Brit. J. Pharmacol. (1959), **14**, 99.

ACTIONS OF SOME ANALOGUES OF TRYPTAMINE ON THE ISOLATED RAT UTERUS AND ON THE ISOLATED RAT FUNDUS STRIP PREPARATIONS

BY

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(RECEIVED OCTOBER 21, 1958)

Some 3-(2-dialkylaminoethyl)-, 3-(2-alkylaminoethyl)-, and 3-(2-dialkylaminoethyl)-2-methylindoles have been synthesized and tested, along with 5-benzyloxygramine, 5-benzyloxy-3-(2-dimethylaminoethyl)-, 3-(2-aminopropyl)- and 3-(2-aminobutyl)-indoles, on the rat uterus and rat fundus strip. 5-Benzyloxy-3-(2-dimethylaminoethyl)indole, even though it contains an ethylene side-chain, was a less potent antagonist of 5-hydroxytryptamine than was 5-benzyloxygramine. The remaining compounds were still less active. There are differences between the ability of some of them to antagonize 5-hydroxytryptamine and to antagonize tryptamine. 3-(2-Dimethylaminoethyl)-2-methylindole, in particular, shows a considerable degree of specific antagonism of 5-hydroxytryptamine on both tissues.

Nearly all the compounds stimulate the preparations, some combining antagonism of 5-hydroxytryptamine at low concentrations with stimulant activity at higher concentrations. The most active compound is 3-(2-dipropylaminoethyl)indole. On the rat fundus strip this has, on a molar basis, 1/40th of the stimulant activity of 5-hydroxytryptamine, and is 20 times as active as tryptamine: on the rat uterus it is only 1/200th as active as 5-hydroxytryptamine, and equal in activity to tryptamine.

From quantitative studies of antagonists of 5-hydroxytryptamine on the rat uterus, Gaddum, Hameed, Hathway, and Stephens (1955) concluded that, in compounds related to 3-aminomethylindole and 3-(2-aminoethyl)indole (tryptamine), antagonist activity was increased by: (1) Introduction of two methyl groups on the amino group in the side-chain. (2) Introduction of a methyl group in the 2-position of the indole nucleus (this effect was also noted by Woolley and Shaw, 1953, in another series of substituted indoles). (3) Introduction of a benzyloxy group in the 5-position of the indole nucleus of gramine [they did not study 5-benzyloxy-3-(2-dimethylaminoethyl)indole].

In a search for other antagonists of 5-hydroxytryptamine, we have prepared and tested a number of analogues of tryptamine, some of which contain these structural features (Table I). There are three types of compound: (1) 3-(2-Dialkylaminoethyl)indoles, (2) 3-(2-alkylaminoethyl)indoles and (3) 3-(2-dialkylaminoethyl)-2-methylindoles. We have included in our work 3-(2-aminopropyl)indole (α-methyltryptamine), 3-(2-aminobutyl)indole (α-ethyltryptamine) and 5-benzyloxygramine (Gaddum, Hameed, Hathway, and Stephens, 1955) and also 5-benzyloxy-3-(2-dimethylaminoethyl)indole (Speeter and Anthony, 1954), whose pharmacological properties do not appear to have been extensively investigated.

The substances have been tested for both stimulant and antagonistic properties on the rat uterus and on the rat fundus strip (Vane, 1957).

METHODS

Drugs.—The compounds were all prepared by the method of Speeter and Anthony (1954). Indole, 2-methylindole or 5-benzyloxyindole, dissolved in ether and treated with oxalyl chloride, gave the 3-glyoxalyl chloride which was converted to the appropriate amide. This was reduced with lithium aluminium hydride in ether or, more usually (as it is a better solvent for the amide), in tetrahydrofuran. Excess lithium aluminium hydride was destroyed with water, the solvent decanted off and the residue washed with ether. The combined extracts were dried and concentrated and the base was distilled at 10^{-3} to 10^{-4} mm. of Hg pressure. The clear oil or

solid distillate was converted into the hydrochloride or, if this would not crystallize, into the fumarate.

The 5-benzyloxygramine was the sample used by Gaddum, Hameed, Hathway, and Stephens (1955). Although the drugs are referred to as if bases, a suitable salt (Table I) was invariably used.

Preparations.—The isolated uterus was set up in de Jalon solution in a 2 ml. bath, exactly as used by Gaddum and Hameed (1954). The temperature was 30°. It was found that portions of uterus could be stored at 4° for up to two days without loss of sensitivity to either 5-hydroxytryptamine or tryptamine.

The isolated rat fundus strip preparation was cut exactly as described by Vane (1957) but mounted, as subsequently recommended by Vane (personal communication), with a pendular auxotonic lever (Paton, 1957). In some experiments, the magnification of the lever was 16 (as originally described by Vane) and the load on the muscle was 1 g. In other experiments, where it was desired to record a maximum contraction, the magnification was only 4 and only three-quarters of the full length of the dissected fundus strip was used. In these experiments the load on the muscle was 0.5 g. The preparation was suspended in Tyrode solution containing a concentration of 10^{-7} hyoscine hydrobromide which prevented interference from acetylcholine-like effects and also (Vane, personal communication) produced a much steadier base-line. Pure oxygen was bubbled through the bath, which had a volume of 5 ml. The temperature was 37°.

Experimental Procedures

Qualitative

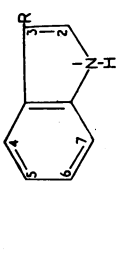
Rat Uterus.—All the drugs were tested on the rat uterus in concentrations varying from below the smallest which produced an effect (usually 10^{-7} to 10^{-6} M) up to 10^{-4} M. The drugs were added directly to the bath in a volume not exceeding 0.2 ml. and allowed to act for 45 sec. The preparation was then washed and allowed 3.25 min. for recovery; the interval between doses was thus 4 min. Control contractions of about 50% of the maximum possible response were obtained with 5-hydroxytryptamine, tryptamine (in some experiments) and acetylcholine. These drugs were added in that order, first in the absence of the test drug, then in its presence (it was maintained in the bath by adding it freshly after each wash), then again in its absence.

After high doses of 5-hydroxytryptamine, the preparation is much less sensitive to smaller ones. To see whether such an antagonism of 5-hydroxytryptamine was shown by these other compounds, the responses to control doses of 5-hydroxytryptamine and of acetylcholine were observed before and after a high concentration (10^{-4} M) of the test drug had been left in the bath for 5 to 10 min.

Rat Fundus Strip.—The drugs were added directly to the bath in a volume not exceeding 0.2 ml. and allowed to act for 90 sec. The preparation was

TABLE I

COMPOUNDS AND STIMULANT ACTIVITY



Comp. No.	Ring Substituent	Side-chain (R) (Trivial Name)	Salt	Crystallized from	m.p. (°C.)	Found C	Found H	Found N	Theory C	Theory H	Theory N	Stimulant Activity Equipotent Molar Ratios: Mean ±s.e. Rat Uterus 30°	No. of Expts.	Rat Fundus Strip 37°	No. of Expts.
1	—	$-CH_2-CH_2-N(CH_3)_2$ (*N':N'*-Dimethyltryptamine)	Fumarate	EtOH/MEK/Et_2O	152–152·5	63·3	6·81	9·40	63·2	6·65	9·21	†1,000 (M)	2	350±100	4
			Picrate	EtOH	169·5–170·5	51·6	4·51		51·8	4·60					
2	—	$-CH_2-CH_2-N(C_2H_5)_2$ (*N':N'*-Diethyltryptamine)	HCl	EtOH/MEK/Et_2O	172–3	66·4	8·37	10·7	66·5	8·40	11·1	500 (M)	2	112±14	3
			Picrate	EtOH	170·5	54·2	5·03		53·9	5·22					
3	—	$-CH_2-CH_2-N(C_3H_7)_2$ (*N':N'*-Dipropyltryptamine)	HCl	EtOH/MEK/Et_2O	176–8	68·7	8·85		68·5	8·64		200±23	3	40±9	3
4	—	$-CH_2-CH_2-N(iso\text{-}C_3H_7)_2$ (*N':N'*-Di*iso*propyltryptamine)	HCl	EtOH/MEK	195·5–196·5	68·6	8·74		68·5	8·64		520±104	3	53±8	4
5	—	$-CH_2-CH_2-N(C_4H_9)_2$ (*N':N'*-Dibutyltryptamine)	HCl	EtOH	184–5	70·1	9·05		70·0	9·17		500 (M)	2	244±65	4
6	—	$-CH_2-CH_2-N$ 	HCl	EtOH/MEK/Et_2O	182–3			11·2			11·2	2,000 (M)	1	470±115	3
			Picrate	EtOH	173–4	54·4	4·93		54·2	4·79					

Microanalyses are by Dr. J. W. Minnis (Department of Biochemistry); melting points were taken on a Kofler hot stage. An asterisk indicates that Speeter and Anthony (1954) record m.p. 154–5°. EtOH=ethanol; MEK=ethyl methyl ketone; Et_2O=ether. † Indicates that the dose/response curve was not parallel to that of 5-hydroxytryptamine and at high doses a value as extreme as 20,000 : 1 was obtained. M indicates that estimates were determined by matching (see text).

7	—	$-CH_2-CH_2-N$	HCl Picrate	EtOH/MEK/Et_2O EtOH	225–7 174–174·5	67·9 55·2	7·90 4·88	10·5	68·1 55·1	7·64 5·08	10·6	1,600 (M)	1	7,500±1,770	2
8	—	$-CH_2-CH_2-N$	HCl Picrate	EtOH/MEK EtOH	214–5 202–3	63·2 52·4	7·02 4·43		63·1 52·3	7·20 4·62		> 10,000 (M)	2	> 75,000	2
9	—	$-CH_2-CH_2-NH(CH_3)$ (*N'*-Methyltryptamine)	HCl Picrate	EtOH/MEK/Et_2O EtOH	181·5–182·5 193–4	62·8 51·2	6·91 4·45	13·3	62·7 50·6	7·20 4·26	13·3	2,000 (M)	2	2,400±98	3
10	—	$-CH_2-CH_2-NH(C_2H_5)$ (*N'*-Ethyltryptamine)	HCl Picrate	EtOH/MEK/Et_2O EtOH	183–4 187–9	64·2 51·9	7·56 4·40	12·7	64·1 51·8	7·65 4·60	12·5	2,000 (M)	2	350±29	3
11	—	$-CH_2-CH_2-NH(C_3H_7)$ (*N'*-Propyltryptamine)	HCl	EtOH/MEK/Et_2O	184–185·5	65·5	8·07		65·4	8·05		2,000 (M)	2	490±85	4
12	2-CH_3	$-CH_2-CH_2-N(CH_3)_2$ (2: *N'N'*-Trimethyltryptamine)	HCl Picrate	EtOH/MEK EtOH	211–2 176·5	65·2 52·8	7·60 4·93		65·4 52·9	8·05 4·92		> 20,000 (M)	2	6,665±1,330	3
13	2-CH_3	$-CH_2-CH_2-N(C_2H_5)_2$ (*N'N'*-Diethyl-2-methyltryptamine)	HCl Picrate	EtOH/MEK/Et_2O EtOH	217–8 174–5	67·8 55·0	8·87 5·30		67·5 54·9	8·72 5·50		1,000 (M)	2	280±42	4
14	2-CH_3	$-CH_2-CH_2-N(C_3H_7)_2$ (2-Methyl-*N'N'*-dipropyltryptamine)	HCl	EtOH/MEK	227–8	69·4	9·27		69·3	9·26		630 (M)	2	70±10	3
15	5-OC_7H_7	$-CH_2-CH_2-N(CH_3)_2$ (5-Benzyloxy-*N'N'*-dimethyltryptamine)	HCl	EtOH/MEK/Et_2O	159–161*	69·3	6·73	8·42	68·9	7·03	8·47	> 20,000	2	625±135	4
16	5-OC_7H_7	$-CH_2-N(CH_3)_2$ (5-Benzyloxygramine)												25,000	1
17	—	$-CH_2-CH(CH_3)-NH_2$ (α-Methyltryptamine)	HCl									800		50±14	3
18	—	$-CH_2-CH(C_2H_5)-NH_2$ (α-Ethyltryptamine)	HCl									> 20,000	2	10,000	2
19	—	$-CH_2-CH_2-NH_2$ (Tryptamine)	HCl									210±30	4	933±33	3
20	5-OH	$-CH_2-CH_2-NH_2$ (5-Hydroxytryptamine)										1		1	

washed and stretched for 60 sec. and allowed a further 2.5 min. for recovery; the interval between doses was thus 5 min. On this preparation, the effects of low concentrations of the drugs were of particular interest. A concentration, too small to produce a contraction (though often disturbing the base line), was maintained in the bath for from 15 to 45 min. to see how this affected the responses to repeated doses of 5-hydroxytryptamine and tryptamine.

The highest concentrations tested on this preparation were not usually greater than those which produced a maximum contraction (around 10^{-5} M) as, on the occasions when they were tested, concentrations above this did not give rise to repeated contractions or to non-specific inhibition, such as was observed on the rat uterus.

Quantitative

Antagonistic Activity. — This was determined by measuring the dose ratio and, if possible, the drug ratio of the drug to 5-hydroxytryptamine exactly as described by Gaddum, Hameed, Hathway, and Stephens (1955). As in their experiments, the antagonists were maintained in the bath for 1 hr. The procedure with the rat fundus strip only differed from that with the rat uterus in that the interval between doses was 5 min. instead of 4 min. (see above). In all experiments on antagonistic activity with the rat fundus strip, control responses were obtained with acetylcholine (in large doses because of the hyoscine present).

Stimulant Activity.—Dose/response curves for all the compounds were obtained on the rat fundus strip and for some of the compounds on the rat uterus. On any one preparation only two curves were obtained, one for 5-hydroxytryptamine and one for the test drug. In these experiments it was necessary with some of the drugs to modify the cycle of events. On the rat uterus it was a long time before the effects of certain compounds wore off and the controls returned to normal. On the rat fundus strip it was sometimes necessary to allow up to 3 min. for the contraction to reach its maximum and also to allow even up to 45 min. stretching to assist recovery. If insufficient time were allowed for recovery, the responses were reduced and the dose/response curves consequently appeared to flatten out. This

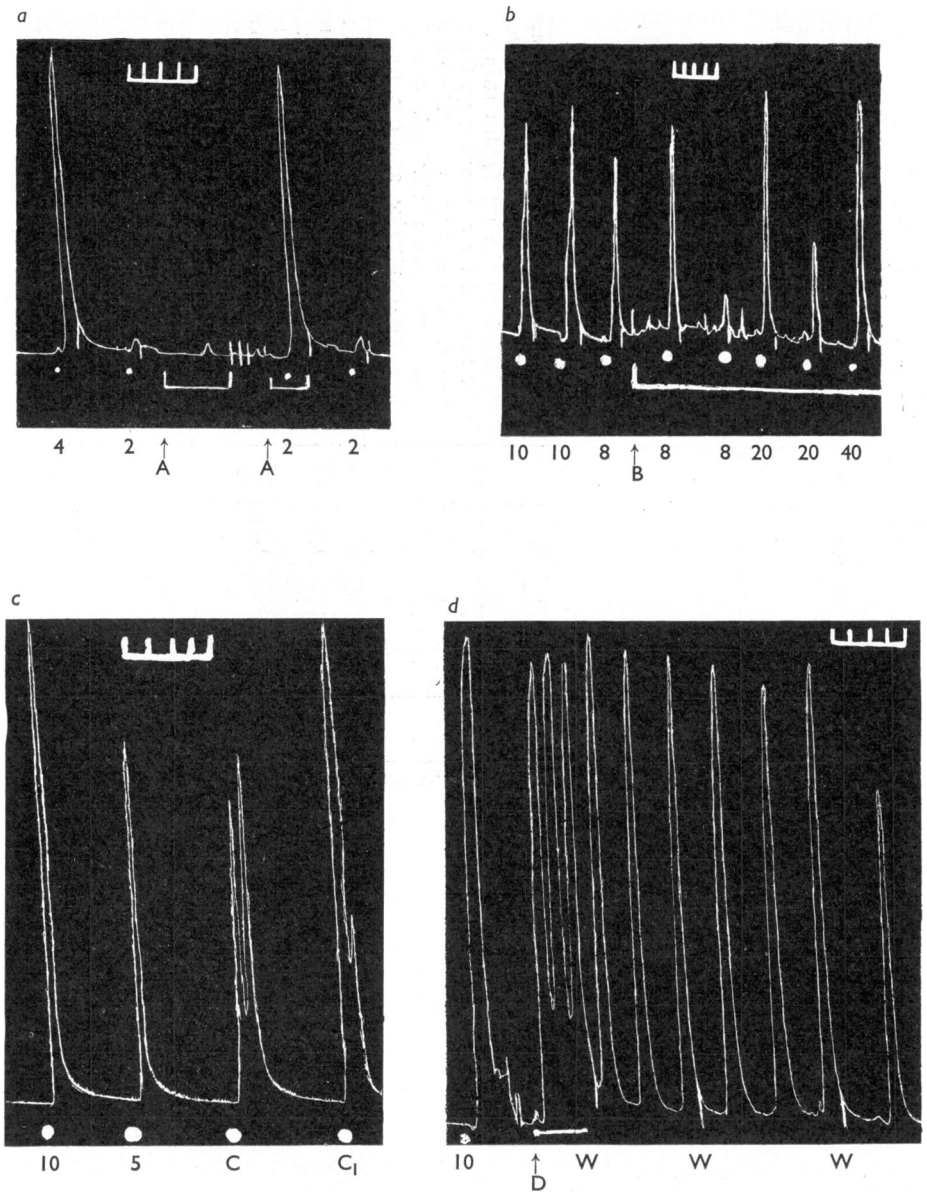


FIG. 1.—Isolated rat uterus preparation. Time, min. (*a*)—Synergism with 5-hydroxytryptamine (**5-HT**) added at white dots. At **A**, a concentration of $4{\cdot}8\times10^{-6}$ M 3-(2-dibutylaminoethyl)indole was maintained in the bath for the period indicated. By itself it had no effect, but when added with 2 ng. of **5-HT** (**4**) the response was about as big as that to 4 ng. of **5-HT** (**2**) previously. (*b*)—Antagonism of 5-hydroxytryptamine (**5-HT**) added at white dots. At **B** a concentration of 4×10^{-6} M 3-(2-diethylaminoethyl)indole was maintained in the bath. After 18 min. 40 ng. of **5-HT** (**40**) produced a response equivalent to 10 ng. of **5-HT** (**10**) previously. 8 = 8 ng. of **5-HT**. (*c*)—Stimulant action. 3-(2-di*iso*propylaminoethyl)indole was added in a concentration $4{\cdot}5\times10^{-6}$ M at **C** and 9×10^{-6} M at $\mathbf{C_1}$. Numerals indicate ng. of 5-hydroxytryptamine added. (*d*)—Repeated contractions. At **D** a concentration of 10^{-5} M 3-(2-dipropylaminoethyl)indole maintained in the bath for the period indicated. The repeated contractions persisted in spite of washing at **W**. **10** = 10 ng. of 5-hydroxytryptamine. (*e*)—Profound depression. Responses to 500 ng. of carbachol (at white dots with **Cc** below them) and 5-hydroxytryptamine (**5-HT**) at white dots, the numerals below which indicate ng. of **5-HT**, are shown before and after treatment with 3-(2-dipropylaminoethyl)indole in a concentration at **E** of $3{\cdot}6\times10^{-4}$ M and $\mathbf{E_1}$ of $7{\cdot}2\times10^{-4}$ M maintained for the period indicated.

gave the misleading impression that the compounds were partial antagonists (Stephenson, 1956). With the rat fundus strip it was necessary to reduce the magnification of the lever, as described above.

After every test contraction (whether of 5-hydroxytryptamine or of the drug), small control doses of 5-hydroxytryptamine were given and the experiment was continued only when the responses to these were normal. Because of the persistence of the effects of high doses, the doses were given in increasing order of magnitude rather than in any random manner.

The dose/response curves were examined by eye to see if they were parallel, and, as they were (with one dubious exception), it was possible to evaluate the equipotent molar ratio, namely the number of molecules of the drug required to produce the same effect as that of one molecule of 5-hydroxytryptamine.

After many of the compounds, the rat uterus only returned to normal very slowly and the determination of these dose/response curves became very tedious, particularly with high doses. With such compounds, simple matching experiments were performed and the equipotent molar ratio was calculated by comparing the doses of 5-hydroxytryptamine and of the test drug which produced roughly 50% of the maximal contraction.

RESULTS

Effects on the Isolated Rat Uterus

The effects varied with the concentration as well as with the nature of the drug. Starting with those of low concentrations, they could be divided into: (1) Synergism with 5-hydroxytryptamine, tryptamine and acetylcholine, (2) antagonism of 5-hydroxytryptamine and tryptamine, (3) stimulant action, (4) production of repeated contractions, and (5) profound depression. These are illustrated in Fig. 1.

Synergism.—Many of the compounds showed synergism with 5-hydroxytryptamine, tryptamine and also with acetylcholine. This was observed when a dose of one of these was applied first alone, and then in the presence of a dose of the test drug which was too small to produce a visible effect by itself. The contraction which ensued was greater than that caused by the 5-hydroxytryptamine, tryptamine or acetylcholine alone.

This phenomenon was never observed with 5-benzyloxy-3 - (2 - dimethylaminoethyl) - indole (Table I, No. 15) or with 3-(2-dimethylaminoethyl)indole (No. 1) and was difficult to demonstrate with 3-(2-methylaminoethyl)indole (No. 9), 3-(2-dimethylaminoethyl)-2-methylindole (No. 12) and 3-(2-diethylaminoethyl)indole (No. 2). It was shown by all the other substances, but only over a narrow range of concentration. A 5-fold increase was usually adequate for the transition from no effect at all to stimulation of the preparation by the drug itself (action 3).

This type of experiment was designed to show any potentiation which might be caused by an action of the compounds in inhibiting the destruction of tryptamine or 5-hydroxytryptamine. By comparison with eserine and acetylcholine, for instance, such a potentiation might be expected to increase the effects of tryptamine or 5-hydroxytryptamine by a factor of at least ten or more, which is not what was observed here. The increase in the responses to tryptamine, 5-hydroxytryptamine and acetylcholine was noticeable, but such observations do not by themselves prove that the combined action of the two drugs was more than additive. It was possible to produce similar effects with 5-hydroxytryptamine itself (Fig. 2). When the dose/effect curve was steep it is not surprising that the response to a small dose should be increased by adding a paeneliminal dose of the same drug, or of some other stimulant drug. There was no evidence that these compounds had more effect than would be expected on this basis: all of them caused stimulation in slightly higher doses.

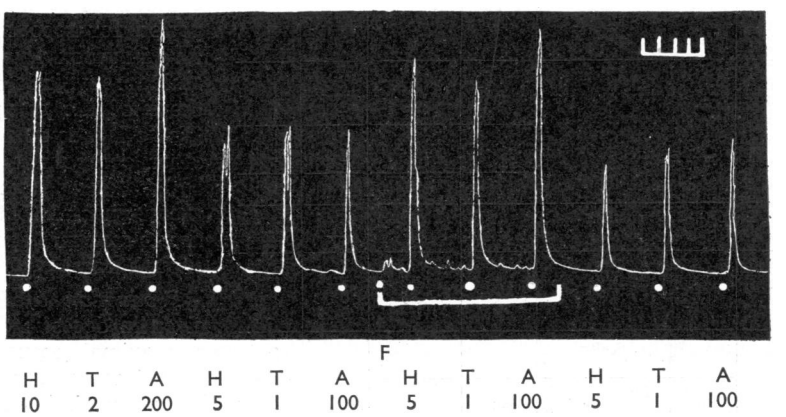


FIG. 2.—Isolated rat uterus preparation. Responses to 5-hydroxytryptamine (H), tryptamine (T) and acetylcholine (A) before, during, and after maintenance at F of a dose of 2·5 ng. of 5-hydroxytryptamine in the bath for the period indicated. This shows the synergism of all three drugs with the small background dose of 5-hydroxytryptamine. The numerals below H and A give the doses in ng., while those below T in μg. Time, min.

Antagonism of 5-Hydroxytryptamine and Tryptamine.—This was shown only by the compounds listed in Table II, which summarizes the results of quantitative experiments on the antagonistic properties of the compounds on this preparation. Gaddum, Hameed, Hathway, and Stephens (1955) obtained drug ratios of 0.66 for 5-benzyloxygramine (No. 16) and 0.027 for 3-(2-dimethylaminoethyl)indole (calculated on a molar basis), which agree quite well with our results. It is convenient to classify the compounds into antagonists comparable with lysergic acid diethylamide (which has a drug ratio of 37 on a molar basis), moderate antagonists such as 5-benzyloxygramine, 5-benzyloxy-3-(2-dimethylaminoethyl)indole and 3-(2-dimethylaminoethyl)-indole, and feeble antagonists, such as those shown in Table IIB. These latter have such weak activity that the dose ratio only was determined: the highest values of this were below the limit (about 5) at which it is possible to measure the drug ratio. In these experiments the acetylcholine controls were unaffected by the moderate antagonists, and tryptamine, in the few experiments performed with it, was antagonized in much the same way as 5-hydroxytryptamine. One of the feeble antagonists however, 3-(2-dimethylaminoethyl)-2-methylindole, increased the responses to acetylcholine and tryptamine slightly and, on this preparation, was quite a specific antagonist of 5-hydroxytryptamine.

TABLE II

ANTAGONISTIC ACTIVITY ON THE RAT UTERUS AT 36°

A—Drug Ratio Experiments using 5-Hydroxytryptamine. Note: The drug ratio is the ratio of the concentration of the agonist to the concentration of the antagonist when the response is 50% of the initial maximum. In this work, the antagonist was allowed to act for one hour: all concentrations are expressed in terms of molarity. The asterisk indicates that, at this high concentration, Gaddum, Hameed, Hathway and Stephens (1955) obtained similar high drug ratios which they ascribed to the development of an unsurmountable block.

Comp. No.	Drug	Conc. (M)	Drug Ratio (Mean±s.e.)	No. of Expts.
16	5-Benzyloxygramine ..	9.0×10^{-7} 18·0 *72·0	0·77 0·56 6·4	1 1 1
15	5-Benzyloxy-3-(2-dimethylaminoethyl)indole ..	90·0	0·066±0·008	4
1	3-(2-Dimethylaminoethyl)-indole	99·0	0·042±0·014	4

B—Dose Ratio Experiments. Note: The dose 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 one hour. It should obviously increase with increasing the concentration of antagonist. The values for the dose ratio (below) and drug ratio (above in Table IIA) should 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. * Indicates potentiation: after washing this effect passed off much more quickly than did the antagonism of 5-hydroxytryptamine. † Indicates that the compound was allowed to act for 30 min. only. — Signifies not tested.

Comp. No.	Drug	Conc. (M)	Dose Ratios for: 5-Hydroxytryptamine	Tryptamine	Acetylcholine
1	3-(2-Dimethylaminoethyl)indole	1×10^{-6}	4 3·5	2·5 2	1 1
		3	7 5·5	2·5 1·5	1 1
		10	10	4	1
2	3-(2-Diethylaminoethyl)indole	4	4	—	1
12	3-(2-Dimethylaminoethyl)-2-methylindole	10	1·5 1·5 1	— <0·5* 0·25*	1 <0·5* 0·25*
9	3-(2-Methylaminoethyl)indole	12·5	3	—	1
7	3-(2-Piperidinoethyl)-indole	15†	2	—	1
8	3-(2-Morpholinoethyl)-indole	20†	2	1·5	1
18	3-(2-Aminobutyl)indole	42	4	—	1

Stimulant Action. — All the drugs except 5-benzyloxy-3-(2-dimethylaminoethyl)indole, 3-(2-aminobutyl)indole (No. 18) and 3-(2-morpholinoethyl)indole (No. 8) caused contraction of the rat uterus when given in suitable concentration. The equipotent molar ratios are shown in Table I. As with 5-hydroxytryptamine and tryptamine, there was a latent period of 5 to 10 sec. before the muscle started to respond, the contraction lasted 20 to 30 sec. and then passed off. 5-Hydroxytryptamine, tryptamine and 3-(2-aminopropyl)-indole were easily washed out and the preparation rapidly returned to normal, but the other compounds required more washing and a longer period for recovery. The dose/response curves for 5-hydroxytryptamine, tryptamine and 3-(2-aminopropyl)indole appeared to be parallel, as did those of the compounds whose agonist activity (Table I) was obtained from such curves. Of the other compounds, whose activity was estimated by matching (see above), all, with the sole exception of 3-(2-dimethylaminoethyl)indole, gave rise to maximal contractions when given in a large enough dose.

Production of Repeated Contractions.—When given in doses in excess of those which produced a maximal response, all the stimulant drugs caused repeated contractions of the preparation. Even though large doses of 3-(2-dimethylaminoethyl)indole failed to produce a maximal contraction they caused repeated (submaximal) responses. After treatment with 3-(2-dimethylaminoethyl)indole the preparation was noticeably

less sensitive to 5-hydroxytryptamine for a short time as it was also after similar treatment with 5-hydroxytryptamine, or with any of the drugs possessing antagonistic activity. After repeated contractions produced by some of the compounds which were purely stimulant, the tissue was more sensitive to 5-hydroxytryptamine for a short time.

Profound Depression.—In still higher concentrations, the preparation was quiet and insensitive to 5-hydroxytryptamine. The responses to acetylcholine were not always affected, but could be blocked by raising the concentration of the drug still further.

Effects on the Isolated Rat Fundus Strip Preparations

Modification of the Responses to 5-Hydroxytryptamine and Tryptamine by Very Low Concentrations.—When low concentrations of the drugs acted on the rat fundus strip, there did not appear to be any synergism with 5-hydroxytryptamine such as was found on the rat uterus. Some of the compounds enhanced the initial responses to tryptamine, and two substances, 3-(2-dimethylaminoethyl)indole and 3-(2-dimethylaminoethyl) - 2 - methylindole, antagonized 5 - hydroxytryptamine. The dose ratios at the end of 60 min. are shown in Table III. The 2-methyl compound, like some of the others, enhanced the responses to tryptamine in concentrations which antagonized 5 - hydroxytryptamine but 3 - (2 - dimethylaminoethyl)indole itself did not.

TABLE III

ANTAGONISTIC ACTIVITY ON THE RAT FUNDUS STRIP AT 37°

* Indicates potentiation.

Comp. No.	Drug	Conc. (M)	Dose Ratios for:	
			5-Hydroxytryptamine	Tryptamine
1	3-(2-Dimethylaminoethyl)indole	1×10^{-7}	2	1
		3·3	10	2
12	3-(2-Dimethylaminoethyl)-2-methylindole	20	10	0·5–1*
		20	5	0·5–1*

Stimulant Action.—In slightly higher concentrations, all the compounds stimulated the preparation, but some of them, particularly 5-benzyloxy-3-(2-dimethylaminoethyl)indole, 3-(2-aminopropyl)indole and 3-(2-dipropylaminoethyl)-indole (No. 3) produced a much slower contraction of the muscle than did 5-hydroxytryptamine or tryptamine. Recovery, also, was a much slower process. The equipotent molar ratios are shown in Table I.

The dose/response curves of all the compounds appeared to be parallel to those of 5-hydroxytryptamine and tryptamine, though there was some doubt about the results for 5-benzyloxy-3-(2-dimethylaminoethyl)indole because of its slow onset and long duration of action.

Depression.—After stimulation with these compounds, the preparation was insensitive to 5-hydroxytryptamine. When dose/response curves were being obtained it was particularly necessary to allow time for this depression to pass off and to wait until control responses to 5-hydroxytryptamine returned to normal before proceeding.

Differentiation Between the Actions of Tryptamine and 5-Hydroxytryptamine.—In the course of testing these compounds it became apparent that there were differences between the antagonism of tryptamine and the antagonism of 5-hydroxytryptamine. Although, on both the rat uterus and the rat fundus strip, the dose/response curves for tryptamine and 5-hydroxytryptamine were roughly parallel, the existence of substances which antagonized one rather than the other made it seem likely that tryptamine and 5-hydroxytryptamine were not acting in the same way or at the same site. It was therefore necessary to see whether the stimulant action of our substances was tryptamine-like or 5-hydroxytryptamine-like.

In spite of the fact that some of the substances tested, particularly 3-(2-dimethylaminoethyl-2-methyl)indole, were fairly specific in their antagonism of 5-hydroxytryptamine, they could not be used for analysing the stimulant action of the drugs on the rat fundus strip because they were themselves stimulant in higher concentrations. Woolley and Shaw (1957) found that 1-benzyl-3-(2-dimethylaminoethyl)-5 - methoxy - 2 - methylindole (BAS) was much more effective as an antagonist of 5-hydroxytryptamine than as an antagonist of tryptamine when tested on the blood pressure of the anaesthetized dog. Table IV shows that, on the rat fundus strip, it had relatively little antagonistic activity, whereas 2-bromolysergic acid diethylamide was suitable for studying specifically the antagonism of 5-hydroxytryptamine. Concentrations of this compound which affected the responses to tryptamine only slightly had a pronounced effect on the responses to 5-hydroxytryptamine and (unlike similar concentrations of lysergic acid diethylamide) did not themselves cause any contraction or even disturb the base-line. It was, however, found, in the presence of 2-bromolysergic acid diethylamide, that the contraction of the muscle took longer to develop, even in response to 5-hydroxytryptamine

TABLE IV

ANTAGONISM OF 5-HYDROXYTRYPTAMINE, TRYPTAMINE, 3-(2-DIMETHYLAMINOETHYL)INDOLE AND 3-(2-DIPROPYLAMINOETHYL)INDOLE ON THE RAT FUNDUS STRIP AT 37°

The antagonistic effect required 30 to 40 min. to develop completely: this period was allowed for the action of BAS, but in the experiments with Brom-LSD this antagonist was allowed to act for 60 min. BAS = 1-Benzyl-3-(2-dimethylaminoethyl)-5-methoxy-2-methylindole (Woolley and Shaw, 1957). Brom-LSD = 2-Bromolysergic acid diethylamide. 5-HT = 5-Hydroxytryptamine. Me_2N = 3-(2-Dimethylaminoethyl)indole (No. 1). nPr_2N = 3-(2-Dipropylaminoethyl)indole (No. 3).

		Dose Ratios with			
		5-HT	Me_2N	nPr_2N	Tryptamine
BAS					
$3{\cdot}1\times10^{-7}$ M	..	1	—	—	1
$3{\cdot}1\times10^{-6}$	..	2	—	—	1
$7{\cdot}8\times10^{-6}$	..	4	—	—	1
Brom-LSD					
5×10^{-8} M	..	1·5	—	—	1
$1{\cdot}2\times10^{-7}$	(i)	40	—	—	4
	(ii)	50	40	—	7
	(iii)	15	—	15	—
	(iv)	5	>10	—	2
	(v)	10	—	10	5
$2{\cdot}5\times10^{-7}$	..	20	—	—	7
$3{\cdot}7\times10^{-7}$	(i)	40	—	40	10
	(ii)	25	53	—	6
$7{\cdot}5\times10^{-7}$	..	100	—	—	10
$2{\cdot}5\times10^{-6}$	(i)	130	—	—	>10
	(ii)	>200	—	—	6

or tryptamine, and in these experiments a period of up to 3 min. was allowed (instead of 90 sec.) after the addition of the stimulant drugs.

The contractions produced by 3-(2-dimethylaminoethyl)indole and 3-(2-dipropylaminoethyl)-indole were depressed in much the same way as those produced by 5-hydroxytryptamine, the dose ratios being quite similar to those of 5-hydroxytryptamine and different from those of tryptamine. Another interesting fact which emerges from Table IV is that, above a certain level (roughly 2×10^{-7} M), increasing the concentration of 2-bromolysergic acid diethylamide by as much as a factor of 10 did not increase the block of tryptamine, although it greatly increased the block of 5-hydroxytryptamine.

DISCUSSION

If synergism on the rat uterus is accepted as a simple addition of two actions, the results from the two preparations are not dissimilar. Most of the compounds were purely stimulant, but some combined an antagonism of 5-hydroxytryptamine (and, to varying extents, of tryptamine) with a stimulant action at higher concentrations. 5-Benzyloxygramine and 5-benzyloxy-3-(2-dimethylaminoethyl)indole alone were purely antagonists on the rat uterus, but even they could cause stimulation of the rat fundus strip.

Antagonistic activity is confined to relatively few of the compounds; the most active are those with the dimethylamino group in the side-chain. In contrast to the results of Gaddum, Hameed, Hathway, and Stephens (1955) and of Woolley and Shaw (1953), introduction of a methyl group in the 2 position of the indole nucleus of these simple analogues of tryptamine did not increase the antagonistic activity although it did seem to endow it with a certain degree of specificity for 5-hydroxytryptamine. Another unexpected discovery was that 5-benzyloxy-3-(2-dimethylaminoethyl)indole was much less active than 5-benzyloxygramine. It was anticipated that the former, being a substituted tryptamine, would be more active than the latter, which has only one methylene group in the side-chain. One of the most interesting results was the existence among compounds tested of differences between antagonism of 5-hydroxytryptamine and tryptamine. It is odd that these tryptamines without a 5-hydroxyl group, such as 3-(2-dimethylaminoethyl)-2-methylindole, should have antagonized 5-hydroxytryptamine much more effectively than they antagonized tryptamine.

Stimulant activity was shown by many of the compounds and seemed to vary in quite a definite manner with chemical structure. It is disappointing, though probably significant, that the high activity of 3-(2-dipropylaminoethyl)indole on the rat fundus strip was not so marked on the rat uterus. It is not clear whether this compound is the most active member of the 3-(2-dialkylaminoethyl)indoles because the propyl group is specifically associated with activity or simply because the size of that part of the molecule has reached a critical limit. It would be interesting to know if the activity of the 2-methyl substituted compounds is also maximal in the dipropylamino member of the series. It is astonishing that, although these compounds lack a 5-hydroxyl group, their stimulant activity appears to resemble that of 5-hydroxytryptamine rather than that of tryptamine, and so, both in antagonistic and stimulant properties, the drugs influenced the actions of 5-hydroxytryptamine rather than those of tryptamine. Another important finding was that those of the compounds which have antagonistic activity cause stimulation in concentrations greater than those which cause antagonism: this combination of antagonistic and stimulant properties is the reverse of what would be expected if the compounds were partial agonists (Stephenson,

1956). The present evidence does not justify extensive speculation about the modes of action of 5-hydroxytryptamine and tryptamine. This problem is being studied further, and it must be better understood before any attempt is made to discuss relationships between chemical structure and pharmacological properties either antagonistic or stimulant. It is possible, for instance, that the anomalously high activity of 5-benzyloxygramine compared with 5-benzyloxy-3-(2-dimethylaminoethyl)indole may be due to some differences between the modes of action of the two. It is likewise tempting to look for a difference between the actions of really powerful antagonists, such as 2-bromolysergic acid diethylamide, and the moderate antagonists described here, which have a greater formal resemblance to tryptamine and 5-hydroxytryptamine.

We wish to express our most sincere thanks to Professor J. H. Gaddum, who suggested the problem to us and who has given us much helpful advice, to Dr. J. R. Vane and Mr. R. P. Stephenson, with whom we have had many useful discussions, and to Dr. Vane particularly for initiating us into the use of the rat fundus strip. This work was done during the tenure by one of us (I. K.) of a Pakistan Government Scholarship.

The specimens of 3-(2-aminopropyl)indole and 3-(2-aminobutyl)indole hydrochlorides were kindly sent us by Dr. H. C. Carrington of Imperial Chemical (Pharmaceuticals) Ltd. We thank Dr. M. E. Speeter, The Upjohn Company, for the gift of 5-hydroxytryptamine creatinine sulphate, Dr. D. W. Woolley, The Rockefeller Institute, New York, for 1-benzyl-3-(2 - dimethylaminoethyl) - 5 - methoxy - 2 - methyl - indole, and Sandoz A. G. for 2-bromolysergic acid diethylamide.

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