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THE CONTROL OF TRANSEPITHELIAL POTENTIAL OSCILLATIONS IN THE SALIVARY GLAND OF *CALLIPHORA ERYTHROCEPHALA*

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SUMMARY

The effects of the hormone 5-hydroxytryptamine, its analogues and its antagonists on the electrical activity and secretion rate of isolated salivary glands of the blowfly *Calliphora erythrocephala* were investigated. The secretion rate increases linearly with the logarithm of hormone concentration between 10^{-9} and 10^{-8} M. At $>10^{-8}$ M the transepithelial potential depolarizes and rapidly attains a new stable value. However, at intermediate hormone concentrations, the potential does not maintain a stable intermediate value but displays sustained oscillations. These oscillations are not an artifact resulting from periodic variations in hormone concentration. The frequency of the oscillations increases with hormone concentration and with the concentration of external calcium and hormone analogues. The frequency decreases following the addition of lanthanum to the perfusion medium.

The experimental results suggest that the potential oscillation may be driven by an oscillation in the intracellular concentrations of cyclic AMP and calcium. It is argued that oscillatory control provides a reliable, noise-resistant strategy for controlling secretion rate.

INTRODUCTION

Sustained oscillatory behaviour characterizes many important biological processes. Typical examples include smooth muscle and cardiac pacemaker cells. Recently, oscillations have been observed in the membrane potential of secretory cells such as pancreatic beta cells (Matthews & O'Connor, 1979) and anterior pituitary cells (Kidokoro, 1975; Poulsen & Williams, 1976). In this contribution we report observations of similar oscillatory behaviour in isolated, perfused, salivary glands from the blowfly *Calliphora erythrocephala*. It is possible in this preparation to measure both the electrical signal and the rate of fluid secretion in response to hormone challenge and variations in ionic composition of the fluid perfusing the gland. This in turn has made possible to correlate electrical events with secretory activity.

The stimulating hormone employed in these experiments was 5-hydroxytryptamine (5-HT). At concentrations $< 10^{-9}$ M no secretory response was observed. At all concentrations $> 10^{-8}$ M a maximal secretion rate was obtained. Intermediate rates of fluid transport were obtained between 10^{-9} to 10^{-8} M, a similar range to that which stimulates sustained oscillations in transepithelial potential. The frequency increases with hormone concentration and thus secretion rate is seen to increase with the frequency.

It has previously been proposed that electrical oscillations in secretory cells may be generated by an instability in the calcium-cyclic AMP network that results in an oscillation in the internal concentration of these second messengers (Rapp & Berridge, 1977). Data presented in this paper (specifically the dependence of frequency on the concentration of calcium in the perfusing solution) clearly demonstrate that the original simplified mathematical realization of the calcium-cyclic AMP oscillator is untenable. However, the data do indicate that the central qualitative idea of a sustained oscillation in the internal concentration of calcium and cyclic AMP may be correct. While the functional role of cellular oscillations may be apparent in cardiac muscle cells, the advantages of oscillatory regulation in secretory cells is less obvious. It has been suggested that oscillatory control systems can provide efficient and precise control of intermediate secretion rates (Rapp, Mees & Sparrow, 1981). It will be argued that the results presented here offer support to this hypothesis.

METHODS

The salivary gland of the blowfly consists of a single layer of epithelial cells which form a hollow tube that is closed at the posterior end. The exterior basal membrane is exposed to the abdominal cavity and the interior apical membrane faces the central core of the gland, the lumen. Glands were dissected from adult flies 15 to 21 days after hatching.

In all experiments, an unmodified control Ringer consisted of NaCl (130 mM), KCl (10 mM), $CaCl_2$ (2 mM), $MgCl_2$ (2 mM), NaH_2PO_4 (4 mM), malic acid (3 mM), sodium glutamate (3 mM), glucose (10 mM) and tris(hydroxymethyl)methylamine (10 mM). In some experiments, the chloride level of the Ringer was reduced and isotonicity was maintained by adding an equivalent amount of isethionate. Phenol red was added to all solutions and a pH of 7·2–7·4 was maintained. Secretion rates were measured as described previously (Berridge & Patel, 1968).

The procedure used in measuring the transepithelial potential has been described in detail by Berridge & Prince (1972). The electrical effects of variations in hormone and ion concentrations at the basal membrane were established by altering the composition of the perfusing solutions. Calcium concentrations ranging from 0·01 to 10 mM were obtained by serial dilution with a calcium-free solution without using any calcium chelating agents. Rapid changes in perfusate composition were effected by a six-way ball and socket tap connecting the perfusion chamber input to one of six possible solutions.

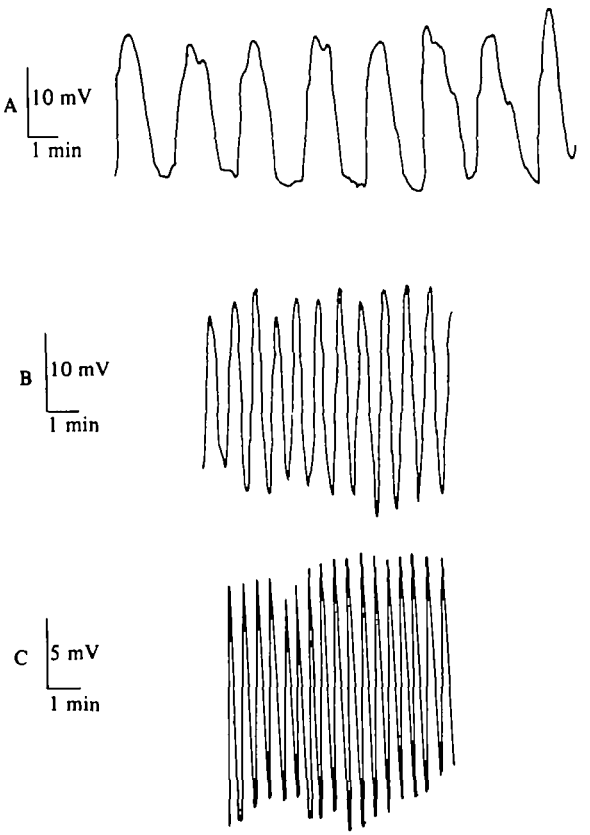

Fig. 1. Typical traces of transepithelial potential versus time. (*a*) A low frequency oscillation displaying irregularities typical of this frequency range. Control Ringer and $0{\cdot}2 \times 10^{-8}$ M 5-HT. (*b*) An intermediate frequency oscillation, irregularities are largely suppressed at this frequency. Control Ringer with 5×10^{-6} M-gramine and 10^{-8} M 5-HT. (*c*) A high frequency oscillation displaying increased nonlinear character. Control Ringer with 10^{-8} M 5-HT and 10^{-3} M lanthanum, immediately after introduction of lanthanum.

RESULTS

The properties of the transepithelial potential oscillation and its relation to the secretion rate are examined in this section. The results fall into three general groups. In the first, the qualitative features of the oscillation are described. The second group of experiments established the control characteristics of the oscillator; the oscillator's sensitivity to the concentration of challenging hormone, external calcium and other agents will be described. The final set of experiments were performed to eliminate two possible explanations of the origin of the oscillation.

1. *Potential oscillations at intermediate* 5-*HT concentrations are reproducible and autonomous*

In previously published experiments (Berridge, 1970) it was found that a near linear relationship between secretion rate and log (5-HT) was obtained for intermediate concentrations between 10^{-9} and 10^{-8} M 5-HT. Similarly the transepithelial

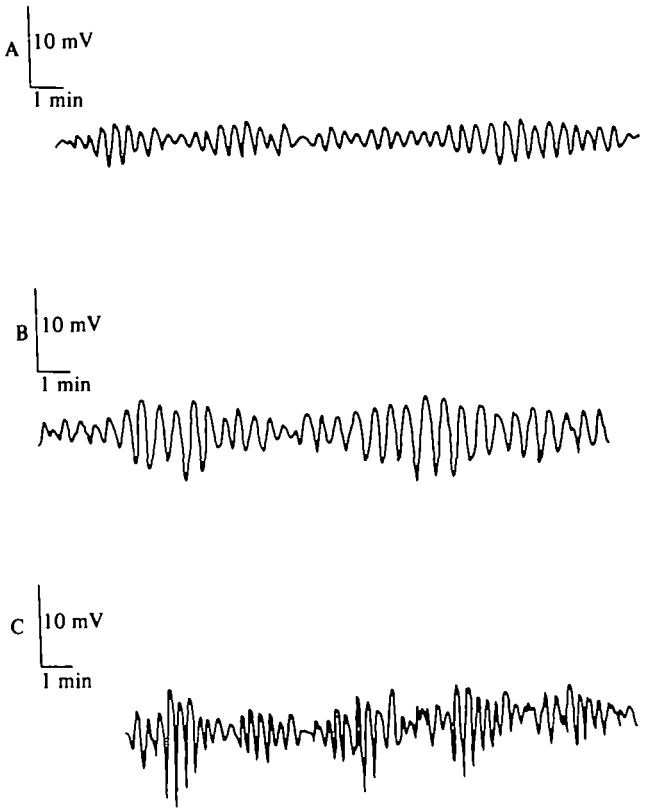

Fig. 2. Examples of experiments in which a superimposed low frequency signal was present (*a*) Control Ringer and 10^{-8} M 5-HT. (*b*) Control Ringer with 5×10^{-4} M gramine and 10^{-8} M 5-HT. (*c*) Control Ringer with glucose deleted and $0{\cdot}5 \times 10^{-8}$ M 5-HT.

potential difference responds to 5-HT. In isolated glands perfused with control Ringer, the lumen is usually 15 to 20 mV positive with respect to the outside of the gland. Stimulation by 5-HT concentrations greater than 10^{-8} M causes the potential to depolarize rapidly towards 0 mV. However, in the intermediate range of $0{\cdot}2 \times 10^{-8}$ to 1×10^{-8} M 5-HT, the potential does not attain a stable intermediate value but rather exhibits oscillations. Irregular oscillations had been observed previously for these stimulation levels (Berridge & Prince, 1971, 1972). However, the maximum duration of these experiments was 5 min and thus it was impossible to determine if the oscillations were a stable nondecaying periodic signal or if they were a transient artifact.

The simplest way to distinguish between transient ringing and autonomous oscillations is to determine if it is possible to consistently obtain an oscillation that can continue for the life of the preparation. Oscillations were systematically obtained in glands in the intermediate 5-HT range. These oscillations were sustained for more than 100 cycles at constant perfusion rates with undiminished amplitude and unaltered frequency. The longest trace obtained ran for 6 h (450 cycles) and glands were routinely found to be oscillating at the termination of any given experiment (typically lasting about 2 h, $n = 105$). Accordingly, it is concluded that the oscillations are autonomous and not due to a transient switching effect or due to periodic changes in the composition of the perfusing fluid.

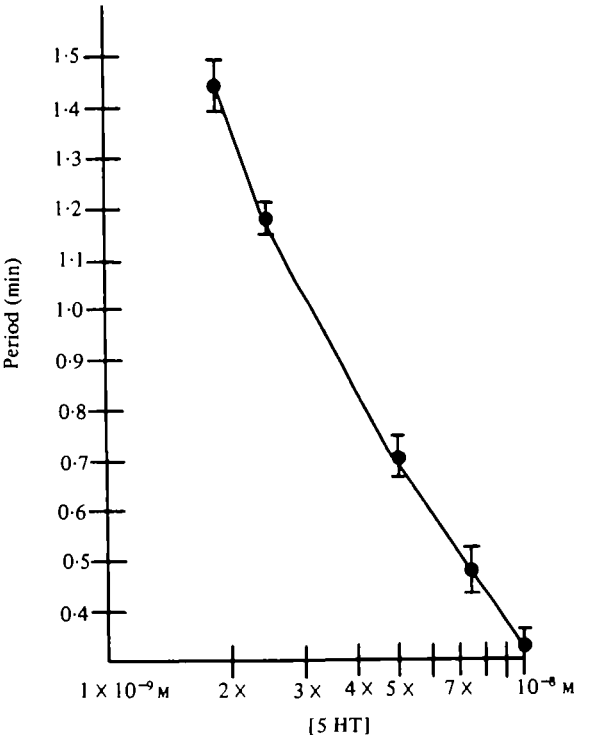

Fig. 3. Period (in minutes) of the oscillation as a function of log(5-HT) (in moles/l). The perfusate consists of control Ringer and 5-HT only. Throughout any given experiment, perfusion rate and 5-HT were constant. The detailed quantitative data are as follows: 5-HT = $0{\cdot}2 \times 10^{-8}$ M, period = $1{\cdot}44 \pm 0{\cdot}05$ min ($n = 3$); 5-HT = $0{\cdot}25 \times 10^{-8}$ M, period = $1{\cdot}18 \pm 0{\cdot}03$ min ($n = 5$); 5-HT = $0{\cdot}5 \times 10^{-8}$ M, period = $0{\cdot}70 \pm 0{\cdot}04$ min ($n = 5$), 5-HT = $0{\cdot}75 \times 10^{-8}$ M, period = $0{\cdot}48 \pm 0{\cdot}05$ min ($n = 5$); 5-HT = $1{\cdot}0 \times 10^{-8}$ M, period = $0{\cdot}32 \pm 0{\cdot}03$ min ($n = 5$). Not shown: 5-HT = $0{\cdot}125 \times 10^{-8}$ M, no oscillations ($n = 3$); 5-HT = $1{\cdot}06 \times 10^{-8}$ M, no oscillations ($n = 3$).

Qualitatively three classes of waveform were usually observed. At lower frequencies (periods of the order of 1 min) irregularities were imposed on the principal signal (Fig. 1*a*). These irregularities took the form of notches on both the ascending and descending part of the wave. Though the central frequency was reproducible (see Result 3 below), the irregularities were not predictable. At intermediate frequencies (periods of 0·5 to 1 min) a smoother sinusoidal signal was obtained (Fig. 1*b*) and at higher frequencies (periods of 0·2 to 0·5 min) a regular but more nonlinear wave was produced (Fig. 1*c*).

2. *Some oscillations display a superimposed low frequency signal*

In most experiments, a simple waveform with a dominating central frequency was obtained. However, in some cases ($n = 5$) a low frequency periodic signal was superimposed on the primary oscillation. Examples are given in Fig. 2. It was not possible to obtain this behaviour systematically, but it was noted that it was more likely at higher 5-HT concentrations (usually 5-HT = 10^{-8} M). No explanation or physiological significance can be associated with this observation.

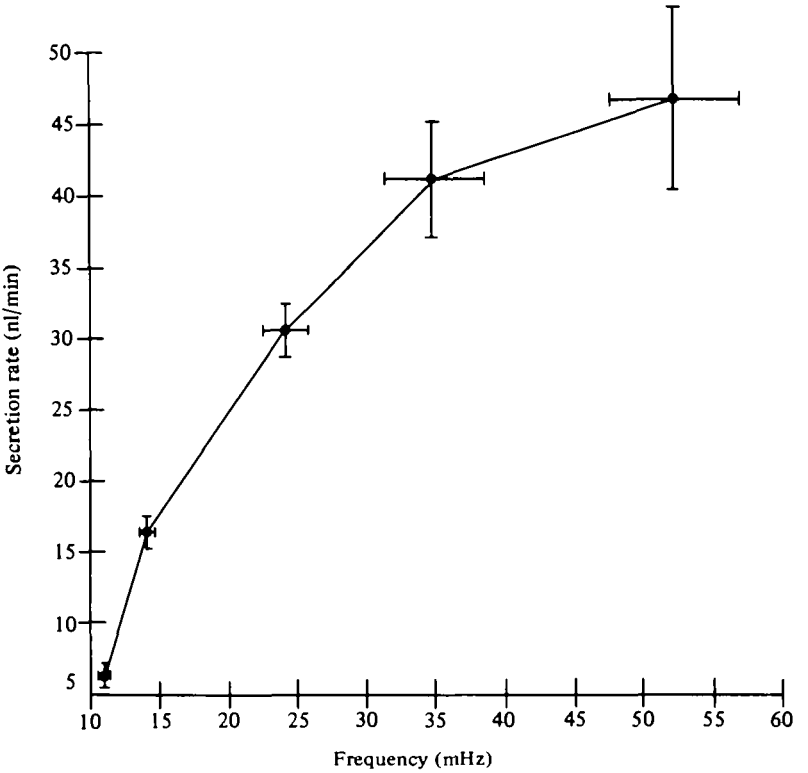

Fig. 4. Secretion rate (in nl/min) for a given concentration of 5-HT versus the oscillator frequency (in mHz) corresponding to that 5-HT concentration. Reading from left to right the common 5-HT concentration value is: $0{\cdot}2 \times 10^{-8}$ M, $0{\cdot}25 \times 10^{-8}$ M, $0{\cdot}5 \times 10^{-8}$ M, $0{\cdot}75 \times 10^{-8}$ M and $1{\cdot}0 \times 10^{-8}$ M. The details of the frequency data are given in the caption to Fig. 3. The secretion rates are as follows: 5-HT = $0{\cdot}2 \times 10^{-8}$ M, secretion rate = $6{\cdot}7 \pm 0{\cdot}7$ nl/min ($n = 5$); 5-HT = $0{\cdot}25 \times 10^{-8}$ M, secretion rate = $16{\cdot}3 \pm 1{\cdot}1$ nl/min ($n = 5$); 5-HT = $0{\cdot}5 \times 10^{-8}$ M, secretion rate = $30{\cdot}7 \pm 6{\cdot}8$ nl/min ($n = 5$); 5-HT = $0{\cdot}75 \times 10^{-8}$ M, secretion rate = $41{\cdot}2 \pm 4{\cdot}2$ nl/min ($n = 5$); 5-HT = $1{\cdot}0 \times 10^{-8}$ M, secretion rate = $46{\cdot}7 \pm 6{\cdot}1$ nl/min ($n = 5$).

3. *Frequency and secretion rate increase with 5-HT*

As previously indicated, potential oscillations were obtained in preparations perfused with control Ringer only if the 5-HT concentration was in the $0{\cdot}2 \times 10^{-8}$ M to 1×10^{-8} M range. The oscillatory range of 5-HT concentration appears to be sharply defined. It was impossible to obtain oscillatory behaviour at $0{\cdot}125 \times 10^{-8}$ M ($n = 3$) and $1{\cdot}06 \times 10^{-8}$ M 5-HT ($n = 3$). In the oscillatory range, the period decreases with 5-HT concentration. When 5-HT = $0{\cdot}2 \times 10^{-8}$ M, period was $1{\cdot}44 \pm 0{\cdot}05$ min ($n = 3$, in all cases the reported statistic is the standard deviation of the mean) and at $1{\cdot}0 \times 10^{-8}$ M 5-HT the period was $0{\cdot}32 \pm 0{\cdot}03$ min ($n = 5$). The graph relating period and log (5-HT) is almost linear (Fig. 3). It was possible to reliably tune ($\pm 10\%$) the period to any value in this range by administering the appropriate 5-HT concentration. (Note: This period and 5-HT concentration range are obtained if the perfusing solution contains 5-HT and control Ringer only. As outlined below, treatment with additional agents can alter both the period and the range of 5-HT values that yield oscillations.)

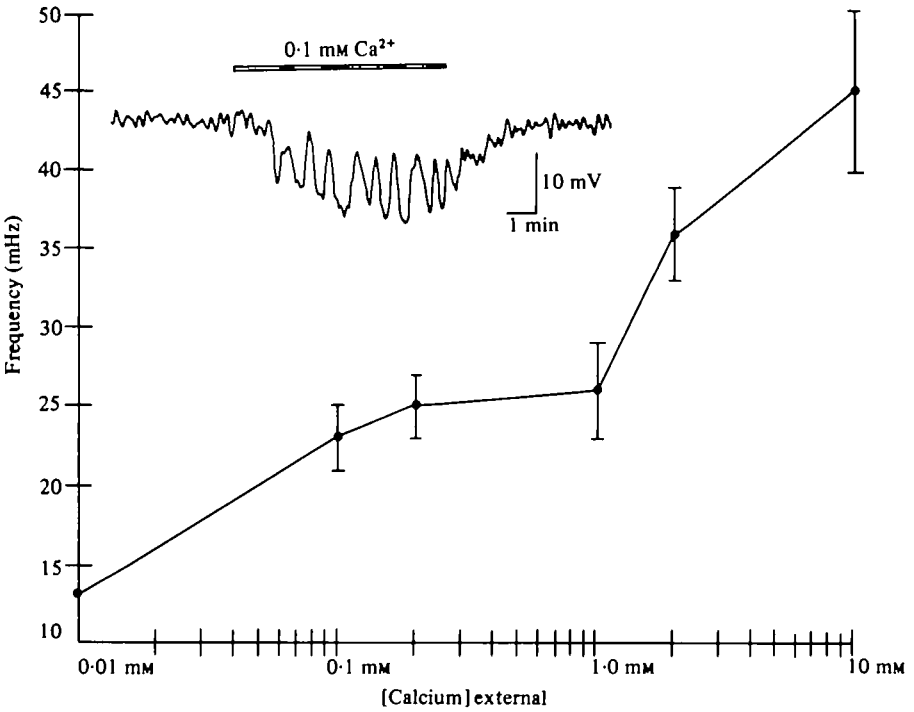

Fig. 5. Frequency (in mHz) versus the logarithm of external calcium concentration (in mM). 5-HT = $0{\cdot}25 \times 10^{-8}$ M for all experiments shown. The quantitative details are as follows (calcium concentrations refer to extracellular concentrations): Ca^{2+} = 0·01 mM, frequency = $18{\cdot}0 \pm 3{\cdot}0$ mHz (n = 6); Ca^{2+} = 0·1 mM, frequency = $28{\cdot}0 \pm 2{\cdot}0$ mHz (n = 10); Ca^{2+} = 0·2 mM, frequency = $30{\cdot}0 \pm 2{\cdot}0$ mHz (n = 4); Ca^{2+} = 1·0 mM, frequency = $31{\cdot} \pm 3{\cdot}0$ mHz (n = 9); Ca^{2+} = 2·0 mM, frequency = $41{\cdot}0 \pm 3{\cdot}0$ mHz (n = 5); Ca^{2+} = 10·0 mM, frequency = $50{\cdot}0 \pm 5{\cdot}0$ mHz (n = 6). The inset shows the rapid and reversible response to changes in the concentration of external calcium. The open bar represents the period when the calcium concentration was reduced from 10 mM to 0·1 mM.

The secretion rate is also 5-HT sensitive. The range of 5-HT concentration that produces oscillations is identical to the range of 5-HT concentration that produces secretion rates intermediate between a negligible unstimulated rate and the saturated maximally stimulated rate. At $0{\cdot}2 \times 10^{-8}$ M 5-HT, the secretion rate was $6{\cdot}4 \pm 0{\cdot}7$ nl/min (n = 5). At 10^{-8} M 5-HT the secretion rate was $46{\cdot}7 \pm 6{\cdot}1$ nl/min (n = 5). These results indicate that both secretion rate and frequency increase with 5-HT. The relationship between oscillator frequency and the secretion rate for a given 5-HT concentration is shown in Fig. 4. The resulting function is a saturating, monotonically increasing, hyperbolic function. The simplicity of this function suggests that there is a functional relationship between the oscillator and the secretion mechanism. This possibility will be developed in the discussion section of this paper.

4. *Frequency increases with external calcium*

In this salivary gland preparation, 5-HT has two principal effects. It stimulates adenylate cyclase and it increases the rate of calcium entry into gland cells through the basal membrane (Prince, Berridge & Rasmussen, 1972). The effect of 5-HT on gating external calcium suggested that the concentration of external calcium may have

an important effect on the oscillation. The frequency of the oscillation was determined as a function of external calcium for a constant 5-HT concentration. It was found that the frequency increases with increased extracellular calcium. External calcium effects frequency in a dose dependent manner throughout the investigated concentration range of 0·01 to 10 mM (Fig. 5). The effect of altering calcium concentration on frequency was rapid and reversible (Fig. 5, inset).

Modifications of the standard perfusion solution were necessary in solutions containing elevated calcium concentrations. Calcium was added as $CaCl_2$. To keep a constant chloride concentration, a corresponding amount of sodium chloride was replaced with sodium isethionate. At elevated calcium concentrations, an insoluble calcium phosphate precipitate was formed. This was prevented by deleting sodium phosphate from these solutions. The pH was then adjusted to the standard value (7·2–7·4) with HCl. The removal of phosphate from some solutions generates an additional question: is phosphate primarily involved in pH adjustment or does it have other effects? To answer this question, it was necessary to determine the effect of phosphate removal on the frequency. The period was first determined for standard Ringer ($CaCl_2$ = 2 mM, NaH_2PO_4 = 4 mM) at $0{\cdot}5 \times 10^{-8}$ M 5-HT. The solution was then switched to one containing the same calcium and 5-HT concentrations but not containing phosphate. Periods were found by averaging oscillations over a 30 min trial period. Removing phosphate from the perfusate resulted in a small acceleration (6·4% ± 1·9%, n = 4).

In previous experiments it was found that there was no substantial change in either amplitude or frequency during experiments lasting at least 100 cycles (Result 1). However, some compounds, notably lanthanum (see next section), express their effects only after prolonged exposure. Accordingly, the effects of the prolonged presence of 10 mM calcium (500% standard) and 20 mM calcium (1000% standard) were determined. Recordings were made for 1·5 h for each gland and the periods averaged over the first, second and third 0·5 h. The relative changes in period were then determined. For glands exposed to 10 mM calcium, there was a nonsignificant acceleration between the first and second 0·5 h (2·1% ± 2·6%, n = 6) and the first and third half hour (1·1% ± 2·9%, n = 6). Similarly for glands run at 20 mM calcium, the difference between the first and second 0·5 h was 0·3% ± 2·1% (n = 5) and between the first and third 0·5 h −3·7% ± 5·4% (n = 5). Thus there was no significant effect of calcium exposure that could not be identified during the first 0·5 h of perfusion.

5. *Frequency and secretion rate decrease with lanthanum*

External lanthanum is thought to act by blocking calcium conductances at the basal membrane. A confirmation of the experiments in which external calcium was varied would be obtained if increasing the concentration of lanthanum in solutions of constant 5-HT and calcium concentrations parallels the effect of reducing external calcium.

As expected, the addition of lanthanum causes an immediate, dose-dependent, increase in period (Table 1). At higher concentrations, the period continued to increase during prolonged experiments. In the case of $La^{3+} = 10^{-3}$ M, additional experiments were performed for 90 min sample periods. The average period over t

Table 1. *Effect of lanthanum on period*

La^{3+} (M)	Period 1st half h (min)	Period 2nd half h (min)	Period averaged over entire experiment (min)
0	0·32 ± 0·03	0·31 ± 0·03	0·31 ± 0·03 (n = 5)
10^{-5}	0·47 ± 0·04	0·47 ± 0·04	0·47 ± 0·04 (n = 3)
10^{-4}	0·53 ± 0·04	0·58 ± 0·11	0·53 ± 0·07 (n = 3)
10^{-3}	0·54 ± 0·06	0·74 ± 0·06	0·75 ± 0·07* (n = 3)
10^{-2}	Oscillations suppressed (n = 3)		

* Includes data for the 3rd half h 5HT = 10^{-6} M for all cases.

Table 2. *Effect of lanthanum on secretion rate*

Lanthanum (M)	Secretion rate nl/min
10^{-5}	16·0 ± 2·4 (n = 4)
10^{-4}	13·5 ± 0·5 (n = 4)
10^{-3}	11·0 ± 2·1 (n = 4)
10^{-2}	0·8 ± 0·2 (n = 4)

5-HT = 10^{-6} M in all cases.

third 0·5 h was 0·98 ± 0·07 min (n = 4). This long-term effect of high lanthanum concentration may have been due to a slow accumulation of the metal within the cell. While it is generally supposed that biological membranes are impermeable to lanthanum, it does seem probable that these aberrantly high external concentrations did force some lanthanum into the cell.

At concentrations of 10^{-2} M external lanthanum, the oscillations were immediately suppressed (n = 3), which might indicate that calcium movement through the basal membrane is an essential aspect of the oscillatory mechanism.

The effect of external lanthanum on the secretion rate is consistent with its effect on the period of the oscillation. The lanthanum concentrations which alter frequency also cause a marked decrease in the secretion rate (Table 2).

6. *Oscillations induced by analogues of 5-HT*

Certain analogues of 5-HT such as 4-fluoro alpha methyltryptamine, 5-fluoro alpha methyltryptamine and histamine can activate adenylate cyclase without causing any increase in calcium entry (Berridge, 1981; Berridge & Heslop, 1981). Such analogues were found to induce oscillations similar to those produced by 5-HT. The relationship between histamine concentration, fluid secretion and oscillation frequency is shown in Fig. 6. As the concentration was increased, the rate of fluid secretion was increased to a level identical to that obtained with a maximal dose of 5-HT. Oscillations were observed throughout this range, and the frequency increased as the concentration was elevated (insets on Fig. 6).

7. *The oscillation is not a mechanical artifact due to intermediate secretion rates.*

The rate of secretion in the oscillatory range of 5-HT may be as little as $\frac{1}{10}$ of the maximal rate. Therefore, it seemed possible that the oscillation might be due to a discontinuous release of drops of saliva from the long cylindrical gland into the saliva duct. This possibility was excluded by demonstrating that the frequency of the

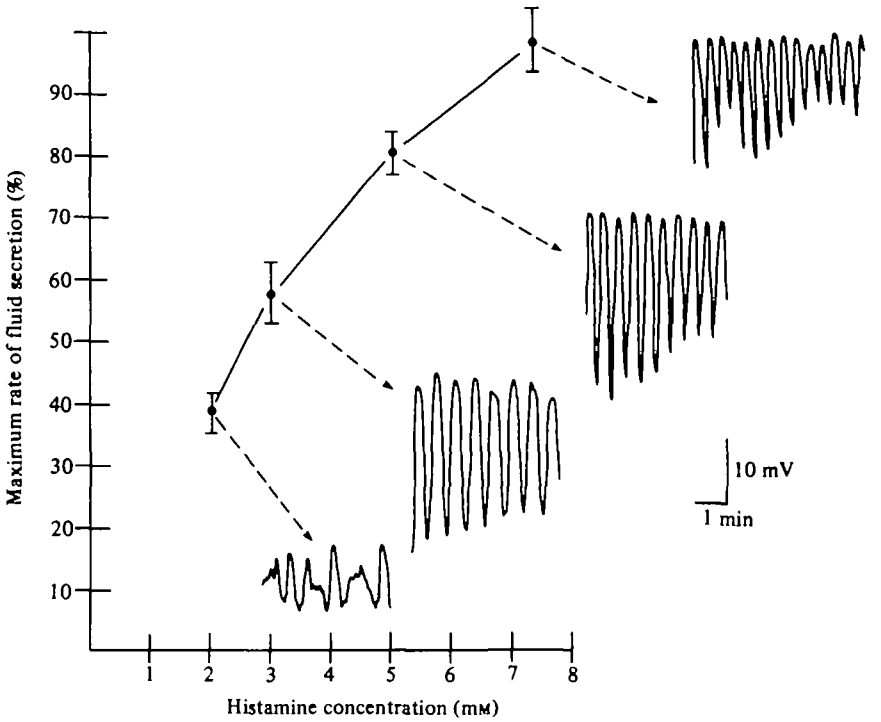

Fig. 6. Histamine-induced oscillatory activity. The effects of various doses of histamine on fluid secretion and on transepithelial potential were tested. The four insets illustrate the potential oscillations obtained at four different histamine concentrations. The corresponding rates of fluid secretion have been plotted as a percentage of the maximum rate obtained with $2{\cdot}0 \times 10^{-4}$ M 5-HT.

oscillation can be uncoupled from the secretion rate. Prince & Berridge (1973) have shown that chloride is required to maintain secretion. By reducing the chloride content of the perfusing solution, it is possible to reduce the secretion rate.

The period of the oscillation was found to be independent of the external chloride concentration. Oscillations were first obtained at the standard chloride concentration (148 mM) and were continued after rapid switching to lower concentrations (60 and 20 mM). The 5-HT concentration was constant ($0{\cdot}5 \times 10^{-8}$ M). The change in period on switching to (and from) 60 mM chloride was $1{\cdot}6\% \pm 3{\cdot}3\%$ ($n = 6$) and $-1{\cdot}2\%$ $2{\cdot}7\%$ ($n = 6$) on switching to 20 mM chloride, that is, there was no significant effect on the period. This result indicates that the oscillation in transepithelial potential is not the result of periodic release of saliva.

8. *Oscillations are not due to an instability in the glycolytic pathway*

Smooth sustained biological oscillations with periods in the 0·3 to 1·5 min range are immediately reminiscent of the glycolytic oscillator (Hess & Boiteux, 1971; Hess, 1979). An obvious question suggests itself: are the potential oscillations driven by an internal glycolytic oscillator?

Glycolytic oscillations in yeast cell-free extracts are sensitive to the rate of glucose input. Hess, Boiteux & Kruger (1969) found that oscillations were sustained only

the glucose input rate was held between 20 and 160 mM/h. This contrasts with the salivary gland oscillator. Periodic behaviour persisted when the glucose concentration was switched from 10 mM (standard) to 0 mM or to 100 mM. A shift to 0 mM glucose caused an acceleration in the period ($5{\cdot}9\% \pm 3{\cdot}8\%$, $n = 4$) as did a shift to 100 mM glucose ($15{\cdot}0\% \pm 4{\cdot}6\%$, $n = 4$). The maintenance of oscillations in a zero glucose medium and the relative insensitivity of the frequency to glucose argues against a glycolytic origin for the potential oscillation.

This conclusion is further substantiated by a previous result (Berridge, 1970) where it was found that while glutamate and glutamine can sustain secretion, glucose and trehalose can not. It is thought that the principal energy source in these cells is the Krebs cycle and not the glycolytic pathway.

DISCUSSION

The experimental results indicate that the oscillation is not a decaying transient response. The stability and reproducibility of the periodic signal suggests that these autonomous oscillations may be a normal feature of the response of the gland to hormone challenge. If this is indeed the case, then two questions follow. What mechanism generates the oscillation? What functional purpose is served by the oscillation?

As mentioned above, the results appear to eliminate a purly mechanical origin to the oscillation. Another obvious possibility, an oscillatory instability in the glycolytic pathway, would appear to be excluded by the oscillator's observed insensitivity to the availability of glycolytic intermediates. In an attempt to identify the source of an oscillation, it is often helpful to examine the factors that can reversibly alter the frequency. The experiments have identified several agents: 5-HT, 5-HT analogues, external calcium and lanthanum. A previous study has established that frequency is also sensitive to the phosphodiesterase inhibitor isobutyl methylxanthine (Berridge & Rapp, 1979). The identified actions of 5-HT in this preparation are the stimulation of adenylate cyclase and the stimulation of calcium entry into cells through the basal membrane. The importance of extracellular calcium was established by demonstrating that the frequency can be tuned by variations in its concentration. This was confirmed by the experiments with the calcium antagonist lanthanum. Taken together, these experiments indicate that elevated intracellular calcium is probably integral to the oscillatory process. The importance of elevated intracellular cyclic AMP is suggested, but not conclusively confirmed, by the previous experiments with isobutyl methylxanthine (Berridge & Rapp, 1979). This phosphodiesterase inhibitor, which will potentiate the effect of 5-HT on the level of cyclic AMP, caused a marked increase in frequency. The importance of cyclic AMP is also apparent from the studies with histamine and 5-fluoro alpha methyltryptamine which act specifically to increase the level of cyclic AMP without stimulating the entry of calcium. Since these analogues can all induce oscillations, it is apparent that an increase in the level of cyclic AMP is also important for initiating periodic activity.

These results directed attention to a further examination of calcium and cyclic AMP in the internal compartment. It is important to recall that these second messengers do not exist independently of each other but mutually regulate their intra-

cellular concentrations (Rasmussen, Goodman & Tenenhouse, 1972; Berridge, 1975). Many of these interactions may exist in the form of closed feedback loops (Rapp & Berridge, 1977). It has been argued that closed calcium-cyclic AMP feedback loops of this form are dynamically analogous to feedback loops of engineering systems that are known to exhibit sustained oscillations (Rapp & Berridge, 1977). If the calcium-cyclic AMP control loop is unstable, the intracellular concentrations of these compounds would oscillate. Oscillations in intracellular calcium and cyclic AMP could produce an observable potential oscillation since both compounds are coupled to the membrane. Cytosol calcium causes an increase in chloride permeability at both the basal and apical membrane (Berridge, Lindley & Prince, 1975; Prince & Berridge, 1972, 1973), and internal cyclic AMP effects the apical membrane potential by stimulating a potassium pump in that membrane (Prince & Berridge, 1972; Berridge & Schlue, 1978).

While the mechanism that generates the sustained oscillation in transepithelial potential has not been identified with certainty, the precise modulation of its frequency (by agents that exert their effects on the calcium-cyclic AMP system) is consistent with the hypothesized intracellular calcium-cyclic AMP oscillator. The question of the functional role of the oscillator will now be considered.

A preliminary question which must be resolved is whether the oscillator is functionally related to the hormonal control of secretion or if the secretory mechanism and the oscillator are independent systems. The experimental evidence suggests that they are related. First, the 5-HT concentration range that stimulates oscillations is coincident with the range of hormone that elicits secretion rates that are intermediate between the basal unstimulated and the saturated maximal rate. Second, agents that cause an increase in frequency, 5-HT, external calcium and phosphodiesterase inhibitors, cause an increase in secretion rate. Third, an agent that causes a decrease in frequency, lanthanum, causes a decrease in secretion rate.

A further argument for a causal connection between the oscillator and the secretion mechanism follows from an examination of the analysis which lead to the calcium-cyclic AMP oscillator model. These two chemicals are believed to control secretion rates in this preparation. As previously noted, cyclic AMP stimulates a potassium pump in the apical membrane. (Potassium is the principal cation in saliva). Calcium controls exocytotic release of proteins into the saliva (principally amylase) and controls basal and apical membrane permeability to chloride which follows potassium. Water is drawn into the lumen by virtue of the movement of these ions. According to this scheme, cyclic AMP and calcium determine the secretion rate and thus any oscillation in calcium and cyclic AMP would be functionally coupled to secretion.

The strongest single piece of evidence that supports the proposition that the secretion rate is determined by the oscillator frequency is given in Fig. 4 which shows the secretion rate as a function of frequency. Since both frequency and secretion rate increase with 5-HT, an increasing function is expected. However, there was no *a priori* basis for supposing that this function would be a simple one. The demonstration that the secretion versus frequency relation is an increasing, monotonic and saturating, hyperbolic function clearly suggests that the oscillation and secretion mechanism are not independent phenomena and that the secretion rate is frequency controlled.

This discussion began with the question, what functional purpose is fulfilled by the oscillator? It has now been proposed that the frequency of the oscillation may determine the secretion rate. If this should be the case, a subsidiary question follows: what are the functional advantages of frequency dependent control? This question has been considered in greater detail elsewhere (Rapp, Mees & Sparrow, 1981). The principal advantage of oscillatory regulation concerns the accuracy of control. In this system an input signal, 5-HT concentration, results in an output signal, the secretion rate. The purpose of regulation is to produce a secretion rate that accurately meets the ideal rate specified by hormone concentration. In any system, and most particularly in biological systems, the input signal will be corrupted by noise. It has been argued (Rapp *et al.* 1981) that a process in which the information expressed in the external hormone concentration is converted to an intracellular oscillator frequency will be more resistant to noise-induced control defects than an amplitude dependent system. This conclusion is not a surprising one since it is consistent with long standing practice in engineering systems. The salivary gland presents a biological analogue of frequency encoded control.

There is another more speculative argument in support of a frequency control hypothesis that concerns the system's ability to function adequately in the event of a transition to more complex forms of dynamical behaviour in which the system generates aperiodic signals that do not converge to a stable oscillation. Sparrow (1980) has argued that this transition could occur in calcium-cyclic AMP control systems. A theoretical analysis of these systems (Rapp *et al.* 1981) indicates that, unlike amplitude-dependent control systems, frequency encoded control networks may be able to adequately regulate output even after this transition has occurred.

We have expressed these arguments in terms of specific application to the control of salivary secretion in *Calliphora*, but we think it possible that they may prove to be more generally applicable.

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