(CH_3)_2$	HCl	(I)						>20,000 (2)	625±135 (4)
9	5-OC_7H_7	$-CH_2-CH_2-N(CH_3)_2$	Methiodide	EtOH	210–11°	55·5	6.09	55·1	5·80	>10,000 (2)	>5,000 (2)
10	5-OC_7H_7	$-CH_2-CH_2-N(CH_3)_2$	Ethiodide	EtOH	198–9°	55·7	5·81	56·0	6·06	>20,000 (2)	>10,000 (2)
11	5-OC_7H_7	$-CH_2-CH_2-N(C_2H_5)_2$	HCl	EtOH/EMK/Et_2O	171–2°	70·8	7·22	70·2	7·61	>10,000 (1)	470±60 (4)
12	5-OC_7H_7	$-CH_2-CH_2-N(C_3H_7)_2$	HCl	EtOH	209–11°	71·7	7·65	71·4	8·10	>20,000 (1)	614±80 (3)
13	5-OC_7H_7	$-CH_2-CH_2-N(isoC_3H_7)_2$	HCl	EtOH/EMK	215–18° (dec)	71·3	7·96	71·4	8·10	>10,000 (1)	867±183 (2)
14	5-OC_7H_7	$-CH_2-CH_2-N(C_4H_9)_2$	HCl	EtOH/EMK	220–2° (dec)	72·1	7·81	72·4	8·52	>20,000 (1)	>20,000 (1)
15	5-OC_7H_7		HCl	EtOH	227–9° (dec)	67·4	6·23	67·6	6·77	>20,000 (1)	>5,000 (2)

16	5-OH	$-CH_2-CH(CH_3)-NH_2$ (5-Hydroxy-α-methyltryptamine)	Creatinine sulphate HCl	(S) (C)						2±0 (2)	1·7±0·2 (5)
17	$5-OC_7H_7$	$-CH_2-CH(CH_3)-NH_2$	HCl	(S)						>20,000 (1)	63±11 (2)
18	$5-CH_3$	$-CH_2-CH_2-NH_2$ (5-Methyltryptamine)	HCl	(C)						173±80 (3)	620±180 (3)
19	$5-CH_3$	$-CH_2-CH(CH_3)-NH_2$ (5,α-Dimethyltryptamine)	HCl	(C)						1,300±17 (3)	22·7±1·5 (4)
20	$5-OCH_3$	$-CH_2-CH_2-NH_2$ (5-Methoxytryptamine)	HCl	(Cu)						8±1·7 (3)	39±9·7 (3)
21	$5-OCH_3$	$-CH_2-CH(CH_3)-NH_2$ (5-Methoxy-α-methyltryptamine)	HCl	(V)						4·3±1·4 (3)	1·4±0·25 (3)
22	$1-CH_3$	$-CH_2-CH(CH_3)-NH_2$	HCl	(S)						1,350±450 (2)	69±14 (3)
		$-CH_2-CH_2-NH_2$ (Tryptamine)	HCl	(I)						210±30 (4)	933±33 (3)
	5-OH	$-CH_2-CH_2-NH_2$ (5-Hydroxytryptamine)	Creatinine sulphate							1	1

bufotenine methiodide and ethiodide were obtained by alkylation of bufotenine base [3-(2-dimethylaminoethyl)-5-hydroxyindole] at room temperature.

The bufotenine synthesized, even after repeated crystallization, had a much lower melting point (125 to 126°) than that recorded by Wieland, Konz, and Mittasch (1934) and Speeter and Anthony (1954), namely 146 to 147°. Stoll *et al.* (1955), however, recorded m.p. 138 to 140°. As our material was distilled satisfactorily (b.p. 166 to $167°/10^{-2}$ mm. Hg pressure) and gave a reasonable analysis and fumarate, we suggest that it is a different crystalline modification. The bufotenine prepared by us was compared on the rat fundus strip preparation with a sample of bufotenine used by Gaddum *et al.* (1955). The two specimens were equiactive.

Preparations and Experimental Procedures.—These were identical with those previously described (Barlow and Khan, 1959) except that, in the initial qualitative tests on the rat uterus, no attempt was made to produce repeated contractions with all the compounds.

Results

Effects on the Isolated Rat Uterus Preparation

These were similar in character to those obtained with the tryptamines and can be divided into: (1) synergism with 5-hydroxytryptamine, tryptamine, and acetylcholine, (2) antagonism of 5-hydroxytryptamine and tryptamine, and (3) stimulant action. The effects of higher concentrations, which produced repeated contractions and profound depression, were not studied in any detail.

Synergism.—In our previous paper (Barlow and Khan, 1959), we interpreted this phenomenon as an addition, and showed that the presence of a small concentration of 5-hydroxytryptamine, which did not in itself produce any visible effect, caused increased responses to 5-hydroxytryptamine itself and to tryptamine and acetylcholine. We have found that a paeneliminal concentration (5×10^{-7}) of acetylcholine behaved similarly in that it enhanced the responses to 5-hydroxytryptamine, tryptamine, and acetylcholine.

Further evidence that the effect is little, if any, more than would be expected from simple addition was obtained by adding a dose of 5 ng. of 5-hydroxytryptamine at the same time as 100 ng. of acetylcholine. The contraction produced (Fig. 1) was only slightly larger than those produced either by 10 ng. of 5-hydroxytryptamine or by 200 ng. of acetylcholine acting alone. It was certainly less than the contractions produced by 20 ng. of 5-hydroxytryptamine or by 400 ng. of acetylcholine. This experiment was

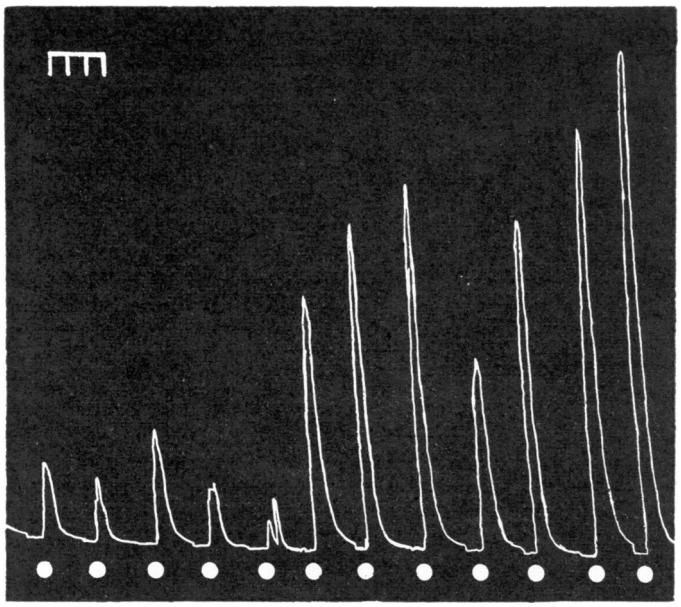


FIG. 1.—Isolated rat uterus preparation. Synergism between acetylcholine and 5-hydroxytryptamine. Contractions are shown in response to 10, 20, and 40 ng. of 5-hydroxytryptamine (**10**, **20**, and **40**) and to 200, 400, and 800 ng. of acetylcholine (**200**, **400**, and **800**). The response to a combined dose of 5 ng. of 5-hydroxytryptamine plus 100 ng. of acetylcholine (**5+100**) is not significantly greater than that to 10 ng. of 5-hydroxytryptamine or to 200 ng. of acetylcholine alone. Similarly the response to a combined dose of 10 ng. of 5-hydroxytryptamine plus 200 ng. of acetylcholine (**10+200**) is not significantly greater than that to 20 ng. of 5-hydroxytryptamine or to 400 ng. of acetylcholine alone. Bath vol., 2 ml. Time, min.

repeated, using twice the doses, with the same result, and also with tryptamine (1 μg. and 2 μg.) in place of 5-hydroxytryptamine. This type of addition was shown by all our compounds which possessed stimulant, but not antagonistic, activity.

Antagonism of 5-Hydroxytryptamine and Tryptamine.—As in previous work (Gaddum *et al.*, 1955; Barlow and Khan, 1959), it was necessary to allow 1 hr. for the full development of the antagonistic effect, and the reversal of this effect after washing took about the same time. The antagonistic activities of the compounds are shown in Table II. When some antagonists were present in high concentrations, the responses of 5-hydroxytryptamine did not increase as the concentration of 5-hydroxytryptamine was increased. In these circumstances the drug ratio cannot properly be determined; it appears to be very high and varies with the concentration of antagonist. This situation has been described by Gaddum *et al.* (1955), who call this type of block "unsurmountable." High concentrations of some of the compounds also depressed the responses to acetylcholine. There was no evidence, however, that they antagonized 5-hydroxytryptamine more than tryptamine (Table IIB).

5-Benzyloxy-3-(2-dialkylaminoethyl)-indoles all seemed to have the same order of activity although there was an increase up the series to the dipropyl compound (No. 12). The dibutyl compound (No. 14) was not very soluble and in the highest concentration tested (about 6×10^{-5} M) did not have any effect. The corresponding 5-hydroxy compound (No. 7) had quite marked effects and was the only 5-hydroxy compound which antagonized 5-hydroxytryptamine. The most active antagonist was 3-(2-aminopropyl)-5-benzyloxyindole (No. 17), but even this was less active than 5-benzyloxygramine.

Stimulant Action—The stimulant activity of the compounds is shown in Table I. The dose/response curves were all parallel to those of 5-hydroxytryptamine (and tryptamine) except those obtained with 3-(2-dibutylaminoethyl)-5-hydroxyindole (No. 7). This compound was the only one which definitely combined antagonistic and stimulant properties on this preparation (compare with 3-(2-dimethylaminoethyl)indole, Barlow and Khan, 1959) and in high concentration (10^{-5} M) usually produced repeated contractions and subsequent profound depression.

In one experiment 3-(2-aminopropyl)-1-methylindole (No. 22) antagonized 5-hydroxytryptamine, but in a second experiment (in the same concentration, 3×10^{-6} M, but on a different preparation) produced synergism with 5-hydroxytryptamine. In yet another experiment the same concentration produced a contraction by itself. Although this substance, therefore, combined antagonistic and stimulant properties, the stimulant effects predominated and masked the antagonistic ones (compare with 3-(2-diethylaminoethyl)indole, Barlow and Khan, 1959). Like 3-(2-dibutylaminoethyl)-5-hydroxyindole, this compound (No. 22) easily produced repeated contractions of the preparation but, after these, the tissue was more sensitive to 5-hydroxytryptamine for about 10 min. In this respect it resembled the analogues of tryptamine previously studied.

The most active compound was 3-(2-aminopropyl)-5-hydroxyindole (No. 16), and the next most active were 3-(2-aminopropyl)-5-methoxyindole (No. 21) and bufotenine (No. 1). Activity in the series of 3-(2-dialkylaminoethyl)-5-hydroxyindoles declined as the length of the alkyl group was increased, in contrast to the variation of activity with structure in the series of 3-(2-dialkylaminoethyl)indoles. The 5-methyl group and, to a much greater extent, the 5-benzyloxy group, reduced the stimulant activity. The quaternary salts tested were inactive as were also, although these are not included in Table I, the glyoxylamides, (5-benzyloxyindol-3-yl)glyoxyldimethylamide and (2-methylindol-3-yl)glyoxyldimethylamide, which are intermediates in the synthesis of the tryptamines.

Effects on the Isolated Rat Fundus Strip Preparation

Antagonism.—A few of the compounds, in low concentrations, antagonized the effects of tryptamine, and often, to a greater extent, of 5-hydroxytryptamine. This is shown in Table III, which includes results obtained with 5-benzyloxygramine and 5-benzyloxy-3-(2-dimethylaminoethyl)indole not reported in the previous paper. On this preparation, 5-benzyloxygramine was a less potent antagonist than 5-benzyloxy-3-(2-dimethylaminoethyl)indole or even than 3-(2-dimethylaminoethyl)indole itself (Table III). The action of 5-hydroxytryptamine on this preparation differed from that on the rat uterus in that it was much less sensitive to antagonism by 2-bromolysergic acid diethylamide and by 5-benzyloxygramine. For instance, on the rat uterus a concentration of 10^{-8} M-2-bromolysergic acid diethylamide produced an effect roughly comparable with that of a 10^{-7} M solution on the rat fundus strip. Similarly 5-benzyloxygramine, in a concentration of 9×10^{-7} M, gave effects on the rat uterus comparable with those of a concentration of 2×10^{-5} M on the rat fundus strip. On the other hand 3-(2-dimethylaminoethyl)indole in a concentration of 10^{-5} M produced a dose ratio for 5-hydroxytryptamine of 10 on the rat uterus, whereas it produced the same effect on the rat fundus strip in a concentration of 3×10^{-7} M. The antagonistic effects of 5-benzyloxy-3-(2-dimethylaminoethyl)indole also appeared to be greater on the rat fundus than on the rat uterus. On the rat fundus strip, 3-(2-dimethylaminoethyl)indole and 5-benzyloxy-3-(2-dimethylaminoethyl)indole were about equiactive whereas on the rat uterus the latter was more powerful.

TABLE II

ANTAGONISTIC ACTIVITY ON THE RAT UTERUS AT 30°

‡ Included from previous work for comparison (Barlow and Khan, 1959). † Indicates unsurmountable block (see text). * Indicates potentiation. In the drug ratio experiments the ratio of the concentration of the agonist to the concentration of the antagonist when the response was 50% of the initial maximum was used and the antagonist was allowed to act for 1 hr.: all concentrations are expressed in terms of molarity. In the dose ratio experiments the ratio is the number of times the concentration of the agonist must be increased in order to produce a 50% response after an antagonist has been allowed to act on the preparation for 1 hr. It should obviously increase with increasing the concentration of antagonist. The values for the dose ratio and drug ratio in the two parts of the Table must not be confused. It is only possible to proceed to determine the drug ratio if a value above 5 can be obtained for the dose ratio.

A. Drug Ratio Experiments using 5-Hydroxytryptamine

No.	Compound	Conc. (M)	Drug Ratio (Mean±S.E.)	No. of Expts.
8	5-Benzyloxy-3-(2-dimethylaminoethyl)indole	90×10^{-7}	0·066±0·008	4‡
11	5-Benzyloxy-3-(2-diethylaminoethyl)indole	7 21 55	0·074 0·093 0·47†, 9·25†	1 1 2
12	5-Benzyloxy-3-(2-dipropylaminoethyl)indole	5·2 13 26	0·095 0·107±0·069 0·095	1 2 1
13	5-Benzyloxy-3-(2-di-isopropylaminoethyl)indole	6·5 13	0·076±0 0·57†, 0·95†	2 2
15	5-Benzyloxy-3-(2-morpholinoethyl)indole	13 26	0·06 0·03	1 1
17	3-(2-Aminopropyl)-5-benzyloxyindole	7·8 32 78	0·16 0·27 0·12	1 1 1
7	3-(2-Dibutylaminoethyl)-5-hydroxyindole	30 75	0·10±0·035 0·054	2 1

B. Dose Ratio Experiments

No.	Compound	Conc. (M)	Dose Ratio		
			5-Hydroxytryptamine	Tryptamine	Acetylcholine
12	5-Benzyloxy-3-(2-dipropylaminoethyl)-indole	$5\cdot2\times10^{-7}$	4	2	1
		13	20 4	27 8	1 1
		26	20	40	1
13	5-Benzyloxy-3-(2-di-isopropylamino-ethyl)indole	6·5	4 20	2 20	1 2
15	5-Benzyloxy-3-(2-morpholinoethyl)-indole	13 26	4 4	2 4	1 1·5
17	3-(2-Aminopropyl)-5-benzyloxyindole	7·8	5 4	8 3	1·5 1
		32	37	50	2
7	3-(2-Dibutylamino-ethyl)-5-hydroxy-indole	30	10 20	5 10	0·3* 0·6*
		75	20	25	0·5*

Although the dose ratios obtained in some instances were enough to justify attempts to measure the drug ratio, this was not done.

TABLE III

ANTAGONISTIC ACTIVITY ON THE RAT FUNDUS STRIP AT 37°

† Indicates potentiation. ‡ Indicates that these results confirm our previous estimates with this compound.

No.	Compound	Conc. (M)	Dose Ratios			
			5-Hydroxy-trypt-amine	Trypt-amine	5-Methyl-trypt-amine	Acetyl-choline
8	5-Benzyloxy-3-(2-dimethyl-aminoethyl)-indole	6×10^{-7}	10	2	—	<1†
			10	2	—	<1†
	5-Benzyloxy-gramine	18	2·5	1	—	1
		36	5	2	—	1
			5	2	—	1
		130	15	2	—	1
		180	27	8	—	2
		360	100	20	—	1
	3-(2-Dimethyl-aminoethyl)-indole‡	3·3	10	<1†	—	1
			10	1·5	—	1
	2-Bromolysergic acid diethyl-amide	1·2	75	10	15	1
		2·5	60	8	8	1
		3·7	400	20	20	2

Estimation of the drug ratio requires the production of a maximal response, which on this tissue would render the preparation insensitive for a considerable time.

Stimulant Action.—All the compounds (except 5-benzyloxy-3-(2-morpholinoethyl)indole and the intermediate glyoxylamides mentioned above) stimulated the preparation. Those compounds listed above as antagonists showed this effect at concentrations greater than those which produced antagonism. In low concentrations the substances produced a gradual shifting of the base-line but, as was found with the tryptamines, there was no apparent synergism with 5-hydroxytryptamine, such as was seen on the rat uterus. There was no sign of any potentiation of tryptamine by these compounds (compare with 3-(2-dimethylaminoethyl)-2-methylindole, Barlow and Khan, 1959). The stimulant activity is summarized in Table I.

The dose/response curves of all the compounds, except those containing a 5-benzyloxy group, were parallel to those of 5-hydroxytryptamine (and tryptamine). The 5-benzyloxy compounds did not give a maximal response, although at lower concentrations the curves were parallel to those of 5-hydroxytryptamine, and it was at these concentrations that the equipotent molar ratios were determined.

5-Hydroxytryptamine, tryptamine, 3-(2-aminopropyl)-5-hydroxyindole (No. 16), 5-methoxytryptamine (No. 20), and 5-methyltryptamine (No. 18) caused contractions which reached a maximum within 90 to 120 sec. and which passed off after washing and stretching for about 1 min. The 5-benzyloxy compounds and 3-(2-aminopropyl)-1-methylindole (No. 22) required 2 or 3 min. for completion of the contraction, and 30 to 35 min. for stretching and recovery. The remaining compounds fell between these two extremes.

The most active compounds were 3-(2-aminopropyl)-5-hydroxyindole (No. 16) and 3-(2-aminopropyl)-5-methoxyindole (No. 21). The next most active were bufotenine and 3-(2-di-isopropylaminoethyl)-5-hydroxyindole. The variation of structure and activity in the series of 3-(2-dialkylaminoethyl)-5-hydroxyindoles was slightly different on the rat fundus from what it was on the rat uterus. Relative to the others, the dipropyl (No. 5) and di-isopropyl (No. 6) compounds were more active on the rat fundus than on the rat uterus.

In two instances (Nos. 19 and 21), substances containing a methyl group in the α position relative to the amino group were more active than the simple aminoethyl derivatives (Nos. 18 and 20), but this is not true for 5-hydroxytryptamine and 3-(2-aminopropyl)-5-hydroxyindole (No. 16). This result confirms the work of Vane (1959). The 5-methoxy compounds are again, as on the rat uterus, more active than their 5-methyl analogues.

Of the 5-benzyloxy compounds, the 3-(2-aminopropyl) member (No. 17) was the most active, but potency in the series, though declining in the higher members, did not follow the same pattern as in the corresponding 5-hydroxy compounds. As these compounds did not give a maximal response, we wished to see if they acted on the same receptors as 5-hydroxytryptamine. We therefore added simultaneously a 5-benzyloxy compound in a concentration which produced the biggest response obtainable (we used 6×10^{-5} M of the 3-(2-dimethylaminoethyl) compound (No. 8) or 5×10^{-5} M of the 3-(2-diethylaminoethyl) compound (No. 11)) and a dose of 5-hydroxytryptamine (100 ng. in a 5 ml. bath) which produced by itself a bigger effect than the 5-benzyloxy compound. In these circumstances (Fig. 2) the response was smaller than that to the dose of 5-hydroxytryptamine alone. When a dose of acetylcholine (200 μg.) was used in place of the 5-hydroxytryptamine, there was no depression of the response. This suggested that the 5-benzyloxy compounds occupied the same receptors as 5-hydroxytryptamine but not those acted on by acetylcholine.

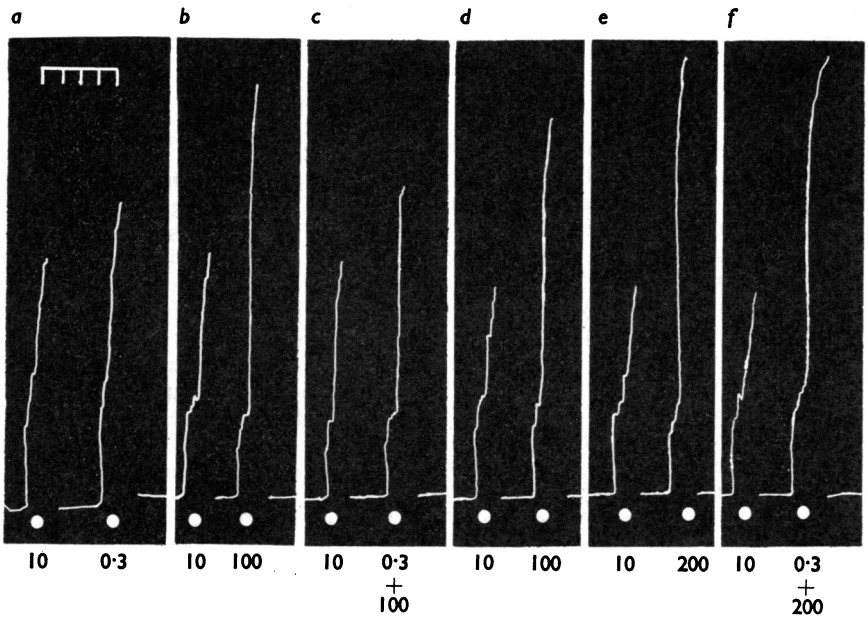


FIG. 2.—Isolated rat fundus strip preparation. (*a*), Responses to 10 ng. of 5-hydroxytryptamine (**10**) and 0.3 μMoles of 5-benzyloxy-3-(2-dimethylaminoethyl)indole (**0.3**) at time 12.28. (*b*), Responses to 10 ng. and 100 ng. of 5-hydroxytryptamine (**10** and **100**) at 14.05. (*c*), Responses to 10 ng. of 5-hydroxytryptamine (**10**) and a combined dose of 0.3 μMoles 5-benzyloxy-3-(2-dimethylaminoethyl)indole *plus* 100 ng. of 5-hydroxytryptamine (**0.3+100**) at 14.20. (*d*), Responses to 10 ng. and 100 ng. of 5-hydroxytryptamine at 15.45. (*e*), Responses to 10 ng. of 5-hydroxytryptamine (**10**) and to 200 μg. of acetylcholine (**200**) at 16.15. (*f*), Responses to 10 ng. of 5-hydroxytryptamine (**10**) and to a combined dose of 0·3 μMoles 5-benzyloxy-3-(2-dimethylaminoethyl)indole *plus* 200 μg. of acetylcholine (**0.3+200**). The results suggest that the 5-benzyloxy compound acts at the same receptors as 5-hydroxytryptamine but not at those acted upon by acetylcholine. Time, min.

Differentiation Between the Actions of Tryptamine and 5-Hydroxytryptamine

We again obtained evidence of differentiation between the antagonism of 5-hydroxytryptamine and of tryptamine on the rat fundus strip (Table III) but not on the rat uterus (Table IIB). 5-Benzyloxygramine and 5-benzyloxy-3-(2-dimethylaminoethyl)indole (No. 8) behaved like 2-bromolysergic acid diethylamide (and the compounds previously described, Barlow and Khan, 1959) in antagonizing 5-hydroxytryptamine more than tryptamine. Contractions produced by 5-methyltryptamine, however, unlike those produced by dimethyltryptamine and dipropyltryptamine, but like those produced by tryptamine, were much more resistant to the action of 2-bromolysergic acid diethylamide (Table III).

DISCUSSION

Antagonistic activity was confined to only a few compounds, those containing a 5-benzyloxy group and 3-(2-dibutylaminoethyl)-5-hydroxyindole (No. 7). Even the most active of these was not very powerful on the rat uterus, and on the rat fundus strip only 5-benzyloxy-3-(2-dimethylaminoethyl)-indole (No. 8) was an antagonist. On the rat fundus, however, the latter was more active than 5-benzyloxygramine and this draws attention to the different sensitivity of the two preparations. For activity as an antagonist of 5-hydroxytryptamine this difference is revealed by the fact that greater concentrations of 2-bromolysergic acid diethylamide and of 5-benzyloxygramine were needed on the rat fundus strip, whereas smaller concentrations of 5-benzyloxy-3-(2-dimethylaminoethyl)indole (No. 8) were needed on the rat fundus strip than on the rat uterus. Also, nearly all the antagonists (including 2-bromolysergic acid diethylamide and 5-benzyloxygramine) were more effective against 5-hydroxytryptamine than against tryptamine on the rat fundus strip whereas they do not differentiate between the two on the rat uterus.

Variations of activity with structure are also quite different with the two preparations. In the 5-hydroxy compounds, in particular, stimulant activity on the rat fundus strip after dropping from bufotenine (No. 1) to the diethyl compound (No. 4) increased again in the dipropyl and di-iso-

propyl compounds (Nos. 5 and 6); this is reminiscent of the maximum in activity on this preparation at the dipropyl compound in the simple tryptamines. With the rat uterus the relationships between structure and activity of the 5-hydroxy compounds were quite different from those of the analogues which lacked the 5-hydroxyl group. Again, apart from the 5-hydroxy compounds, most of the substances were, relative to 5-hydroxytryptamine, much more active stimulants on the rat fundus strip than on the rat uterus (this was particularly noticeable with dipropyltryptamine). The stimulant activity of the 5-hydroxy compounds relative to 5-hydroxytryptamine, however, was not greatly different on the two preparations.

Although, therefore, the general effects of the compounds on the rat uterus and rat fundus strip were not dissimilar, there were substantial differences which were brought to light especially by the 5-hydroxy compounds. Vane (1959) has suggested that the 5-hydroxy compounds are not able to penetrate cells but act at the surface, whereas non-phenolic compounds can penetrate the cell and so are subject to the action, inside the cell, of amine oxidase. Vane (1959) has shown that homogenates of rat fundus strip will oxidize 5-hydroxytryptamine and tryptamine to about the same extent and explains in this way why an α-methyl group enhances the activity of 5-methoxy- and 5-methyl-tryptamines and of tryptamine itself, but not that of 5-hydroxytryptamine. It would seem from our results with the rat uterus that this tissue should be deficient in amine oxidase and we have found that the presence of iproniazid (Marsilid), in concentrations of up to 3×10^{-5} M and allowed to act for over 30 min., had no effect on responses to 5-hydroxytryptamine and tryptamine. In such a concentration Vane (1959) found that iproniazid potentiated the action of tryptamine on the rat fundus strip by a factor of more than 10 but had no effect on the responses to 5-hydroxytryptamine.

The activity of the compounds may well depend upon their actions (either as substrates or inhibitors) on amine oxidase as well as their actions on the tryptamine and/or 5-hydroxytryptamine receptors. We have no systematic information about the action of the compounds on the enzyme, nor is it clear whether there are separate tryptamine and 5-hydroxytryptamine receptors. Furthermore, we might expect that substances which antagonize 5-hydroxytryptamine at one site would do so at others, also, perhaps, centrally. Similarly substances which stimulate, say, the rat uterus might be expected to be stimulants at other sites. In fact our results with the rat uterus and rat fundus strip do not justify extending the character of the action from one site to another. Until information has been obtained about all these points, we do not feel justified in speculating further about the actions, peripheral or central, of these compounds.

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