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The electrical response of isolated salivary glands during stimulation with 5-hydroxytryptamine and cyclic AMP

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Rate of fluid secretion by the salivary glands of the blowfly *Calliphora* is regulated by 5-hydroxytryptamine (5-HT) working in conjunction with cyclic AMP. Although cyclic AMP can exactly mimic the acceleration of fluid secretion produced by 5-HT, the underlying electrical events are completely different. Transepithelial potentials were measured by a liquid paraffin-gap technique which permits continuous potential recordings during rapid changes of the bathing medium. The potential of the lumen of unstimulated glands is +5 mV with respect to the bathing medium but becomes −10 to 20 mV after applying 5-HT. After stimulation with cyclic AMP, however, the luminal potential becomes more positive (+30 to 40 mV). A similar effect is obtained with theophylline or when glands are treated with 5-HT in the presence of an impermeant anion such as isethionate.

These observations suggest that in addition to stimulating the synthesis of cyclic AMP, 5-HT may also act directly to increase anion movement. Cyclic AMP appears to stimulate cation transport, which explains the increase in positive potential obtained when this compound (or theophylline) is applied in the absence of 5-HT.

Introduction

Neurotransmitters and peptide hormones play an important role in regulating ion and water movement across epithelia. For example, fluid secretion by salivary glands, pancreas and avian salt gland is regulated by acetylcholine, while the absorption of ions and water by toad bladder and collecting ducts of the mammalian kidney is regulated by vasopressin. For most of these epithelia the exact mechanism of fluid movement and its control remains to be determined. However, there is increasing evidence that adenosine 3′,5′-monophosphate (cyclic AMP) plays a central role in the action of many of the agents which regulate fluid transport (Robison, Butcher & Sutherland 1968). Cyclic AMP is a naturally occurring intracellular constituent which is synthesized from ATP by the enzyme adenyl cyclase which has been localized on the plasma membrane (Robison *et al.* 1968). The current concept is that hormones act on adenyl cyclase to accelerate the synthesis of cyclic AMP and the subsequent increase in intracellular cyclic AMP concentration is then responsible for mediating the further action of the hormone. However, the exact nature of the informational transfer between cyclic AMP and the effector system remains to be explored. We have used the isolated salivary glands of an insect to study the role of cyclic AMP in the control of fluid movement. A brief description of the structure and function of this secretory organ will illustrate the many advantages of this tissue as a model system for studying how hormones regulate ion and water movement.

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The structure and function of *Calliphora* salivary glands

The salivary glands of the blowfly, *Calliphora erythrocephala*, consist of a pair of long tubes which extend down the length of the animal (figure 1). Most of the gland is made up of a single cell type (stippling) which secretes an isotonic fluid rich in potassium (Oschman & Berridge 1970). A short length of the gland (clear region) immediately preceding the cuticle-lined ducts in the thorax is composed of a different cell type which dilutes this primary isotonic secretion by reabsorbing potassium (Oschman & Berridge 1970). Most of the studies performed so far have used the secretory part of the gland which lies in the abdomen. The cells in this region are

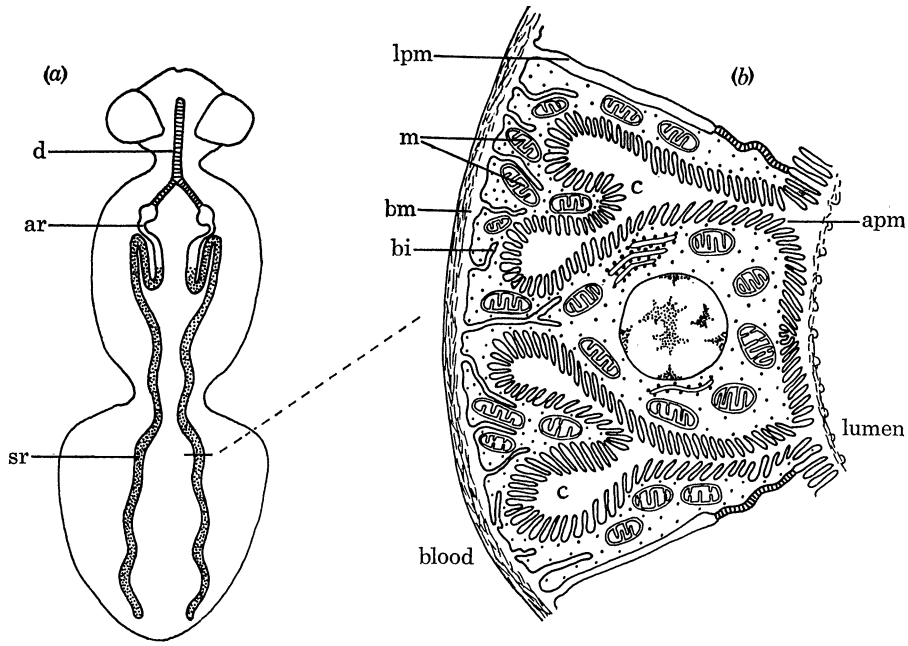

Figure 1. (*a*) The dorsal view of an adult fly showing the arrangement of the salivary glands. d, duct; ar, absorptive region; sr, secretory region. (*b*) The ultrastructural organization of a secretory cell. c, canaliculi; lpm, lateral plasma membrane; m, mitochondria; bm, basement membrane; bi, basal infolds; apm, apical plasma membrane.

characterized by the presence of extensive secretory canaliculi formed by deep invaginations of the apical plasma membrane (Oschman & Berridge 1970). In this respect they closely resemble the parietal cell found in the gastric mucosa of mammals (Ito & Winchester 1963). The surface area of the apical membrane is increased through further invaginations to form flattened plate-like microvilli. The lateral plasma membrane is straight and the cells are held together by septate desmosomes formed between lateral membranes in the apical region. The basal plasma membrane has a number of irregular infoldings which are closely associated with mitochondria. The presence of a continuous epithelium composed of a single cell type, and the complete absence of nerve, muscle or connective tissue elements greatly facilitate the interpretation of physiological and pharmacological studies.

Control of fluid secretion has been investigated using a technique originally devised to study Malpighian tubules (Ramsay 1954). The gland was dissected out of the abdomen and placed in a drop of saline contained under liquid paraffin in the well of a watch-glass (figure 2). The cut end of the gland was ligated with a silk thread and pulled a short distance out of the saline

drop. Saliva escaped from a nick made immediately behind the ligature and rate of secretion was measured by estimating the volume of fluid removed from the cut end at regular intervals. A series of pharmacological studies suggested that 5-hydroxytryptamine (5-HT), or a closely related compound, controlled the secretory activity of isolated salivary glands (Berridge & Patel 1968).

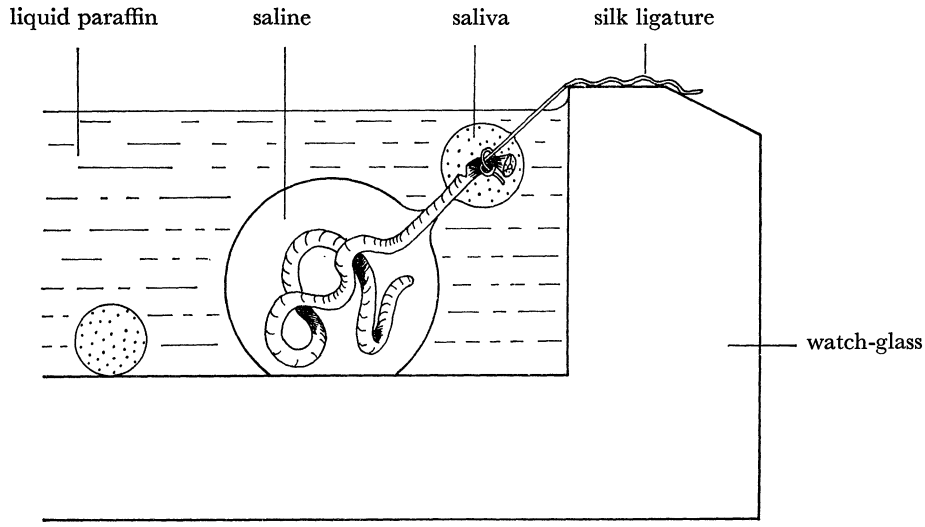

FIGURE 2. The technique used for measuring rate of fluid secretion by isolated salivary glands.

The ability of 5-HT to stimulate fluid secretion can be mimicked by cyclic AMP (figure 3). The osmotic pressure and composition of the saliva secreted during stimulation with 5-HT or cyclic AMP are identical (Oschman & Berridge 1970). The specificity of the response to cyclic AMP can be analysed by testing the effect of a wide range of nucleotides. Although the receptor can accommodate certain modifications of the base region, the conformation of the sugar and phosphate ring are inviolate (unpublished observation). As in other systems, very high concentrations of these cyclic nucleotides are required to duplicate the effect of 5-HT (figure 3). It is generally considered that these high concentrations are necessary because cell membranes are relatively impermeable to these large nucleotides. However, at these high concentrations sufficient cyclic AMP will flood into the cell to raise the intracellular concentration enough to stimulate secretion.

Another way of raising the intracellular cyclic AMP level is to use methyl xanthines (theophylline or caffeine) to inhibit the enzyme phosphodiesterase (PDE) which degrades cyclic AMP to 5′-AMP (Butcher & Sutherland 1962). The ability of theophylline to stimulate the glands (Berridge 1970) provides further evidence to implicate cyclic AMP in the control of secretion. Theophylline is also capable of greatly potentiating the effects of both 5-HT and cyclic AMP (Berridge 1970).

More direct evidence for a role of cyclic AMP in the control of secretion has been obtained by measuring the change in the level of cyclic AMP when cellular activity is increased with 5-HT. During stimulation with 5-HT there is a fivefold increase in the intracellular cyclic AMP concentration (Prince & H. Rasmussen, unpublished observation). There thus appears to be little doubt that cyclic AMP plays a central role in mediating the action of 5-HT.

In salivary glands, therefore, the hormonal response begins with a specific interaction

between 5-HT and its receptor (figure 4). The chemical signal input resulting from a successful hormone-receptor interaction is then transduced into a secondary message in the form of an elevated level of cyclic AMP. The next problem is to determine how this increase in cyclic AMP concentration is translated into a change in the rate of saliva production.

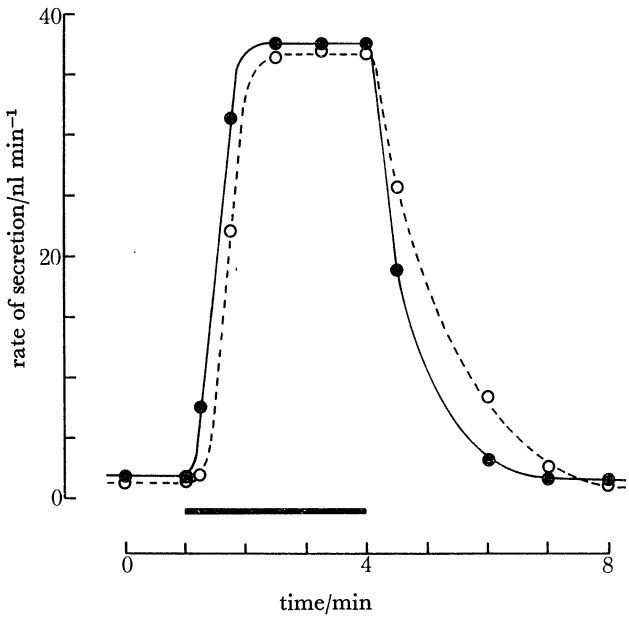

FIGURE 3. The effect of 10^{-8} mol/l 5-HT (●) and 10^{-2} mol/l cyclic AMP (○) on rate of fluid secretion. The horizontal bar represents the length of treatment with these two compounds.

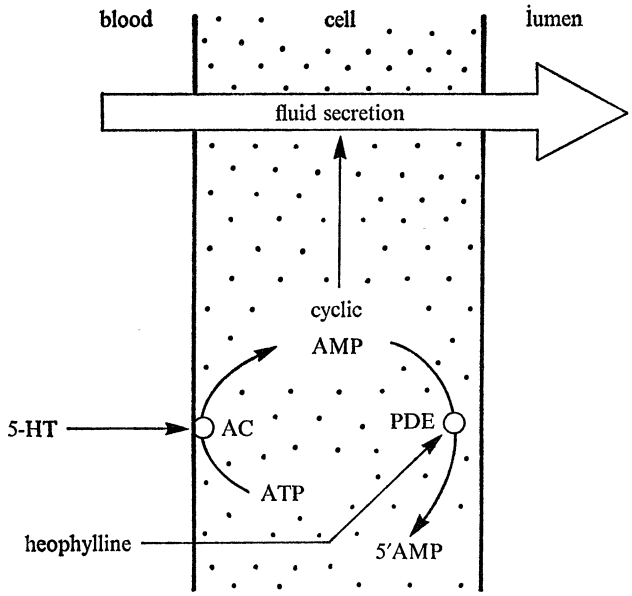

FIGURE 4. The role of cyclic AMP in the control of fluid secretion. The intracellular concentration of cyclic AMP depends on the balance which exists between its synthesis by adenyl cyclase (AC) and its destruction by phosphodiesterase (PDE). 5-HT may act by stimulating adenyl cyclase.

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The electrical events associated with stimulation of secretion

On the basis of present concepts concerning the mechanism of isotonic fluid transport, we can assume that the increased flow of water which occurs during stimulation with 5-HT depends on an increased movement of both cations and anions. Consequently the ionic basis of fluid secretion is being studied in the hope of uncovering the site and mode of action of cyclic AMP. We have made a start on this problem by studying the electrical events associated with the changes in rate of fluid secretion induced by 5-HT or cyclic AMP (Berridge & Prince 1971).

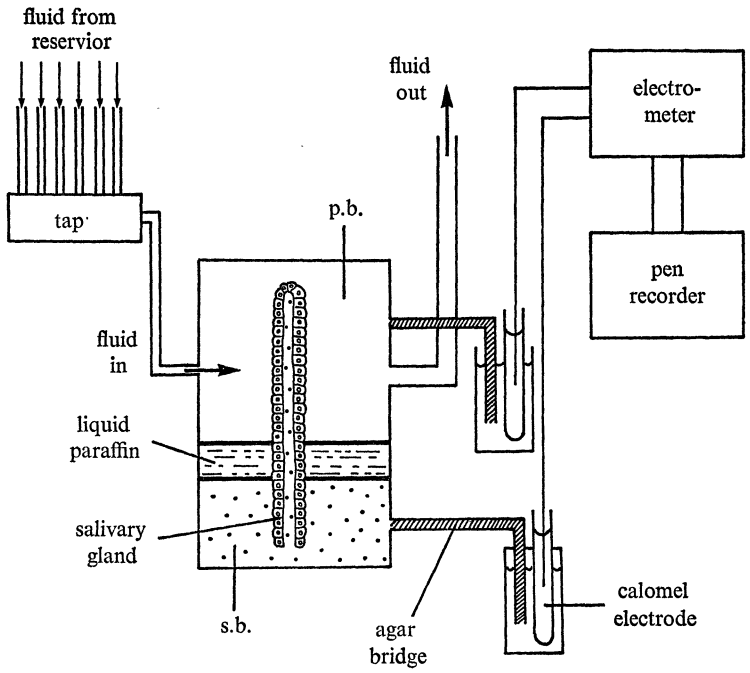

Figure 5. Schematic diagram of the perfusion bath (viewed from above) and potential recording system. The closed end of the salivary gland lay in the perfusion bath (p.b.) while the open end lay in the saliva bath (s.b.).

Since the increase in rate of fluid secretion is very rapid, an apparatus was designed to permit continuous potential recordings during the application of different test substances. The gland was placed in a groove which connected three parallel perspex chambers (figure 5). The closed end of the gland lay in one outer chamber which was constantly perfused with saline. A six-way tap placed close to the inlet tube reduced the dead space during changes in the perfusion fluid. By measuring the change in the resting potential of salivary glands during infusion of solutions with different potassium concentrations, it is possible to show that a new solution reaches the gland 1 s after opening the tap. The cut end of the gland lay in the other outer chamber (saliva bath) which also contained saline. Liquid paraffin contained in the narrow middle chamber provided an effective means of insulating the two outer chambers. Agar bridges connected each outer chamber to a calomel electrode and the transepithelial potential difference was measured by a Keithley electrometer and recorded on a Servoscribe.

The lumen of an unstimulated gland is usually about 5 mV positive with respect to the outside. When stimulated with 5-HT, however, the lumen goes negative (10 to 20 mV) within 3 to 5 s (figure 6). After a short (10 s) treatment with 5-HT the potential soon returns towards the

unstimulated level and often displays a large positive undershoot. If glands are treated with 5-HT for a longer period, the luminal potential remains negative but returns to the unstimulated level as soon as 5-HT is withdrawn (figure 6). In this case the recovery takes much longer and the undershoot is either absent or much reduced. Salivary glands will continue to respond to 5-HT in such a constant and repeatable fashion for several hours.

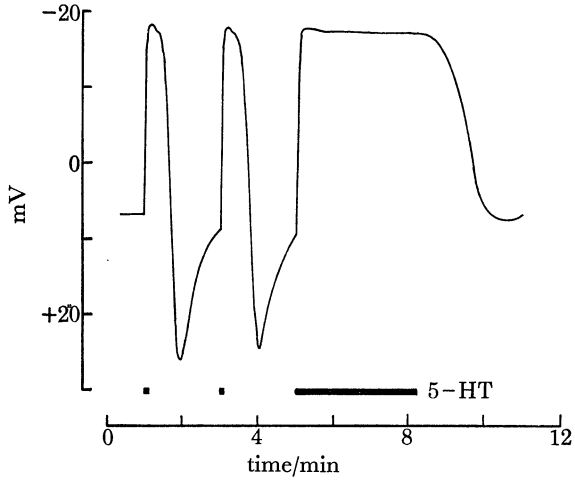

FIGURE 6. The electrical response of an isolated salivary gland to 10^{-8} mol/l 5-HT applied for 10 s (short bars) or 100 s (long bar).

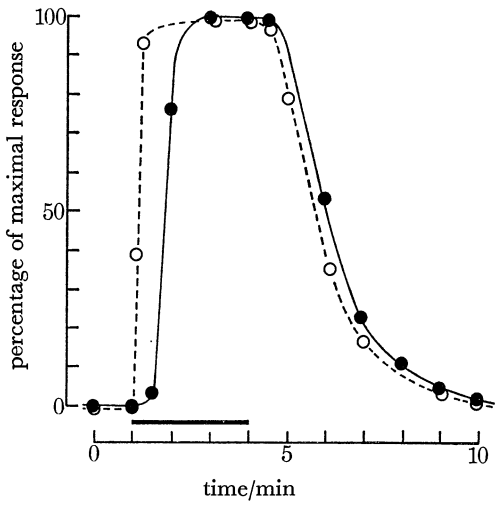

FIGURE 7. Comparison of the electrical response (○) and the change in rate of fluid secretion (●) induced by a 4 min infusion of 10^{-8} mol/l 5-HT (horizontal bar). All the values were expressed as a percentage of the maximal response.

The electrical response to 5-HT was very much faster than the increase in rate of fluid secretion (figure 3) measured using the *in vitro* method described earlier (figure 2). In order to make this comparison more accurate, rate of fluid secretion has been measured in the same perfusion bath used to record potentials. These experiments confirm that the electrical response is completed long before the acceleration of secretion begins (figure 7). This lag may be caused by a dead space in the lumen which must be filled with fluid before there is enough secretion pressure to force saliva out into the liquid paraffin. Another possibility is that after 5-HT has

acted on the gland additional events, such as an increase in the level of cyclic AMP, must be completed before an increase in fluid secretion can begin. Further experiments will be necessary to decide between these alternatives. The rate of recovery after removing 5-HT, however, is the same for both the potential and rate of secretion (figure 7).

Having characterized the electrical events associated with 5-HT stimulation, we then tried cyclic AMP fully expecting to obtain an exact replica of the 5-HT response. Such expectations were short-lived because cyclic AMP produced a change in electrical potential which was almost the exact opposite of that produced by 5-HT (figure 8). Instead of going negative, the

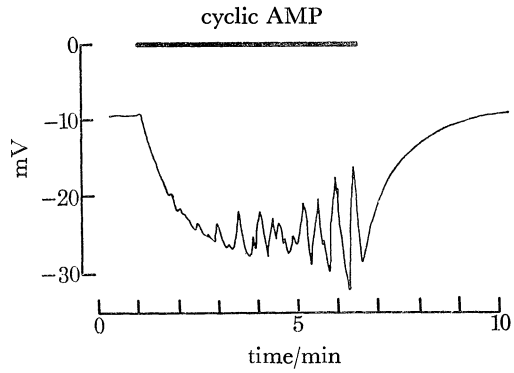

FIGURE 8. The electrical response of an isolated salivary gland to 10^{-2} mol/l cyclic AMP.

luminal potential went more positive and once the potential had levelled off, oscillations of variable frequency and amplitude were sometimes observed. These oscillations rapidly cease and the potential returns to the unstimulated level 2 min after cyclic AMP is withdrawn. Therefore, although cyclic AMP can mimic the ability of 5-HT to stimulate secretion (figure 3), the associated electrical events are very different.

One way to account for this discrepancy on the basis of the cyclic AMP hypothesis, is to assume that 5-HT has a direct effect on ion transport in addition to stimulating the synthesis of cyclic AMP. The simplest interpretation of the electrical recordings is that cyclic AMP increases cation movement while 5-HT increase the transport of anions. Therefore, when 5-HT is added to a gland, it not only increases anion transport directly, but it also acts to increase cation transport indirectly by stimulating adenyl cyclase to synthesize more cyclic AMP. Since the overall electrical change is an increase in negativity, the increase in anion transport must override the increase in cation transport. However, when cyclic AMP acts in the absence of 5-HT, there is an increased positivity because cation transport will be accelerated in the absence of a corresponding increase in anion transport.

A simple way of testing this hypothesis is to study the change in electrical events which occur when theophylline stimulates fluid secretion (figure 9). Since theophylline inhibits the enzyme responsible for degrading cyclic AMP, an elevated cyclic AMP level will develop without the mediation of 5-HT. The above hypothesis would predict an increase in positivity which is precisely what is found experimentally (figure 9). The change in potential has a very similar time course to the change in rate of fluid secretion which is also illustrated in figure 9. Five minutes after applying theophylline there is little visible change in either parameter. This long delay probably depends on a slow synthesis of cyclic AMP by the unstimulated adenyl cyclase. Salivary glands soon recover from the prolonged treatment with theophylline and apparently suffer no ill-effects because subsequent 5-HT responses are quite normal.

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Another way of illustrating the independent action of 5-HT and cyclic AMP is to test the effect of 5-HT when all the chloride in the bathing medium is replaced with a less permeable anion such as isethionate (figure 10). Perfusion with the control isethionate medium has little effect on the potential of unstimulated glands, but when 5-HT is added there is a sudden and large increase in positivity instead of the increase in negativity produced in a chloride medium (figure 6). The direct effect of 5-HT on anion transport is prevented by the presence of an

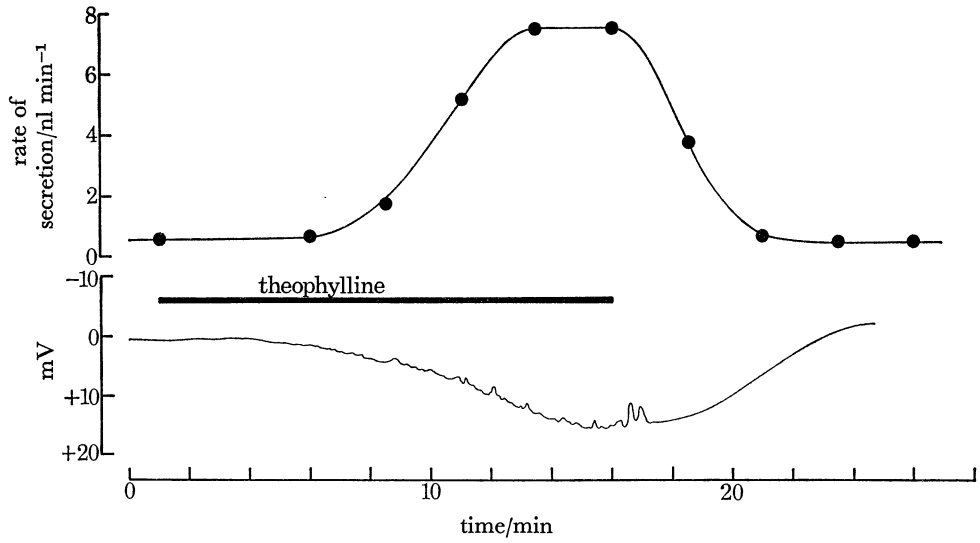

FIGURE 9. The effect of 10^{-2} mol/l theophylline (horizontal bar) on rate of fluid secretion (nl/min) and the potential (mV) across isolated salivary glands.

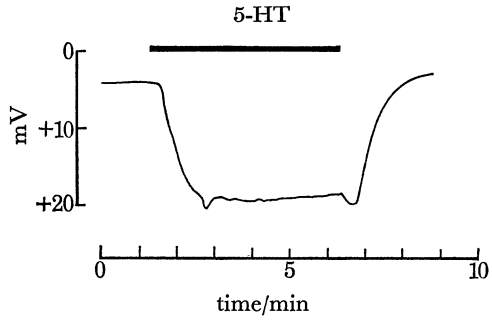

FIGURE 10. The electrical response of isolated salivary glands to 10^{-8} mol/l 5-HT (horizontal bar) when all the chloride in the saline was replaced with isethionate.

impermeant anion while the indirect effect of 5-HT on increasing the level of cyclic AMP results in the increased positivity which appears to characterize the independent action of the latter compound. The potential soon returns to the unstimulated level when 5-HT is withdrawn (figure 10).

All our current observations (summarized in figure 11) are consistent with the idea that 5-HT has a direct effect on ion movement in addition to its ability to stimulate adenyl cyclase. In an unstimulated cell there is a slow synthesis of cyclic AMP by adenyl cyclase (1), and the PDE (2) is capable of keeping the intracellular concentration at an unstimulated level (u.l.) which is below the threshold level necessary to stimulate secretion (figure 11*a*). 5-HT acts to increase the transport of both cations and anions (figure 11*b*). The first effect is achieved

through stimulating adenyl cyclase (1) to raise the concentration of cyclic AMP to a stimulated level (s.l.) and cation transport is increased. At the same time, 5-HT also increases anion transport directly which accounts for the sudden increase in negativity induced by 5-HT. The intracellular level of cyclic AMP can be raised artificially by either adding this compound to the bathing medium (figure 11*c*) or by using theophylline to inhibit its breakdown by PDE

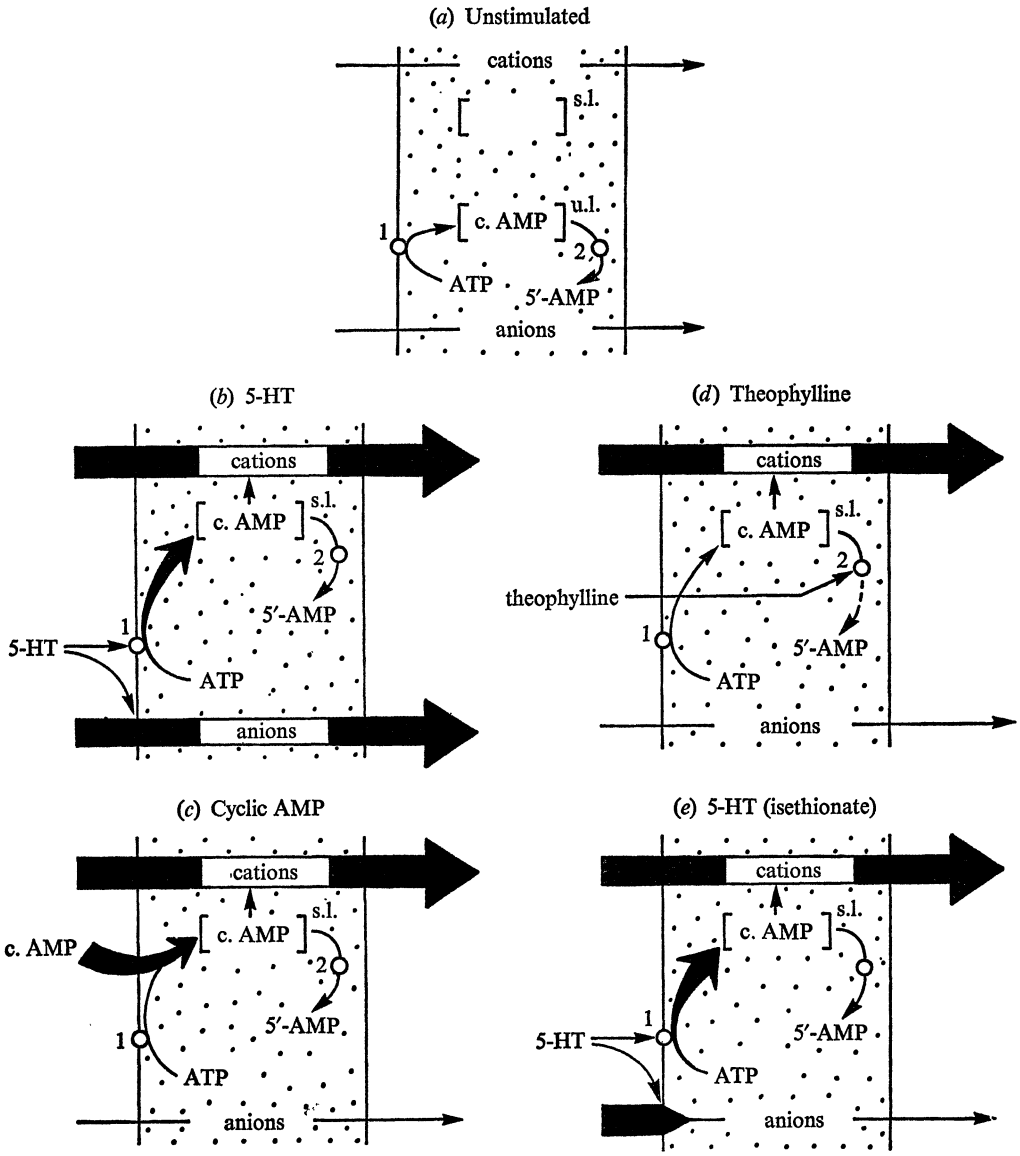

FIGURE 11. Diagram to represent the proposed mode of action of 5-HT and cyclic AMP (c. AMP). (For further details see text.)

(figure 11*d*). In both cases, the observed increase in positivity is consistent with an action of cyclic AMP on cation transport in the absence of the 5-HT-dependent increase in anion movement. In the last case (figure 11*e*), 5-HT acts normally to increase both the cyclic AMP level and anion transport, but the latter effect cannot be expressed because chloride has been replaced by isethionate. However, the unimpeded increase in cation transport results in a large increase in positivity (figure 11*e*).

Conclusion

The action of most hormones depends on a series of sequential events which lead to a carefully regulated change in cellular activity. Specific receptors on the cell are responsible for detecting the hormonal signal and transmitting the information to the cell. In many cells the transduction of a successful hormone–receptor interaction involves a stimulation of the enzyme adenyl cyclase which synthesizes cyclic AMP from ATP. The resulting increase in intracellular cyclic AMP concentration is then responsible for mediating the further actions of the hormone. In the case of salivary glands, therefore, the apparent role of cyclic AMP in mediating the action of 5-HT has much in common with the control mechanisms found in many other systems.

The most important feature to emerge from the present studies is that 5-HT may have a direct effect on membrane function in addition to stimulating the synthesis of cyclic AMP. The simple hypothesis that 5-HT affects anion transport whereas cyclic AMP increases cation transport appears to satisfy all our current observations. Further information on the ionic basis of fluid secretion will be required before the electrical events recorded during the action of 5-HT and cyclic AMP can be discussed in greater detail. However, these observations do indicate that although cyclic AMP undoubtedly plays a central role in regulating the activity of many cells, the possibility that the primary hormone may have a direct effect on cell function should not be ignored.

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