Advances in

Insect Physiology

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J. E. TREHERNE, M. J. BERRIDGE
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I. INTRODUCTION

Coordination of cell activity in higher animals is achieved through various chemical messengers which are released by one cell population to influence another. Most hormones function in long range communication where the blood system carries the chemical messages to the target cells. However, communication can also occur over a short distance, such as in the synapse, where chemicals diffuse across the narrow synaptic gaps to transfer information between two

excitable cells. In both forms of communication the basic mechanism is the same – a simple chemical message is used to transfer information from one cell to another. This review attempts to summarize the progress which has been made in understanding how one particular cell system, the salivary gland of an insect, receives a chemical stimulus and translates the information into a change in activity.

The salivary glands of the adult blowfly, *Calliphora erythrocephala* have unique structural and physiological properties which have enabled us to analyse the sequence of events which occur during cell activation by a specific hormone. Berridge and Patel (1968) found that fluid secretion by isolated salivary glands was regulated by 5-hydroxytryptamine (5-HT). This simple biogenic amine may be an important secretogogue in insects because Whitehead (1971) has evidence that 5-HT may be released from the nerves which innervate the salivary glands of the cockroach, *Periplaneta americana*. In the cockroach, the increase in fluid secretion observed during nerve stimulation can be mimicked by externally applied 5-HT. Three major events have been recognized during stimulation of fluid secretion by 5-HT in the salivary glands of *Calliphora* (Berridge, 1970, 1972; Berridge and Prince, 1971, 1972a, b; Prince and Berridge, 1972; Prince *et al.*, 1972). Firstly, 5-HT recognizes and interacts with a specific cellular receptor. Secondly, the result of a successful 5-HT-receptor interaction is decoded into an increase in the concentration of two intracellular messengers, cyclic AMP and calcium. Thirdly, cyclic AMP and calcium are then responsible for stimulating the transport processes of the cell to produce an increase in fluid secretion.

This work on *Calliphora* salivary glands which has led to a simple hypothesis of the mode of action of 5-HT will form the first part of this review. In the second part, some of the general concepts which have emerged from these salivary gland studies will be discussed in relation to the mode of action of other hormones.

II. THE STRUCTURE AND FUNCTION OF *CALLIPHORA* SALIVARY GLANDS

The salivary glands of the adult blowfly are paired tubules which extend down the length of the animal. That part of the gland which lies in the abdomen is uncoiled but after entering the thorax the gland forms a tangled mass which lies on either side of the

proventriculus. Each tube empties into a small elongated bulb which is connected anteriorly to a cuticle-lined duct. The two salivary ducts converge and unite in the thorax to form a common duct which conveys saliva to the mouthparts. The gland has three major cell types: secretory, reabsorptive and duct cells (Oschman and Berridge, 1970).

The abdominal region of the gland and most of the convolutions in the thorax consist of a single cell type (Fig. 1A). These secretory cells are characterized by extensive canaliculi formed by elaborate infoldings of the apical plasma membrane (Oschman and Berridge, 1970). These canaliculi and the free apical surface are lined with sheet-like microvilli arranged in parallel arrays. The lateral plasma membrane is relatively straight and there are septate desmosomes in the apical region. The basal plasma membrane has infoldings which are closely associated with mitochondria. These basal infoldings often come very close (less than 1 μ) to the bottom of the canaliculi. Large mitochondria with perforated cristae are scattered throughout the cytoplasm. When stimulated with 5-HT these secretory cells produce an isosmotic fluid consisting primarily of potassium and chloride (Fig. 1) (Oschman and Berridge, 1970; Berridge and Prince, unpublished). This primary secretion then passes down through a short region of the gland which is lined with a simple squamous epithelium (reabsorptive cells).

Reabsorptive cells (Fig. 1B) are much less elaborate in that there are no secretory canaliculi. The apical surface has a few short microvilli resembling those of the secretory cells. The short wide basal infoldings are often closely associated with mitochondria. Various experimental procedures have revealed that this region of the gland reabsorbs potassium thus diluting the saliva (Fig. 1). This mechanism of isosmotic fluid secretion followed by hyperosmotic reabsorption to produce a dilute fluid closely resembles the mechanism of saliva production in mammalian salivary glands (Mangos *et al.*, 1966; Young and Schögel, 1966).

That part of the gland which lies in the abdomen and is responsible for secreting the primary saliva has been used to study the mode of action of 5-HT. Secretory activity can be monitored *in vitro* using a technique originally devised by Ramsay (1954) to study isolated Malpighian tubules. When set up in control saline salivary glands secrete very slowly (0.5-1.0 nl/min) but 1 min after addition of 1×10^{-8} M 5-HT the rate suddenly accelerates to more than 40 nl/min (Berridge, 1970). This high rate of secretion is maintained

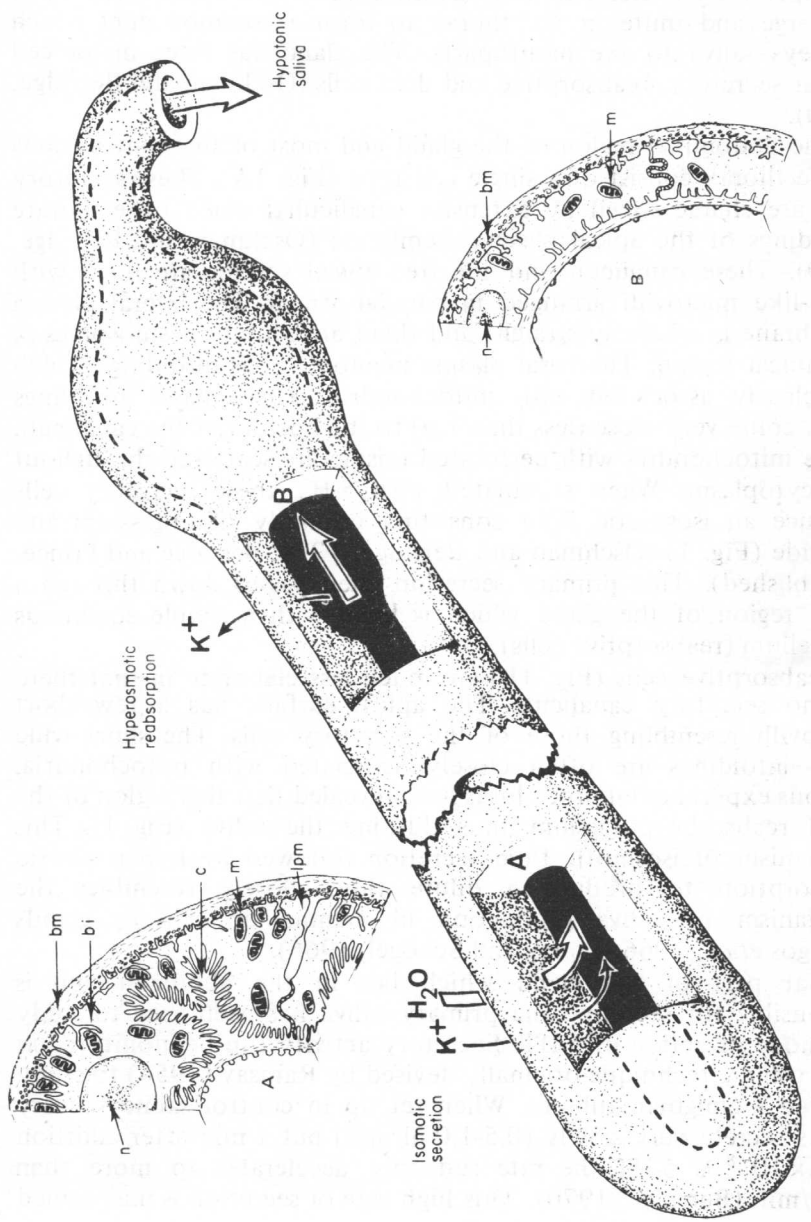
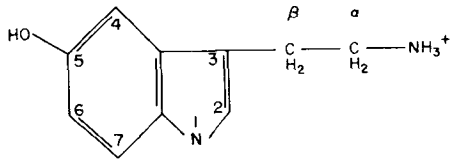


Fig. 1. The salivary gland of the blowfly. Each gland is a tubular structure divided into a secretory (A) and a reabsorptive (B) region. Schematic diagrams of the cells found in these two regions are shown. In the secretory region (A) water and chloride follow the transport of potassium resulting in the formation of an isosmotic fluid. In the reabsorptive region (B) potassium and chloride are reabsorbed so that the secreted saliva is hypotonic. bm, basement membrane; bi, basal infold; c, canaliculus; lpm, lateral plasma membrane; m, mitochondria; n, nucleus.

for as long as 5-HT is present but returns to the resting level 1-2 min after 5-HT is withdrawn. This very rapid response and recovery implies that the 5-HT-receptor interaction is readily reversible. Since these glands are composed of a single cell type whose secretory activity can be monitored continuously, they are particularly suited for analysing the sequence of events which occur during hormone action. The first of these events is the molecular interaction between the stimulating molecule, in this case 5-HT, and its receptor.

III. THE 5-HT-RECEPTOR INTERACTION

The molecular pharmacology of 5-HT has been approached by a study of the structure-activity relationships of a wide range of analogues of 5-HT (Berridge, 1972).



I

5-HT (I) consists of an indole ring nucleus with an ethylamine chain at the 3-position and a hydroxyl group at the 5-position. The indole ring system has a high electron density with the delocalized π electrons smeared over the two rings to form a rigid planar structure. The positions of the hydroxyl group and the β-carbon atom are fixed by their connection to this rigid ring system. The only parts of the molecule which have any degree of freedom are the α-carbon atom and the terminal nitrogen atom of the ethylamine side chain. The activity of such compounds is usually determined by the charge distribution over the molecule. The main centre of charge resides on the ethylamine nitrogen atom which is basic and accepts a proton at physiological hydrogen ion concentrations to become positively charged (I). The hydroxyl group at the 5-position has a partial negative charge resulting from its negative inductive effect on the π electrons of the indole ring. These two centres of charge will be separated from each other by the hydrophobic indole ring.

The activity of molecules such as 5-HT depends on two important features:

(i) a high affinity for the receptor which enables them to recognize and interact with the receptor at very low concentrations and

(ii) once attached to the receptor they must be capable of inducing an effect often referred to as intrinsic activity.

Antagonists have a high affinity for the receptor but cannot induce an effect, i.e. they have no intrinsic activity. The first experiments on 5-HT pharmacology determined which part of the molecule was responsible for its intrinsic activity (i.e. the active site on the molecule) and which part confers upon it such a high degree of specificity.

A positively charged ethylamine side-chain is an important component of a wide range of biologically active compounds including histamine, γ-aminobutyric acid and dopamine and is probably the active site on all these molecules. When the two major parts of the 5-HT molecule, 5-hydroxyindole and ethylamine, were tested separately they failed to activate salivary glands. When these two major parts were presented together at very high concentrations (10^{-2} M) they still failed to stimulate secretion. The action of 5-HT thus depends on the corporate effect of both these moieties. The importance of the ethylamine chain can be demonstrated by testing a range of homologues in which the length of the chain is increased by the addition of extra carbon atoms (Fig. 2). Increasing the chain length causes a progressive increase in activity so that amylamine, which has a 5-carbon chain, stimulates the glands maximally (i.e. it has the same intrinsic activity as 5-HT). However, very high concentrations were required to stimulate secretion indicating that these simple straight chain compounds have a very low affinity for the receptor. The groups used for increasing the chain length must be hydrophobic because there is no activity if the chain terminates with a hydrophilic group as in ethanolamine (II), ethylenediamine (III) or γ-aminobutyric acid (GABA) (IV).

$HOCH_2CH_2NH_3^+$ (II) $\quad {}^+NH_3CH_2CH_2NH_3^+$ (III) $\quad COOHCH_2CH_2CH_2NH_3^+$ (IV)

The requirement for a hydrophobic group at one end of the ethylamine chain implies that interaction of the positively charged quartenary nitrogen atom with an anionic site on the receptor requires hydrophobic interaction with the opposite end of the molecule. A progressive increase in the length of the carbon chain permits this hydrophobic interaction to develop with a resulting increase in activity (Fig. 2). That high concentrations of these compounds are needed to produce activity suggests that they have low affinities for the receptor possibly because their interaction with the hydrophobic site is unspecific.

The specificity of the hydrophobic site was investigated by testing compounds where ethylamine was attached to various ring structures (Berridge, 1972). Molecules containing a benzene ring (phenethylamine) or an imidazole ring (histamine) were more active than the straight chain homologues of ethylamine, but were still very much less active then tryptamine or 5-HT which have an indole ring (Fig. 2). Thus we can conclude that the specificity of 5-HT depends on a high degree of complementarity between the indole ring and a hydrophobic site on the receptor.

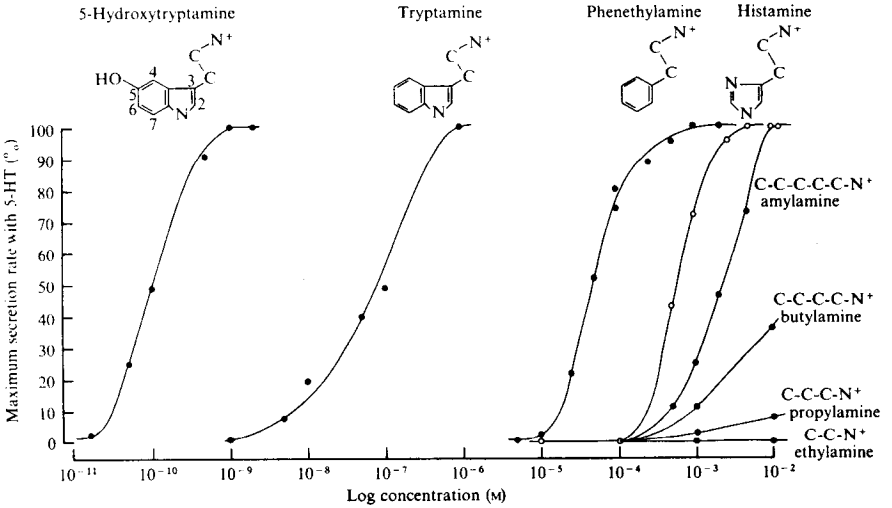
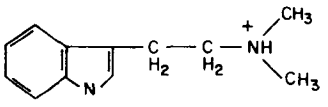


Fig. 2. The dose-response curves of isolated salivary glands to 5-HT and some 5-HT-like compounds. (From Berridge, 1972.)

Another problem to consider is the role of the hydroxyl group in the 5-HT-receptor interaction. Removal of the hydroxyl group causes a thousand-fold reduction in affinity but no change in intrinsic activity because tryptamine is capable of inducing a maximal response (Fig. 2). A similar reduction in activity is observed when dimethyl tryptamine (V) is compared with the hydroxylated derivative bufotenine (VI) (Berridge, 1972). The effect of the hydroxyl group was also assessed by comparing the activity of tryptophane (VII) and 5-hydroxytryptophane (VIII). The former is totally inactive whereas the latter is capable of stimulating secretion although both the affinity and intrinsic activity are reduced. The ability of the hydroxyl group to increase the activity of the three combinations tested implies that this group increases the affinity



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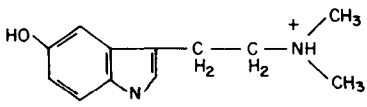

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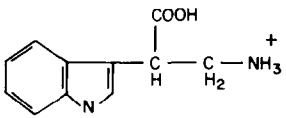

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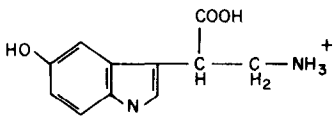

VIII

possibly through hydrogen bonding. The position of the hydroxyl group is critical because its displacement to the 4- or 6-positions causes a thousand-fold decrease in activity similar to that observed in the absence of the hydroxyl group. These results imply that there is a hydrogen bonding site on the receptor which has a precise orientation with respect to the hydrophobic portion of the receptor which reacts with the indole ring.

Having determined the contribution of the different regions of the molecule to its interaction with the receptor, it remains to establish the conformation of the ethylamine side chain as the molecule attaches itself to the receptor. As mentioned earlier, the α-carbon atom and the terminal nitrogen atom have considerable degrees of freedom and could assume various configurations as shown in Fig. 3. The α-carbon atom is free to rotate around the β-carbon atom and can thus assume any position represented by the base of the cone I. Similarly, for most of the possible positions of the α-carbon atom, the nitrogen atom could assume a number of positions represented by the base of the second cone II. A clue to the natural configuration of 5-HT can be obtained by testing certain molecules where this ethylamine side chain is fixed in a rigid configuration by being incorporated into additional ring systems. Preliminary unpublished experiments have shown that the lysergic acid derivative bromolysergic acid diethylamide (BOL) (IX) can stimulate secretion whereas various harmala alkaloids such as harmaline (X) are totally inactive. However, these harmala alkaloids have a high affinity for the receptor because they are potent competitors; this indicates that they have most of the attributes for a successful 5-HT-receptor interaction but once attached to the receptor they cannot induce an effect. This can be explained by a comparison of the position of the