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STRUCTURE-ACTIVITY RELATIONSHIP OF TRYPTAMINE ANALOGUES ON THE HEART OF *VENUS MERCENARIA*

BY

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A number of tryptamine analogues and other exciter agents have been tested on the heart of *Venus mercenaria*. The method of estimation of potency, especially for irreversibly acting compounds, is discussed. Specificity of action with respect to the site of action of 5-hydroxytryptamine is defined experimentally. The specific activity of tyramine and phenethylamine and the non-specific excitatory action of indole and skatole indicate that the indole ring is neither necessary nor sufficient for 5-hydroxytryptamine-like activity. Tryptamine analogues differ in mode of action as well as potency. Congeners without a 5-hydroxyl group tend to act more slowly and irreversibly as well as less strongly than 5-hydroxytryptamine. Methyl substitution also increases the time of action and difficulty of reversal. However, the potency of such compounds may be increased or decreased depending upon the position of substitution and the presence of the 5-hydroxyl group. The relations between structure and potency and mode of action are discussed. Suggestions are made concerning the effective conformation of the 5-hydroxytryptamine molecule and the nature of its receptor.

5-Hydroxytryptamine might be a chemical transmitter in mollusca (Bacq, Fischer and Ghiretti, 1952 ; Welsh, 1953, 1954 ; Twarog, 1954 ; Hill, 1958). The evidence supporting this contention has recently been summarized by Welsh (1957).

The structure-activity relations of 5-hydroxytryptamine have been explored only rarely in preparations of mollusc (Marczyński, 1959), although isolated mammalian organs have often been used for this purpose (Page, 1958 ; Barlow and Khan, 1959a, b ; Vane, 1959). The great sensitivity to 5-hydroxytryptamine of the isolated ventricle of the clam, *Venus mercenaria,* makes it an excellent preparation for such a study.

In the course of this work a number of indole analogues and other excitor agents have been tested on the *Venus* heart preparation. Some suggestions are made regarding the binding sites and effective conformation of the 5-hydroxytryptamine molecule as well as the shape of its receptor.

METHODS

Preparation.—Hearts of *Venus mercenaria* (the quahog) were removed from the animals by the method of Welsh and Taub (1948). The hearts were set up in a 10 ml. perfusion bath at 15°. Treatment of the hearts in the bath, drug administration and recording were as previously described (Greenberg, 1960).

The Effect and its Measurement.—5-Hydroxytryptamine increases the force of contraction of the *Venus* heart and, at high concentrations, augments the tone as well. The measure of exciter effect used in this study is the difference, in mm., between the amplitude of beat before and after the response to an agent. Any increase of tone which occurs is included in the measurement of the final amplitude. A given effect of 5-hydroxytryptamine, or of any other compound tested, was considered completed, and therefore measurable, when there was no longer any change of amplitude following administration of a given dose ; that is, when the drug bound to the receptors was in equilibrium with the drug in the perfusion fluid. Consequently, time of action varies with dose, drug and preparation. However, the time of action for a given dose of any drug, relative to that of 5-hydroxytryptamine, is approximately the same in every preparation.

The log dose-response curve for 5-hydroxytryptamine is sigmoid between about 10^{-9} and 10^{-6} M (Greenberg, 1960). Between about 10^{-8} M and 3×10^{-7} M the curve is nearly linear and the increase in tone is never more than 15% of the response. In the experiments to be described, concentrations of 5-hydroxytryptamine above 3×10^{-7} M were rarely used.

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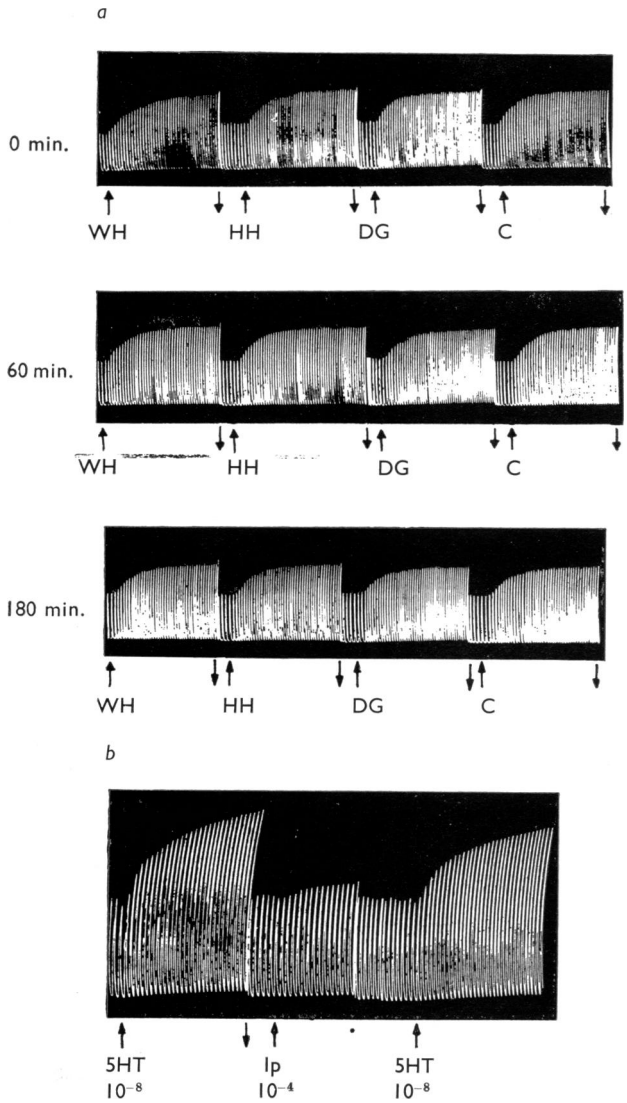


FIG. 1.—Absence of amine oxidase activity in *Venus* heart. (a) The effect, on a heart, of 3×10^{-8} M 5-hydroxytryptamine incubated with whole heart (WH), homogenized heart (HH) and homogenized digestive gland (DG) at room temperature. (C) is the untreated control dose of 5-hydroxytryptamine. Length of incubation period is indicated. (b) Ineffectiveness of iproniazid (Ip) in enhancing the effect of 5-hydroxytryptamine (5HT). (●) indicates that the drum was stopped for 1·5 hr. Washing is at downward-pointing arrows. Benzoquinonium chloride (10^{-5} g./ml.) added to bath after each washing. Dosage: moles/l. in the bath. Tension: 500 mg. Bath temperature: 15°.

The potency of the various compounds tested is expressed as the equiactive molar ratio. 5-Hydroxytryptamine is the standard and has a value of 1.0. The ratios are obtained directly from the log dose-response curves of 5-hydroxytryptamine and the test analogue from the same preparation. The points for these curves, except in the special cases mentioned below, are obtained by adding, and then washing out, successively larger doses of the agent. When the relative potency is independent of concentration, these curves are parallel. Often, however, they are not parallel. In such instances the estimation of the equiactive molar ratio is made at the inflection point of the curve of the test compound. In a few instances individual responses of the test analogue were matched by equiactive doses of 5-hydroxytryptamine to obtain the equiactive molar ratio.

Some tryptamine congeners produce irreversible effects so that relative potency is dependent upon concentration. The points for the dose-response curves of such compounds cannot be obtained by washing out a series of successively larger doses. Estimation of potency by matching is also impossible. Therefore in such cases the following method was employed to obtain the equiactive molar ratio. A low concentration of the irreversibly acting drug was added to the bath. After 20 min. with no response, or after a completed response, another dose-increment was added to the bath without washing to make the next concentration in the series, and so on. For any dose, the initial amplitude taken for computation was that prior to the first small dose.

This method of demonstrating the dose-response relationships of slow, irreversibly acting compounds is justified as follows. Any amine oxidase which is present in *Venus* heart and liver tissue is pharmacologically undemonstrable. 5-Hydroxytryptamine incubated with whole heart, homogenized heart, or homogenized digestive gland in no instance decreased in potency over 3 hr. incubation at room temperature (Fig. 1a). Also, pretreatment of hearts with an amine oxidase inhibitor, iproniazid phosphate (Hoffmann-LaRoche) (10^{-4} M), for 1 hr. has no effect on the response to 5-hydroxytryptamine (Fig. 1b). Therefore, there is no need to suppose that the drugs are slowly destroyed during the course of the experiment. Finally, 5-hydroxytryptamine dose-response curves produced on the same heart are similar, whether or not the previous dose was washed out (Fig. 4).

Specificity.—Three tests have been applied to determine whether an exciter agent is acting at the 5-hydroxytryptamine site.

(1) The only effective inhibitor of 5-hydroxytryptamine on the *Venus* heart is (+)-2-bromolysergic acid diethylamide (Brom LSD). A concentration of 10^{-5} g./ml. of this compound present in the bath for 20 min. will usually completely inhibit the action of even high doses of 5-hydroxytryptamine (Welsh and McCoy, 1957). On the other hand, adrenaline, noradrenaline, histamine and n-alkylamines excite the *Venus* heart, but are not antagonized by Brom LSD (Greenberg, 1960).

(2) Drugs which are rendered ineffective by tachyphylaxis of the preparation to 5-hydroxytryptamine are thought to act specifically (Greenberg, 1960). Such compounds in high concentrations can, furthermore, make the heart insensitive to 5-hydroxytryptamine or other specifically acting analogues.

(3) When specific tryptamine analogues in concentrations greater than 10^{-6} M have been left on the heart for over 2 hr., the addition of 10^{-5} M or 2×10^{-5} M 5-hydroxytryptamine will cause a decrease in amplitude. This is usually accompanied by a small increase in tone and a chronotropic effect characteristic of the action of the catechol amines on this preparation (Greenberg, 1960).

Drugs Used.—The compounds used in this study were 5-hydroxytryptamine creatinine sulphate, gramine, 5-hydroxytryptophan, indol-3-ylacetic acid, indol-3-ylpropionic acid (Nutritional Biochemicals); tryptamine [3-(2-aminoethyl)indole] hydrochloride, 5-hydroxyindol-3-ylacetic acid (Mann Research Laboratories); (+)-lysergic acid diethylamide, (+)-2-bromolysergic acid diethylamide (Sandoz Pharmaceuticals); indole, skatole (3-methylindole), phenethylamine (Eastman Kodak Co.); tyramine (*p*-hydroxyphenethylamine) (Abbott Laboratories); tryptophan (California Foundation for Biochemical Research); bufotenine [5-hydroxy-*N'N'*-dimethyltryptamine; 3-(2-dimethylaminoethyl)-5-hydroxyindole], α-methyltryptamine [3-(2-aminopropyl)indole], 5-hydroxy-α-methyltryptamine [3-(2-aminopropyl)-5-hydroxyindole] creatinine sulphate, *N'*-methyltryptamine [3-(2-methylaminoethyl)-indole], *N'N'*-dimethyltryptamine [3-(2-dimethylaminoethyl)indole], *N'*-ethyltryptamine [3-(2-ethylaminoethyl)indole], *N'N'*-diethyltryptamine [3-(2-diethylaminoethyl)indole], 3-(3-dimethylaminopropyl)indole (Upjohn); 5-methoxy-2-methyltryptamine [3-(2-aminoethyl)-5-methoxy-2-methylindole] hydrochloride, 5-hydroxy-2-methyltryptamine [3-(2-aminoethyl)-5-hydroxy-2-methylindole] hydrochloride (Merck Sharp and Dohme); benzoquinonium chloride (Mytolon) (Sterling-Winthrop).

Drugs were made up in M/10 to M/1,000 stock solutions and diluted with distilled or sea water. All doses are expressed as molar concentrations in the organ bath.

RESULTS

The Effects of Phenethylamine and Tyramine.—The action of phenethylamine is a positive inotropic effect, the threshold concentration being about 10^{-5} M. At 10^{-4} M the increase is large and takes about 75 min. to develop completely. Its potency compared with that of 5-hydroxytryptamine is small; the equiactive molar ratio is 2,000. Tyramine, the *p*-hydroxy derivative of phenethylamine, is about 4 times more potent and 1.5 times faster acting than the latter. The actions of both compounds are antagonized by Brom LSD (10^{-5} g./ml.), indicating that they act at the same site as 5-hydroxytryptamine (Fig. 2a, b).

The Effects of Indole and Skatole.—Indole and its 3-methyl derivative, skatole, elicit a positive inotropic response from the *Venus* heart. Threshold is about 3×10^{-6} M. The effect at 10^{-4} M is large and equal to that of 3×10^{-8} M 5-hydroxytryptamine; however, this increase is not blocked by Brom LSD (Fig. 2c, d). Consequently, the action of the indole ring is non-specific.

Close Congeners of 5-Hydroxytryptamine.—The compounds to be discussed here are all closely related in structure to 5-hydroxytryptamine. The actions have, in most cases, been shown to be specific by one or more of the tests previously mentioned. The experimental results are listed in Table I.

Group A.—Tryptamine is about 10 times less active than 5-hydroxytryptamine. Furthermore, the effect of an equiactive dose of tryptamine takes about twice as long to develop (Fig. 3a) and to wash out. The slow recovery from the response is especially evident at higher concentrations. The log dose-response curves for 5-hydroxytryptamine and tryptamine are usually parallel or only slightly divergent (Fig. 4a).

Group B.—Tryptamine analogues with no 5-hydroxyl group, and with methyl or ethyl groups substituted on either the nitrogen or carbon atoms of the side-chain, produce a response which is in certain respects different from that to 5-hydroxytryptamine.

Firstly, the effects are very slow to develop. For example, the response to 3×10^{-9} M *N'*-methyltryptamine may take 5 min. to become noticeable and between 30 and 40 min. to be completed (Fig. 3a). At higher doses this time is often longer.

Secondly, the response is irreversible. While prolonged washing decreases the effect slightly, the original amplitude is never regained. Due to the irreversibility of these compounds, points for dose-response curves were found by adding successively larger doses without interposed washing, as described under methods.

Thirdly, the relationship between concentration and effect of the *N'*-alkyl-substituted compounds is unlike that of 5-hydroxytryptamine. Threshold

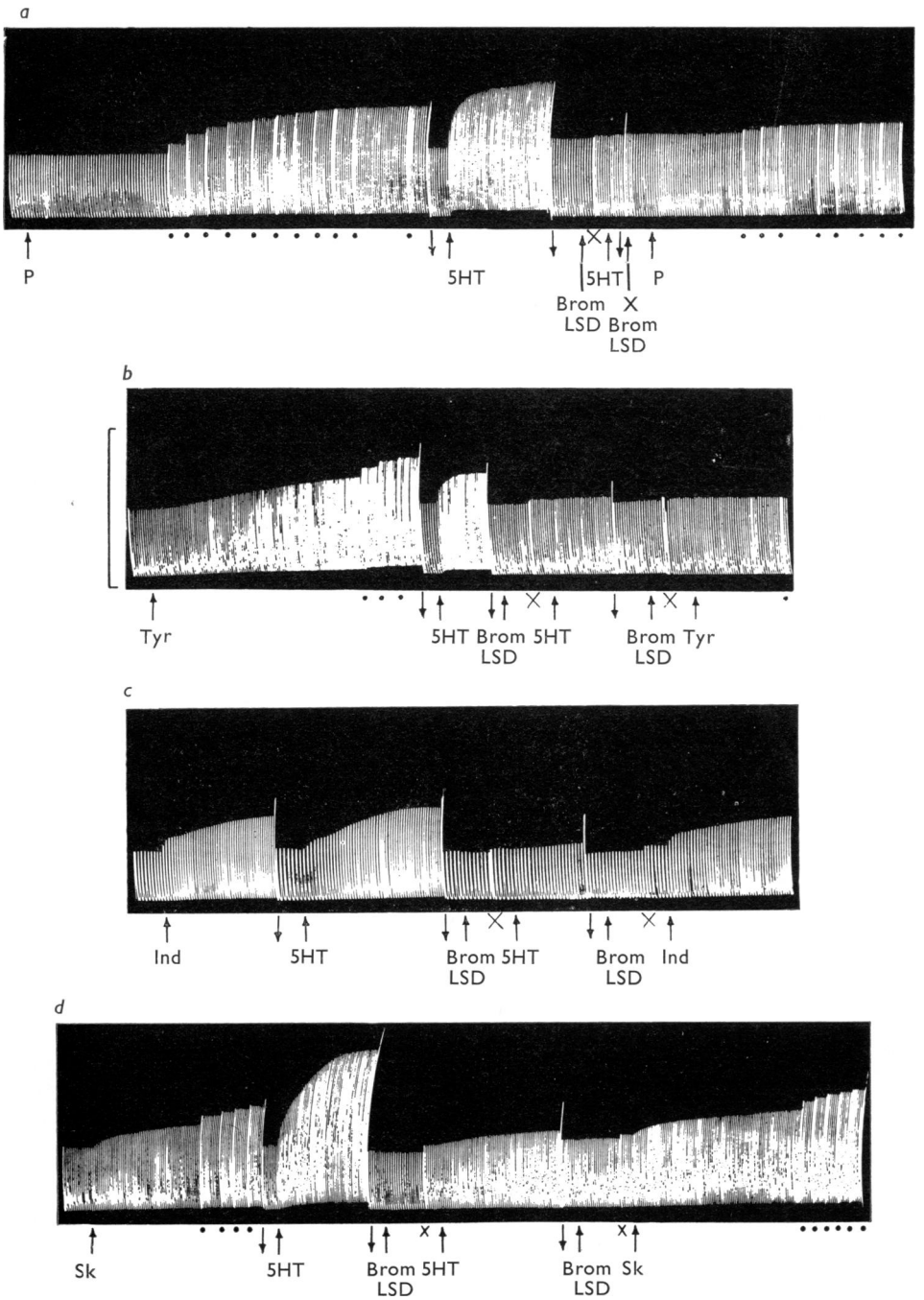
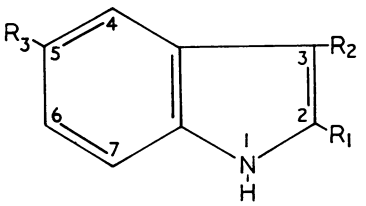


FIG. 2.—The effect of 10^{-5} g./ml. (+)-2-bromolysergic acid diethylamide (Brom LSD) on the responses of the *Venus* heart to (a) 5-hydroxytryptamine 3×10^{-7} M (5HT) and phenethylamine 10^{-4} M (P); (b) 5-hydroxytryptamine 3×10^{-7} M (5HT) and tyramine 10^{-7} M (Tyr); (c) 5-hydroxytryptamine 3×10^{-8} M (5HT) and indole 10^{-4} M (Ind); (d) 5-hydroxytryptamine 10^{-7} M (5HT) and skatole 10^{-4} M (Sk). Following each dose of Brom LSD the drum was stopped (at X) for 20 min. (●) indicates drum was stopped; total elapsed time is 60 min. in (a) and (b) and 40 min. in (d). Downward-pointing arrows indicate washing. Benzoquinonium chloride (10^{-5} g./ml.) added to bath after washing. Tension: 1,000 mg. Temperature: 15°. Heart rate: 10/min. Amplitude scale: 6 cm.

TABLE I

RELATIVE POTENCIES OF TRYPTAMINE ANALOGUES ON *VENUS* HEART

The equiactive molar ratio of 5-hydroxytryptamine is taken as unity. N.E. means that no effect could be elicited from the preparation.



Group	Compound	R_1	R_2	R_3	Equiactive Molar Ratio: Individual Values	Equiactive Molar Ratio: Mean
A	Tryptamine	H	$-CH_2-CH_2-NH_2$	H	15, 14, 13, 11, 10, 6, 6, 4	9·9
B	*N'*-Methyltryptamine	H	$-CH_2-CH_2-NH(CH_3)$	H	6, 5, 4, 4, 3·3, 2·2, 1·4	3·7
	N'-Ethyltryptamine	H	$-CH_2-CH_2-NH(C_2H_5)$	H	15, 14, 10, 10, 7, 4·5, 3	9·1
	N'N'-Dimethyltryptamine	H	$-CH_2-CH_2-N(CH_3)_2$	H	30, 15, 12·5, 10, 4, 2, 1·7	10·7
	N'N'-Diethyltryptamine	H	$-CH_2CH_2-N(C_2H_5)_2$	H	12, 12, 10, 10, 8·6, 1·7, 1	7·9
	α-Methyltryptamine	H	$-CH_2-CH(CH_3)-NH_2$	H	10, 8·7, 7	8·6
C	Bufotenine	H	$-CH_2-CH_2-N(CH_3)_2$	-OH	0·04, 0·03, 0·025, 0·017	0·028
	5-Hydroxy-α-methyltryptamine	H	$-CH_2-CH(CH_3)-NH_2$	-OH	6, 6	6
D	5-Hydroxy-2-methyltryptamine	$-CH_3$	$-CH_2-CH_2-NH_2$	-OH	60, 35, 30, 17, 15	31·4
	5-Methoxy-2-methyltryptamine	$-CH_3$	$-CH_2-CH_2-NH_2$	$-OCH_3$	65, 50, 35, 25	43·8
E	Gramine	H	$-CH_2-N(CH_3)_2$	H	N.E., N.E., N.E., 10,000, 7,000, 170	—
	N'N'-Dimethyltryptamine	H	$-CH_2-CH_2-N(CH_3)_2$	H	30, 15, 12·5, 10, 4, 2, 1·7	10·7
	3-(3-Dimethylaminopropyl)indole	H	$-CH_2-CH_2-CH_2-N(CH_3)_2$	H	3,300, 1,700, 1,000	2,000

is usually 10^{-9} M or 3×10^{-9} M. In 82% of the experiments the response to low concentrations (10^{-9} to 10^{-8} M) of the *N'*-alkyltryptamines was equal to, or greater than, that to the same dose of 5-hydroxytryptamine. The maximum effect was achieved at 10^{-5} to 10^{-4} M. However, both the inotropic effect and the increased tone resulting from this maximal response are only equivalent to those of 3×10^{-8} M to 3×10^{-7} M 5-hydroxytryptamine.

The log dose-response curves which result vary greatly in shape (Fig. 4). In general the curves are sigmoid with a lower limb which may cross the 5-hydroxytryptamine curve of the same preparation (Fig. 4b). The middle, steeply-rising, portion of the *N'*-alkyltryptamine curves starts at about 10^{-7} M and is usually divergent from that of 5-hydroxytryptamine. On the other hand, with *N'N'*-diethyl- or *N'*-ethyl-tryptamine this portion of the curves is often parallel to the standard (Fig. 4c). In about a third to a half of the experiments, once a response at low concentrations was established, further increases in dose (up to about 10^{-7} M) produced either small or negligible increases in amplitude. The log dose-response curves which resulted were biphasic with a

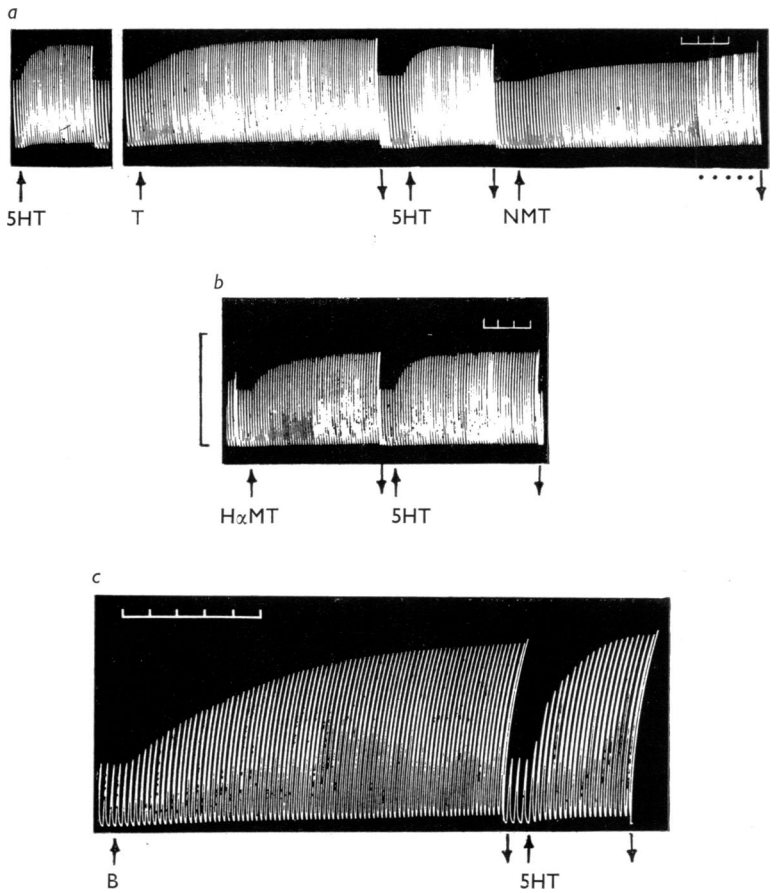


FIG. 3.—Comparison of velocities of *Venus* heart preparations in response to (a) 5-hydroxytryptamine 3×10^{-7} M (5HT), tyramine 3×10^{-6} M (T), 5-hydroxytryptamine 10^{-7} M (5HT) and *N'*-methyltryptamine 10^{-8} M (NMT); (b) 5-hydroxy-α-methyltryptamine 10^{-7} M (HαMT) and 5-hydroxytryptamine 2×10^{-8} M (5HT); (c) bufotenine 10^{-8} M (B) and 5-hydroxytryptamine 10^{-7} M (5HT). At (●) the drum was stopped for 5 min. Washing is at downward-pointing arrows. Benzoquinonium chloride (10^{-5} g./ml.) added to the bath immediately after washing and 5 min. before succeeding dose. Tension: 500 mg. Temperature: 15°. Time scale: 30 sec. Amplitude: 3 cm.

plateau between about 10^{-8} M and 10^{-7} M (Fig. 4c). Such variation in the shape of the curves, and their divergence from those of 5-hydroxytryptamine, suggest that there should be some uncertainty in potency measurements. This is confirmed by the results shown in Table I. The equiactive molar ratios of α-methyl-, *N'*-ethyl-, *N'N'*-diethyl- and *N'N'*-dimethyl-tryptamine are of the same order of magnitude as that of tryptamine itself. However, *N'*-methyltryptamine has a potency about double that of tryptamine.

Group C.—5-Hydroxy-α-methyltryptamine and bufotenine are the 5-hydroxy analogues of two methyl-substituted amines previously discussed (Group B). The presence of the hydroxyl group results in some marked changes in action. First, the responses are reversible. Second, the speed of action increases greatly.

5-Hydroxy-α-methyltryptamine is not significantly more active than α-methyltryptamine, but bufotenine is about 500 times as active as *N'N'*-dimethyltryptamine.

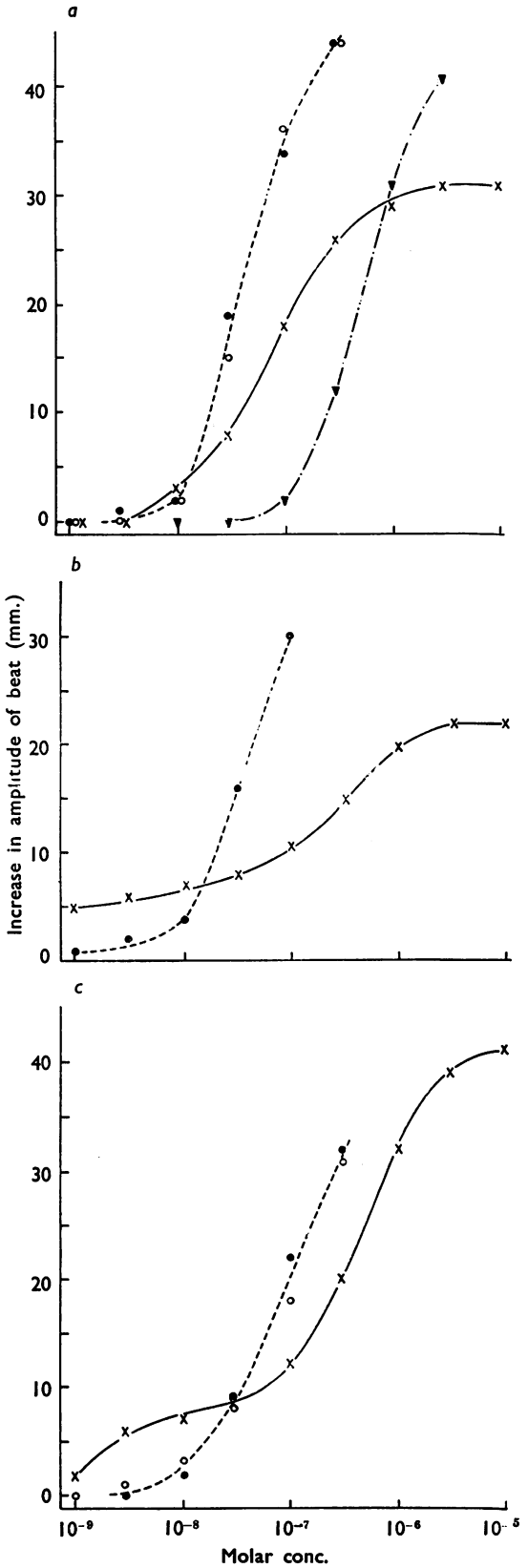


FIG. 4.—Dose-response curves illustrating the variation in the effects of non-hydroxylated tryptamine analogues on three *Venus* heart preparations. The effects of 5-hydroxytryptamine (— — —) are compared with those of: (a) *N'*-methyltryptamine (X——X) and tryptamine (▼—.—▼); (b) *N'N'*-diethyltryptamine (X——X); and (c) *N'*-ethyltryptamine (X——X). In preparations (a) and (c) one set of 5HT points were obtained by washing between successive doses (**O**) and another by making additions without washing (●).

Of all the compounds which have been tested, 5-hydroxy-α-methyltryptamine produces effects which are most similar to those of 5-hydroxytryptamine. Its time of action is only slightly longer than that of 5-hydroxytryptamine (Fig. 3b). The effects, even of relatively high doses (3×10^{-7} M), are quickly washed out. The log dose-response of the two substances are parallel (Fig. 5a); this is similar to the results with tryptamine.

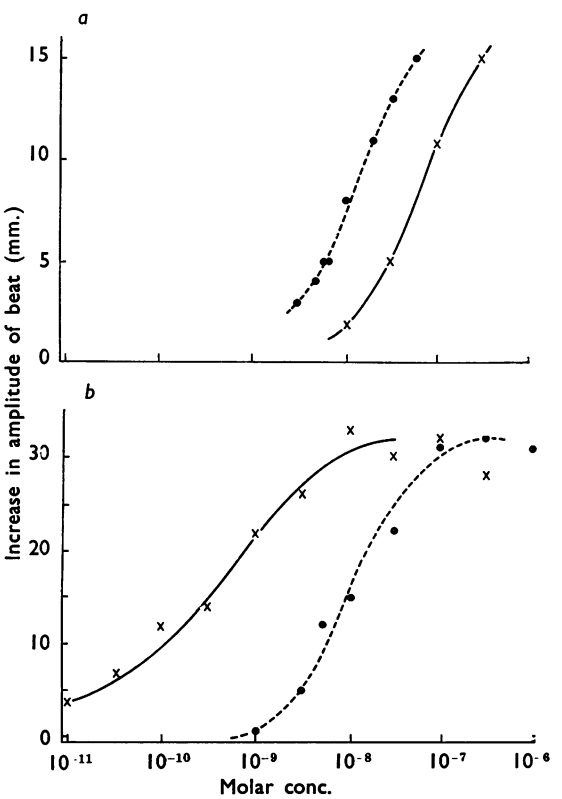


FIG. 5.—Dose-response curves contrasting the effects on *Venus* hearts of 5-hydroxytryptamine (●— — —●) and (a) 5-hydroxy-α-methyltryptamine (X——X) and (b) bufotenine (X——X).

However, compared with tryptamine, 5-hydroxy-α-methyltryptamine is more potent, faster in action, and easier to wash out.

Bufotenine is more potent than 5-hydroxytryptamine. Its structure is intermediate between that of 5-hydroxytryptamine and *N'N'*-dimethyltryptamine, and its mode of action is also intermediate. First, while its effects are not irreversible, the washing time for a similar response is almost twice that of 5-hydroxytryptamine. Second, at threshold or slightly higher concentrations (10^{-11} M to 10^{-9} M), the response to bufotenine may take between 20 and 30 min. to develop and at any concentration it is slower than the equipotent 5-hydroxytryptamine response (Fig. 3c). Third, the log dose-response curve of bufotenine is always less steep than that of 5-hydroxytryptamine (Fig. 5b). Accordingly, the equiactive molar ratio varies with concentration. At threshold it is 1/100. Near the maximum response of bufotenine, at about 3×10^{-8} M, it is approximately 1/10.

Group D.—The compounds of this group have a substituent in the 2-position of the indole ring. This substitution serves to decrease the potency greatly. Thus, 5-hydroxy-2-methyltryptamine is about 30 times less potent than 5-hydroxytryptamine. Its threshold is 3×10^{-7} M. Also, the time of action is slow and the effect is difficult to wash out, especially at high concentrations. 5-Methoxy-2-methyltryptamine is slightly less potent than its 5-hydroxy congener, although its threshold is about the same. It is also far more difficult to wash out. The effects are, in fact, not completely reversible.

Brom LSD might be included here in its capacity as an excitor agent (Greenberg, 1960) with a substituent in the 2-position. The exciter action is extremely undependable. When it occurs, the equiactive molar ratio of a 3×10^{-5} M dose varies between 300 and 30,000 depending upon the preparation. The time of action is slow and the effect is irreversible.

Group E.—The substances included here differ only in the length of the side-chain at the 3-position. In gramine it is one, in *N'N'*-dimethyltryptamine it is two, and in 3-(3-dimethylaminopropyl)indole it is three carbon atoms long. All three compounds have two methyl groups on the amino-nitrogen atom. None has a 5-hydroxyl group in the indole ring.

It is clear that increasing or decreasing the length of the side-chain results in a profound loss of activity (Table I). This loss is greatest with gramine. Gramine is well known as a 5-hydroxytryptamine antagonist in a variety of preparations. It also acts in this way, although weakly, on *Venus* heart. Applied to the heart for an hour, 10^{-4} M gramine decreases the response by more than half. 3-(3-Dimethylaminopropyl)indole is only slightly inhibitory. Its excitatory action at high concentrations usually masks any inhibition which might occur.

No attempt was made to block the action of gramine or 3-(3-dimethylaminopropyl)indole with Brom LSD. Since these two compounds antagonize 5-hydroxytryptamine, it was assumed that they act at the 5-hydroxytryptamine site. However, the specificity of the antagonism has not been demonstrated.

The Effects of Indol-3-yl Acids.—Of the indol-3-yl acids which have been examined, some are in the 5-hydroxytryptamine metabolic pathway and others are not. It might be expected that tryptophan (α-aminoindol-3-ylpropionic acid) and 5-hydroxytryptophan, which are precursors of 5-hydroxytryptamine (Udenfriend, 1958), would be converted by enzymes in the heart. Their effect should then be a slow increase in the amplitude of the heart. In fact, tryptophan is completely inactive up to 10^{-3} M. 5-Hydroxytryptophan excites feebly and occasionally at 10^{-3} M. Its potency relative to 5-hydroxytryptamine is about 100,000.

5-Hydroxyindol-3-ylacetic acid is a product of the enzymic breakdown of 5-hydroxytryptamine (Udenfriend, Wiessbach, and Bogdanski, 1957). This acid does excite feebly at 10^{-4} M. The equiactive molar ratio is 20,000.

Indol-3-ylacetic acid and indol-3-ylpropionic acid are essentially inert although indol-3-ylacetic acid excites at 10^{-3} M. The equiactive molar ratio is over 100,000.

These acids are so feebly active that no attempt was made to antagonize their action with Brom LSD. Indeed, the exciter response to the latter at 2×10^{-5} g./ml. is usually of greater magnitude.

At least two attempts were made, using each of these acids, to block the actions of 5-hydroxytryptamine. None were successful.

Lysergic Acid Diethylamide.—Characteristics encountered in the *N'*-alkyltryptamines are emphasized in lysergic acid diethylamide. The usual threshold concentrations (10^{-16} M–10^{-15} M) often result in a nearly maximal response which, however, takes 4 hr. to be completed (Welsh and McCoy, 1957). The log dose-response curves from such preparations cross, almost perpendicularly, those of 5-hydroxytryptamine (Fig. 6a). Estimation of potency in such cases is meaningless. However, not all hearts respond in this

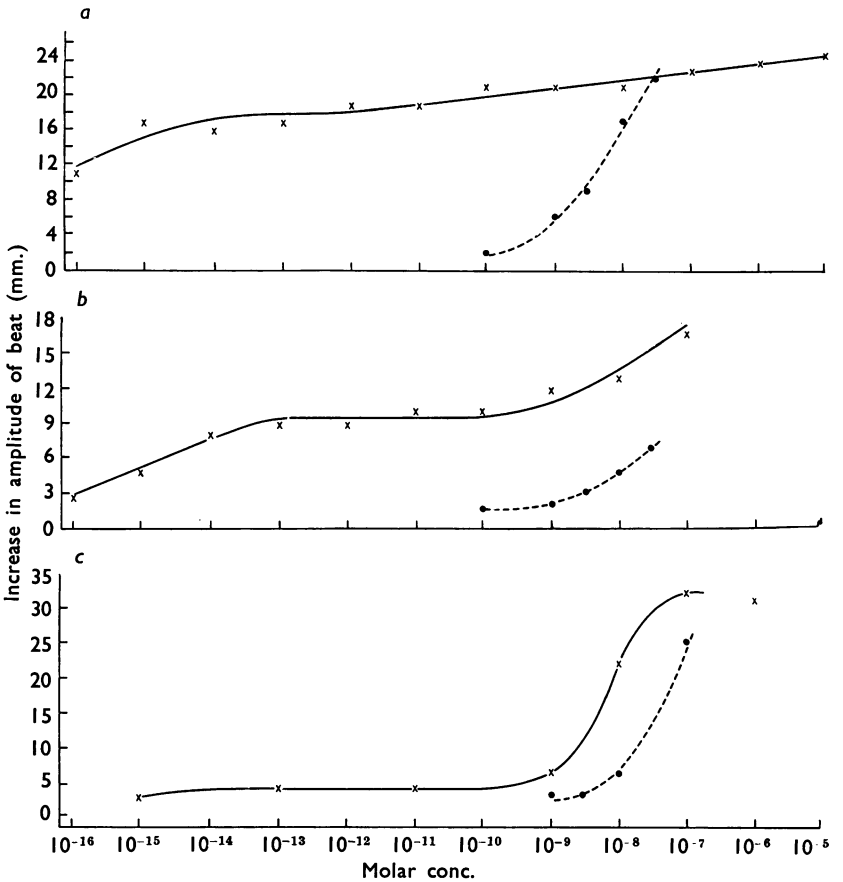


FIG. 6.—Dose-response curves showing variations in the responses of different *Venus* heart preparations to (+)-lysergic acid diethylamide (X——X) and to 5-hydroxytryptamine (● — — — ●).

manner. Some preparations are affected less strongly by low concentrations of lysergic acid diethylamide. The curve in these cases may turn upward at about 10^{-10} M (Fig. 6b). In two experiments, there was almost complete insensitivity to low concentrations. The log dose-response curve rose abruptly, at about 10^{-10} M, parallel to that of 5-hydroxytryptamine on the same heart (Fig. 6c). In both experiments, the equiactive molar ratio in this parallel portion of the curve was 0.1.

Yohimbine.—This alkaloid excites the *Venus* heart weakly. The threshold concentration is between 10^{-6} M and 10^{-5} M. The mean equipotent molar ratio is 630. The response is slow, taking about 30 min. for completion. It is also not entirely reversible. Evidence of specificity from tachyphylaxis experiments is negative but inconclusive.

DISCUSSION

The simplest structural requirement for 5-hydroxytryptamine-like activity on the *Venus* heart preparation seems to be embodied in phenethylamine and tryptamine: a flat aromatic nucleus with a 2-aminoethyl side-chain. These requirements are established largely by the ineffectiveness of some of the substances tested.

Neither the primary alkylamines, nor indole alone, have a 5-hydroxytryptamine-like action on the *Venus* heart. The n-alkylamines have been shown to be excitatory but unspecific in their action relative to 5-hydroxytryptamine (Greenberg, 1960). Evidently an alkyl side-chain cannot take the place of a proper ring substituent. Indole and its close congener skatole are likewise excitatory but unspecific in action. Indeed, these two compounds have been shown to be inactive

(Erspamer, 1952), or unspecifically depressant (Izquierdo and Stoppani, 1953), on a variety of smooth muscle preparations.

The characteristically different actions of histamine and dopamine on *Venus* heart (Greenberg, 1960) point to the ineffectiveness of imidazolyl- or dihydroxyphenyl-ethylamines in producing 5-hydroxytryptamine-like effects. However, if these compounds had such an action, it would probably be no stronger than that of tyramine. Hence, it might be completely masked by the strong generic effects of histamine and dopamine.

The importance of the amino-group itself is illustrated, firstly, by the ineffectiveness of indol-3-ylacetic acid, in which the amino-group is absent.

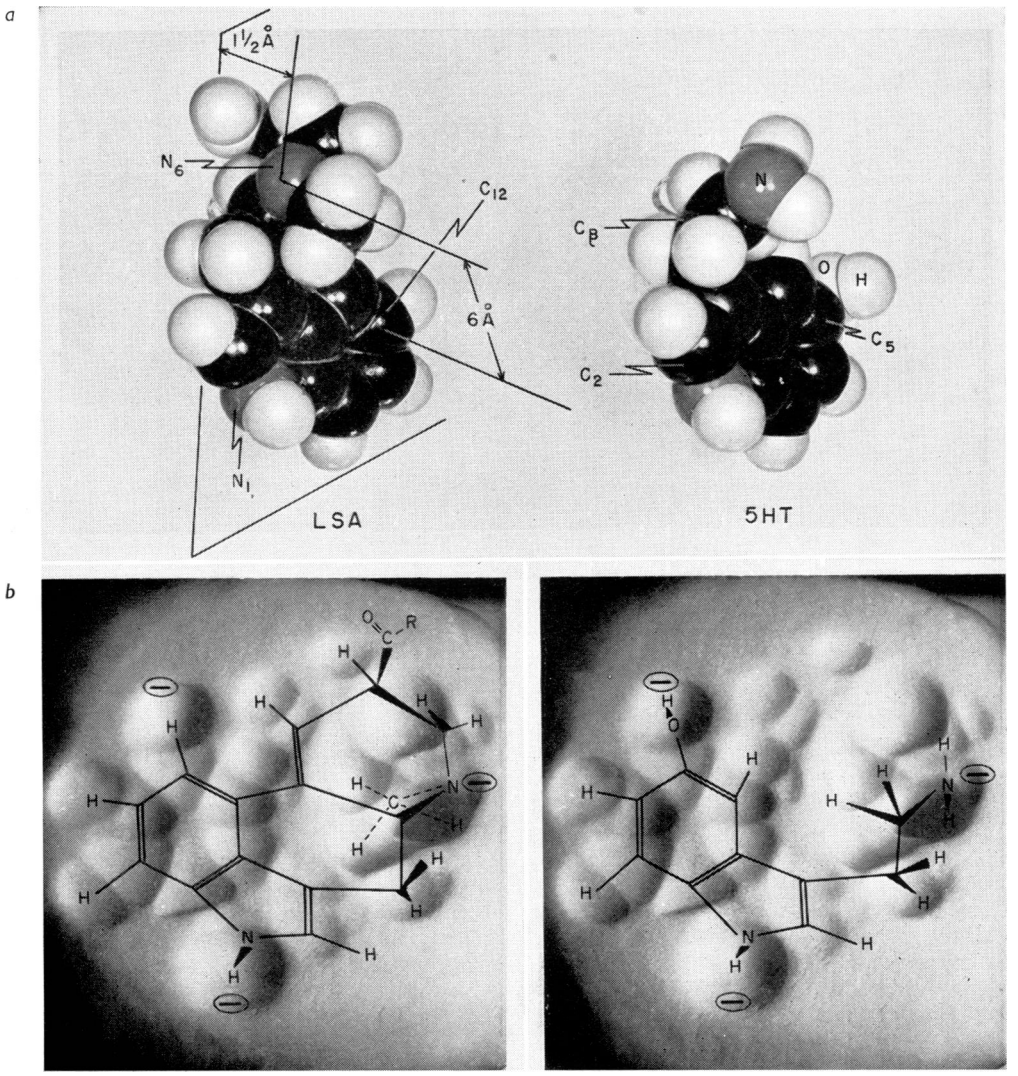


FIG. 7.—(a) Taylor-Hirschfelder models of (+)-lysergic acid (LSA) and 5-hydroxytryptamine (5HT). (b) Composite negative imprint of the molecules in (a) as a model of 5-hydroxytryptamine receptor area. Structural formulae of (+)-lysergic acid diethylamide (left) and 5-hydroxytryptamine (right) show orientation of molecules. The H at C_5 of (+)-lysergic acid diethylamide is omitted; R is $-N(C_2H_5)_2$. Three proposed negative binding sites for the 5-hydroxyl group, 1-nitrogen atom and terminal amino-group of 5-hydroxytryptamine are indicated.

Secondly, in tryptophan and 5-hydroxytryptophan solutions, the α-amino-groups are hydrogen-bonded to the carboxyl groups. These two tryptamine analogues are also feeble exciter agents on the *Venus* heart. Finally, the amino-nitrogen atom must be two carbon atoms distant from the ring. Pronounced reduction in potency accompanies either a decrease (gramine) or an increase [3-3-(dimethylaminopropyl)indole] of only about 1.5 Å. in the length of the aminoalkyl side-chain. That gramine excitation might not even be specific is suggested by the fact that it antagonizes the effect of adrenaline as well as 5-hydroxytryptamine in *Spisula solida* (Gaddum and Paasonen, 1955).

The arrangement in space of the aminoethyl side-chain of tryptamine and phenethylamine analogues is variable. Consequently, the effective conformations of these molecules and the corresponding shapes of the receptors are in question. A partial answer resides in two properties of (+)-lysergic acid diethylamide: Firstly, its extremely low threshold on the *Venus* heart suggests that the molecule must fit some cellular receptor site precisely. Secondly, the dimensions and conformation of the (+)-lysergic acid nucleus are fixed by closure of the C and D rings. Thus, lysergic acid diethylamide might well be used as a tool in establishing at least one active conformation and complementary receptor of tryptamine and its congeners.

Three salient points define the spatial arrangement of lysergic acid diethylamide (Cookson, 1953): (1) The lysergic acid nucleus is planar up to a line joining C_5 and C_8 across the upper (D) ring. (2) Along this line the D ring is folded so that N_6 (corresponding to the primary amino-nitrogen atom of 5-hydroxytryptamine) is out of the plane by 1 to 1.5 Å. (3) The distance between C_{12} (corresponding to C_5 in 5-hydroxytryptamine) and N_6 is between 5.5 and 6 Å.

Of the many possible conformations of 5-hydroxytryptamine, one exists (Fig. 7) which satisfies the spatial requirements defined by lysergic acid. (1) Only one of the three most probable positions of C_β, that in which the C_β hydrogen atoms are symmetrically disposed about the C_2 hydrogen atom, permits the carbon atoms of the aminoethyl side-chain of 5-hydroxytryptamine to lie in the same plane as the indole ring. Such a conformation is energetically favoured by the positioning of the C_2 and C_β hydrogen atoms at an energy minimum which, however, is not necessarily the lowest minimum (Dauben and Pitzer, 1956). (2) With C_β in the position described, the primary amino-nitrogen atom can be displaced 1 to 1.5 Å. out of the plane of the molecule by the rotation of C_α. (3) The distance between C_5 and the primary amino-nitrogen atom then varies between 4.5 and 6.5 Å. Thus, a receptor (for example, Fig. 7) which accommodates lysergic acid diethylamide will also accept 5-hydroxytryptamine—but only when the latter molecule is in the conformation described above. This conformation, it must be mentioned, is not the most probable one which exists for (2-aminoethyl)indole analogues. However, if the free energy barrier between this conformation and the most probable one is between 4,000 and 6,500 cal./mole, then for every 10^3 to 10^5 molecules having the probable conformation there will be one in the planar conformation hypothesized. Now, lysergic acid diethylamide requires about 10^6 to 10^7 times fewer molecules to produce a threshold response than do its tryptamine congeners. If we assume (comparing Table I and Fig. 6c) that lysergic acid diethylamide is between 10 and 100 times more potent than these analogues, then 1 out of 10^3 to 10^5 tryptamine molecules would indeed be sufficient to produce the observed effects. Thus, the improbability of the proposed conformation would seem to explain, in part, the great difference in the number of molecules required for the responses of lysergic acid diethylamide and its tryptamine analogues.

Two regions are seen to be necessary in the receptor area. Firstly, there must be a flat surface, about 11 Å. by 9 Å., which is complementary to the indole, or benzene, ring. Secondly, a contiguous ovoid depression, 6 Å. by 4 Å. and up to 3.5 Å. deep, should be present. The depression accepts the groups in the lysergic acid D ring and the terminal amino-groups of tryptamines or phenethylamines which are folded out of the plane of the indole ring.

On the basis of this model some deductions can be made regarding the binding forces between the various compounds employed and the receptor. The forces which bind the aromatic nuclei to the receptor are probably of the weak, unspecific van der Waals type which depend for strength on exact conformity between many atoms in the drug and its receptor. Also, the possibility of hydrogen bonding between the 1-nitrogen of the indole ring and the receptor exists. The relative impotency of the benzene nucleus in phenethylamine (compared to indole in tryptamine) could be attributed to a deficiency in either of these sorts of binding forces.

Since the pK_a of 5-hydroxytryptamine is 10.0, the amino-group is mostly ionized at physiological *p*H (Vane, 1959). This fact, together with the

loss of potency with lengthening of the side-chain (and presumably with distance from a binding site), suggests that the terminal amino-group of tryptamines, and probably of phenethylamines, is hydrogen bonded to some negative site in the receptor.

Structural modifications of the basic compounds, tryptamine and phenethylamine, affect both the potency and mode of action of the resulting analogues. The presence of the 5-hydroxyl group in 5-hydroxytryptamine, bufotenine and 5 - hydroxy - α - methyltryptamine renders these analogues more potent, faster acting, and more rapidly reversible than their unhydroxylated congeners. The potency relationship has been observed in other molluscan preparations. Gaddum and Paasonen (1955) found that tryptamine was about one thousand times less effective than 5-hydroxytryptamine in stimulating the heart of *Spisula solida*. The threshold of both 5-hydroxytryptamine and bufotenine excitation of *Anodonta* heart is 7×10^{-9} g./ml., while that of *N'N'*-dimethyltryptamine is found to be only 10^{-5} g./ml. (Marczyński, 1959). These data suggest the possibility of a hydrogen bridge between a negative site in the receptor (Fig. 7) and the 5-hydroxyl group which is unionized at physiological hydrogen-ion concentrations (Vane, 1959).

While tyramine is more potent and has a smaller response time than phenethylamine, it cannot be assumed that the *p*-hydroxyl group of tyramine necessarily occupies the same binding site as the 5-hydroxyl group of 5-hydroxytryptamine. A position of the molecule in which the benzene ring is flat on the planar portion of the receptor and the amino-group is in its appropriate site is compatible with binding of the *p*-hydroxyl group of tyramine either at the 5-hydroxyl site or the 1-nitrogen site of 5-hydroxytryptamine. The conformation of tyramine in either position is relatively improbable, which helps account for its low potency. Incidentally, if binding of the *p*-hydroxyl group at the 5-hydroxyl site occurs, an additional planar receptor area must be hypothesized. Both receptor positions, as well as others, are probably assumed by these small, flexible molecules.

Tryptamine analogues which have alkyl substituents on the primary amino-nitrogen atom have a lower potency than 5-hydroxytryptamine. However, in no case is potency significantly lower than that of tryptamine; in one instance (*N'*-methyltryptamine) it is higher. In this regard, an *N'*-methyl substituent, homologous with that of lysergic acid, would be expected to provide the most complementary fit to the hypothesized receptor.

Since the *N'*-alkyltryptamine effects are also irreversible, it becomes clear that increased binding alone seems to lead to little or no increase in potency if there is, at the same time, no 5-hydroxyl group. The hydrogen bridge at the latter location is essential for the precise positioning of the indole ring in the receptor area. If this is correct, then *N'*-alkyltryptamine analogues which have a 5-hydroxyl group should have a greater potency than 5-hydroxytryptamine. In fact, bufotenine is about thirty-five times as potent as 5-hydroxytryptamine, while 5-hydroxy-*N'*-methyltryptamine is about ten times as potent (Bumpus and Page, 1955). On the other hand, bufotenidine (5-hydroxy - *N'N'N'* - trimethyltryptamine) is only about as active as tryptamine on *Venus* heart (Twarog and Page, 1953). The amino-nitrogen atom in bufotenidine is quaternary. Consequently, it probably also acts on the acetylcholine receptors of the ventricle which would result in an observed effect with a negative inotropic component (Welsh and Taub, 1948).

Substitution of a methyl group in the tryptamine molecule, in positions other than on the primary amino-group, either lowers, or has little effect on, the potency of the resulting compound. Although such compounds are also irreversible indicating strong binding, the analogues with 5-hydroxyl groups, unlike the *N'*-alkyl tryptamines, are not much more potent than 5-hydroxytryptamine. Thus, 5-hydroxy-2-methyltryptamine is barely more potent than 5-methoxy-2-methyltryptamine. Again, while the presence of a 5-hydroxyl group in α-methyltryptamine changes the character of the response, the potency remains essentially unchanged. Substitution of a methyl group in positions other than on the primary amino-group probably sterically hinders the binding of the side-chain (α-methyltryptamines) or the indole nucleus (2-methyltryptamines) by lifting them off the planar portion of the receptor. For example, a methyl group or bromine atom in the 2-position would raise C_2 and the adjacent tertiary nitrogen atom almost an Ångstrom, thus, of course, decreasing the strength of the interaction. If the displacement is sufficient, the presence or absence of the 5-hydroxyl group seems to have relatively little further effect on the positioning, and hence the potency, of the analogue.

Conformations of 5-hydroxytryptamine other than that represented by the hypothetical receptor exist, of course. Some of these might also be

effective on the *Venus* heart. For example, another spatial arrangement is represented in the structure of the harmala and rauwolfia alkaloids. As is well known, both 5-hydroxytryptamine-like action and inhibition of 5-hydroxytryptamine-like action by these compounds have often been correlated with the presence of the 3-(2-aminoethyl)-indole configuration (Woolley and Shaw, 1957). However, two features of yohimbine, in particular, suggest that such a conformation of 5-hydroxytryptamine is ineffective on *Venus* heart.

First, it is obvious that when the planar indole rings of lysergic acid diethylamide and yohimbine are congruent, the important ring nitrogen atoms (N_6 and N_4 respectively) are 4 to 5 Å apart. Thus, judging from the lack of potency of gramine and 3-(3-dimethylaminopropyl)indole, yohimbine and similar alkaloids cannot be effective at the *Venus* heart receptors with which lysergic acid diethylamide interacts. It follows that 5-hydroxytryptamine in a conformation corresponding to that of yohimbine (and involving, at least, rotation of C_β through about 90°) would also be inactive at the lysergic-acid-like site. Second, not only is the structure of yohimbine theoretically incompatible with effective action at lysergic-acid-like sites but also it is, *in fact,* relatively inactive. This suggests that, in *Venus* heart, there is no receptor area complementary to yohimbine or to 5-hydroxytryptamine in this conformation.

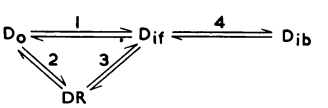
It seems possible that the well-known differences in action between the rauwolfia and ergot alkaloids (Brodie, 1958) are due to the fact that these two groups of compounds are structurally incapable of acting at the same cellular receptors. The relative smallness and flexibility of 5-hydroxytryptamine, on the other hand, would permit interaction of this molecule with the sites of both sorts of alkaloids. However, differences between such interactions would be expected and, in fact, occur in a variety of preparations (Woolley and Shaw, 1953; Gaddum and Hameed, 1954; Salmoiraghi and Page, 1957).

Tissues may have a population of cellular receptors representing the different conformations (and presumably the different effects) of small, active molecules such as 5-hydroxytryptamine. The relative effectiveness of large molecules with fixed conformations would be a measure of the distribution of such a population. The *Venus* heart receptors, in contrast to those of mammalian brain, apparently respond to a smaller range of possible conformations of 5-hydroxytryptamine.

Many analogues of tryptamine are more or less irreversible. The ease with which a drug will leave the tissue for the bath solution is determined, in part, by the oil-water partition coefficient of the compound. Vane (1959) showed that the coefficient of tryptamine is about twenty times that of 5-hydroxytryptamine due to the absence, in the former, of the polar hydroxyl group. It is well known that alkyl substitution increases the partition coefficient and it is in these terms that the irreversibility of such compounds can most readily be explained.

There is no evidence from experiments on the *Venus* heart preparation which indicates whether the *N'*-alkyltryptamines are merely strongly bound to the cell membrane or whether they actually penetrate. However, data from other studies suggest that these compounds enter the cell. Vane (1959), for example, using the enhancement of the excitor activity by amine oxidase inhibition as a test, was able to show that tryptamine analogues with high oil-water partition coefficients (for example, tryptamine, *N'*-ethyltryptamine, *N'*-methyltryptamine) entered the cells of the rat fundus while 5-hydroxytryptamine did not. Similarly, lysergic acid diethylamide and bufotenine, as well as *N'N'*-dimethyltryptamine and *N'N'*-diethyltryptamine (Szara, 1957), are hallucinogens in man and thus cross the blood-brain barrier. 5-Hydroxytryptamine, which cannot cross, is not a hallucinogen.

If the alkyl-substituted tryptamines enter the cell, then the effective drug molecules must be considered as being divided into two pools: those inside (D_i) and those outside (D_o) the cell. The following equilibria might obtain at any bath concentration:



where D_{if} and D_{ib} are, respectively, free and bound drug inside the cell. DR is drug-receptor complex at the cell surface. The following points need to be made regarding the above model:

(1) The action of lysergic acid diethylamide and the *N'*-alkyltryptamines suggests that the drug accumulates slowly in the cells from low bath concentrations. However, it is impossible, on present evidence, to distinguish between steps (1) and (2+3). It is important to note that the response is assumed to be a measure of the concentration of drug-receptor complex. Consequently, even if step (1) does occur, only internal

free drug accumulating in this manner in close proximity to the receptor can be measured.

(2) The biphasic dose-response curves which are obtained in a third of *N'*-alkyltryptamine experiments (Fig. 4c) suggest that the "tryptamine space" of the cell can be saturated. Thus, when the bath concentration is about 10^{-8} M the net movement of drug into the cells ceases. The ratio of dose-response becomes constant until the bath concentration is made large enough (about 10^{-7} M) so that $d(D_oR)/dt$ becomes larger than $d(D_iR)/dt$. Naturally, compounds such as the 2-methyl analogues, whose thresholds are greater than the maximum internal drug concentration, will have no effect even though they enter the cell and accumulate there to the same extent as do the *N'*-alkyltryptamines.

(3) If drug is bound in the cell, it is not bound to the receptors (that is, $D_{ib} \neq DR$). As drug concentration is increased above the levels which saturate the cell interior, the response again increases, indicating the availability of more free receptors. Lipid associated with the cell membrane is probably responsible for intracellular binding if the latter occurs.

The relationship between doses of lipid-soluble tryptamine analogues and their effects on the *Venus* heart is obviously a complex one. The individual variation found in the responses of different hearts to the *N'*-alkyltryptamines clearly reflects this.

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