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SEPARATE 5-HYDROXYTRYPTAMINE RECEPTORS ON THE SALIVARY GLAND OF THE BLOWFLY ARE LINKED TO THE GENERATION OF EITHER CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE OR CALCIUM SIGNALS

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1 5-Hydroxytryptamine (5-HT) stimulates the formation of two separate second messengers in the salivary gland of the blowfly. Activation of adenylate cyclase raises cyclic adenosine 3′,5′-monophosphate (cyclic AMP) whereas the hydrolysis of phosphatidylinositol (PI) is associated with an increase in calcium permeability. The possibility that these two signal pathways might be controlled by separate 5-HT receptors was studied by testing the specificity of 5-HT analogues and antagonists.

2 The parent compound 5-HT was found to stimulate both cyclic AMP formation and the related parameters of PI hydrolysis and calcium transport with similar dose-response relationships.

3 Certain analogues such as 4- and 5-fluoro-α-methyltryptamine were capable of raising cyclic AMP levels and stimulating fluid secretion but did not stimulate the hydrolysis of PI or the entry of calcium.

4 Other analogues, which had chloro or methyl substituents at the 5-position, were found to stimulate the hydrolysis of PI and the transport of calcium at much lower doses than those required to stimulate the formation of cyclic AMP.

5 Antagonists were also found to exert selective effects. Methysergide was a potent inhibitor of PI hydrolysis whereas cinanserin was far more selective in blocking the stimulatory effect of 5-HT on cyclic AMP formation.

6 It is concluded that 5-HT acts on two separate receptors, a 5-HT_1 receptor acting through calcium and a 5-HT_2 receptor which mediates its effects through cyclic AMP.

Introduction

Many neurotransmitters and hormones can act on more than one type of receptor. A good example of such multiplicity of receptor types is the adrenergic system which has both α- and β-receptors. These two groups have been further sub-divided to give α_1, α_2 and β_1, β_2. Similarly, pairs of receptors for acetylcholine, histamine, and dopamine have also been well-defined (Snyder & Goodman, 1980). The identification of such separate receptors usually depends upon pharmacological criteria obtained by studying the effects of specific agonists and antagonists. For example, β-adrenoceptors can be activated by isoprenaline whereas α-receptors respond to phenylephrine. Such separate receptors may function to regulate different cellular processes in that they are often linked to the generation of different second messengers. β-Adrenoceptors and H_2-histamine receptors, for example, activate adenylate cyclase whereas α_1-adrenoceptors and H_1-histamine receptors seem to generate calcium signals. Even the peptide hormone, vasopressin, has been shown to act either through cyclic adenosine 3′,5′-monophosphate (cyclic AMP) (Orloff & Handler, 1967) or through calcium (Keppens, Vandenheede & DeWulf, 1977; van de Werve, Hue & Hers, 1977) which may indicate the existence of separate receptors.

The existence of functionally separate 5-hydroxytryptamine (5-HT) receptors has not been as well-defined as the receptors for many other neurotransmitters. Gaddum & Picarelli (1957) were the first to propose the existence of separate 5-HT receptors in guinea-pig ileum based on the differential inhibitory effects of various antagonists. The nervous system contained M receptors, which were blocked by morphine, whereas the muscle cells had D receptors sensitive to dibenzyline. Separate 5-HT receptors have also been identified in NCB-20 neuroblastoma-brain hybrid cells (MacDermot, Higashida, Wilson, Matsuzawa, Minna & Nirenberg, 1979) and in brain membranes (Peroutka & Snyder, 1979). The first indication that these separate 5-HT

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receptors might perform different functions emerged from the studies on the NCB-20 cells where one receptor was linked to adenylate cyclase whereas the other receptor induced membrane depolarization and acetylcholine release (MacDermot *et al.*, 1979). Such functionally distinct receptors seem to exist on the salivary glands of the blowfly which secrete fluid in response to 5-HT. An electrophysiological study has revealed that certain 5-HT analogues produce a hyperpolarizing response whereas other analogues have the opposite effect (Berridge, unpublished observations). In this study we show that analogues which give hyperpolarizing responses seem to activate receptors that are linked to adenylate cyclase whereas activation of receptors which induce depolarizing responses brings about an influx of calcium that is associated with the hydrolysis of phosphatidylinositol.

Methods

All experiments were performed on salivary glands isolated from adult blowflies (*Calliphora erythrocephala*). All incubations were done in a standard medium which had the following composition (mM): Na^+ 155, K^+ 10, Ca^{2+} 2, Mg^{2+} 2, Tris 10, Cl^- 156, malate 2.7, glutamate 2.7 and glucose 10. The pH was adjusted to 7.3 with Tris/HCl.

Measurements of fluid secretion

Salivary glands were set up in 50 μl of medium under liquid paraffin. A single silk thread was tied around the cut end which was pulled a short distance out into the liquid paraffin. The glands were nicked behind the ligature and the saliva which accumulated around this end was removed at set intervals. Microscopic measurement of the diameters of these fluid drops was used to determine their volumes and hence the rate of fluid secretion.

Inositol release

Glands were first set up for measuring fluid secretion as described. They were then labelled by incubation with *myo*-[2-^{3}H]-inositol (sp. act. 5 Ci/mmol; The Radiochemical Centre, Amersham). Three glands were incubated for 1 h in 50 μl of medium containing 4.2×10^6 ct/min, which gave an inositol concentration of 20 μM. The labelled medium was removed and the gland was washed 6 times with control medium before beginning the efflux experiment.

The release of [^{3}H]-inositol to the saliva was determined by removing the secreted fluid from under liquid paraffin with Pasteur pipettes at 3 min intervals and adding the drops to counting vials. At similar 3 min intervals, the medium surrounding the gland was removed and counted for radioactivity while fresh medium was added to the gland to measure efflux over the next interval.

At the end of the efflux experiment, the label left in the gland was measured by first homogenizing the gland in 0.5 ml of chloroform/methanol (2:1,v/v). The homogenate was transferred to a counting vial and after evaporating to dryness, radioactivity was measured after adding 4 ml of Biofluor (New England Nuclear). The amount of label entering the saliva and medium over the 3 min intervals was combined and has been expressed as a percentage of the label present in the gland at the beginning of each efflux period. When preparing a dose-response curve, the agonist was applied to the gland cumulatively. Each dose was applied to the glands for a total time of 12 min which enabled four measurements to be made. The amount of label released during the last 3 min collection period was used to construct the dose-response curves.

Calcium transport

The transport of calcium was measured by adding $^{45}CaCl_2$ (sp. act. 12.7 Ci/mg; The Radiochemical Centre, Amersham) to the medium bathing glands set up for measurement of fluid secretion. The control medium contained 10 mM cyclic AMP to stimulate fluid secretion so that $^{45}Ca^{2+}$ transport could be measured in the absence of any drug. Three glands were incubated in medium containing 7000 ct/min of $^{45}Ca^{2+}$/μl. Drops of saliva were removed from under liquid paraffin at 3 min intervals and transferred to counting vials containing 4 ml of Biofluor by Pasteur pipettes. When preparing a dose-response curve, the agonist was applied to the gland cumulatively. Since calcium transport inactivates at high 5-HT concentrations (Berridge & Fain, 1979), a maximum of three measurements was made on each group of three glands. In control experiments where no agonist was added there was a slight increase in $^{45}Ca^{2+}$ transport with time as the label equilibrated with internal pools. No correction was made for this small drift in the baseline value.

Cyclic AMP concentrations in glands

Groups of six glands were taken from 7 to 21 day old adults of either sex so that in any group no two glands came from the same animal. They were exposed to drugs in open dishes at room temperature. The glands were grasped with forceps and transferred to the different experimental solutions for 20 s. They were then rapidly homogenized in 0.1 ml of 0.4 M ice-cold perchloric acid. The extractions with perchloric acid and subsequent radioimmunoassays for cyclic AMP have been previously described (Heslop

& Berridge, 1980). During the first 25 s after treatment with 5-HT or a similar agonist there is negligible cyclic nucleotide phosphodiesterase activity and the change in cyclic AMP concentration in the glands is a measure of adenylate cyclase activity (Heslop & Berridge, 1980).

Results

Dose-response relationships for fluid secretion, cyclic AMP formation, [^{3}H]-inositol release and $^{45}Ca^{2+}$ transport

The effect of the parent compound 5-HT on four separate processes is shown in Figure 1. The dose-response curve for the onset of fluid secretion is steeper than for the other processes. The results shown in Figure 1 are the average of three separate experiments. The sensitivity of the glands to 5-HT and other drugs was found to be very consistent. The concentration necessary to induce a 50% increase in rate of secretion (vertical dashed line) is used as a reference point for comparison with the other responses. High rates of fluid secretion were evident with small increases in cyclic AMP level (Figure 1a),

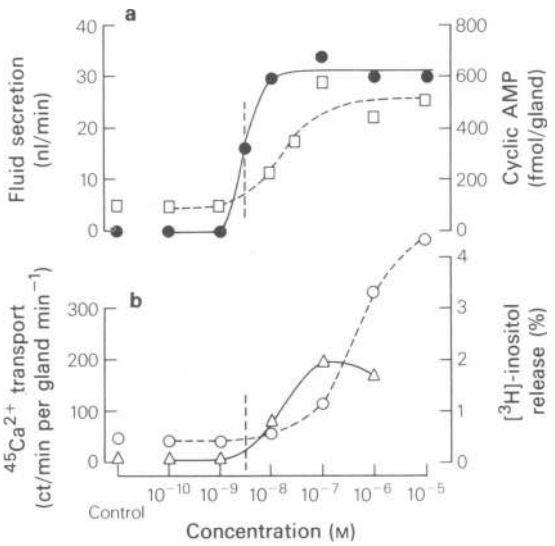

Figure 1 The effect of 5-hydroxytryptamine (5-HT) on fluid secretion (●), cyclic AMP formation (□), [^{3}H]-inositol release (○) and $^{45}Ca^{2+}$ transport (△) by salivary glands of *Calliphora* studied *in vitro*. The vertical dashed line on this figure, and on all subsequent figures, represents the concentration of 5-HT which gives a 50% increase in fluid secretion and thus provides a useful reference for comparing the dose-response relationships of all four responses. The resting or control value for each parameter before the addition of 5-HT is also shown.

[^{3}H]-inositol release and $^{45}Ca^{2+}$ transport (Figure 1b). These last three processes continued to increase as the concentration was raised whereas fluid secretion was maximal at 1×10^{-8} M 5-HT. Tryptamine, *N*-dimethyltryptamine and 5-hydroxy-*N*-dimethyltryptamine closely resembled 5-HT with regard to the relationship between fluid secretion, [^{3}H]-inositol release and $^{45}Ca^{2+}$ transport. Previous studies showed that all four analogues produced identical electrophysiological responses (Berridge, 1981).

Another group of analogues displayed a relationship between these four parameters which was very different from that just described for 5-HT. A typical example of this group is 4-fluoro-α-methyltryptamine (Figure 2) where the onset of fluid

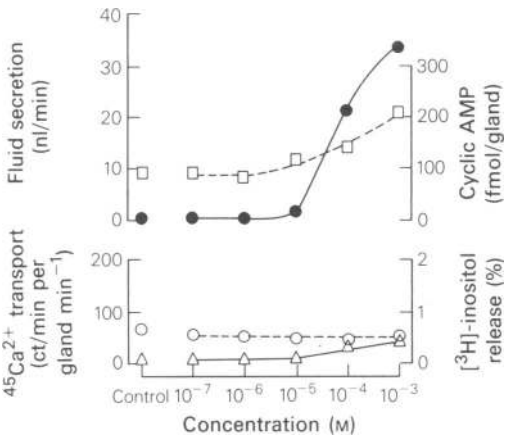

Figure 2 The effect of 4-fluoro-α-methyltryptamine on fluid secretion (●), cyclic AMP formation (□), [^{3}H]-inositol release (○) and $^{45}Ca^{2+}$ transport (△).

secretion was associated with an increase in cyclic AMP but there was no change in [^{3}H]-inositol release or $^{45}Ca^{2+}$ transport. The slight increment in $^{45}Ca^{2+}$ transport was not significant as it can be accounted for by a shift in the baseline value which always occurred even in control glands. A similar lack of effect on [^{3}H]-inositol release and calcium transport was found for 5-fluoro-α-methyltryptamine and also for histamine. These agents thus retain the capacity to stimulate the formation of cyclic AMP but do not generate a calcium signal unless the concentration is greatly increased.

The specificity of these 5-HT derivatives seems to depend on the methyl substituent at the α-position of the ethylamine side chain. The significance of α-methyl substitution was further tested for the parent compound 5-HT (Figure 3). There was a parallel shift in the cyclic AMP dose-response curve for 5-hydroxy-α-methyltryptamine as compared to 5-HT.

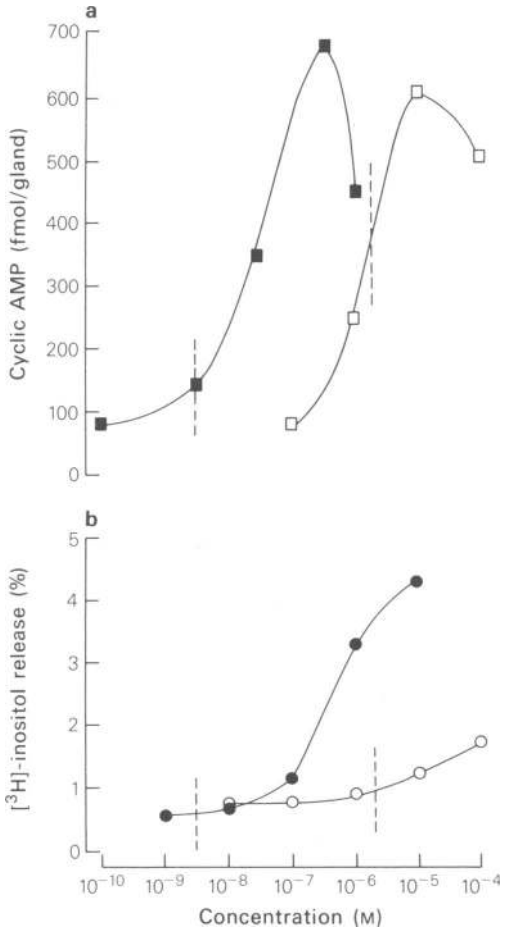

Figure 3 A comparison of the effects of 5-hydroxytryptamine (solid symbols) and 5-hydroxy-α-methyltryptamine (open symbols) on either cyclic AMP formation (a) or [^{3}H]-inositol release (b). The fluid secretory response is not shown but the vertical dashed lines represent the concentrations which give a 50% increase in fluid secretion for each agonist.

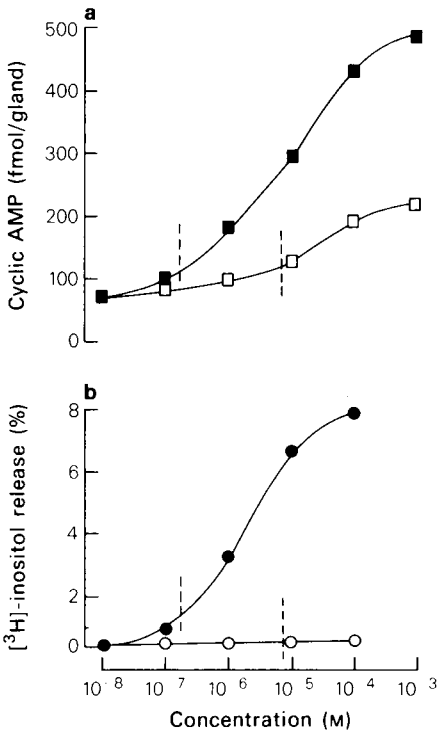

Figure 4 A comparison of the effects of 5-fluorotryptamine (solid symbols) and 5-fluoro-α-methyltryptamine (open symbols) on either cyclic AMP formation (a) or [^{3}H]-inositol release (b). The vertical dashed lines represent the concentrations which produce a 50% increase in fluid secretion for each agonist.

There was a more dramatic effect on [^{3}H]-inositol release in that the methylated derivative caused very little release of [^{3}H]-inositol even at concentrations which induced a maximal cyclic AMP response (Figure 3). A similar relationship was observed when 5-fluorotryptamine was compared with 5-fluoro-α-methyltryptamine (Figure 4). Once again the effect of α-methyl substitution on cyclic AMP formation was much less than the effect on [^{3}H]-inositol release which was completely suppressed.

A group of analogues with altered ring substituents showed the opposite specificity in that the effect on [^{3}H]-inositol release and $^{45}Ca^{2+}$ transport was largely unchanged whereas the ability to form cyclic AMP was reduced. A typical example was 5-chlorotryptamine (Figure 5) where the release of [^{3}H]-inositol and the transport of $^{45}Ca^{2+}$ began to increase at 1×10^{-8} M whereas the threshold for fluid secretion and cyclic AMP formation occurred at higher doses. The dose-response relationship between the last two parameters was very similar to that seen for 5-HT (Figure 1a). The main difference between 5-HT and 5-chlorotryptamine appears to be a reduced ability of the latter to stimulate cyclic AMP formation whereas the ability to promote [^{3}H]-inositol release was unchanged. Very similar alterations in specificity were found for 5-methyltryptamine and 4-hydroxytryptamine. When compared to 5-HT, the dose-response curve for fluid secretion for these derivatives with ring substituents were shifted equally to the right whereas their ability to stimulate the release of [^{3}H]-inositol was unchanged (Figure 6).

If a chloro substituent on the 5-position is combined with a carboxyl group on the 1-position of the indole ring, the ability to stimulate fluid secretion is

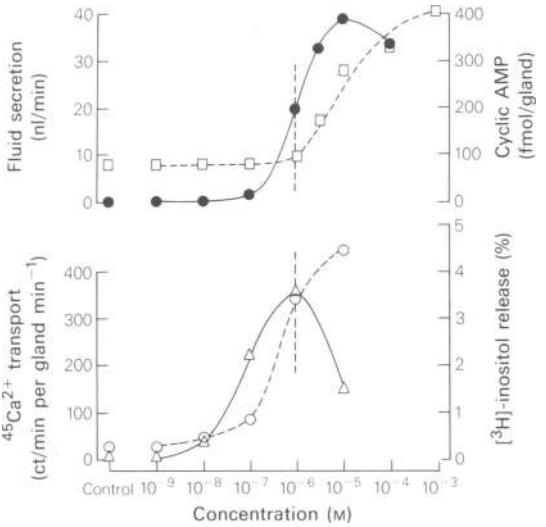

Figure 5 The effect of 5-chlorotryptamine on fluid secretion (●), cyclic AMP formation (□), [^{3}H]-inositol release (○), and $^{45}Ca^{2+}$ transport (△).

almost totally abolished whereas this analogue retains the ability to stimulate the hydrolysis of [^{3}H]-inositol and to gate $^{45}Ca^{2+}$ (Figure 7).

The effect of antagonists

To determine whether antagonists might discriminate between separate receptors, their effects on fluid secretion and [^{3}H]-inositol release were studied. Their inhibitory effects were compared by determining the concentration that produced a 50% reduction in the response of the gland to a standard dose of 3×10^{-8} M 5-HT (Table 1). All the antagonists tested fell into two main groups. Those antagonists in group 1 were found to have a more pronounced effect on [^{3}H]-inositol release than on fluid secretion. This specific effect was particularly pronounced in the case of methysergide which was studied in more detail by measuring cyclic AMP formation (Figure 8a). At the lowest concentration tested, methysergide caused some reduction of cyclic AMP formation but further increases in concentration did not affect about 50% of the response whereas the release of [^{3}H]-inositol was completely inhibited (Figure 8a).

Antagonists of the second group were more effective against fluid secretion than against the release of [^{3}H]-inositol. This difference was even more pronounced when cyclic AMP formation was measured. For phentolamine and cinanserin, cyclic AMP formation was found to be two orders of magnitude more sensitive to these inhibitors than was the release of [^{3}H]-inositol (Table 1). The differential effect of

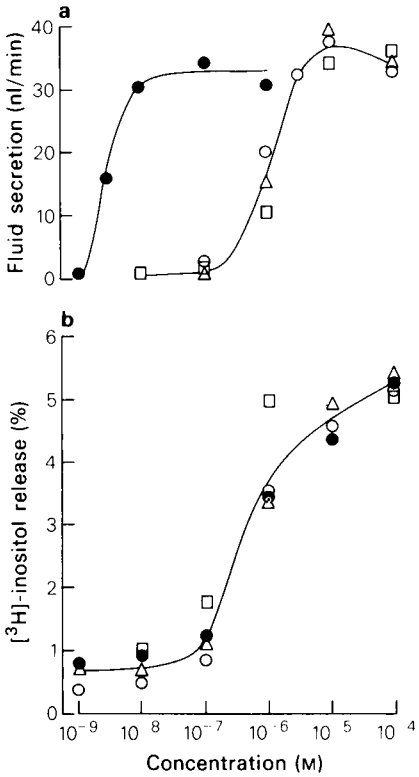

Figure 6 The effect of different agonists on either fluid secretion (a) or [^{3}H]-inositol release (b): 5-hydroxytryptamine (●); 5-methyltryptamine (□); 5-chlorotryptamine (○) and 4-hydroxytryptamine (△).

cinanserin on cyclic AMP formation, fluid secretion and [^{3}H]-inositol release is shown in Figure 8b.

Morphine, which was used by Gaddum & Picarelli (1957) to distinguish between peripheral receptors, had no inhibitory effect either on fluid secretion or on [^{3}H]-inositol release even at doses as high as 10^{-3} M.

Interactions between separate analogues

The results described above indicate that certain analogues such as 4-fluoro-α-methyltryptamine can stimulate cyclic AMP formation without altering the release of [^{3}H]-inositol whereas low concentrations of 5-chlorotryptamine had the opposite effect. The next series of experiments was designed to establish to what extent these two agonists interact with each other. In the first experiment, the effect of combining these two agonists was studied on cyclic AMP formation and the release of [^{3}H]-inositol (Figure 9). The specificity of these two agonists is evident from the fact that 4-fluoro-α-methyltryptamine increased the formation of cyclic AMP but had no effect on

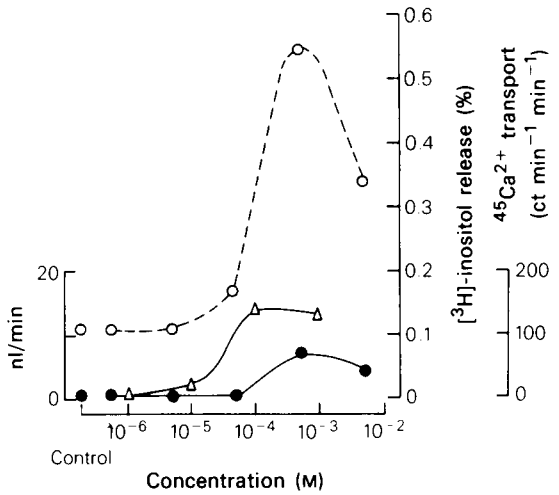

Figure 7 The effect of 5-chloro-1-carboxytryptamine on fluid secretion (●), [^{3}H]-inositol release (○) and $^{45}Ca^{2+}$ transport (△).

[^{3}H]-inositol release. On the other hand, 5-chlorotryptamine stimulated the hydrolysis of phosphatidylinositol but did not significantly affect cyclic AMP formation. In this experiment, therefore, there appeared to be no interaction between these two agonists when they were added simultaneously to the gland for a short period. However, if glands were pre-incubated with 5-chlorotryptamine for variable times before addition of 4-fluoro-α-methyltryptamine, the ability of the latter to stimulate the formation of cyclic AMP was severely curtailed (Table 2). Preliminary experiments showed that the threshold for stimulation of cyclic AMP formation by 5-chlorotryptamine was 0.5 μM. A dose of 0.1 μM of this agonist should therefore produce no stimulation of adenylate cyclase but a marked increase in calcium gating. A much higher concentration of 4-fluoro-α-methyltryptamine was selected. It is a weak agonist but at 0.1 mM caused a rise in the

Table 2 Combined effects of 4-fluoro-α-methylytryptamine (4F CH_3T) 1×10^{-4} M and 5-chlorotryptamine (5-CIT) 1×10^{-7} M on the cyclic AMP content of salivary glands

Incubation conditions	*Cyclic AMP content* (fmol/gland above control values)
5-CIT (0 to 20 s)	8
5-CIT (0 to 65 s)	9
5-CIT (0 to 145 s)	8
4F CH_3T (0 to 20 s)	59
4F CH_3T (0 to 24 s)	69
5-CIT (0 to 65 s) + 4F CH_3T (40 to 65 s)	24
5-CIT (0 to 145 s) + 4F CH_3T (120 to 125 s)	20

Incubations with both compounds added separately, compared with experiments in which 4F CH_3T was added after variable periods of pre-incubation with 5-CIT. Each figure is the mean of duplicate estimations on extracts of at least three groups each of six salivary glands.

Table 1 Summary of the inhibitory effect of antagonists on fluid secretion, [^{3}H]-inositol release and cyclic AMP formation.

Antagonist	*Fluid secretion IC_{50}* (M)	*[^{3}H]-inositol release IC_{50}* (M)	*Cyclic AMP formation IC_{50}* (M)
Group 1			
Methysergide	10^{-3}	1×10^{-5}	—
Yohimbine	2×10^{-5}	6×10^{-5}	—
(±)-Propranolol	7×10^{-5}	8×10^{-6}	—
Harmaline	5×10^{-5}	1×10^{-5}	—
Gramine	3×10^{-5}	4×10^{-6}	—
Methergoline	1×10^{-6}	8×10^{-7}	—
Trifluoperazine	9×10^{-7}	4×10^{-7}	—
Cyproheptadine	1.5×10^{-7}	3×10^{-8}	—
Pizotifen	1.5×10^{-7}	2×10^{-8}	—
Group 2			
Phentolamine	6×10^{-5}	3×10^{-4}	3×10^{-6}
Cinanserin	2×10^{-5}	1×10^{-4}	1×10^{-6}
Spiperene	3×10^{-6}	3×10^{-5}	—
Phenoxybenzamine (dibenzyline)	1.5×10^{-6}	8×10^{-6}	—

IC_{50} represents the molar concentration of the antagonist which induces a 50% inhibition of fluid secretion, [^{3}H]-inositol release or cyclic AMP formation due to 3×10^{-8} M 5-HT.

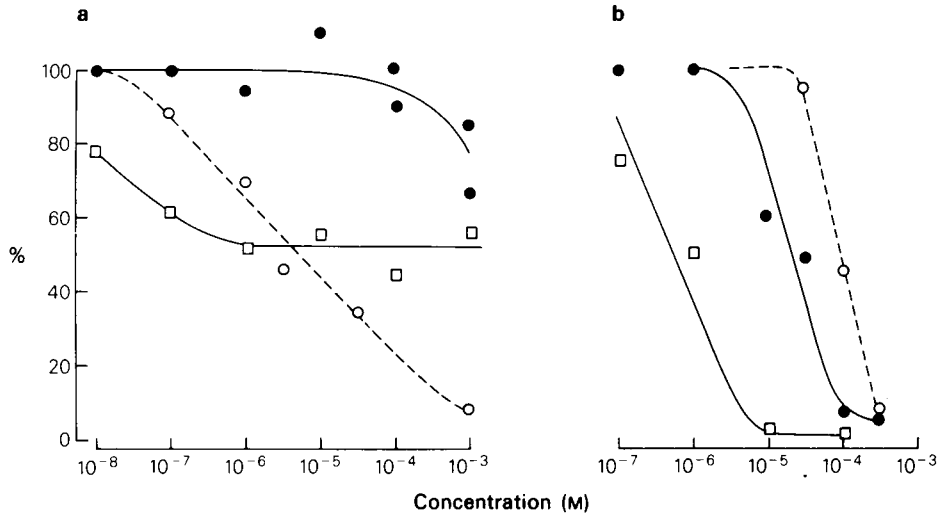

Figure 8 A comparison of the inhibitory effect of methysergide (a) and cinanserin (b) on the rate of fluid secretion (●), cyclic AMP formation (□), and [^{3}H]-inositol release (○), induced by 3×10^{-8} M 5-hydroxytryptamine. Inhibition is depicted as a percentage of the control value obtained with 3×10^{-8} M 5-HT.

cyclic AMP content of salivary glands which was linear with respect to time for the first 30 s after treatment. The content of cyclic AMP in the glands 20 or 25 s after exposure to 4-fluoro-α-methyltryptamine is a useful measure of the cyclic AMP-mediated effect and was estimated in at least three groups of six glands in each case. Figure 9 shows that 4-fluoro-α-methyltryptamine at 0.1 mM did induce an increase of cyclic AMP above control levels and that in the presence of 5-chlorotryptamine this rise was slightly reduced. If however, there was a pre-incubation with 5-chlorotryptamine of 40 s or 120 s followed by exposure to both agonists for a further 25 s, the inhibition was more pronounced (Table 2). Extension of the pre-incubation time to 20 min had no further effect.

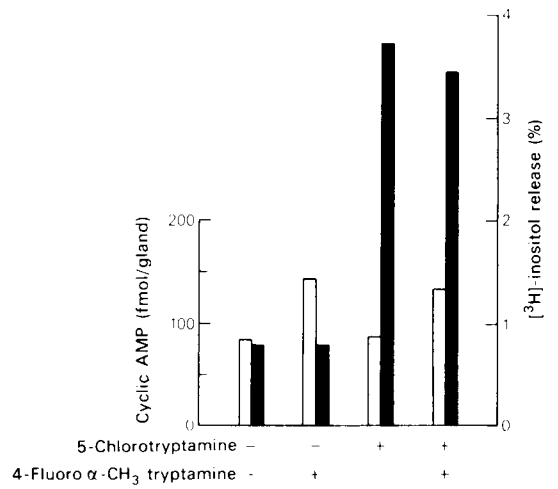

Figure 9 The effect of combining 5-chlorotryptamine (5×10^{-7} M) and 4-fluoro-α-methyltryptamine (1×10^{-4} M)on cyclic AMP formation (open columns) and [^{3}H]-inositol release (closed columns).

Discussion

5-HT can control separate physiological processes in the insect salivary gland. Previous studies showed that there was an increase in potassium transport in addition to changes in chloride conductance (Prince & Berridge, 1973; Berridge, Lindley & Prince, 1975). These two effects are thus reminiscent of the action of 5-HT on frog skin where there is an increase in sodium transport and chloride permeability (Dalton, 1979). In a pharmacological study on frog skin, Dalton concluded that these two ionic events were mediated by a single 5-HT receptor. This apparently is not the case in the insect salivary gland where the effects on potassium and chloride movement are controlled through separate 5-HT receptors.

Preliminary evidence for the existence of separate 5-HT receptors on the insect salivary gland was obtained from an electrophysiological study where it was possible to separate effects on potassium transport from those on chloride conductance (Berridge, unpublished observations). Certain analogues, such as 5-chlorotryptamine, gave a predominantly depolarizing response due to a calcium-dependent increase in chloride permeability. On the other hand,

analogues such as 5-fluoro-α-methyltryptamine had no effect on chloride conductance but produced a hyperpolarizing response due to an increase in a cyclic AMP-dependent potassium transport. These two ionic mechanisms thus appeared to be regulated through separate receptors using different second messengers. One receptor acted through calcium to produce a chloride-dependent depolarizing response whereas the other receptor acted through cyclic AMP to induce a potassium-dependent hyperpolarizing response. The observations described in this paper provide more direct biochemical evidence for the existence of separate receptors.

The ability of 5-HT to generate a calcium signal in the insect salivary gland seems to depend on the hydrolysis of phosphatidylinositol (Berridge & Fain, 1979; Fain & Berridge, 1979a,b). The rate of breakdown of this phospholipid, which is monitored by measuring the release of [^{3}H]-inositol from prelabelled glands, was closely related to calcium permeability. In this study we show that analogues which gave the calcium-dependent depolarizing response were extremely active in promoting both the release of [^{3}H]-inositol and the gating of calcium. There was no significant difference between these two parameters for 5-chlorotryptamine and for 5-HT (Figure 1) but the threshold of fluid secretion is raised about 300 fold. Similar responses to 5-chlorotryptamine were observed for 5-methylytryptamine and for 4-hydroxytryptamine. An interesting feature about these compounds was that their effect on [^{3}H]-inositol release was greater than that of 5-HT whereas their ability to induce fluid secretion was less (Figure 6). Since fluid secretion depends primarily on the generation of cyclic AMP, this suggests that in these analogues carrying alterations on the 5-position there has been a marked reduction in their ability to generate cyclic AMP. This was confirmed for 5-chlorotryptamine where the dose-response curve for cyclic AMP formation (Figure 5) was displaced to the right of that found for 5-HT (Figure 1).

In contrast to these analogues which were particularly effective at generating calcium signals, other analogues were found which acted almost exclusively through cyclic AMP. As described earlier, the sensitive electrophysiological test had revealed that analogues such as 4-fluoro-α-methyltryptamine induced a cyclic AMP-like hyperpolarizing response with no evidence of the typical calcium-dependent depolarization (Berridge, unpublished). The present study has confirmed that this agent had no effect on [^{3}H]-inositol release or calcium transport, but was able to generate cyclic AMP and to stimulate fluid secretion. It appears as if the addition of a methyl substituted on the α-carbon position of the ethylamine side-chain is primarily responsible for this change in specificity. This was confirmed by studying the introduction of an α- methyl substituent into 5-HT (Figure 3) and 5-fluorotryptamine (Figure 4). In both cases, it was clear that the release of [^{3}H]-inositol was inhibited to a much greater extent than was the synthesis of cyclic AMP.

Further evidence for the possible existence of separate receptors was obtained by studying some of the antagonists which have been reported to block the action of 5-HT (Gaddum & Picarelli, 1957; Haigler & Aghajanian, 1977; Connell, Middlemiss & Stone, 1980; Fuller, 1980). When applied to the insect salivary gland, most of these antagonists were found to inhibit fluid secretion and the release of [^{3}H]-inositol. The fact that the IC_{50} was sometimes markedly different for these two processes suggested that some of these antagonists might be specific for separate receptors. This possibility was confirmed by further studies with methysergide and cinanserin (Figure 8). Methysergide had almost no effect on fluid secretion while the release of [^{3}H]-inositol was markedly reduced. Jafferji & Michell (1976) have also found that methysergide was a potent antagonist against the PI response induced by 5-HT in smooth muscle. Its effect on cyclic AMP formation was much less severe in that there was only a partial inhibition even at high doses.

This partial inhibition suggests that there are two components, one of which is sensitive and one very insensitive to methysergide, raising the possibility that two receptors acting through cyclic AMP may be present, a possibility that requires further exploration. In contrast, cinanserin exerted a much greater inhibitory effect against cyclic AMP formation than against the release of [^{3}H]-inositol. The fact that these two antagonists seem to be selective against the two receptors in the fly salivary gland is interesting because their effects in other systems are often very different. For example, when tested on pyramidal cells of the hippocampus, cinanserin was much less effective in blocking the action of 5-HT than was methysergide (Segal, 1976). Similarly, methysergide was much more potent than cinanserin in inhibiting the binding of 5-HT to synaptic membranes (Connell *et al.*, 1980). When tested on rats, methysergide was also more effective than cinanserin in antagonizing tryptamine-induced seizures (Leysen, Niemegeers, Tollenaere & Laduron, 1979). The most effective antagonists against these seizures were cyproheptadine and pizotifen which turned out to be the most potent antagonists for inhibiting the action of 5-HT in this insect salivary gland (Table 1). These studies with antagonists thus add further support to the proposal that there are separate 5-HT receptors on the insect salivary gland.

As evidence for the existence of separate 5-HT receptors begins to grow, there arises the difficult problem of terminology. In their original paper on

separate 5-HT receptors, Gaddum & Picarelli (1957) used D and M to distinguish between them, based on their sensitivity to morphine and dibenzyline respectively. However, recent studies have not confirmed the inhibitory action of morphine which has led to the suggestion that these receptors should be reclassified (Wallis & Nash, 1980). Another form of classification based on using the first letters of the alphabet has been introduced by Gerschenfeld (1971) to distinguish between the multiple effects of 5-HT in snail neurones. The A-, B-, and C-receptors are thought to regulate sodium, potassium and chloride permeabilities respectively. However, it is not apparent whether these effects of 5-HT on ionic conductances are direct effects of receptor activation or whether they develop indirectly due to the formation of second messengers such as calcium. This classification also bears no relationship to the numerical system which has been used to distinguish between the multiple receptors that have been identified for other neurotransmitters (Snyder & Goodman, 1980). Peroutka & Snyder (1979) have used 5-HT_1 and 5-HT_2 to distinguish between the separate binding sites which have been identified in brain membranes. Separate receptors are often linked to the formation of either cyclic AMP or calcium (Berridge, 1980). For example, histamine H_1-receptors act through calcium whereas the H_2-receptors mediate their effects through cyclic AMP. To maintain some degree of conformity, this precedent has been followed in classifying the 5-HT receptors on the insect salivary gland. 5-HT_1 is proposed for the receptors which act to hydrolyse phosphatidylinositol to gate calcium and 5-HT_2 for the receptors which act through cyclic AMP. In proposing this nomenclature the authors withdraw their suggested classification based on homology with dopamine receptors (Heslop & Berridge, 1981).

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