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THE MODE OF ACTION OF 5-HYDROXYTRYPTAMINE

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INTRODUCTION

The action of a hormone is initiated through a chemical reaction with a cellular receptor. Specificity depends on the high degree of complementarity which exists between the two reacting molecules. The molecular configuration of the hormone can provide important information about the nature of its chemical interaction with the receptor. We can gather further clues by studying the change in activity produced by modifying the chemical conformation of the hormonal molecule. I have used this approach to study the mode of action of 5-hydroxytryptamine (5-HT).

The isolated salivary glands of the blowfly *Calliphora* have been used as an assay system because they show a high degree of sensitivity and specificity towards 5-HT (Berridge & Patel, 1968). Rate of fluid secretion by unstimulated glands is very slow (0·5–1·0 nl/min) but increases enormously (40 nl/min) when a low concentration of 5-HT is added to the bathing medium. The response and recovery time to 5-HT are also very rapid (Berridge, 1970), indicating that the hormone–receptor interaction is readily reversible. The interpretation of pharmacological experiments is greatly facilitated by the structural simplicity of these glands. They consist of a single layer of homogeneous cells which are completely free of nerves, muscles or connective tissue elements (Oschman & Berridge, 1970). These isolated salivary glands thus provide an ideal system for studying the molecular pharmacology of 5-HT.

The 5-HT molecule (Fig. 1) consists of an indole ring nucleus with an ethylamine chain connected at the 3-position and a hydroxyl group at the 5-position. The significance of these different regions has been determined by testing a wide range of molecules closely related to 5-HT. The results indicate that the 5-hydroxyindole moiety is mainly concerned with attaching the molecule to the receptor while the positively charged quaternary nitrogen on the end of the ethylamine side chain is the active site which induces the hormonal effect.

METHODS

The techniques used to study the isolated salivary glands of adult blowflies (*Calliphora erythrocephala*) were identical to those described previously (Berridge & Patel, 1968; Berridge, 1970).

A standard procedure was adopted to test the activity of each compound. Two groups of six salivary glands were used in each experiment. In order to reduce natural variation, the two salivary glands obtained from each animal were assigned one to

each of the two groups. One group was then used to test the activity of a new compound while the activity of 5-HT was determined on the other group which served as control. The concentration of the test compounds was gradually increased from a minimal dose of 1×10^{-10}M up to a maximum of 1×10^{-2}M. If the glands began to respond, the response to each further increase in concentration was measured until the rate of fluid secretion reached a plateau. At the end of this procedure the control glands were stimulated with 1×10^{-8}M 5-HT in order to assess the maximal response of the glands. The rate of secretion obtained for each concentration of test substance was then expressed as a percentage of this maximal rate obtained with 5-HT.

If salivary glands failed to respond to a test substance at 1×10^{-2}M, a simple process was used to screen for possible competitors of 5-HT. After increasing the concentration of the test compound to 1×10^{-2}M, 5-HT (1×10^{-8}M) was added and the response was compared with that of adding 5-HT to the control glands. If the action of 5-HT was inhibited, the activity of these competitors was characterized further by measuring their ability to alter the response of salivary glands to 5-HT. Dose–response curves for 5-HT were thus prepared in the presence of a fixed concentration (1×10^{-4}M) of these competitors.

RESULTS

(*a*) *Significance of the ethylamine chain and indole ring*

Most of the ethylamine analogues tested were totally inactive (Table 1) and there was no evidence of any competition with 5-HT. Ethylamine itself was inactive but longer-chain amines in this series were active. Activity was found to increase with an increase in the length of the hydrocarbon chain. This relationship is shown more clearly in Fig. 1, which shows that amylamine is capable of stimulating salivary glands maximally. The ability of these simple amines to stimulate secretion suggests that the positively charged quaternary nitrogen atom may be the active site on the molecule. Since most of the other amines were inactive, it is apparent that this positive charge must be connected to a suitable moiety in order to interact with the receptor. The increase in chain length must involve hydrophobic components because the addition of hydrophilic groups (e.g. monoethanolamine and GABA) had no effect. Although the progressive addition of carbon atoms to ethylamine leads to an increase in activity, the most active molecule (amylamine) was very much less active than 5-HT. These experiments suggest that the very high sensitivity of 5-HT depends on the 5-hydroxyindole moiety.

5-Hydroxyindole alone is not capable of interacting with the receptor because it is neither an agonist nor an antagonist (Table 1). Two other indole analogues were also inactive. Since ethylamine is inactive even when tested in combination with 5-hydroxyindole (Table 1), the activity of 5-HT must depend on the combined effect of both these moieties.

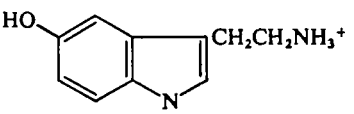
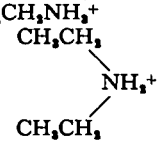
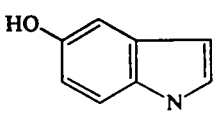
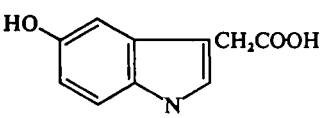
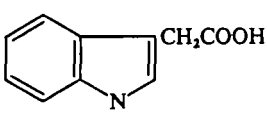
(*b*) *Specificity of the indole ring*

The preceding experiments suggested that the 5-hydroxyindole nucleus was responsible for the high sensitivity of 5-HT while the positively charged ethylamine side-chain was concerned with inducing the effect. The specificity of the indole nucleus was probed by testing a range of molecules which had different ring systems connected to an ethylamine chain (Fig. 1). Molecules having an imidazole ring (histamine) or a

benzene ring (phenethylamine) are able to stimulate salivary glands maximally but are still very much less active than 5-HT (Fig. 1).

Since phenethylamine showed considerable activity, other catecholamines were tested (Fig. 2). Dopamine, which has two hydroxyl groups on the benzene ring, was slightly less active than phenethylamine. The addition of a hydroxyl group on the β-carbon atom of the ethylamine chain reduces the activity further and norepinephrine is incapable of producing a maximal response even at very high concentrations (Fig. 2). The further addition of a methyl group on the nitrogen atom at the

Table 1. *The effect of various ethylamine and indole analogues on rate of fluid secretion by isolated salivary glands (except for 5-hydroxytryptamine (5-HT), the final concentration of each substance tested was* 10^{-2}M. *None of these compounds had any effect on the subsequent response of salivary glands to* 10^{-8}M *5-HT)*

Compound	Structure	Rate of secretion (nl/min)
A. Control – bathing medium		0·5–1·0
B. 5-Hydroxytryptamine (1×10^{-8}M)		40·0
C. Ethylamine analogues		
Ammonium	NH_4^+	0·6
Methylamine	$CH_3NH_3^+$	0·6
Ethylamine	$CH_3CH_2NH_3^+$	0·6
Propylamine	$CH_3CH_2CH_2NH_3^+$	2·0
Butylamine	$CH_3CH_2CH_2CH_2NH_3^+$	12·0
Amylamine	$CH_3CH_2CH_2CH_2CH_2NH_3^+$	37·0
Monoethanolamine	$HOCH_2CH_2NH_3^+$	0·4
2-Methoxyethylamine	$CH_3OCH_2CH_2NH_3^+$	0·5
Ethylenediamine	$NH_3^+CH_2CH_2NH_3^+$	0·7
γ-Aminobutyric Acid (GABA)	$COOHCH_2CH_2CH_2NH_3^+$	0·6
Diethylamine		0·6
D. Indole analogues		
5-Hydroxyindole		0·5
5-Hydroxyindole acetic acid		0·6
3-Indole acetic acid		0·5
E. Synergism		
5-hydroxyindole + ethylamine	As above	0·7

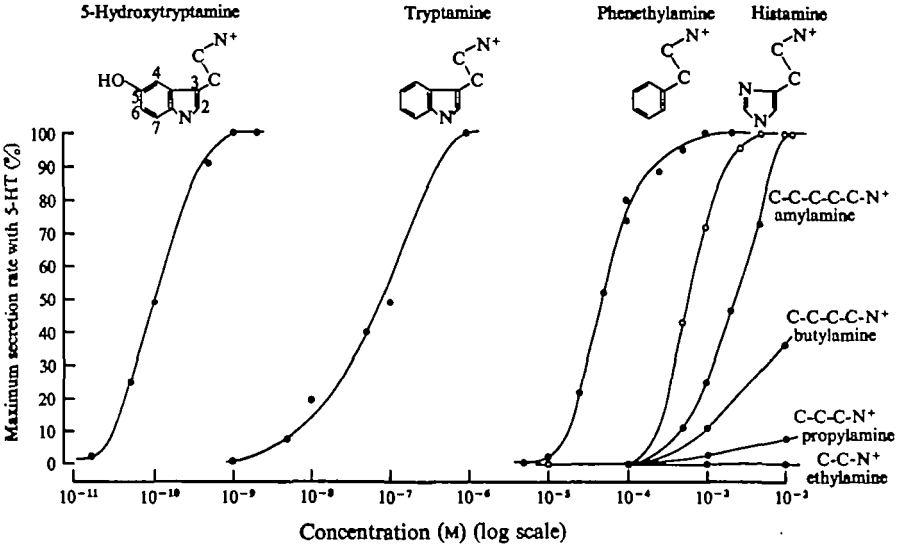


Fig. 1. The ability of different molecules containing an ethylamine chain (C–C–N+) to stimulate fluid secretion by isolated salivary glands. All compounds were compared with 5-hydroxytryptamine (5-HT) and fluid secretion has been expressed as a percentage of the maximum rate obtained with this parent compound.

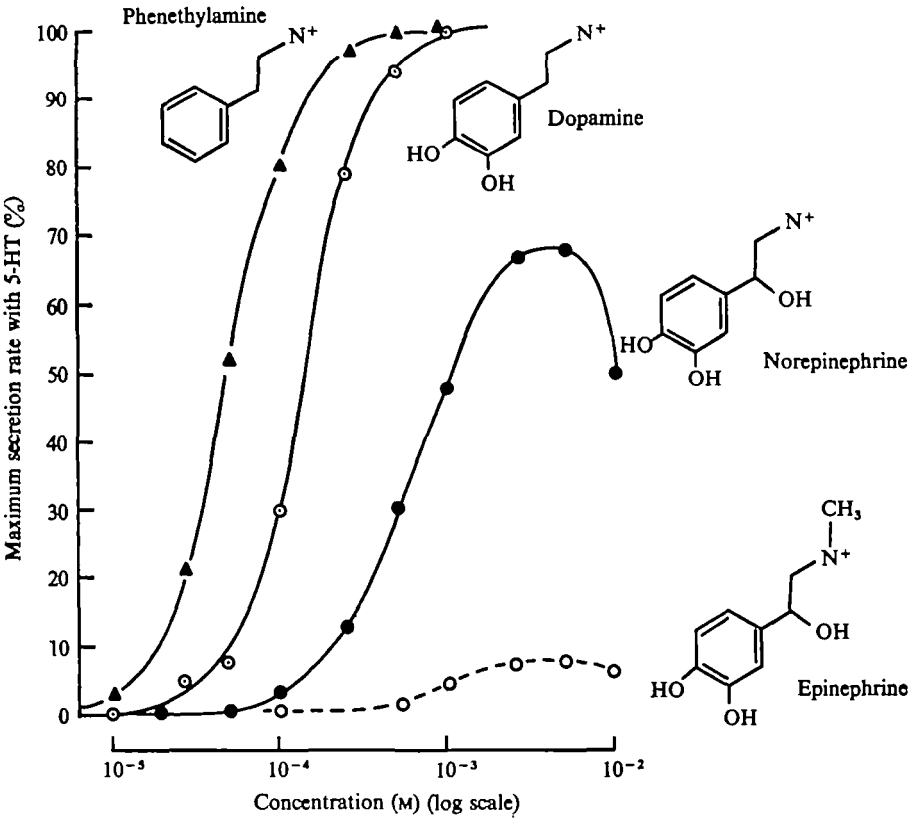


Fig. 2. The effects of different catecholamines on isolated salivary glands.

end of the chain reduces the activity even more, and epinephrine is almost totally inactive. These experiments show that the receptor will accommodate moieties other than 5-hydroxyindole, but much higher concentrations are required to stimulate secretion.

(*c*) *Significance of the hydroxyl group*

The hydroxyl group of 5-HT appears to play an important role because tryptamine, which lacks this group, is much less active than 5-HT (Fig. 1). A similar decrease in activity is observed when the hydroxyl group is omitted from two other tryptamine analogues (Fig. 3). Bufotenine, which has two methyl groups on the nitrogen atom of the ethylamine side-chain, is slightly less active than 5-HT. However, dimethyl tryptamine which lacks the 5-hydroxyl group is much less active (Fig. 3). The effect of the hydroxyl group can also be tested by comparing the activity of tryptophane

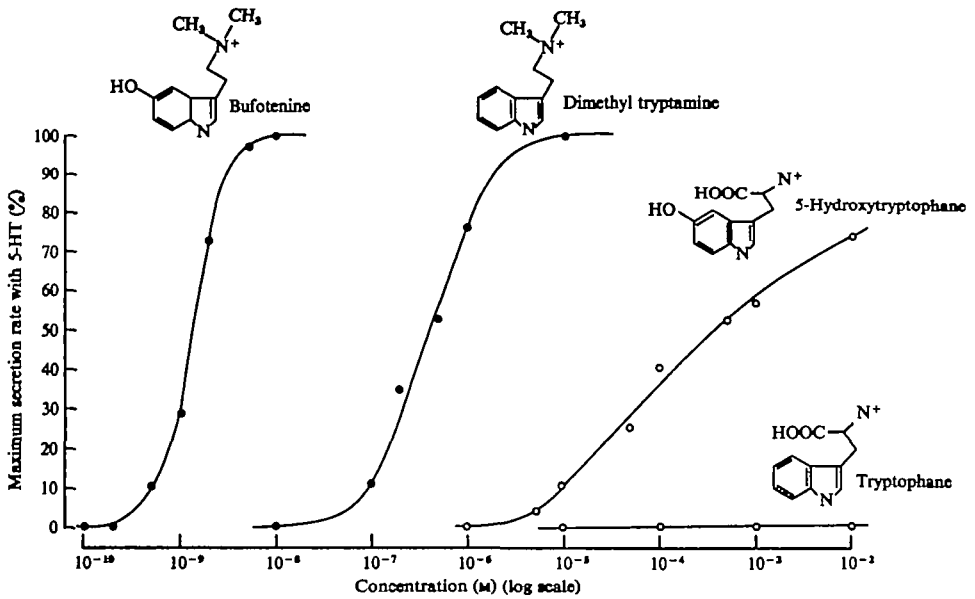


Fig. 3. The ability of the hydroxyl group at the 5-position to increase the activity of dimethyltryptamine and tryptophane.

with 5-hydroxytryptophane (Fig. 3). The former is totally inactive, but the addition of a hydroxyl group does confer some activity to the molecule. The inability of 5-hydroxytryptophane to produce a maximal effect probably depends on having the negatively charged carboxyl group on the α-carbon atom. This negative charge will not only reduce the positive charge on the nitrogen atom but may also introduce considerable steric hindrance during the approach of the positive charge towards its active site on the receptor.

The 5-position of the hydroxyl group is preferred because both 4-hydroxytryptamine (4-HT) and 6-hydroxytryptamine (6-HT) were much less active than the parent compound (Fig. 4). The decrease in activity observed with 4-HT and 6-HT resembles that found in tryptamine where the hydroxyl group is omitted completely (Fig. 2).

Replacing the hydroxyl group at the 5-position with other substituents results in a

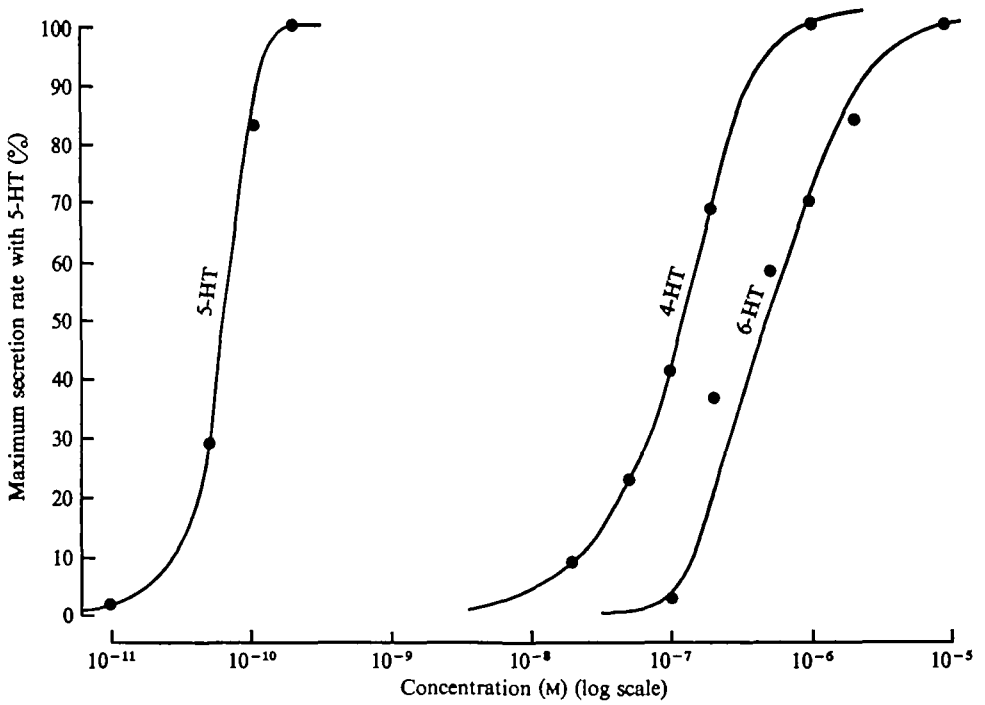


Fig. 4. The effect of varying the position of the hydroxyl group.

reduction in activity. Chloride was found to be a more effective replacement than a methoxy or a methyl group (Fig. 5).

(*d*) *The effect of varying the distance between the positive charge and the indole ring*

The foregoing experiments showed that a molecule must have a positively charged nitrogen atom connected to a suitable hydrophobic moiety in order to produce an effect. The most active compounds were those where the hydrophobic group was a ring structure, in particular the indole ring. In order to determine whether the distance between the ring and the positive charge was critical, two compounds were tested where the length of the chain was either decreased (gramine) or increased (3-aminopropyl indole) by a single carbon atom.

Gramine did not stimulate secretion (Fig. 6) but was a most effective competitor in that it displaced the 5-HT dose–response curve to the right by at least two orders of magnitude (Fig. 7). 3-Aminopropyl indole functioned as a partial agonist because it caused a slight stimulation of secretion (Fig. 6) but was also capable of inhibiting the action of 5-HT (Fig. 7).

DISCUSSION

The positively charged quaternary nitrogen of the ethylamine side-chain appears to be the active site on the 5-HT molecule. Since ethylamine alone cannot activate the receptor, the remaining 5-hydroxyindole moiety is required for a successful 5-HT–receptor interaction. The receptor thus appears to have an anionic site which reacts

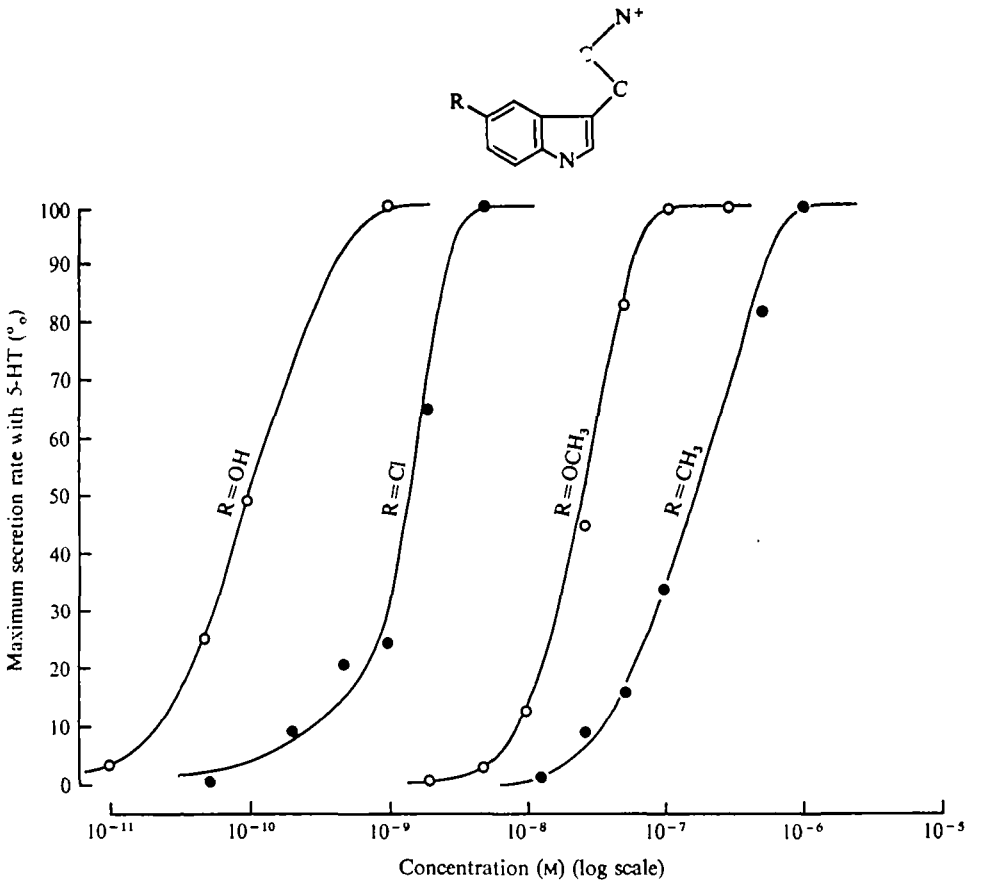


Fig. 5. The effects of different substituents at the 5-position.

with the positively charged nitrogen atom and a hydrophobic region which accepts the indole ring (Fig. 8). A further centre of charge may exist on the periphery of this hydrophobic area to account for the increased affinity conferred on various tryptamine analogues possessing a hydroxyl group at the 5-position (Figs. 1, 2).

The anionic site may not be freely accessible at the surface but appears to be protected from all the non-specific positively charged molecules circulating in the blood. In order to penetrate the protective shield surrounding the anionic site, a molecule must have a suitable hydrophobic moiety. Ethylamine, which has only two carbon atoms connected to the positively charged nitrogen atom, cannot react with the receptor. However, the addition of extra carbon atoms or the introduction of an imidazole or benzene ring can confer some activity by enabling part of the molecule to interact with the hydrophobic region of the receptor, but very high concentrations are required to elicit a response. The much greater activity of certain tryptamine analogues indicates that the molecular configuration of the hydrophobic region of the receptor is precisely designed to accept the indole ring. This precision may extend to the position of the receptor area which interacts with the hydroxyl group of 5-HT through hydrogen bonding. The reduced activity of 4-HT and 6-HT may result, in part, from an inability of their hydroxyl groups to interact with this hydrogen-bonding centre.

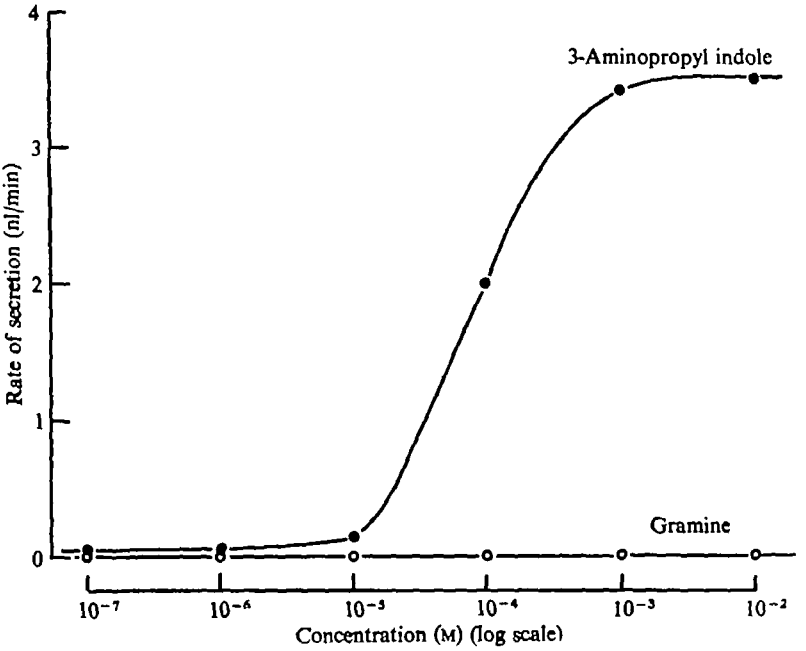


Fig. 6. The effects of gramine and of 3-aminopropyl indole on rate of fluid secretion. The maximum rate of secretion obtained with 3-aminopropyl indole is much less than that observed with 5-HT (see Table 1).

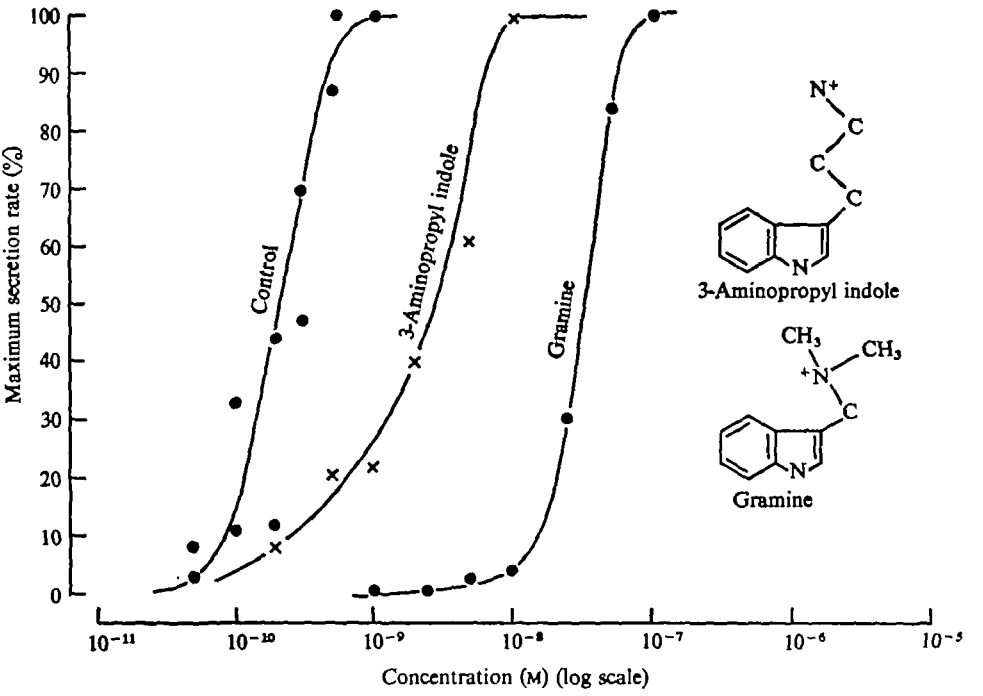


Fig. 7. The ability of gramine and 3-aminopropyl indole, both at 1×10^{-4}M to compete with 5-HT.

The specificity of 5-HT thus depends both on the position of hydrogen bonding and a precise hydrophobic interaction involving the indole ring.

The mode of action of acetylcholine has been interpreted in terms of a series of molecular interactions which become increasingly specific as the stimulating molecule approaches its receptor (Ariëns, Simonis & von Rossum, 1964). A similar series of events may occur during a 5-HT–receptor interaction. The initial attraction between 5-HT and its receptor probably depends on long-range ionic forces operating between

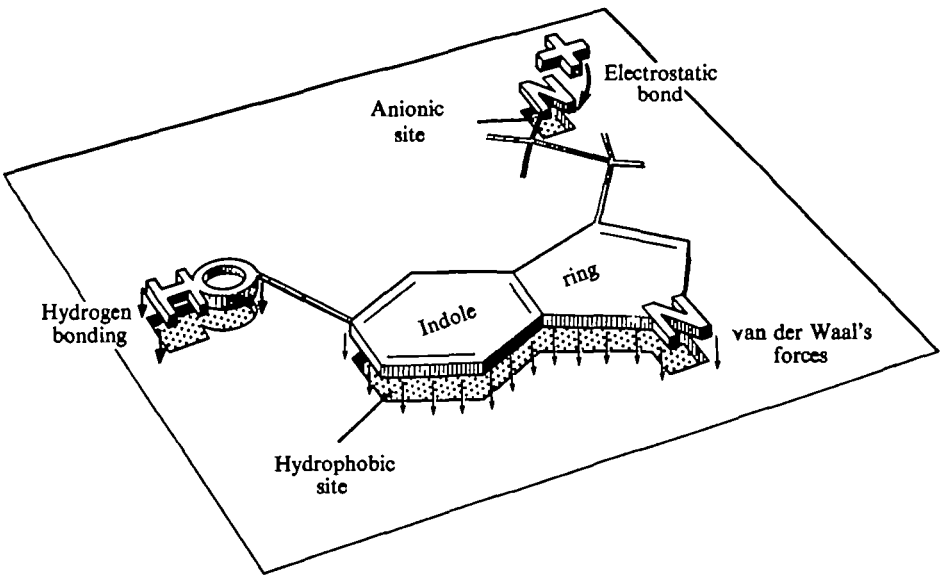


Fig. 8. Diagrammatic representation of the proposed 5-hydroxytryptamine–receptor interaction. The positively charged nitrogen atom at the end of the ethylamine side chain forms an electrostatic bond with an anionic site on the receptor. The specificity of the molecule depends on (1) a precise interaction between the indole ring and the hydrophobic site presumably by van der Waal's forces (small arrows), and (2) hydrogen bonding involving the hydroxyl group at the 5-position.

the positively charged nitrogen of 5-HT and the underlying anionic site of the receptor. Such a long-range ionic attraction is non-specific and a large number of positively charged molecules, other than 5-HT, will probably attempt to reach the anionic site. During the final approach towards the receptor, therefore, more specific forces must take over to allow 5-HT a free access while rigidly excluding all other positively charged molecules. The final approach sequence may thus involve hydrogen bonding and van der Waal's forces both of which operate over very short distances. Apart from contributing to the binding energy, the onset of hydrogen bonding may also play an important role in orientating the molecule so that the indole ring is correctly positioned over the underlying hydrophobic site for a successful interaction. It is during this final approach onto the receptor that there must be a high degree of complementarity between 5-HT and its receptor.

A successful interaction with the receptor probably allows the positive charge on 5-HT to approach close enough to the anionic site for the formation of an effective electrostatic bond (Fig. 8). Neutralization of the anionic site may then induce conformational changes in the receptor which are responsible for initiating the next steps

in the action of the hormone. The ability of gramine and 3-aminopropyl indole to compete with 5-HT implies that these molecules interact with the receptor. Presumably they follow the same approach sequence as 5-HT, but once they are attached to the receptor their positive charges cannot approach the anionic site close enough for an effective interaction. In the case of gramine, which has no stimulatory activity, the side chain has been shortened by one carbon atom; and steric hindrance may also result from having two methyl groups on the nitrogen atom. The presence of an extra carbon atom in 3-aminopropyl indole extends the positive charge beyond the anionic site and again prevents neutralization. However, the increase in chain length permits a greater degree of freedom and may produce certain conformations whereby the positively charged nitrogen atom can interact with the anionic site. These particular conformations are obviously not preferred because only a small stimulation of secretion is observed even at very high concentrations of 3-aminopropyl indole. These experiments emphasize the precision of the 5-HT–receptor interaction since the displacement of the positive charge by a few Angströms is apparently sufficient to prevent a successful neutralization of the anionic site.

Most previous studies on the pharmacology of 5-HT have been concerned with the search for strong antagonists. There have been relatively few structure–activity studies directed towards an understanding of the 5-HT–receptor interaction. Most of our information on the 5-HT receptor has come from studies on the isolated rat stomach strip (Vane, 1959; Offermeier & Ariëns, 1966) or the heart of a mollusc (Greenberg, 1960). Both these assay systems respond to a wide range of tryptamine analogues in much the same way as *Calliphora* salivary glands. The 5-HT receptor at neuromuscular junctions may thus resemble that found in these non-excitable secretory organs.

Once 5-HT has interacted with the receptor, the next step is to translate the chemical signal input from the receptor into a specific cellular response. An earlier study indicated that the action of 5-HT was probably mediated by cyclic AMP (Berridge & Patel, 1968; Berridge, 1970). Activation of the receptor, possibly through a neutralization of the anionic site, may lead to stimulation of the enzyme adenyl cyclase which synthesizes cyclic AMP from ATP. Another consequence of receptor activation appears to be a change in membrane function which is independent of cyclic AMP (Berridge & Prince, 1971 *a*, *b*). At this stage it is not possible to speculate whether the stimulation of adenyl cyclase and the change in membrane function results from a single perturbation in the receptor. Our ignorance on this aspect stems from an almost total lack of information concerning the chemical conformation of receptors (Burgen, 1970). Until we know more about the nature of receptors our understanding of the informational transfer between hormone and receptor will remain a subject of speculation.

SUMMARY

1. The isolated salivary gland of the blowfly *Calliphora* has been used to study the molecular pharmacology of 5-hydroxytryptamine (5-HT).

2. Ethylamine alone is inactive but the addition of extra carbon atoms or ring systems (imidazole or benzene) introduces some activity. A 5-hydroxyindole ring confers most activity.

3. If the hydroxyl group is either removed, replaced with other groups, or displaced to the 4- or 6-position on the indole ring there is a large reduction in activity.

4. The activity of the molecule is greatly reduced if the length of the side chain is decreased (gramine) or increased (3-aminopropyl indole) by a single carbon atom. Such molecules are effective competitors.

5. These structure–activity studies suggest that the specificity of 5-HT resides in the 5-hydroxyindole moiety which binds the molecule to the receptor while the positively charged ethylamine side chain is responsible for inducing the hormonal effect.

The gifts of chemicals from Imperial Chemical Industries Ltd. (5-chlorotryptamine), Parke, Davis and Company (3-aminopropyl indole and 5-methoxytryptamine), Roche Products Ltd. (6-Hydroxytryptamine) and Sandoz Ltd. (4-hydroxytryptamine) are gratefully acknowledged.

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