J. Physiol. (1978), **274**, *pp.* 421–435
With 7 text-figures
Printed in Great Britain

THE CONTROL OF ENZYME SECRETION FROM FLY SALIVARY GLANDS

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(*Received 25 April 1977*)

SUMMARY

1. Stimulation of fluid secretion from fly salivary glands by 5-hydroxytryptamine (5-HT) is known to involve calcium and cyclic AMP. Isolated salivary glands were used to investigate the role of these second messengers in the control of enzyme (sucrase) secretion.

2. The protein component of secretion from isolated glands treated with 5-HT appears to be identical to that of saliva secreted by flies during feeding.

3. Stimulation of fluid secretion by 5-HT follows a definite dose-response curve, but there is no consistent relationship between the rate of enzyme secretion and the stimulating concentration of 5-HT.

4. Exogenous cyclic AMP causes secretion of enzymes as well as of fluid, thus mimicking the action of 5-HT. The phosphodiesterase inhibitor theophylline enhances the rate of 5-HT-stimulated enzyme secretion.

5. Removal of calcium from the bathing medium enhances enzyme secretion in response to 5 or 10 nM-5-HT but has no effect on enzyme secretion stimulated by 100 nM-5-HT or by cyclic AMP.

6. Addition of 0·1 mM-lanthanum to medium containing 2 mM-calcium mimics the effect of calcium-free solution on 5-HT-stimulated enzyme secretion.

7. The ionophore A 23187 causes secretion of both fluid and enzyme. The secretory rate is initially high but soon declines and ceases after about 40 min.

8. Enzyme secretion in response to 5-HT or to cyclic AMP is progressively inhibited as the concentration of potassium is increased from 10 to 80 mM. Secretion in response to A 23187 is initially inhibited by 80 mM-potassium but then partially recovers.

9. The rate of enzyme secretion appears to be affected by the intracellular concentrations of both calcium and cyclic AMP. It is possible that the rate of enzyme secretion increases as the intracellular calcium concentration rises, until the optimal calcium concentration is reached when further increase in the level of calcium progressively inhibits secretion. The optimal calcium concentration for enzyme secretion is lower than that for fluid secretion, and 5-HT normally causes maximal fluid secretion and submaximal enzyme secretion.

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INTRODUCTION

Isolated fly salivary glands have been used to study the role of the second messenger substances calcium and cyclic AMP (adenosine-3′,5′-monophosphate) in the regulation of fluid secretion by 5-hydroxytryptamine (5-HT) (see Berridge, 1975). These glands also secrete digestive enzymes (Hansen Bay, 1976), and the isolated glands have now been used to investigate the factors involved in controlling secretion of these enzymes.

The paired salivary glands of *Calliphora* are simple tubular organs composed of a single layer of cells surrounding a lumen. The distal region of these glands used for studies *in vitro* secrete at least three carbohydrases. Ultrastructural studies show that all the cells in this region are of one type and possess the membrane elaborations typical of fluid-secreting cells in this tissue (Oschman & Berridge, 1970), as well as features characteristic of protein-secreting cells. These features include an elaborate system of rough endoplasmic reticulum, Golgi complexes, and membrane-bound granules which are sometimes seen fused to the luminal plasma membrane, indicating that secretion occurs by exocytosis. The granules probably contain the secretory enzymes, since there is a strong correlation between the disappearance of the granules and of the enzymes during secretion, and of their reappearance during a subsequent fast (Hansen Bay, 1976).

The glands are not innervated, and secretion is controlled by a hormone which is thought to be 5-HT, released from neurosecretory axons into the blood which bathes the glands. There is no evidence to suggest that fluid and enzyme secretion can be regulated independently; saliva collected from the salivary duct of flies while they are feeding always contains enzymes, even if the ingested food requires no digestion (Hansen Bay, 1976). It appears that a single factor controls secretion of both fluid and enzyme from a single type of cell.

Preliminary experiments were performed to compare the secretory response of isolated glands to 5-HT with the secretion from glands *in vivo* in response to the natural stimulus of feeding. No differences could be detected in the response of glands, *in vivo* and *in vitro*, so further experiments were carried out on isolated glands to investigate the roles of calcium and cyclic AMP in the control of enzyme secretion.

Previous studies on fly salivary glands have shown that fluid secretion may be induced by 5-HT, by cyclic AMP and by the calcium ionophore A 23187, and have given rise to the following model for the control of fluid secretion by 5-HT (see Berridge, 1975). Interaction of 5-HT with its receptor on the basal membrane of the cell causes an increase in the intracellular concentrations of both calcium and cyclic AMP. The driving force for fluid secretion is a potassium pump on the luminal membrane which is activated by cyclic AMP. Calcium increases the chloride permeability of this membrane, thus allowing chloride ions to follow the potassium into the lumen and preserve electroneutrality. Cyclic AMP also causes release of calcium from internal stores, and during treatment with 5-HT in calcium-free solution cyclic AMP mobilizes these stores of calcium and secretion continues, although at a reduced rate, for up to 90 min until these stores are exhausted. Secretion then ceases but can be restored to the original level by readdition of calcium to the bathing medium.

METHODS

Animals. Adult female blowflies, *Calliphora erythrocephala* Meig. from the laboratory culture were maintained on water, sucrose and heart. Flies were starved for 16 h before each experiment to allow sufficient enzyme to accumulate in the salivary glands. To reduce variation due to age, only flies aged between 7 and 15 days were used.

Experiments in vivo. Saliva was collected from the salivary ducts of flies as described in detail elsewhere (Hansen Bay, 1976). Flies were restrained, ventral side up, and the cuticle between head and thorax removed to expose the common salivary duct. The duct was caught up on a fine glass needle and severed. Salivation was induced by placing a crystal of sucrose on the open labellar lobe of the proboscis. Saliva accumulated around the glass needle and was collected in a siliconed fine capillary pipette.

Experiments in vitro. The abdominal portions of salivary glands were removed from flies and each gland was placed in a separate drop of Ringer solution under liquid paraffin and the open end drawn out into the paraffin by means of a silk ligature. Secreted fluid collected as a drop around the end of the gland (Oschman & Berridge, 1971). In order to measure the rates of secretion of both fluid and enzyme, the fluid secreted by each gland was collected every 5 min and its volume determined by measuring the diameter of the fluid drop using a calibrated graticule in the eyepiece of the microscope. The fluid was then mixed with 0·1 ml. distilled water in a test-tube and assayed for enzyme activity. Every 5 min throughout the experiment, the saline around each gland was removed and replaced by fresh saline, to ensure a continuous supply of oxygen and to prevent the accumulation of waste products. When saline of a different composition was applied, the gland was washed three times to prevent contamination by the previous saline. At the end of each experiment, each gland was homogenized in 0·1 ml. water in a small glass homogenizer, and its enzyme content determined. If any gland did not contain sufficient enzyme for secretion to continue for at least 30 min, secretion from that gland was discarded. All experiments were performed at room temperature (18–22 °C).

The Ringer solution had the following composition, unless otherwise stated: (m-mole/l.) NaCl, 128; KCl, 20; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4, 4; Na glutamate, 2·7; malic acid, 2·7; glucose, 10; pH adjusted to 7·2 with 27 m-moles NaOH. Phenol red (< 0·01 mM) was included in all solutions to provide a continuous check that the pH remained between 7·2 and 7·4. In 'calcium-free' solution, calcium was omitted and 5 mM-ethylene glycol-bis(β-amino ethyl ether)-N,N'-tetra-acetic acid (EGTA) added to ensure the absence of free calcium.

Enzyme assay. Carbohydrase activity for all three non-reducing substrates was determined by a modification of the amylase assay described by Robyt, Ackerman & Keng (1972). Each reaction tube contained 0·1 ml. diluted enzyme, 0·05 ml. 2 % substrate solution and 0·15 ml. 0·04 M-citric acid-disodium hydrogen phosphate buffer, at pH 6·0 and including 1·5 mM-NaCl for amylase assays, at pH 5·4 for other enzymes (Hansen Bay, 1976). The tubes were incubated for 10 min at 37 °C, and the reaction stopped by the addition of 0·4 ml. KCN solution (5·0 g KCN, 0·5 g Brij 35 (a wetting agent)/l.). After the addition of 0·8 ml. $K_3Fe(CN)_6$, solution (0·6 g $K_3Fe(CN)_6$ 20 g Na_2CO_3/l.), the tubes were incubated at 87 °C for 20 min and the colour read at 420 nm in a spectrocolorimeter (Model 151, Beckman Instruments Inc., Palo Alto, California). Loss of colour was proportional to the amount of reducing sugars up to 170 n-moles. Enzyme activity is expressed as n-moles glucose equivalents (nmole G) produced in 10 min at 37 °C.

Expression of results of experiments in vitro. In most experiments, the rate of enzyme secretion in response to the application of 10 nM-5-HT in normal Ringer was first determined to give the '5-HT-stimulated rate' to which the effects of other treatments could be compared. The samples collected at 5 and 10 min after the application of 5-HT were found to contain a very variable amount of enzyme which did not provide a reliable estimate of the subsequent rate of secretion, so the '5-HT-stimulated rate' was taken to be the mean of the enzyme content of the samples collected at 15 and 20 min. Note that the rates of secretion refer to the absolute amount of enzyme secreted, not to the concentration. Although the qualitative changes in enzyme secretion rate were similar in all glands subjected to a given treatment, there was considerable variation between individual glands in the amount of enzyme secreted (see Fig. 1). In order to minimize this variation and to give equal weight to the results from each gland, the '5-HT-stimulated rate', or some other specified rate, was taken as 100 %, and the amount of enzyme secreted in each 5 min period expressed relative to this value. For each experiment, the mean and standard error

of the mean of the results from n glands (n = 5–12) was calculated using these percentage values. Fluid secretion rates were treated in the same way.

Electrophoresis. Polyacrylamide gel electrophoresis was carried out using the microtechnique described by Amos (1976), using 3 μl. samples of fluid. The samples were diluted 1:1 with 0·02 % fluorescein solution containing 20 % glycerol, and run on 10 % gels, with a Tris-glycine running buffer at pH 8·3. Gels were stained for protein with Coomassie Brilliant Blue R (Sigma), destained, and then scanned with a Joyce-Loebl microdensitometer.

RESULTS

Secretion from salivary glands in vivo and in vitro

To compare secretion by glands *in vitro* and *in vivo*, fluid was collected from isolated glands treated with 10 nM-5-HT, and saliva was collected from the salivary ducts of feeding flies, and the enzymic contents analysed. Using the ferricyanide method to detect hydrolysis of non-reducing carbohydrate substrates, all three secretory enzymes, amylase, α-glucosidase and α-galactosidase (Hansen Bay, 1976) were found in both types of fluid. The contribution of α-galactosidase activity to the total enzyme activity was extremely small (< 2 %) so this enzyme was not assayed in subsequent experiments. Sucrase (α-glucosidase) activity usually exceeded amylase activity, although the ratio of these enzymes varied from fly to fly.

TABLE 1. Ratios of sucrose: amylase in secretion and in gland homogenates. Preparations 1–4 are isolated glands treated with 10 nM-5-HT, 5–8 are *in vivo* preparations in which salivation was induced by feeding

	Preparation no.							
Secretion at	1	2	3	4	5	6	7	8
10 min	1·4	2·4	1·2	2·8	2·4	2·9	1·6	0·8
20 min	1·3	2·5	1·4	2·9	2·3	3·0	1·3	0·8
30 min	1·2	2·5	1·2	2·8	—	—	—	—
40 min	1·4	2·4	1·2	2·9	—	—	—	—
Whole gland	1·3	2·4	1·3	2·8	2·5	3·0	1·4	0·8

Further experiment showed that the ratio of the two enzymes in secreted fluid reflected their ratio in the gland. Fluid was collected every 10 min from isolated glands treated with 10 nM-5-HT. After 40 min the glands were homogenized and the sucrase and amylase content of each sample of fluid and of the gland homogenate was determined. As shown in Table 1, in any one preparation both *in vivo* and *in vitro*, the ratio of enzymes in the secretion was similar to that in the gland homogenate. The ratio varied from 0·8 to 3·0 in different animals but the reason for this is not known. The rate of enzyme secretion also varied from fly to fly, but both *in vivo* and *in vitro* the rate of sucrase secretion was in the range 5–27 n-moles G/salivary gland. min.

In subsequent experiments *in vitro*, fluid was collected from each gland every 5 min instead of every 10 min, and the activity of only one enzyme, sucrase, determined to give a measure of the total enzyme activity.

From these analyses it appeared that the release of secretory enzymes occurred normally from isolated glands. To make sure that only the secretory enzymes were released, saliva and fluid secreted *in vitro* were subjected to microelectrophoresis on polyacrylamide gels and stained for protein. The densitometer scans of the two gels were identical, indicating that isolated glands release only the secretory enzymes.

To check that no release occurred over the basal membrane of the gland, the bathing medium was analysed for enzymes and protein but none were ever found.

Experiments in vitro

The response to 5-HT

Fig. 1 shows the response of five individual glands to three 20 min periods of stimulation by 10 nM-5-HT. After each period of stimulation, the 5-HT was removed and the glands allowed to secrete at the basal rate for 10 or 25 min. In the absence of

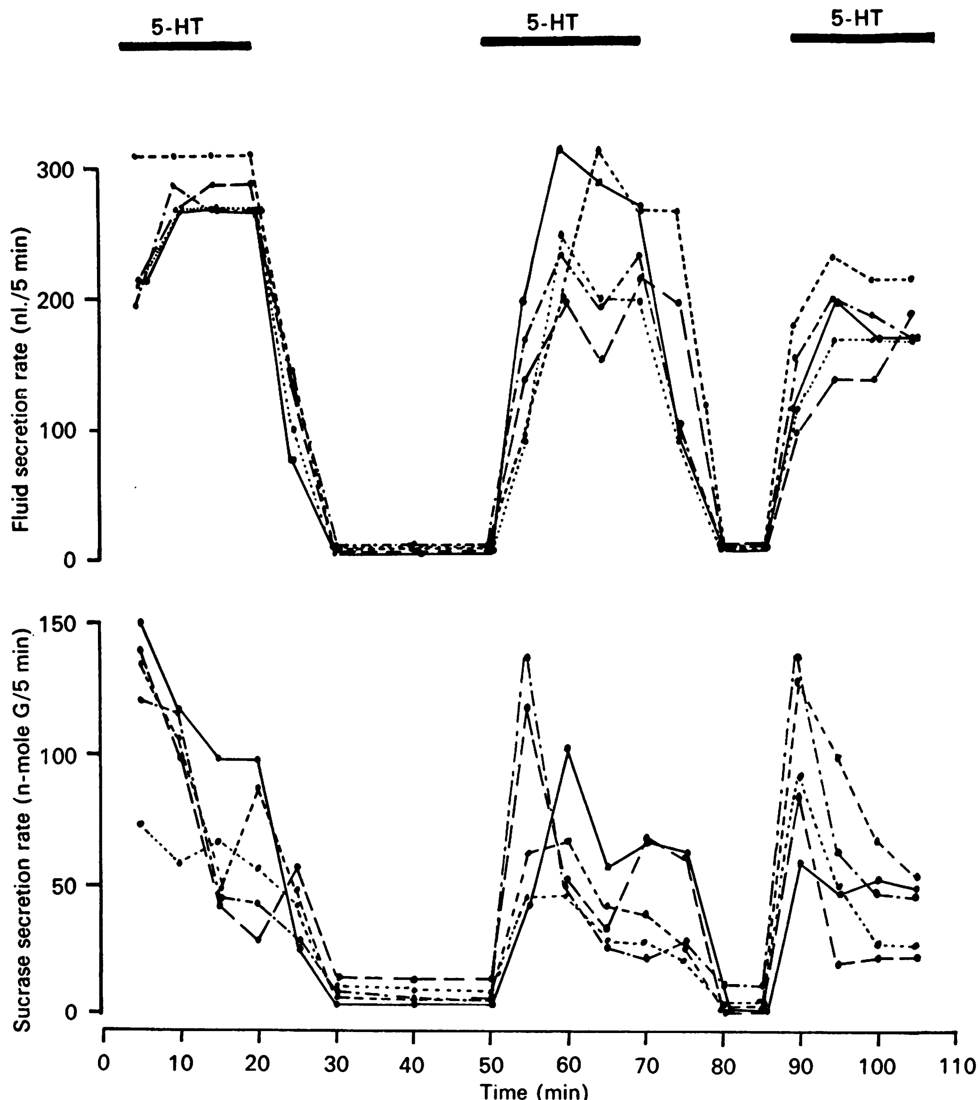

Fig. 1. Secretory responses of five individual glands to 10 nM-5-HT. 5-HT was applied for three 20 min periods as shown by the bars. Fluid was collected every 5 min during periods of stimulation, and at the end of each period of basal secretion. The basal rates of secretion are shown by the points at 40 and 85 min.

any stimulant the glands secreted both fluid and enzyme at a slow rate. Application of 5-HT caused a great increase in the rate of fluid secretion which remained fairly constant throughout the period of stimulation. The rate of enzyme secretion was highest in the first 5 min after treatment with 5-HT and then fell to a relatively steady level.

No consistent relationship could be found between the rate of enzyme secretion and the stimulating concentration of 5-HT, either when the same gland was treated sequentially with two different concentrations of 5-HT, or when the two glands of a pair were treated with two different doses of 5-HT. A given increase in the concentration of 5-HT sometimes increased the rate of enzyme secretion, sometimes decreased it.

Fig. 2 shows the secretory response of glands during an 85 min period of stimulation by 10 nM-5-HT. Application of 5-HT caused an initially high and variable rate of enzyme secretion which fell to a steady rate after 10 min. This steady rate was taken as 100 % for each gland, and secretion rates during the experiment expressed relative to this rate, as described in the Methods section. Secretion continued at the 100 % level for 25 min then gradually declined over the next 45 min. Fluid secretion continued at a steady rate throughout the experiment.

Stimulation by cyclic AMP

Cyclic AMP at a concentration of 10 mM stimulates fluid secretion from salivary glands, thus mimicking the action of 5-HT (Berridge, 1970), and it was also found to stimulate enzyme secretion at a rate very similar to the 5-HT-stimulated rate. Simultaneous application of 5-HT and cyclic AMP did not affect the secretion rate compared to the effect of either stimulant alone. The only apparent difference in the responses to cyclic AMP and 5-HT was the slower onset of secretion of both fluid and enzyme in response to cyclic AMP (Fig. 3).

The effect of theophylline

The methyl xanthine theophylline is a potent inhibitor of the phosphodiesterase responsible for the breakdown of cyclic AMP, and therefore increases the intracellular concentration of cyclic AMP. Fig. 2 shows the effect of 5 mM theophylline on the secretory response to 10 nM-5-HT. Theophylline was added to the bathing medium at 25 min and by 45 min the rate of enzyme secretion had increased 2-fold. Removal of theophylline caused a sharp peak in enzyme secretion before the rate fell to a low level. Fluid secretion was slightly inhibited by theophylline, although it has been shown that the drug potentiates the response to submaximal concentrations of 5-HT (Berridge, 1970).

The influence of cations on enzyme secretion

Calcium. Fig. 3 shows the effect of calcium-free solution on the secretory response to 5-HT and cyclic AMP. Four separate experiments are shown, one with 10 mM-cyclic AMP, three with different concentrations of 5-HT. The absolute values of the 100 % rates were very similar in all four experiments, despite the difference in stimulating agents. With cyclic AMP and 100 nM-5-HT, calcium-free solution had little effect on the rate of enzyme secretion, but as the concentration of 5-HT was reduced,

calcium-free solution markedly enhanced enzyme secretion. Fluid secretion was reduced in all four experiments. Readdition of calcium caused a peak in the rate of enzyme secretion before the rate fell to the initial level, while the rate of fluid secretion was restored to the original level.

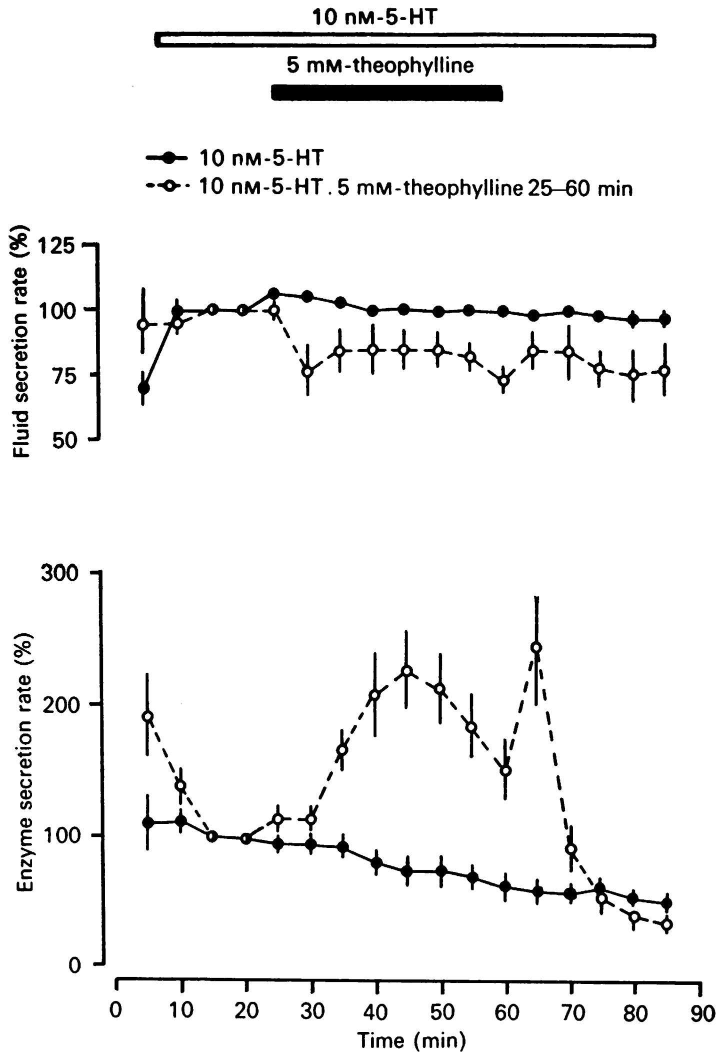

Fig. 2. Secretory responses to continuous stimulation by 10 nM-5-HT (filled circles) and to the addition of 5 mM-theophylline during treatment with 10 nM-5-HT (open circles). Theophylline was present as shown by the solid bar. In this and subsequent Figures, vertical bars represent the S.E. of the mean.

Raising the calcium concentration of the bathing medium from 2 to 20 mM had no effect on the secretory responses either to 5-HT or to cyclic AMP.

Lanthanum. In fly salivary glands the trivalent cation lanthanum appears to block the influx of calcium normally associated with stimulation by 5-HT (Prince & Berridge, 1973). As shown in Fig. 4, addition of 0·1 mM-lanthanum to saline containing

2 mM-calcium and 10 nM-5-HT mimicked the effect of calcium-free solution, by enhancing enzyme secretion and reducing fluid secretion. Removal of lanthanum led to a decline in enzyme secretion, but fluid secretion did not return to the initial level. Higher concentrations of lanthanum, 0·5 and 1 mM, had the same effect as 0·1 mM.

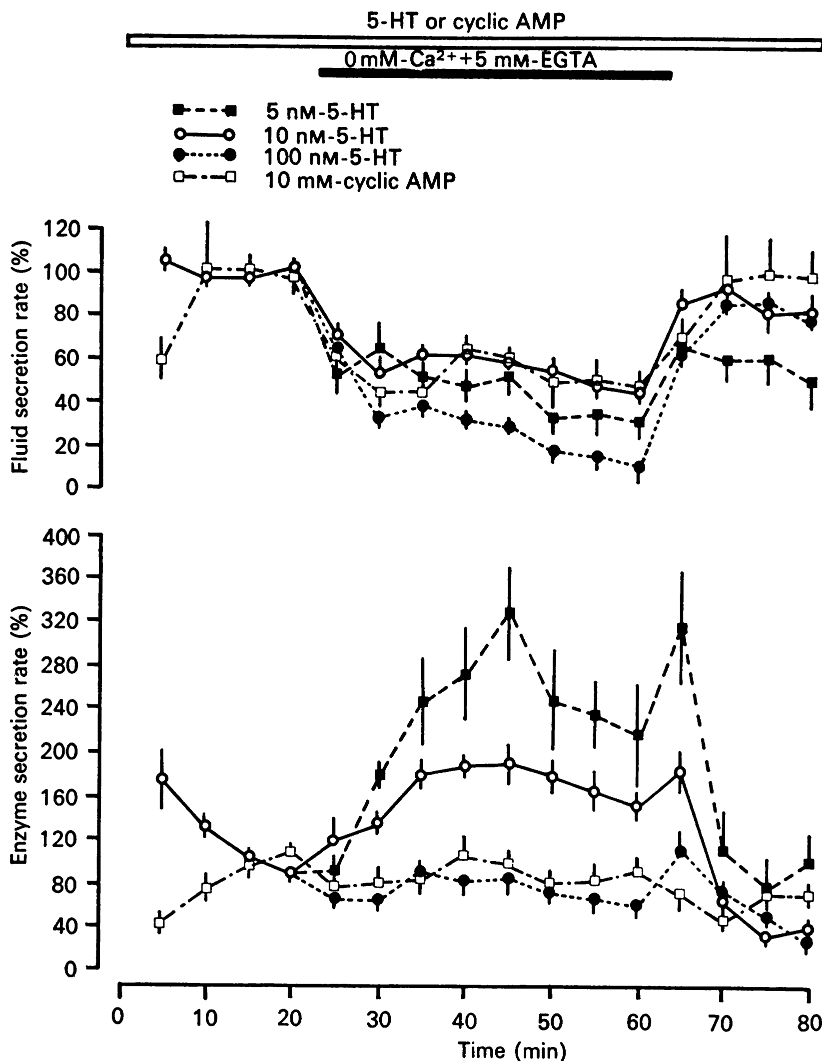

Fig. 3. The effect of calcium-free solution on the secretory response to 5-HT and cyclic AMP. Glands were stimulated throughout by 5-HT (100, 10 or 5 nM) or by 10 mM-cyclic AMP. For the duration of the solid bar, the normal Ringer containing 2 mM-Ca^{2+} was replaced by calcium-free medium containing 5 mM-EGTA. Secretory responses from all glands were 100 % between 10 and 20 min; only those stimulated by 10 nM-5-HT and by cyclic AMP are shown for the sake of clarity.

Magnesium. Secretion from salivary glands is unaffected by changes in the magnesium concentration of the bathing medium. Raising the concentration from 2 to 20 mM had no effect on the secretory responses to 10 nm-5-HT. To test whether the magnesium concentration affected the response to calcium-free solution, the

experiment of Fig. 3 was repeated using 10 nM-5-HT, with one gland of each pair exposed to 20 mM-magnesium, the other to magnesium-free solution. The rates of fluid and enzyme secretion from the two glands of each pair were identical and the normal responses to calcium-free solution were obtained.

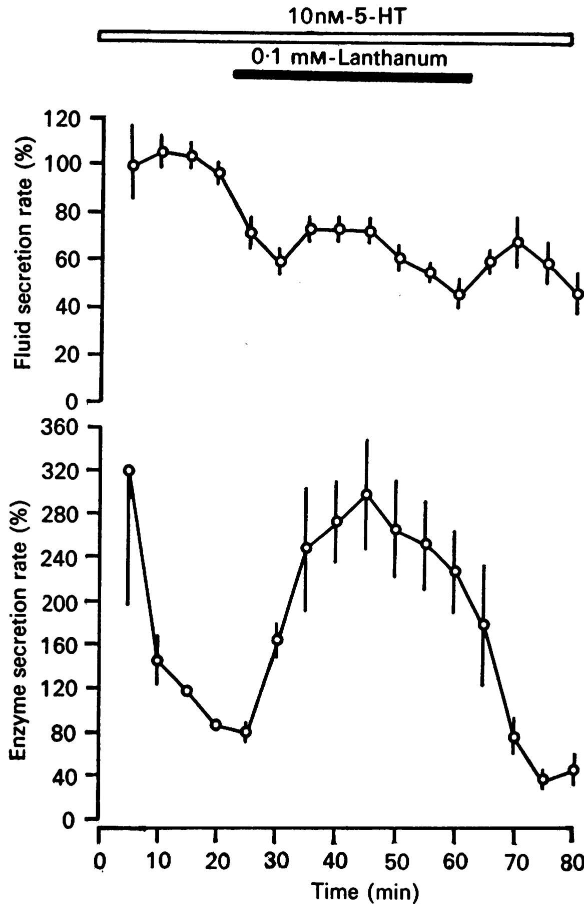

Fig. 4. The effect of lanthanum on the secretory response to 5-HT. Glands were stimulated throughout by 10 nM-5-HT (open bar) in normal Ringer containing 2 mM-Ca^{2+}. For the duration of the solid bar 0·1 mM-lanthanum was also present in the bathing medium.

Potassium. Changes in the potassium concentration of the bathing medium were balanced by changes in the sodium concentration since such changes, from 155 to 95 mM-sodium have no effect on the secretory rates of salivary glands. Lowering the external potassium concentration from 20 to 10 mM markedly enhanced 5-HT-stimulated enzyme secretion without affecting fluid secretion (Fig. 5). Increasing the potassium concentration inhibited enzyme secretion. Glands were treated with 10 nM-5-HT for 70 min. Between 25 and 45 min the saline contained 80, 60, 40, 30 or 10 mM-potassium instead of 20 mM. The results of these experiments were combined to give the relationship between enzyme secretion and potassium concentration (Fig. 6). The rate of enzyme secretion appears to be inversely proportional to the

log of the potassium concentration (Fig. 6*B*). Enzyme secretion stimulated by 10 mM cyclic AMP or by 10 nM-5-HT in the presence of 5 mM-theophylline was also inhibited by elevated potassium concentrations.

Stimulation by the ionophore A 23187. Glands were treated with 10 nM-5-HT for 20 min, washed for 20 min and then treated with 2 μM-A 23187. Fluid was secreted

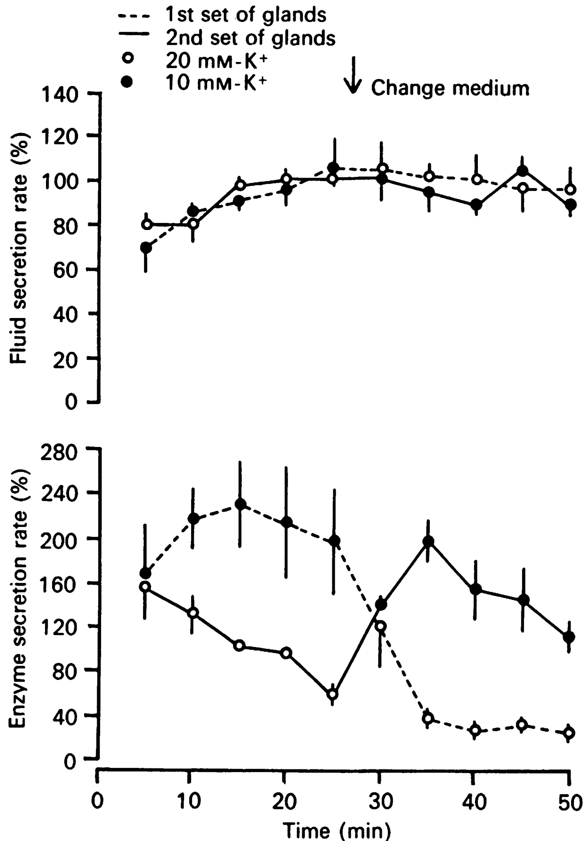

Fig. 5. The effect of potassium concentration on the secretory response to 5-HT. All glands were stimulated throughout by 10 nM-5-HT, one gland from each pair in 20 mM K^+, the other in 10 mM-K^+. The first set of glands was initially exposed to 10 mM-K^+, and at the arrow the medium was changed to 20 mM-K^+. The second set of glands was treated first with 20 mM then with 10 mM-K^+.

for 30 min at 40–50 % of the 5-HT-stimulated rate and then gradually ceased. Enzyme secretion was initially at the 5-HT-stimulated rate, but soon declined to a low level. Fig. 7 shows that A 23187 stimulates fluid and enzyme secretion from glands without prior treatment with 5-HT. Again, the rate of enzyme secretion declined after an initial peak. Fig. 7 also shows the effect of 80 mM-potassium on the response to A 23187. Enzyme secretion was initially much depressed, but recovered a little, while fluid secretion was greatly enhanced by the high potassium and then declined.

Since A 23187 greatly increases the permeability of the cell membrane to calcium, it was possible that the membrane might become leaky and permit release of enzymes over the basal membrane. However, no enzyme was detected in the bathing medium, indicating that enzyme secretion was still restricted to the luminal membrane.

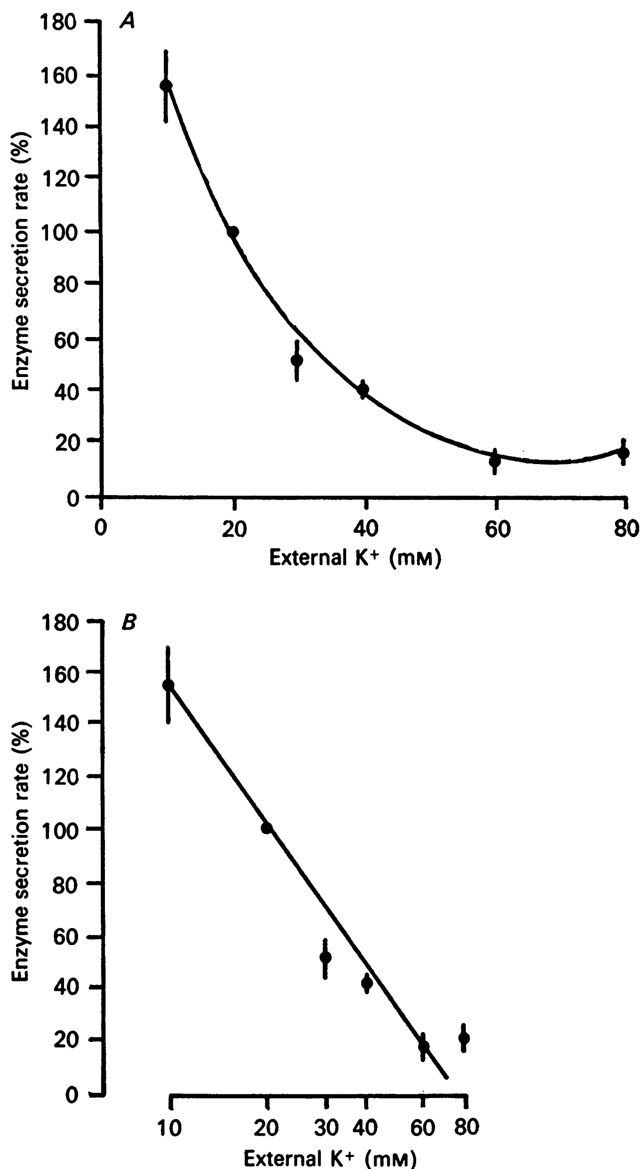

Fig. 6. 5-HT-stimulated rate of enzyme secretion (% rate in 20 mM-K^+) as a function of the potassium concentration (mM) in the bathing medium.

DISCUSSION

The experimental system

As in mammalian exocrine glands, control of secretion in fly salivary glands appears to be mediated by the second messenger substances, calcium and cyclic AMP, and isolated fly glands provide a simple experimental system for the study of the role of these substances. It is possible to measure rates of fluid and enzyme secretion simultaneously, and to follow the changes in potential and resistance across the cell membranes (Berridge, Lindley & Prince, 1975). The glands are made up of only one

type of cell, which simplifies the interpretation of biochemical data. Although the natural secretagogue has not been identified, all available pharmacological evidence indicates that it is 5-HT (see Hansen Bay, 1976). Secretion by isolated glands in response to 5-HT appears to mimic secretion in the natural situation, i.e. salivation in response to feeding, and it seems probable that the intracellular factors regulating secretion *in vivo* are also operating *in vitro*.

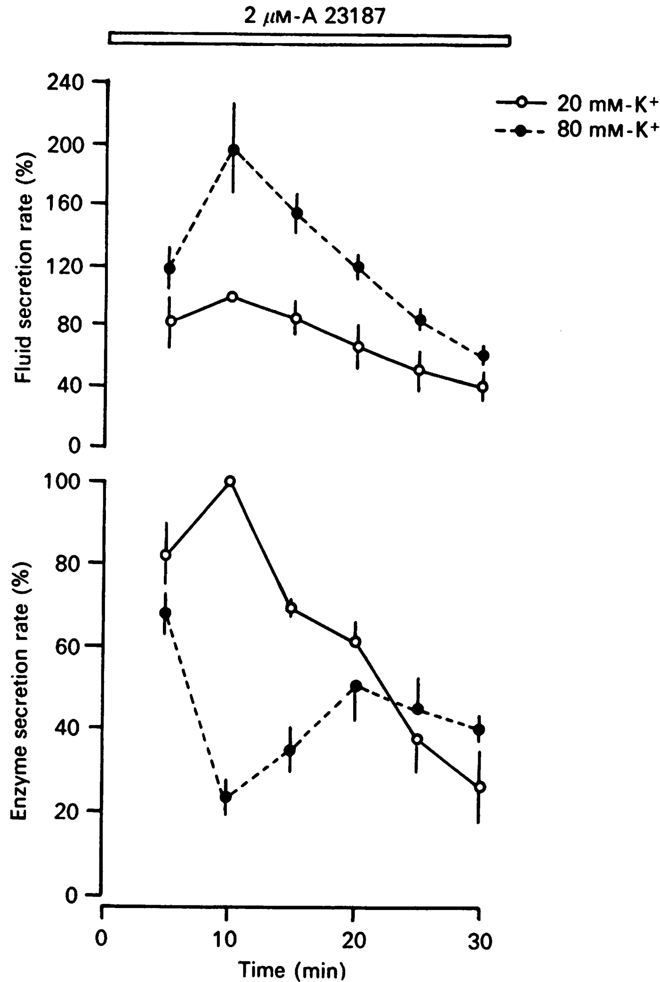

Fig. 7. The effect of potassium concentration on the secretory responses to A 23187. All glands were treated throughout by 2 μM-A 23187, one from each pair in 20 mM-K^+ (open circles), the other in 80 mM-K^+ (filled circles).

The response to 5-HT

Treatment of isolated glands with 5-HT stimulates secretion of both fluid and enzymes. There is an initial peak of secretion lasting 5–10 min after 5-HT is applied, before secretion falls to steady level. A fly seldom salivates continuously for more than a few minutes so the period of salivation corresponds to the period of maximal enzyme secretion. Over a 90 min period of secretion enzyme secretion declines to

about 50% of the initial steady rate. Homogenates of these glands still contain plenty of enzyme, but it is possible that it is not available for secretion, although prolonged stimulation can entirely deplete the glands of enzyme. There appears to be no consistent relationship between the rate of enzyme secretion and the concentration of 5-HT, although the rate of fluid secretion varies linearly with the log of the 5-HT concentration between 0·2 and 10 nM (Berridge & Prince, 1972). Thus the rate of enzyme secretion does not always parallel the rate of fluid secretion, suggesting that enzyme is not merely washed out of the gland with secreted fluid, but is under specific cellular control.

Calcium and cyclic AMP in the control of enzyme secretion

Several observations suggest that cyclic AMP may be involved in the control of enzyme secretion. Treatment with 5-HT causes a two to threefold increase in cyclic AMP concentration (Prince, Berridge & Rasmussen, 1972), and also causes enzyme secretion. Exogenous cyclic AMP stimulates enzyme secretion, thus mimicking the action of 5-HT. Secretion of enzyme in response to 5-HT is enhanced by theophylline which is thought to increase cyclic AMP concentration, although the drug may also have other actions such as changing the movement of calcium within the cell, as suggested for heart muscle (Blinks, Olson, Jewell & Braveny, 1972). It is possible that the stimulatory effect of calcium-free solution is actually due to increased cyclic AMP concentration, since the increase in cyclic AMP caused by 5-HT is greater in calcium-free solution than in the presence of 2 mM-calcium (Prince *et al.* 1972). However, enzyme secretion can also occur when the intracellular level of cyclic AMP is below the resting level, as during treatment with the ionophore A 23187 (Prince, Rasmussen & Berridge, 1973), suggesting that cyclic AMP has only an indirect effect on enzyme secretion, perhaps through its effect on the internal calcium concentration.

The fact that A 23187 causes enzyme secretion strongly suggests that calcium is directly involved in controlling enzyme secretion, since the only known action of the ionophore is to increase membrane permeability to calcium, thus raising the internal calcium concentration (Prince *et al.* 1973). However, the rate of secretion declines after about 10 min and secretion ceases after about 40 min, so perhaps some other factor is also required for secretion to continue, or it is possible that too much calcium enters the cell and inhibits cellular activities.

As in mammalian salivary gland (Selinger, 1975) and exocrine pancreas (Argent, Case & Scratcherd, 1973), enzyme secretion from fly salivary glands is not dependent on external calcium. Enzyme secretion induced by cyclic AMP or by 100 nM-5-HT is not affected by removal of external calcium, while secretion in response to 10 or 5 nM-5-HT is considerably enhanced (Fig. 3). This effect appears to be due to the cessation of calcium influx, rather than to the absence of calcium from the bathing medium, since the addition of a low concentration of lanthanum caused the same effect in the presence of calcium (Fig. 4). Lanthanum has been shown to block calcium fluxes (van Breeman, Farinas, Casteels, Gerba, Wuytach & Deth, 1973), and to mimic the effects of calcium-free solution in parotid glands (Leslie, Putney & Sherman, 1976), and to block calcium influx in fly glands (Prince & Berridge, 1973). The effect of calcium-free solution on fly glands is a specific effect of calcium, and not a general property of divalent cations, since the removal of magnesium had no effect on enzyme

secretion. The rate of fluid secretion is reduced in calcium-free solution and this is thought to be a consequence of lowered levels of internal calcium (Prince & Berridge, 1973).

The effect of potassium on enzyme secretion might be due to its effect on the calcium permeability of the basal membrane. The potential across this membrane, like the rate of enzyme secretion, is inversely proportional to the log of the potassium concentration, and depolarization of the membrane in high potassium causes increased influx of calcium (Berridge, Lindley & Prince, 1976). Thus increased calcium influx would be associated with a decreased rate of enzyme secretion. Potassium affects enzyme secretion induced by 5-HT, cyclic AMP and A 23187, so appears to act either directly, or indirectly via calcium, on the secretory process itself.

Results of the experiments with A 23187 and with calcium-free solution indicate that enzyme secretion is very sensitive to the intracellular calcium concentration. Berridge (1975) has proposed a model of cell activation for fluid secretion in which an increase in the internal level of free calcium activates the luminal potassium pump and causes an increase in anion permeability, with the pump sensitive to a lower level of calcium. If the secretory response to a single concentration of 5-HT is considered, this model may be extended to include enzyme secretion. As the calcium concentration rises, first enzyme secretion is activated, then the potassium pump and finally anion permeability. But the calcium concentration which fully activates anion permeability is above the optimal concentration for enzyme secretion and may actually inhibit enzyme secretion. Cessation of calcium influx lowers the internal calcium concentration, thus reducing fluid secretion but increasing enzyme secretion. Elevation of external potassium may increase the internal level of calcium, thus reducing enzyme secretion. During stimulation by 5-HT, therefore, fluid secretion would be maximal and enzyme secretion submaximal.

Exocytosis in mast cells also appears to have an optimal calcium concentration. Release of histamine from mast cells in sodium-free medium is inhibited by calcium, although it is abolished by total removal of calcium by incubation in EGTA (Cochrane & Douglas, 1976), suggesting that secretion requires calcium but is inhibited by excessive influx of calcium.

The role of cyclic AMP in the control of enzyme secretion is not clear. The situation resembles that in mammalian salivary glands in which enzyme secretion induced by β-adrenergic agents appears to be mediated by cyclic AMP, but enzyme secretion can also occur when cyclic AMP is at the unstimulated level (Butcher, 1975). In fly glands secretion can occur for a short time when cyclic AMP is below the resting level, so perhaps exocytosis itself requires only calcium, while preparation of the granules for secretion, either spatially or chemically, also requires cyclic AMP.

I am most grateful to Dr M. J. Berridge for his direction and encouragement, and to Professor H. Lipke for critically reading this manuscript. I thank the Science Research Council for financial support.

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