

## ION-SELECTIVE ELECTRODE STUDIES ON THE EFFECTS OF 5-HYDROXYTRYPTAMINE ON THE INTRACELLULAR LEVEL OF POTASSIUM IN AN INSECT SALIVARY GLAND

BY M. J. BERRIDGE AND W. R. SCHLUE\*

*Agricultural Research Council Unit of Invertebrate Chemistry and Physiology,  
Department of Zoology, University of Cambridge,  
Downing Street, Cambridge CB2 3EJ, England*

(Received 17 June 1977)

### SUMMARY

Potassium-sensitive microelectrodes were used to measure the intracellular level of potassium in *Calliphora* salivary glands. During stimulation with  $1 \times 10^{-8}$  M 5-HT, the potassium concentration increased from a resting level of 133.2 to 139.0 mM. When the external concentration of potassium was reduced below 2 mM there was a rapid loss of potassium which could be partially reversed by 5-HT. During 5-HT stimulation, the addition of ouabain had no effect on a number of parameters including the rate of fluid secretion, the ionic composition of the saliva and the intracellular level of potassium. The possibility that potassium enters the cells passively is discussed.

### INTRODUCTION

The salivary glands of the blowfly *Calliphora* secrete a watery saliva rich in potassium and chloride. A series of physiological and electrophysiological studies have established some of the main features of both the control and the mechanism of salivation (Berridge & Prince, 1972; Prince & Berridge, 1972; Berridge, Lindley & Prince, 1975, 1976). Secretory activity is regulated by 5-hydroxytryptamine (5-HT) (Berridge, 1972). The second messengers cyclic AMP and calcium are responsible for mediating the ability of 5-HT to greatly accelerate the secretion of ions and water (Berridge, 1970; Prince, Berridge & Rasmussen, 1972; Prince & Berridge, 1973). Calcium is thought to increase chloride permeability of both the basal and apical membranes which thus allows chloride ions to rapidly equilibrate with potassium ions which are pumped into the lumen. The control of potassium transport is still in doubt although there are indications that the potassium pump is stimulated by cyclic AMP.

To find out more about how 5-HT regulates secretion it is necessary to have more information on the mechanism of potassium transport. As yet, there has been little consideration of how potassium enters across the basal surface to replace the potassium ions which are being pumped into the lumen across the apical plasma membrane.

\* Present address: Fachbereich Biologie, Universität Konstanz, 775 Konstanz, Postfach 7733, Federal Republic of Germany.

A conventional sodium potassium pump is apparently not involved because potassium secretion can continue in a sodium-free medium (Berridge *et al.* 1976). One possibility is that there is a sodium independent potassium pump. Another possibility is that potassium may enter the cell passively down an electrochemical gradient created when potassium is pumped from the cell into the lumen. The intracellular level of potassium will depend on the balance between rate of entry across the basal membrane and rate of removal from the cell across the apical membrane. In order for potassium to enter passively, the membrane potential ( $E_m$ ) must exceed the potassium equilibrium potential ( $E_k$ ). We have attempted to determine  $E_k$  by measuring the intracellular level of potassium using potassium-sensitive microelectrodes (Walker, 1971; Zeuthen & Monge, 1975).

#### METHODS

##### *Preparation of isolated salivary glands and measurements of salivation*

The salivary glands of adult blowflies were set up in a Perspex chamber as described previously (Berridge & Prince, 1972). The Perspex chamber was perfused with saline. A potassium concentration between 5 and 10 mM was regarded as normal; the sodium concentration was adjusted so that the Na + K concentration always equalled 130 mM. The concentrations of the other ions were (mM): Ca 2, Mg 2, Tris 10, Cl 156, phosphate 2, malate 2.7, glutamate 2.7 and glucose 10. When the potassium concentration was decreased, the sodium concentration was increased proportionately so as to maintain constant tonicity. Secretory rates were measured as described by Berridge & Patel (1968). The concentration of sodium and potassium in the saline was measured by means of a Beckman flame-photometer.

##### *Electrode preparation*

Many aspects of the preparation and calibration of the double-barrelled ion-selective microelectrodes were similar to those described by others (Silver, 1975; Zeuthen & Monge, 1975).

Glass capillaries with an outer diameter of 1.2 and 2.0 mm, respectively, were cleaned in a mixture of ethanol and nitric acid; after drying they were glued together at their ends. The thinner capillary was twisted around the other by 360° and then both capillaries were pulled with a vertical electrode puller (Narishige PE 2). The inner surface of the thicker central barrel was siliconized with pure dimethyl-dichloro-silane vapour; immediately afterwards the electrodes were dried at a temperature of 100 °C. The tip of the central electrode barrel (ion-exchanger barrel) was back filled with a short column of ion exchanger resin (potassium ion exchanger: Corning 477317). The remaining parts of the tip and the shank were filled with 0.5 M-KCl solution. The second electrode barrel was filled with either 2 M potassium chloride, 3 M sodium acetate or with 0.5 M sodium sulphate.

During the course of this study it became apparent that the measurement of intracellular potassium might be affected by the ionic composition of the solution contained in the reference barrel of these double-barrelled electrodes. For example, an overestimate might be obtained when 2 M-KCl is used because potassium leaking from the reference barrel might affect the neighbouring potassium-sensitive barrel especially when the electrode tip is in close apposition to membrane systems within the cell. To

Table 1. Measurements of intracellular potassium concentrations and membrane potential made with double-barrelled electrodes containing different solutions in the reference barrel

Reference barrel	Intracellular potassium concentration		Membrane potential	
	Control	5-HT	Control	5-HT
2 M-KCl	140.0 ± 1.9	149.6 ± 2.1	59.8 ± 0.5	66.4 ± 1.0
3 M-Na acetate	133.2 ± 1.5	139.0 ± 2.0	61.1 ± 0.4	65.9 ± 0.6
0.5 M-Na <sub>2</sub> SO <sub>4</sub>	105.0 ± 1.1	110.0 ± 1.2	54.5 ± 0.8	60.0 ± 0.6

avoid the possibility of such interactions, most of the observations reported in this paper have been repeated using the other reference solutions mentioned above. The intracellular measurements obtained with 2 M-KCl as a reference were slightly higher than those obtained with 3 M sodium acetate (Table 1). There were no effects on the qualitative changes in the intracellular level of potassium induced by either 5-HT or by varying the external potassium concentration.

Very much lower measurements were obtained using 0.5 M-Na<sub>2</sub>SO<sub>4</sub> as a reference solution but these are clearly artifactual because this reference solution produced a large junction potential thus accounting for much lower membrane potential measurements (Table 1).

#### Electrode calibration

After filling, the tips of the double-barrelled electrodes were dipped in a 0.5 M-KCl solution for 2 h to equilibrate the different interfaces. Thereafter the different measurements of the electrode properties were begun.

The d.c. resistance of each barrel was measured in normal physiological saline. These resistances ranged between 5 and 20 × 10<sup>8</sup> Ω in the reference barrel and between 1 and 10 × 10<sup>9</sup> Ω in the ion-exchanger barrel. The slope and the selectivity of the electrodes was measured using the following test solutions: 5, 10, 15, 50 and 150 mM KCl; 15 mM-KCl + 135 mM-NaCl; physiological saline. The potential difference measured with the ion-exchanger barrel ranged between 45 and 56 mV for a tenfold change of potassium concentration. Electrodes measuring potential differences above 50 mV were most often used.

#### Electrical recording

The ion-exchanger barrel was connected to the input of a Keithley amplifier and the KCl barrel to the input of a high input impedance amplifier (W.P.I. M701). These connexions were made with chlorided silver wires. The outputs of both amplifiers were connected to both inputs of a differential amplifier of an oscilloscope (Tektronix 502 A). The difference between the potential of the ion-exchanger barrel and the potential of the KCl barrel was recorded on a pen-recorder. The membrane potential was directly recorded on the second channel via the output of the high input impedance amplifier.

### *Experimental procedure*

The intracellular level of potassium was determined using two separate approaches.

(a) *Short-term measurements.* Measurements were made immediately after penetrating the cells, only those impalements which resulted in stable potentials were used for this analysis.

(b) *Long-term measurements.* In many experiments the intracellular level of potassium was monitored over an extended period during which those impaled cells which had stable potentials were subjected to various treatments. At the end of each impalement the electrode was withdrawn from the cell to ensure that there had been no shift in the two baselines during the course of the experiment. In order to construct a concentration scale (in mM) from the potential output (in mV) from the K-sensitive barrel we carried out an in-bath calibration. The response of the electrode to the potassium concentration of the normal saline (10 K or 5 K) provided one reference point. An additional reference point was obtained by perfusing the electrode with 140 K. Using such reference points it was possible to convert the potential trace into a record of concentration. It is important to stress that the intracellular values represent apparent concentrations; we have assumed that the intracellular activity coefficient for potassium is equal to that of the outer solution.

## RESULTS

### *The effect of 5-HT on intracellular potassium levels*

The intracellular level of potassium always increased when salivary glands were stimulated with  $1 \times 10^{-8}$  M 5-HT (Table 1). This increase was apparent despite the quantitative differences in potassium measurements obtained with the different reference solutions (Table 1). The measurements made with 3 M sodium acetate are probably the most reliable and the changes in membrane potential and intracellular potassium concentration which occur during the action of 5-HT are shown in Fig. 1. The potential across the basal membrane of the cell responded to 5-HT in a similar manner to that described previously (Prince & Berridge, 1972). Initially there was a small but rapid hyperpolarization which was followed, after a short delay, by a further hyperpolarization which developed more slowly (Fig. 1). During the course of this secondary hyperpolarization there was an increase in the intracellular level of potassium. After each 5-HT treatment, the membrane potential and potassium concentration returned to their resting levels.

Using the Nernst equation it is possible to calculate the potassium equilibrium potential ( $E_k$ ) from the average intracellular potassium measurements obtained with the 3 M sodium acetate electrodes (Table 1). In the absence of 5-HT,  $E_k$  ( $65.2 \pm 0.3$  mV) was considerably larger than  $E_m$  ( $61.1 \pm 0.4$  mV). However, during the action of 5-HT  $E_k$  ( $66.3 \pm 0.4$  mV) was not significantly different to  $E_m$  ( $65.9 \pm 0.6$  mV). A response similar to that shown in Fig. 1 was analysed and redrawn in Fig. 2 to illustrate more clearly the relationships between  $E_m$ ,  $E_k$  and the intracellular concentration of potassium during the action of 5-HT. Note that the increase in  $E_k$  is insufficient to account for the increase in  $E_m$  and that the major increase in intracellular potassium concentration occurs when  $E_m$  undergoes its secondary hyperpolarization (Fig. 2).

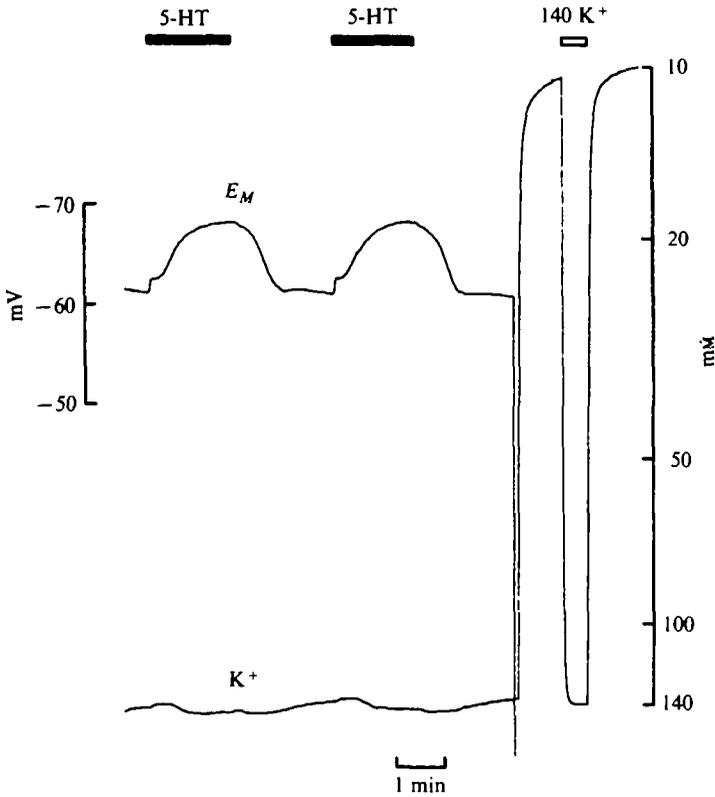


Fig. 1. The effect of  $1 \times 10^{-8}$  M 5-HT on membrane potential ( $E_m$ ) and intracellular potassium concentration ( $K^+$ ) of an isolated salivary gland perfused with 10 K saline. At the end of the experiment, the electrode was removed from the cell, the potassium electrode responded to the potassium level (10 mM) of the bathing medium. While still in the perfusion bath, the electrode was treated with a test saline containing 140 mM potassium to provide another reference point to construct the potassium scale as described in the Methods. (Reference solution: 3 M sodium acetate.)

#### *The effect of varying external potassium levels*

When the external potassium concentration was reduced from 10 to 2 mM there was little change in the intracellular level of potassium and the hyperpolarization of the basal plasma membrane remained steady (Fig. 3). Membrane potential and potassium concentration returned rapidly to their previous levels as soon as the glands were returned to 10 mM potassium. Reduction in potassium concentration below 2 mM caused marked changes in the intracellular level of this ion. When glands were perfused with 0.2 mM potassium, the intracellular concentration fell from 170 mM to approximately 5 mM within 10 min (Figs. 4, 5). There was an equally rapid reaccumulation of potassium when the external potassium concentration was returned to its previous level of 10 mM/l. This very large fluctuation in the intracellular level of potassium has marked effects on membrane potential. Initially there was a large hyperpolarization in response to the low level of external potassium, but as the intracellular level of potassium fell the membrane potential began to fall in unison (Fig. 4). When

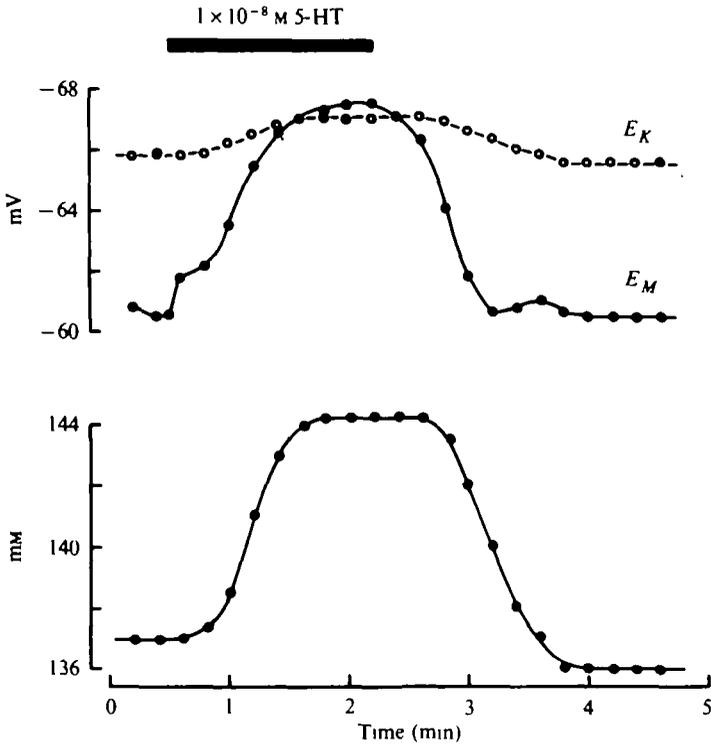


Fig. 2. A comparison of the changes in  $E_m$  and  $E_k$  during the action of 5-HT taken from a recording similar to that shown in Fig. 1. The change in the intracellular level of potassium is shown at the bottom.

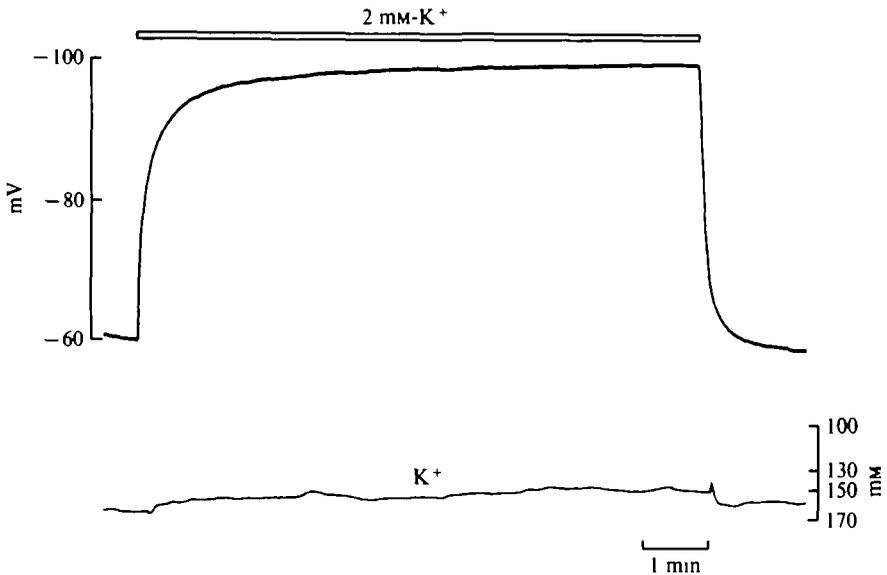


Fig. 3. The effect of lowering the potassium concentration of the perfusing saline from its normal level of 10 to 2 mM on membrane potential (upper trace) and intracellular potassium concentration. (Reference solution: 2 M-KCl.)

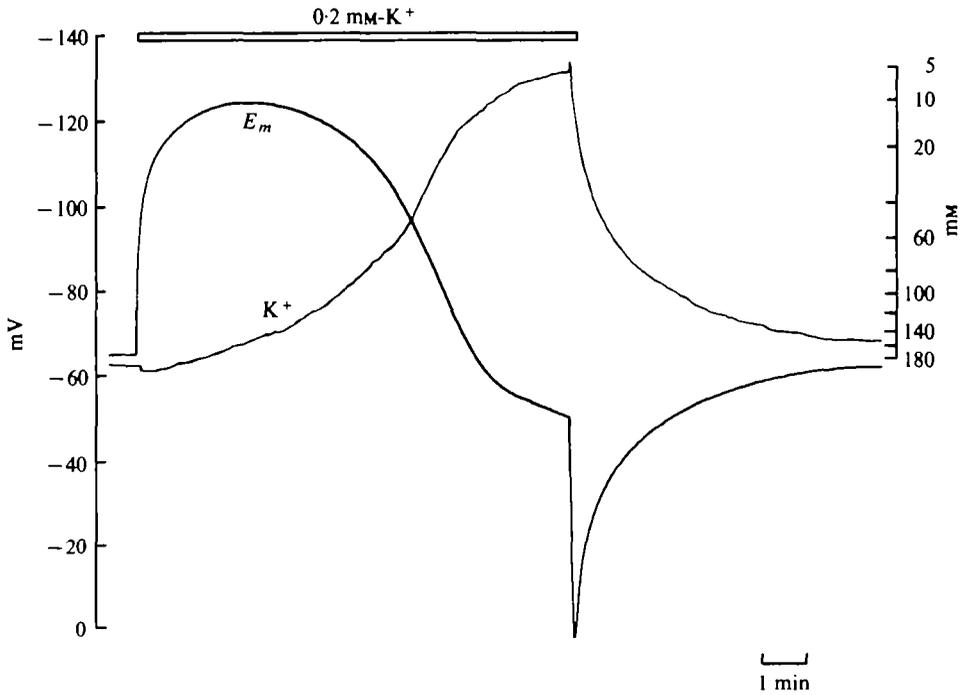


Fig. 4. The effect of lowering the potassium concentration of the perfusing saline from its normal level of 10 to 0.2 mM on membrane potential ( $E_m$ ) and intracellular potassium concentration ( $K^+$ ). Note that  $E_m$  went positive for a short period when the gland was returned to the 10 K saline. (Reference solution: 2 M-KCl.)

the normal saline was returned the potential suddenly depolarized and for a short period it actually reversed and the inside of the cell became positive with respect to the bathing medium. However, as soon as the cell began to reaccumulate potassium the potential slowly returned to normal (Figs. 4, 5). In this low potassium medium, there is a marked discrepancy between  $E_k$  and  $E_m$  which is consistent with previous studies on this gland which showed that the plot of membrane potential against extracellular potassium concentration was not linear in low potassium media apparently because the membrane becomes less responsive to potassium (Berridge *et al.* 1976).

Similar experiments to those just described for 0.2 mM potassium were performed at other external potassium concentrations both in the presence and absence of 5-HT (Fig. 6). When the concentration was reduced below 2 mM there was a regular fall in the intracellular level of potassium. The decline in the intracellular level of potassium was smaller if the glands were treated with 5-HT, thus confirming the earlier observations (Fig. 1, Table 1) that this agent can bring about an increase in the intracellular level of potassium.

This ability of 5-HT to partly alleviate the fall in the intracellular level of potassium which occurs when the glands are in low potassium media is shown in Figs. 7 and 8. The regular decline in the intracellular level of potassium observed when glands were placed in low potassium medium (0.5 mM, Figs. 7, 8) was partially reversed on addition of 5-HT. This reaccumulation of potassium was associated with a large hyper-

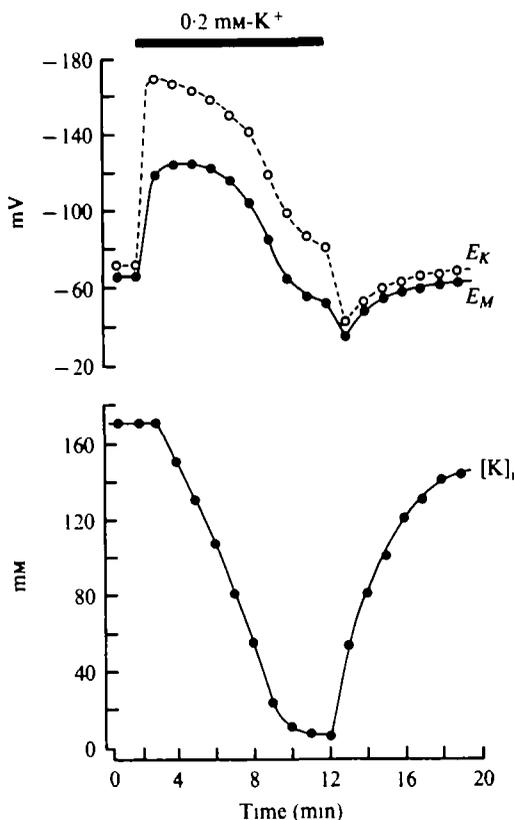


Fig. 5. Analysis of the traces taken from Fig. 4. Note the large discrepancy between  $E_m$  and  $E_k$  when the glands are perfused with 0.2 mM potassium.

polarization of the basal membrane potential ( $E_m$ ). The large discrepancy between  $E_k$  and  $E_m$ , which was found consistently in low potassium media (see Fig. 5) was greatly reduced after stimulation with 5-HT (Fig. 8).

An interesting feature of the membrane potential response in Fig. 7 is the small phasic depolarization which occurs immediately after adding 5-HT. At normal potassium concentrations there is a phasic hyperpolarization (Fig. 1).

#### *The effect of ouabain on intracellular potassium levels and secretory activity*

Some effect of ouabain on membrane potential and internal potassium levels was noted in unstimulated salivary glands (Fig. 9). The intracellular level of potassium was first lowered by perfusing the glands in a potassium-free medium in the presence of  $10^{-4}$  M ouabain. The glands were then returned to a normal potassium level while still in the presence of ouabain (Fig. 9). Although there was some recovery of internal potassium with a concomitant recovery of membrane potential, neither parameter returned to normal. However, on addition of  $1 \times 10^{-8}$  M 5-HT there was a rapid restoration of both potential and potassium level to values exceeding those seen in the resting gland. Once 5-HT was removed both parameters returned close to their initial levels (Fig. 9).

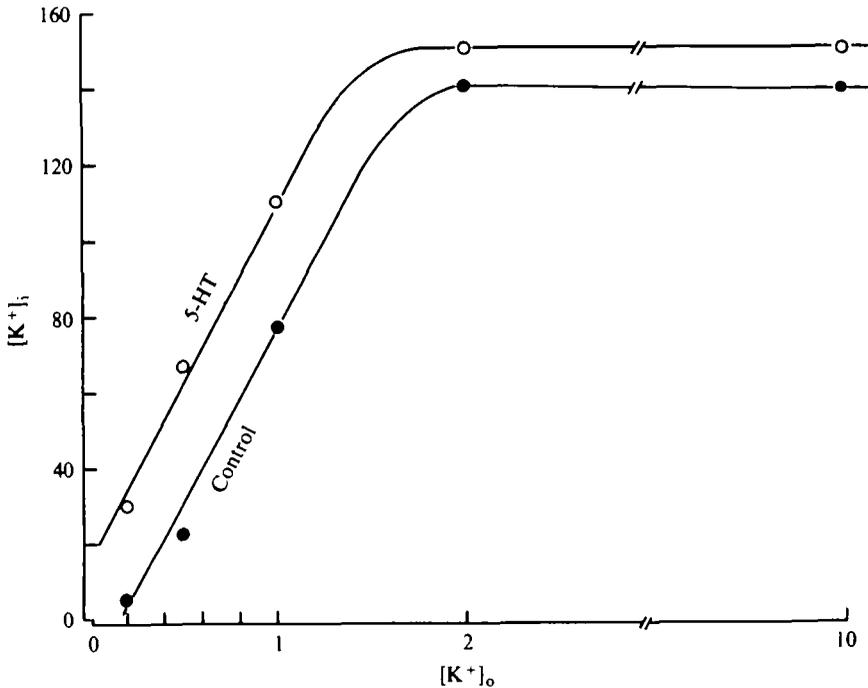


Fig. 6. The relationship between external  $[K^+]_o$  and internal  $[K^+]_i$  potassium concentrations in the presence (○—○) or absence (●—●) of  $1 \times 10^{-8}$  M 5-HT.

The effect of ouabain on the secretory activity of isolated salivary glands was tested. A high concentration of ouabain ( $1 \times 10^{-3}$  M) had no effect either on the rate of fluid secretion or on the concentration of potassium and sodium in the final saliva (Fig. 10). In a stimulated gland, therefore, ouabain seemed to have little effect on the ability of salivary glands to sequester potassium from the bathing medium so as to maintain a high rate of potassium secretion.

#### DISCUSSION

The intracellular level of potassium in the salivary glands of *Calliphora* is increased during the action of 5-HT. This is a surprising observation because the large quantities of potassium which are secreted into the lumen to generate a flow of saliva must be derived from the intracellular compartment. It is necessary to conclude, therefore, that during the onset of secretion, potassium must enter the cell across the basal membrane faster than it leaves the cell across the apical membrane. Once secretion has been established, the rate of entry and extrusion will be exactly matched. When the external potassium concentration was reduced below 2 mM, the entry of potassium from the bathing medium cannot keep pace with the potassium leaving the cell across the luminal membrane and the cell rapidly loses its internal potassium. Even at these low external potassium concentrations, however, 5-HT can still bring about a marked increase in the intracellular level of potassium (Figs. 6–8). Some of the increase in potassium concentration may arise from a decrease in cell volume, but this cannot be the sole mechanism because in the low-potassium medium it would be necessary to

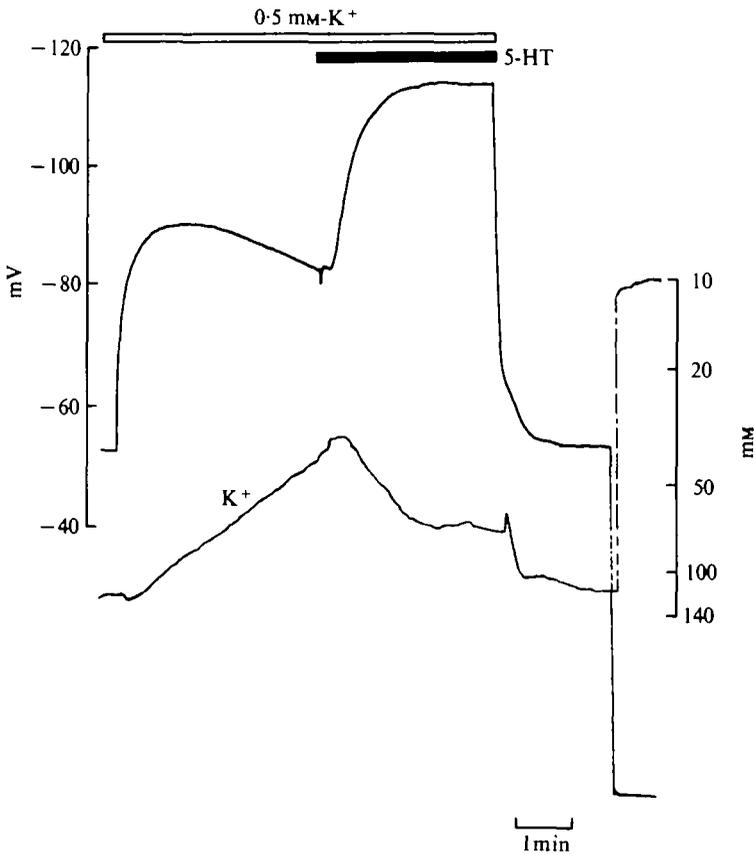


Fig. 7. The ability of 5-HT ( $1 \times 10^{-8}$  M) to partially restore the loss of intracellular potassium which occurs when salivary glands are perfused with a low potassium saline (0.5 mM). Note the large membrane hyperpolarization which develops during the action of 5-HT which apparently precedes the increase in the intracellular level of potassium.

assume that the cell volume decreased by half in order to account for the large increase in potassium level produced by 5-HT (Fig. 8). Such large changes in cell volume were never observed and it seems more likely that the accumulation of potassium is somehow linked to the marked membrane hyperpolarization which is a constant feature of 5-HT action.

If potassium is to enter the cell passively, the membrane potential ( $E_m$ ) must rise above the potassium equilibrium potential ( $E_k$ ). To calculate the latter from the Nernst equation it is necessary to have a precise measure of the intracellular level of potassium. There are certain difficulties in making such precise measurements when using double-barrelled electrodes. In the light of these uncertainties, it is obviously unwise to place too much reliance on the absolute values obtained with these electrodes. There is no doubt that the intracellular level of potassium increases during the action of 5-HT. It is also evident that during this potassium accumulation the membrane potential increases towards  $E_k$ . The uncertainty lies in trying to decide whether  $E_m$  exceeds  $E_k$  to provide the necessary gradient for potassium to enter passively. In

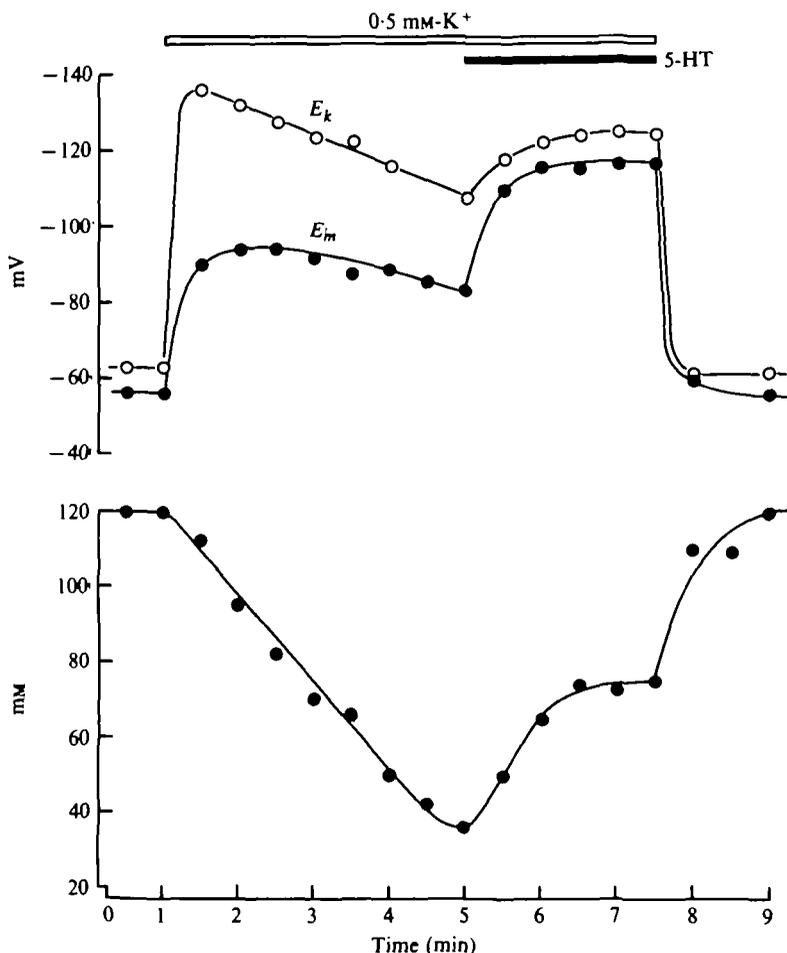


Fig. 8. Analysis of the traces taken from Fig. 7. The large discrepancy between  $E_m$  and  $E_k$ , which is a characteristic feature of glands in low potassium saline (see Fig. 5), is greatly reduced during the action of  $1 \times 10^{-8}$  M 5-HT. The intracellular level of potassium is shown on the bottom trace. (Reference solution: 2M-KCl.)

the experiments using 2 M-KCl in the reference barrel (which may have led to an overestimate of intracellular potassium)  $E_m$  was always less than  $E_k$  (Figs. 5, 8). When 3 M sodium acetate was used,  $E_m$  came close to and sometimes exceeded  $E_k$  (Fig. 2). When considering the significance of these differences between  $E_m$  and  $E_k$  it is also necessary to point out that there may have been errors in measuring membrane potential. The stable membrane potentials recorded during long term experiments were usually a few millivolts less than those recorded immediately after penetrating the cell. The fact that  $E_m$  was found to approach and sometimes exceed  $E_k$  may thus be highly significant and does suggest that the membrane hyperpolarization which occurs during the action of 5-HT could provide the necessary gradient for a passive entry of potassium.

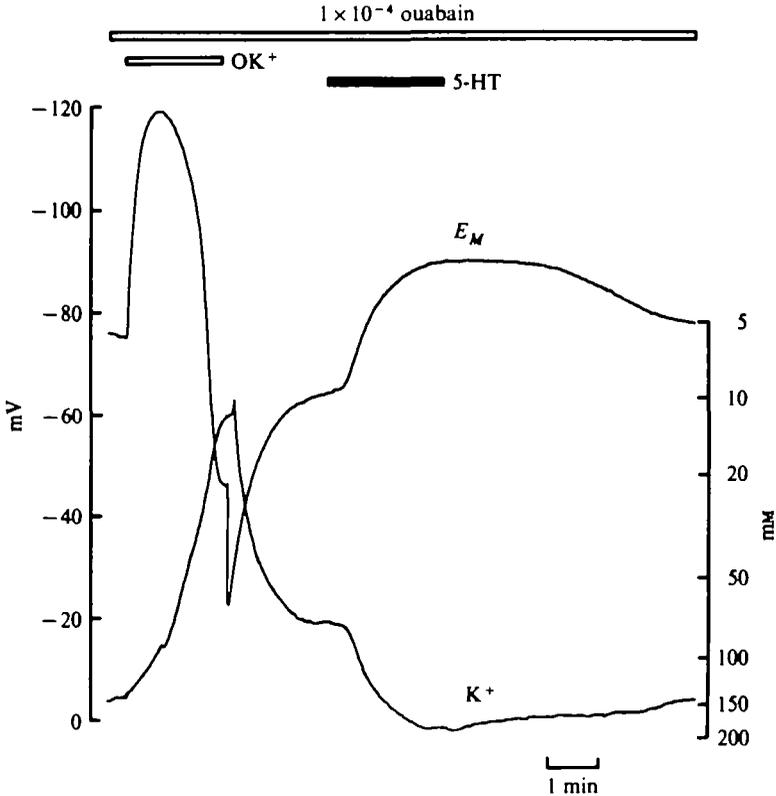


Fig. 9. The effect of ouabain ( $1 \times 10^{-4}$  M) on the ability of depleted glands to reaccumulate potassium when returned to control medium containing 5 mM potassium after a period in K-free medium. The inhibitory effect of ouabain was overcome by adding  $1 \times 10^{-8}$  M 5-HT.

It remains to consider the ionic basis of the membrane hyperpolarization which could be responsible for this passive entry of potassium. It is apparent from Fig. 2 that the increase in potassium concentration is insufficient to account for this hyperpolarization because the increase in  $E_k$  is less than  $E_m$ . Some other ionic mechanism must be responsible for this increase in potential. During the action of 5-HT the membrane hyperpolarizes in two stages (Fig. 1). Initially there is a small but rapid hyperpolarization which is thought to result from the sudden calcium-dependent increase in chloride permeability (Berridge *et al.* 1975). The observation that this early hyperpolarization at normal membrane potentials becomes a depolarization at higher membrane potentials (Fig. 7) suggests a reversal phenomenon and provides further evidence that this early potential change is due to a sudden increase in chloride permeability. There is a further hyperpolarization which develops more slowly with a time course identical to that for the onset of fluid secretion. It has been proposed that this secondary increase in potential results from a decrease in the intracellular level of chloride which occurs when chloride follows the potassium which is being secreted into the lumen to generate the flow of saliva (Berridge *et al.* 1975). Preliminary experiments with a chloride-sensitive electrode have shown that during the action of 5-HT

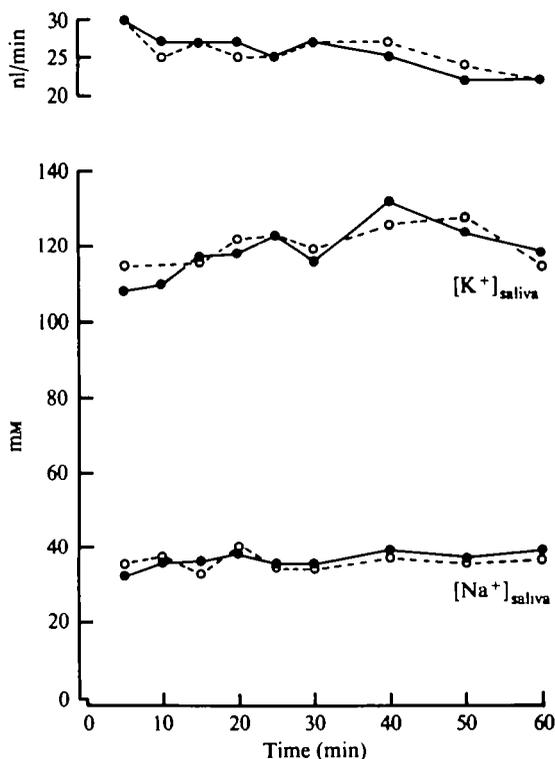


Fig. 10. The absence of any effect of  $1 \times 10^{-3}$  M ouabain on either the rate of fluid secretion (top curves) or on the potassium and sodium content of the saliva. ●—●, Control; ○---○,  $1 \times 10^{-3}$  M ouabain.

the intracellular level of chloride does indeed decrease with a time course similar to the onset of this secondary hyperpolarization. Note that the time course for the increase in the intracellular levels of potassium closely follows the time course for this secondary hyperpolarization. In effect therefore the entry of potassium across the basal membrane is linked to the movement of chloride which, in turn, is linked to the rate of potassium transport into the lumen. Such a mechanism would ensure a precise matching between potassium entry across the basal membrane and its extrusion across the apical membrane which thus enables the cell to maintain relatively constant intracellular potassium concentrations despite wide variations in secretory rates.

The ability of salivary glands to accumulate potassium passively is certainly consistent with several lines of evidence suggesting that a classical Na-K exchange pump is apparently not responsible for potassium entry during 5-HT stimulation. Firstly, *Calliphora* salivary glands continue to secrete a potassium-rich saliva in a medium where sodium is completely replaced with Tris (Berridge *et al.* 1976). Secondly, high concentrations of ouabain had no effect either on the composition of the saliva or on rate of secretion (Fig. 10). However, there was some indication that in unstimulated glands ouabain could prevent the reaccumulation of potassium by depleted glands (Fig. 9). A ouabain-sensitive, Na-K-activated ATPase has been described in another

insect transporting epithelium, the rectal epithelium of the cockroach *Periplaneta* (Tolman & Steele, 1976). O'Riordan (1969) also observed that low concentrations of ouabain will abolish the electrical potential maintained across the midgut of the cockroach. It is conceivable therefore that a similar Na-K exchange pump is responsible for maintaining high potassium levels in resting *Calliphora* salivary glands whereas the potential-dependent mechanism for potassium entry takes over when large amounts of potassium are required to generate the rapid flow of saliva during the action of 5-HT.

Dr Schlue's stay in the ARC Unit at Cambridge was made possible by a grant from the Deutsche Forschungsgemeinschaft, Schl 169/1. We would like to thank Dr Tom Zeuthen and Mr Peter Buchan for their help during the course of these studies.

## REFERENCES

- BERRIDGE, M. J. (1970). The role of 5-hydroxytryptamine and cyclic AMP in the control of fluid secretion by isolated salivary glands. *J. exp. Biol.* **53**, 171-186.
- BERRIDGE, M. J. (1972). The mode of action of 5-hydroxytryptamine. *J. exp. Biol.* **56**, 311-321.
- BERRIDGE, M. J., LINDLEY, B. D. & PRINCE, W. T. (1975). Membrane permeability changes during stimulation of isolated salivary glands of *Calliphora* by 5-hydroxytryptamine. *J. Physiol.* **244**, 549-567.
- BERRIDGE, M. J., LINDLEY, B. D. & PRINCE, W. T. (1976). Studies on the mechanism of fluid secretion by isolated salivary glands of *Calliphora*. *J. exp. Biol.* **64**, 311-322.
- BERRIDGE, M. J. & PATEL, N. E. (1968). Insect salivary glands: stimulation of fluid secretion by 5-hydroxytryptamine and adenosine 3',5'-monophosphate. *Science, N.Y.* **162**, 462-463.
- BERRIDGE, M. J. & PRINCE, W. T. (1972). Transepithelial potential changes during stimulation of isolated salivary glands with 5-hydroxytryptamine and cyclic AMP. *J. exp. Biol.* **56**, 139-153.
- O'RIORDAN, A. M. (1969). Electrolyte movement in the isolated midgut of the cockroach (*Periplaneta americana* L.). *J. exp. Biol.* **51**, 699-714.
- PRINCE, W. T. & BERRIDGE, M. J. (1972). The effects of 5-hydroxytryptamine and cyclic AMP on the potential profile across isolated salivary glands. *J. exp. Biol.* **56**, 323-333.
- PRINCE, W. T. & BERRIDGE, M. J. (1973). The role of calcium in the action of 5-hydroxytryptamine and cyclic AMP on salivary glands. *J. exp. Biol.* **58**, 367-384.
- PRINCE, W. T., BERRIDGE, M. J. & RASMUSSEN, H. (1972). Role of calcium and adenosine-3',5'-cyclic monophosphate in controlling fly salivary gland secretion. *Proc. natn. Acad. Sci. U.S.A.* **69**, 553-557.
- SILVER, I. A. (1975). Measurement of pH and ionic composition of pericellular sites. *Phil. Trans. R. Soc. Lond. B* **271**, 261-272.
- TOLMAN, J. H. & STEELE, J. E. (1976). A ouabain-sensitive, (Na<sup>+</sup>-K<sup>+</sup>)-activated ATPase in the rectal epithelium of the American cockroach, *Periplaneta americana*. *Insect Bioch.* **6**, 513-517.
- WALKER, J. L. (1971). Ion specific liquid ion exchanger microelectrodes. *Analyt. Chem.* **43**, 89A-93A.
- ZEUTHEN, T. & MONGE, C. (1975). Intra- and extracellular gradients of electrical potential and ion activities of the epithelial cells of the rabbit ileum *in vivo* recorded by microelectrodes. *Phil. Trans. R. Soc. B* **71**, 277-281.