

THE EFFECTS OF 5-HYDROXYTRYPTAMINE AND CYCLIC AMP ON THE POTENTIAL PROFILE ACROSS ISOLATED SALIVARY GLANDS

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INTRODUCTION

In two previous papers we have described changes in the transepithelial potential of the salivary gland of the adult blowfly (*Calliphora*) produced by 5-hydroxytryptamine (5-HT), cyclic 3',5'-adenosine monophosphate (cyclic AMP) and theophylline (Berridge & Prince, 1971, 1972).

In the resting state the lumen of the gland was slightly positive (about 4 mV) with respect to the bathing medium but when 5-HT was added the potential became negative. Cyclic AMP is a postulated intermediate in the action of 5-HT (Berridge & Patel, 1968; Berridge, 1970) on this tissue yet it does not produce the same potential responses as 5-HT (Berridge & Prince, 1971, 1972). On addition, cyclic AMP causes increased positivity of the lumen of the gland in contrast to the negativity produced by 5-HT. The response to cyclic AMP is simulated by theophylline and by 5-HT if the chloride in the bathing medium is replaced by less permeant anions such as isethionate and sulphate. On the basis of these results it was concluded that 5-HT has at least two actions on this tissue. One action is to increase cation transport into the lumen through a process regulated by cyclic AMP. The other action is to increase anion movement by a mechanism that does not involve cyclic AMP directly and somehow apparently overrides any effects on potential produced by increased cation transport.

For simplicity, previous discussions treated the epithelium as a single layer; but, by analogy with other transporting epithelia, the salivary gland should be considered as a three-compartment system consisting of the external bathing medium, the intracellular compartment and the saliva separated from one another by membranes. The transepithelial potential changes caused by 5-HT, cyclic AMP and theophylline are the net result of changes at both membranes. Before developing any hypothesis for the mechanism of secretion in this tissue it is essential to determine the contribution made by each membrane to the total transepithelial response.

In this paper the results of an investigation into the site of potential changes produced by 5-HT and cyclic AMP are reported and discussed.

METHOD

The abdominal portion of the salivary glands from adult *Calliphora* were isolated and set up in a Perspex chamber as described previously (Berridge & Prince, 1972).

Transepithelial potential measurements were obtained by connecting each outer chamber of the bath to a Keithley electrometer (type 602) via an agar bridge and calomel half-cell. Responses were displayed on an oscilloscope (Tektronix type 502A) and recorded on a pen recorder (Fig. 1). Potentials were also measured using glass microelectrodes filled with 3M-KCl. The microelectrodes that were selected for use had tip resistances between 15 and 25 M Ω (tip potential < 5 mV) as these were found

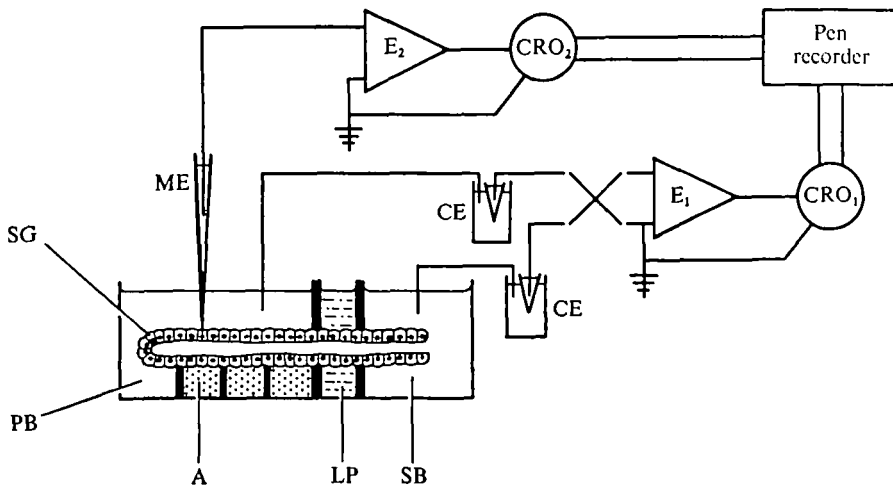


Fig. 1. The circuit diagram of the system used for simultaneous measurement of intracellular and transepithelial potentials. For transepithelial potential measurement the perfusion bath (PB) and salivary bath (SB) were connected via agar bridges and calomel cells (CE) to a Keithley electrometer (E_1). This potential was displayed on one beam of an oscilloscope (CRO_1) and recorded by a pen recorder. Intracellular potentials were recorded using a microelectrode (ME) connected to an impedance converter (E_2), displayed on the second beam of the oscilloscope (CRO_2) and recorded by the pen recorder. Agar blocks (A) supported the salivary gland (SG) during penetrations. LP, Liquid paraffin.

to allow easy penetration and maintenance of intracellular potentials. The criteria for successful penetration were that the potential should appear suddenly, should be maintained and should fall abruptly to zero when the electrode was withdrawn. For recording, a microelectrode was connected to a calomel half-cell. This was connected to an impedance converter whose output was connected to the second channel of the pen recorder and to the oscilloscope (Fig. 1). The potential was measured with reference to earth potential and, because either reference electrode in the two outer baths could be earthed, the potential could be measured across the basal membrane with reference to the perfusion bath or across the apical membrane with reference to the saliva bath. Potentials recorded by the microelectrode could be displayed and recorded simultaneously with the transepithelial potential recorded between the two outer chambers of the tissue bath. Agar blocks, which were held between grooved Perspex baffles, supported the gland during penetration and improved the circulation of fluid within the perfusion bath.

The physiological saline was the same as that used previously (Berridge & Prince, 1972) and had the following composition (mM/l): Na 155, K 20, Ca 2, Mg 2, Cl 156, phosphate 7, malate 2.7, glutamate 2.7, citrate 1.8, glucose 10. Phenol red (< 0.01 mM/l) was routinely included in solutions to provide a continuous check that the pH was maintained between 7.2 and 7.4.

RESULTS

The potential profile. When a microelectrode was inserted into a gland an initial step of -44 mV was recorded with reference to the perfusion medium. In 131 penetrations the average potential of this first step was found to be -44.2 ± 0.2 mV (s.e. of mean). This potential could be held for several hours. Although this potential was

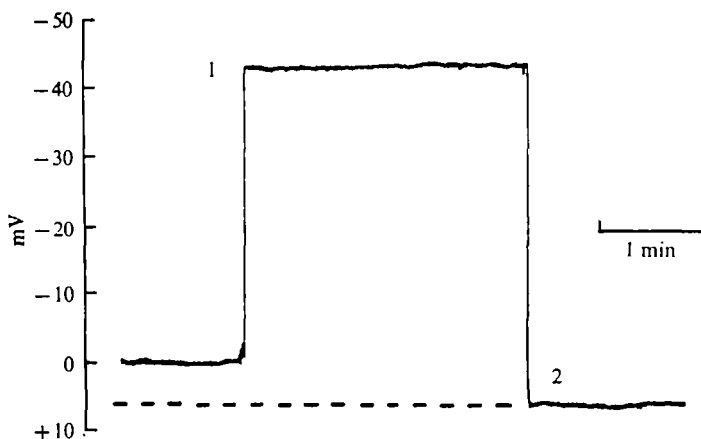


Fig. 2. The potential profile seen by a microelectrode penetrating through the salivary gland epithelium. The first step (1) may correspond to the potential across the basal plasma membrane whereas the second step (2) probably records the potential across the apical membrane as the microelectrode enters the lumen. In the latter position the potential recorded by the microelectrode is identical to the transepithelial potential (dotted line) recorded independently by the two calomel electrodes (see Methods).

about 44 mV, the average transepithelial potential, reported in earlier papers, was only about 4 mV (Berridge & Prince, 1971). The difference between these two figures could be accounted for either by measuring the intracellular potential with reference to the electrode in the saliva bath or by advancing the microelectrode into the lumen.

When a microelectrode recording the first step was advanced towards the lumen of the gland the potential fell from a negative value to one that was equal to the total transepithelial potential so that in penetrating the epithelium the electrode recorded a two-step potential profile (Fig. 2). We believe that the first potential step is recorded on penetrating the cell and so corresponds to the basal membrane potential whilst the second step is recorded across the apical membrane. Dye injected from a microelectrode recording the first potential step was located within the cells, supporting the idea that a microelectrode recording this step was intracellular (Prince, 1971). There are two lines of evidence in further support of this belief. The first comes from experiments in which the potassium concentration in the perfusion medium was varied and the second comes from a comparison of the transepithelial responses with the potential

responses recorded by a microelectrode on addition of 5-HT or cyclic AMP. As described in the 'Methods' the point of reference for the microelectrode could be changed from one chamber of the tissue bath to the other. This procedure allowed constant monitoring of the electrode position in relation to the two-step potential profile.

To simplify writing, the terminology adopted for the remainder of this paper uses 'basal membrane potential' to refer to the potential measured by a microelectrode

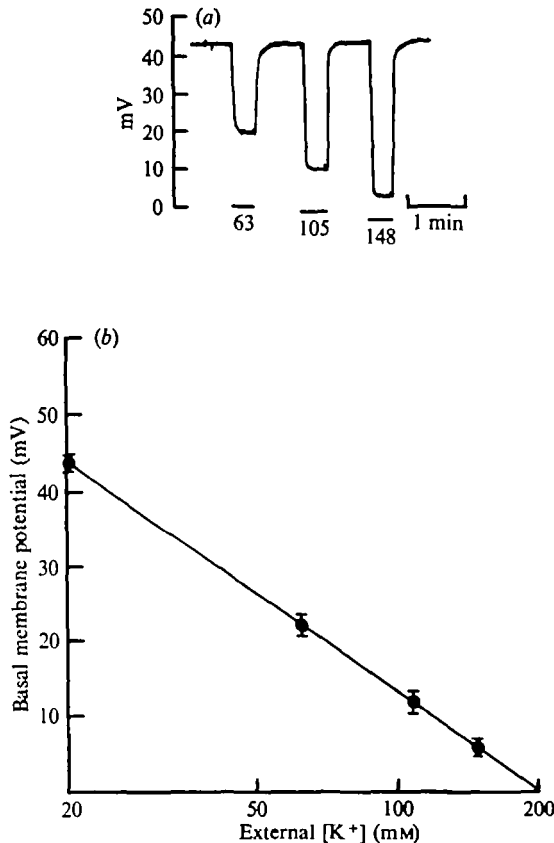


Fig. 3. (a) The effect of changes in the external potassium concentration on the basal membrane potential. Solutions containing 63, 105 and 148 mM potassium replaced the normal saline, containing 20 mM potassium for the duration of the bars. (b) Basal membrane potential (mV) as a function of the potassium concentration (mM) in the bathing medium. Each point represents the average potential of eight cells and the standard errors are shown.

recording the first potential step and, 'apical membrane potential' for the potential recorded when the reference point of a microelectrode recording the first potential step was changed to the saliva bath so that the second step could be recorded without moving the microelectrode.

The effect of changes in potassium concentration. The potassium concentration in the perfusion medium was altered over a range of 20–150 mM and the sodium concentration was altered accordingly to maintain isotonicity. When solutions containing concentrations of potassium higher than 20 mM were perfused over the gland the

basal membrane was rapidly depolarized. A new equilibrium was established in less than 10 sec (Fig. 3*a*). The basal membrane repolarized when the glands were returned to 20 mM potassium. When the potentials observed at different potassium concentrations were plotted as a function of the logarithm of the potassium concentration in the perfusion medium the potentials lay on a straight line with a slope of 43.5 mV for a tenfold change in potassium concentration (Fig. 3*b*).

The response to 5-HT. When the transepithelial potential was recorded, addition of 10^{-8} M 5-HT caused the potential to become negative and to remain negative as long as 5-HT was in contact with the tissue (Fig. 4). This response was similar to that described previously (Berridge & Prince, 1971, 1972).

When the basal membrane potential was recorded by a microelectrode, addition of 5-HT caused this membrane to show a slow hyperpolarization of up to 5 mV (Fig. 4). The hyperpolarization was less than 1 mV with a 5-HT application of 10 sec but with a 150 sec application the membrane became hyperpolarized by 5 mV within 1 min of 5-HT application and remained hyperpolarized until 5-HT was removed. On removal of 5-HT the potential recovered to its resting level. The hyperpolarization across



Fig. 4. Simultaneous records of the responses of the transepithelial (top trace) and basal membrane potentials (bottom trace) to 10^{-8} M 5-HT applied for 10, 20 and 150 sec as indicated by the bars. Note that the bottom trace is recorded at five times the sensitivity of the top trace.

the basal membrane developed more slowly than the transepithelial potential change. Whereas the basal membrane potential increased by up to 5 mV in Fig. 4 the transepithelial potential change produced on addition of 5-HT was up to 25 mV, suggesting that the response recorded across the whole epithelium originated from a change in the apical membrane potential. This was verified by measuring the apical membrane potential.

When the apical membrane potential was measured, addition of 5-HT caused depolarization of this membrane by a value that corresponded to the negative-going step seen in the transepithelial response (Fig. 5*a*). With short applications of 5-HT the apical membrane was first depolarized and then hyperpolarized before returning to the resting potential (Fig. 5*b*). The responses recorded across the apical membrane with the microelectrode were mirror images of the transepithelial responses. Depolarization and hyperpolarization of the luminal membrane corresponded to negativity and increased positivity of the transepithelial potential respectively. Fig. 5*b* also illustrates the repeatability of the responses to 5-HT. When the microelectrode was in the lumen, potential changes recorded with reference to the perfusion medium were the same as

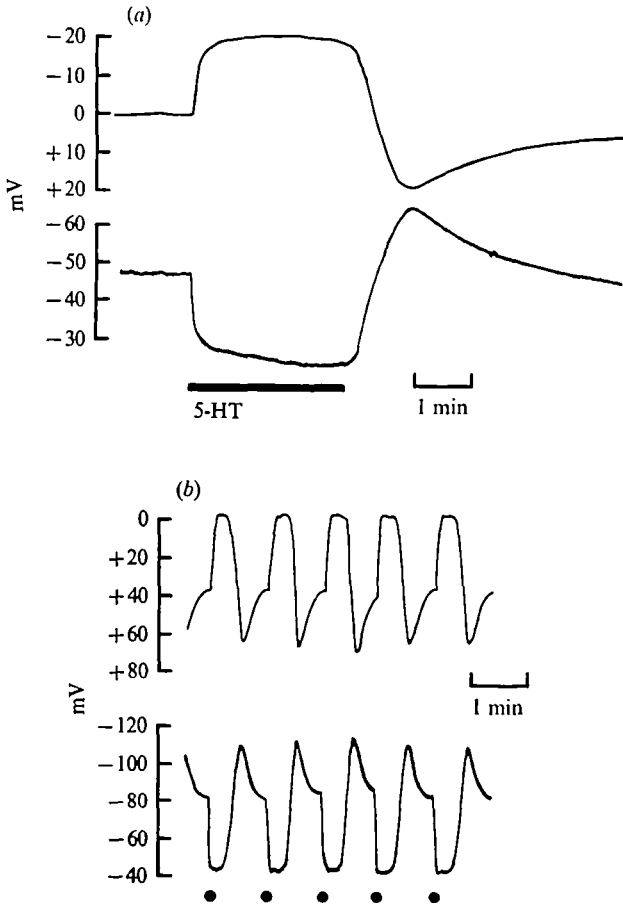


Fig. 5. (a) Simultaneous records of the responses of the transepithelial (top trace) and apical membrane (bottom trace) potentials to 5-HT (10^{-8} M) applied for the duration of the bar. (b) Simultaneous records of the responses of the transepithelial (top trace) and apical membrane (bottom trace) to 10^{-8} M 5-HT applied for 7 sec at each dot.

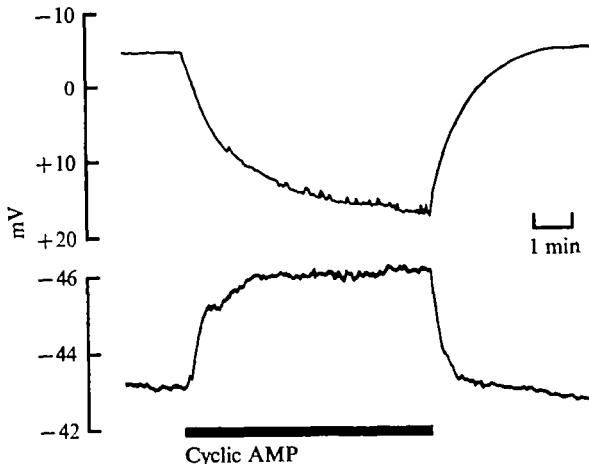


Fig. 6. The effect of 10^{-8} M cyclic AMP on transepithelial (top trace) and basal membrane (bottom trace) potentials. Cyclic AMP was applied for the duration of the bar.

transepithelial changes and no change was recorded when the saliva bath was used as a reference point.

The response to cyclic AMP. As described previously, addition of cyclic AMP caused increased positivity of the transepithelial potential (Fig. 6). As with the response to 5-HT the site of this potential change was found to be at the apical membrane. Thus, when the basal membrane potential was measured, 10^{-8} M cyclic AMP caused a small hyperpolarization of 3 mV. This was similar to that seen on addition of 5-HT. The membrane remained hyperpolarized as long as cyclic AMP was in the perfusion bath and returned slowly to the resting potential on removal of cyclic AMP (Fig. 6).

When the apical membrane potential was measured, addition of cyclic AMP caused a large hyperpolarization of this membrane (Fig. 7). In Fig. 7 hyperpolarization of the apical membrane corresponded to an increase in positivity of the transepithelial potential. The time course of the apical and transepithelial responses were similar. In some preparations (as in Fig. 7) the transepithelial potentials showed oscillations and these were seen as oscillations in the apical membrane potential.

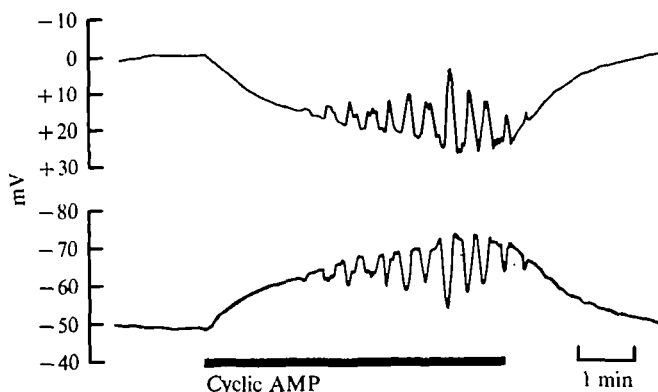


Fig. 7. Simultaneous records of the responses of the transepithelial (top trace) and apical membrane (bottom trace) potentials to 10^{-8} M cyclic AMP applied for the duration of the bar.

DISCUSSION

The effects of 5-HT and cyclic AMP on the potential profile across *Calliphora* salivary glands show that the transepithelial potential changes recorded earlier (Berridge & Prince, 1971, 1972) are mainly restricted to changes across the apical plasma membrane. The potential profiles seen under different conditions by a microelectrode are summarized in Fig. 8. Unstimulated cells are about 44 mV negative to the perfusing medium. On average such cells are slightly more negative with respect to the lumen such that the average transepithelial potential for 84 glands was 4.0 ± 1.0 mV lumen positive with respect to the perfusion medium (Berridge & Prince, 1972). The increase in lumen negativity observed during the action of 5-HT was due to a marked depolarization of the apical plasma membrane whereas cyclic AMP had the opposite effect (Fig. 8). That is, cyclic AMP caused the apical membrane to become hyperpolarized and this resulted in the lumen becoming positive with respect to the perfusion medium. Analysis of these potential profiles provides further information on the mode of action of cyclic AMP and 5-HT and on the ionic mechanisms underlying secretion.

In earlier papers, where only transepithelial potentials were measured, we suggested that 5-HT has at least two actions only one of which is mediated by cyclic AMP (Berridge & Prince, 1971, 1972). One action is to increase anion movement which produces lumen negativity by a mechanism which is independent of cyclic AMP. The second action is to increase cation movement using cyclic AMP as an intermediary. This latter action is produced by 5-HT when the anion dependent action is suppressed by replacing chloride with less permeant anions such as isethionate (Berridge & Prince, 1971, 1972). The positive undershoot observed during responses to short applications of 5-HT (Figs. 4 and 5*b*) may also represent the ability of cyclic AMP to stimulate cation transport. The results summarized in Fig. 8 confirm that 5-HT has two actions and that these effects take place primarily across the apical plasma membrane. The problems that have to be answered are: what causes the potential across this luminal membrane and how is it modified to produce the hyperpolarization and depolarization observed during the action of cyclic AMP and 5-HT?

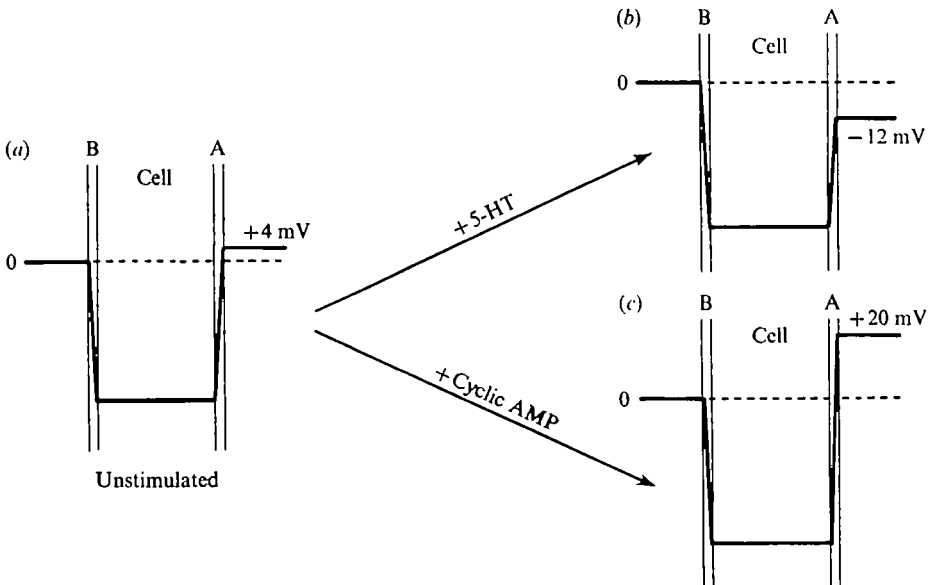


Fig. 8. Diagram illustrating the potential profiles across the salivary gland in the unstimulated state (a) and after addition of 5-HT (b) or cyclic AMP (c). The potential values are approximate. B, Basal membrane; A, apical membrane.

The microelectrode results show that under all conditions the lumen of the gland is always positive with respect to the cell. The very high concentration of potassium in the saliva (155 mM) (Oschman & Berridge, 1970) rules out the possibility that the potential across the apical plasma membrane is a potassium diffusion potential. At present, there are no measurements of the internal potassium concentration but it cannot be much higher than that in the lumen otherwise the cell would be hypertonic to the perfusion medium. Since this would be contrary to current concepts of isosmotic fluid transport (Diamond & Bossert, 1968), we can assume that the concentration of potassium in the cell is similar to or less than that in the saliva. As the lumen is positive to the cell, the distribution of potassium across the apical membrane cannot be passive and the presence of some active process is suggested. The ion pumped is unlikely to be

sodium as there is very little sodium in the saliva (Oschman & Berridge, 1970). If sodium was the prime mover then there would have to be a mechanism exchanging luminal sodium for intracellular potassium in addition to a sodium pump. All the evidence we have, therefore, supports the hypothesis that the origin of the apical membrane potential is an electrogenic potassium pump. Potassium pumps have been postulated in other insect systems (Harvey & Nedergaard, 1964; Nedergaard & Harvey, 1968; Berridge, 1968; Maddrell, 1969). In a resting state the positive potential developed across this membrane would depend on potassium transport out of the cell across a membrane that is relatively impermeable to anions.

The results of earlier experiments indicated that the 5-HT-induced lumen negativity was chloride dependent. Addition of 5-HT in a chloride-free saline caused lumen positivity and not negativity (Berridge & Prince, 1971, 1972). At least two possibilities exist for the mechanism of depolarization. 5-HT could increase the permeability of the apical membrane to chloride so that the membrane goes towards its chloride equilibrium potential. For this to occur, the chloride concentration within the cell would have to be higher than that predicted by the potential across the basal membrane, suggesting that the distribution of chloride across this latter membrane is not entirely passive. Another possibility is that 5-HT allows chloride to neutralize the electrogenic potassium pump by increasing the degree of linkage between potassium and chloride movement. The idea of a separate chloride pump at the apical membrane is excluded because, first, there is a lag time between the production of lumen negativity and secretion, secondly, both 5-HT and cyclic AMP can produce secretion, and as cyclic AMP induces a different potential response another pump, in addition to chloride pump, would have to be proposed to explain the secretion produced by cyclic AMP; and thirdly, the action of 5-HT producing negativity and reversal of the 5-HT response in isethionate by chloride are very rapid (the half time is 5–10 sec) compared with the potential effects of 5-HT in chloride-free saline or of cyclic AMP (the half time of these responses is at least 30–40 sec). Both these latter responses follow the curves for stimulation of secretion produced by these respective compounds.

Hyperpolarization of the apical membrane could be produced by stimulation of the potassium pump by cyclic AMP. Thus, when 5-HT was added in the absence of a permeant anion such as chloride the transepithelial potential went more positive but as soon as chloride was added this effect was abolished with the potential becoming negative (Berridge & Prince, 1972).

On the basis of this model the driving force for secretion would be the potassium pump stimulated by 5-HT using cyclic AMP as an intermediate. In addition, 5-HT has another action, which is independent of cyclic AMP, on chloride movement and allows chloride to follow electrogenically pumped potassium so causing depolarization of the luminal membrane. Water would follow the movement of these ions.

These changes seen at the apical membrane must be matched by changes across the basal membrane since the flux of ions across this membrane must be stimulated to replace ions pumped out of the cell across the apical membrane. Addition of 5-HT or cyclic AMP caused small but consistent hyperpolarizations of the basal membrane. This hyperpolarization might correspond to the secretory potentials recorded across the basal membrane of vertebrate salivary glands (Petersen & Poulsen, 1969) and the potential change produced by noradrenaline in the liver (Haylett & Jenkinson, 1969).

A significant aspect of this hyperpolarization in *Calliphora* salivary glands was its very slow onset compared to the change in potential across the apical membrane. Another important point is that both 5-HT and cyclic AMP produce similar hyperpolarizations even though their effects on the apical membrane are so different. Perhaps the small hyperpolarizations seen at the basal surface during stimulation of secretion simply reflect a redistribution of ions caused by the increase in ion flux across the epithelium.

The only large potentials recorded across this basal membrane were caused by altering the potassium concentration in the bathing medium (Fig. 2). When the different potentials were plotted as a function of the logarithm of the potassium concentration a straight line with a slope of 43.5 mV was obtained. These results show that the potential of this membrane is dependent on the potassium gradient across it and the membrane is therefore permeable to this ion as it is in most other cells. However, as the slope is not 58 mV for a ten-fold change in potassium concentration, as predicted by the Nernst equation, other ions must contribute to the potential. We must await the results of further experiments before we can say more about the ionic basis of the potentials recorded across the basal surface.

SUMMARY

1. The sites of the transepithelial potential changes produced by 5-HT and cyclic AMP in the salivary gland of the blowfly (*Calliphora*) have been investigated.
2. Microelectrodes recorded a two-step potential profile across this epithelium. The cell was negative to both the bathing medium and the saliva.
3. The basal membrane potential was 44.0 ± 0.2 mV and was affected very little by application of either 5-HT or cyclic AMP.
4. The apical membrane was depolarized by 5-HT and in contrast was hyperpolarized by cyclic AMP.
5. On the basis of these results it is concluded that 5-HT has two actions: (a) to stimulate a potassium pump on the apical membrane using cyclic AMP as an intermediary, (b) to increase chloride movement by a mechanism not directly involving cyclic AMP.

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