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STIMULUS-SECRETION COUPLING IN AN INSECT SALIVARY GLAND: CELL ACTIVATION BY ELEVATED POTASSIUM CONCENTRATIONS

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SUMMARY

1. Fluid secretion by isolated salivary glands was stimulated by elevating the external potassium concentration.

2. The stimulatory effect of potassium was dependent on external calcium and was potentiated by a subthreshold dose of 5-hydroxytryptamine (5-HT).

3. During the action of 120 mM potassium there was a large calcium-dependent decrease in transepithelial resistance similar to that produced with 5-HT at normal potassium concentrations.

4. These results on *Calliphora* salivary glands are compared with other cases where cells are activated by high potassium. In most cases, the effect of high potassium is dependent upon calcium, suggesting that the latter plays a primary role in cell activation.

INTRODUCTION

The activity of a wide range of different cell types can be increased by elevating the external potassium concentration. This stimulatory effect was recognized first in excitable tissues: high concentrations of potassium inducing skeletal and smooth muscle contraction (Hodgkin & Horowicz, 1960; Durbin & Jenkinson, 1961) as well as the release of hormones from the neurohypophysis (Douglas & Poisner, 1964). Also, high potassium can stimulate the release of granules from non-excitable tissues such as the β-cells of the endocrine pancreas (Grodsky & Bennett, 1966) or the hormone secreting cells of the anterior pituitary (Samli & Geschwind, 1968; Macleod & Leymeyer, 1970; Vale, Burgus & Guillemin, 1967). The stimulatory effect of potassium is usually dependent on the external calcium concentration. The current hypothesis is that membrane depolarization caused by elevated potassium levels increases the influx of calcium, which then stimulates the appropriate effector systems responsible for cell activation. These observations point to the ubiquitous role of calcium in stimulus-excitation coupling in both muscle contraction and the release of granules by exocytosis. In this paper we extend this generalization by showing that high potassium can lead to a calcium-dependent stimulation of ion transport.

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METHODS

The salivary glands taken from adult blowflies, *Calliphora erythrocephala*, were used throughout this study. The rate of fluid secretion was measured as described by Berridge & Patel (1968). The 'oil-gap' method used to measure various electrical parameters was the same as that described previously (Berridge & Prince, 1972*a*; Prince & Berridge, 1972; Berridge, Lindley & Prince, 1975). Cyclic AMP was assayed using the method described by Prince, Berridge & Rasmussen (1972).

The 'normal saline' used in these experiments was the same as that used before and had the following composition (mM): Na, 155; K, 20; Ca, 2; Mg, 2; Tris, 10; Cl, 156, phosphate, 2; malate, 2·7; glutamate, 2·7; glucose 10. When the potassium concentration was varied the sodium concentration was altered accordingly so as to maintain the sum of these two cations at 175 mM. In the secretory experiments the sum of these two cations was 150 mM (such variations in total cation concentration had no effect on secretory or potential measurements *per se*). Phenol red (< 0·01 mM/l) was routinely added to keep a continuous check that the pH was maintained between 7·2 and 7·4.

In the experiments using calcium-free salines 5 mM-EGTA (ethyleneglycol-bis-(β-amino ethyl ether)*N*,*N*′-tetra-acetic acid) was added to ensure that these salines remained calcium-free.

RESULTS

(*a*) *Secretory responses*

The effect of varying the external potassium concentration on the rate of fluid secretion is illustrated in Fig. 1. Potassium stimulated secretion in a dose-dependent manner. However, the stimulation developed much more slowly (half time of approximately 5 min) than that observed previously during the action of 5-hydroxytryptamine (half time of approximately 30 sec; Berridge, 1970). At the highest dose (150 mM) the rate of secretion was not maintained, the cells became opaque and did not respond when subsequently treated with 5-HT. Above the threshold level of 30–40 mM potassium there was a linear relationship between rate of fluid secretion and potassium concentration (Fig. 1*b*).

The stimulatory effect of potassium was dependent upon external calcium (Fig. 2). In the absence of calcium, 120 mM potassium produced only a slight increase in secretory rate which promptly rose to the control rate when calcium was readmitted.

Previous studies have shown that cyclic AMP plays an important role in the regulation of fluid secretion by these isolated salivary glands. The effect of agents which increase the intracellular level of cyclic AMP were therefore tested in combination with 120 mM potassium. If 10^{-2} M theophylline was added together with 120 mM-K, both the rate of onset and the maximal rate of secretion were increased. A subthreshold dose of 5-HT (1×10^{-9} M) was also capable of decreasing the response time and potentiating the action of potassium (Fig. 3).

A high concentration of gramine (10^{-3} M), which completely inhibited the action of 2×10^{-7} M 5-HT, had no effect on glands stimulated with 120 mM potassium thus ruling out the possibility that the high potassium was releasing endogenous 5-HT.

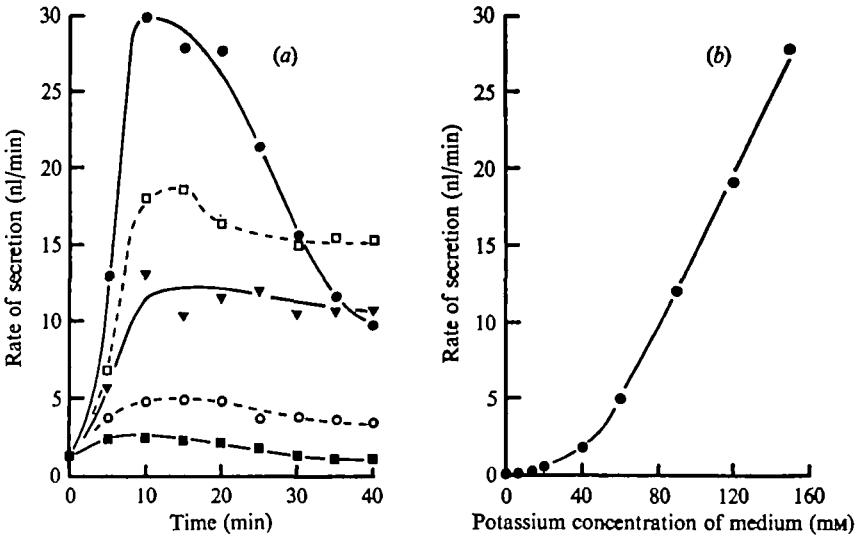


Fig. 1. (*a*) Secretory responses to salines containing 40 (■), 60 (○), 90 (▼), 120 (□), 150 (●) mM potassium (applied at 0 min) in the absence of 5-HT. (*b*) Secretory rates induced by different concentrations of potassium in the bathing medium. These rates were all obtained 10 min after addition of the test solution.

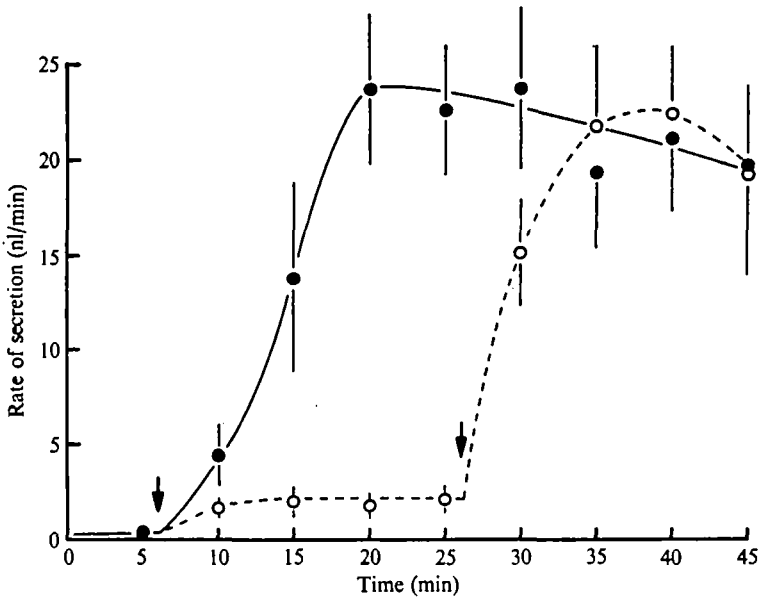


Fig. 2. Secretory responses (± 2 × S.E.M.) to salines containing 120 mM potassium (added at the first arrow) in the presence of 2 mM calcium (●) or in the presence of 5 mM EGTA (○). At the second arrow 2 mM calcium was added to the glands previously bathed in EGTA.

(*b*) *Electrical responses*

The changes in transepithelial potential, lumen relative to the bathing medium, are illustrated in Fig. 4. On addition of 120 mM potassium, the potential became rapidly more positive, probably as a result of depolarizing the basal plasma membrane. The subsequent changes in potential are difficult to interpret, but usually there was a gradual

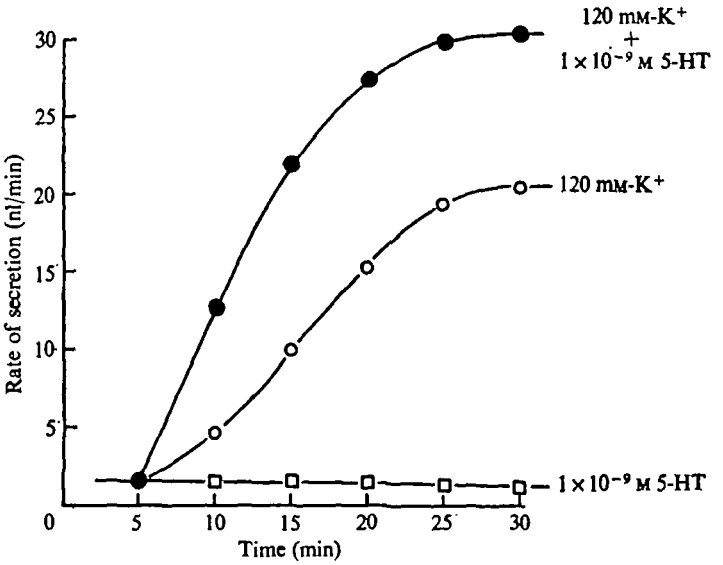


Fig. 3. The ability of a subthreshold dose of 5-HT (1×10^{-9} M) to potentiate the stimulatory effect of 120 mM potassium. The 5-HT and high potassium were applied at 5 min.

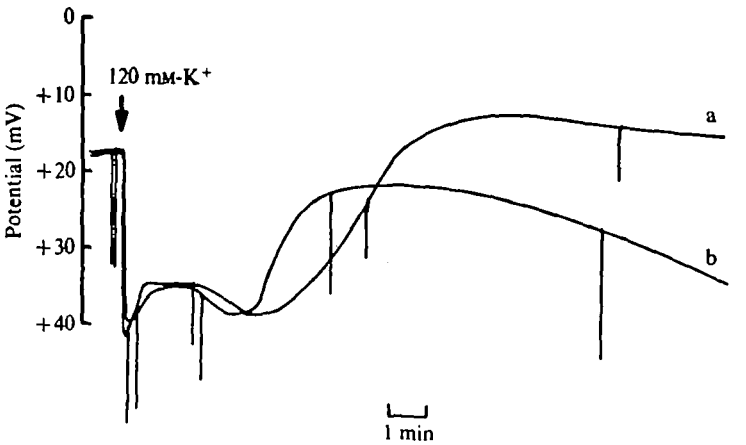
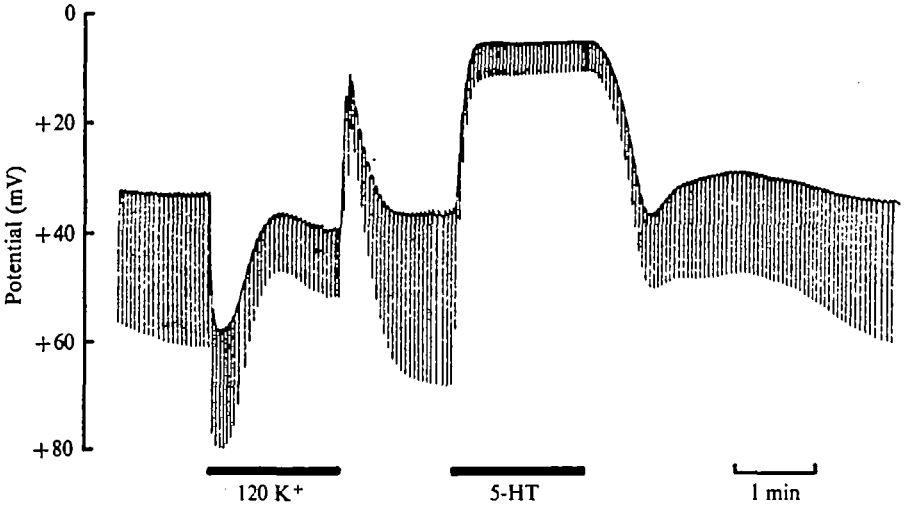


Fig. 4. The effect of 120 mM potassium on transepithelial potential (lumen relative to the bathing medium) either (*a*) in the presence or (*b*) in the absence of calcium (5 mM-EGTA). The vertical lines represent potential deflexions resulting from short current pulses (0·1 μA).

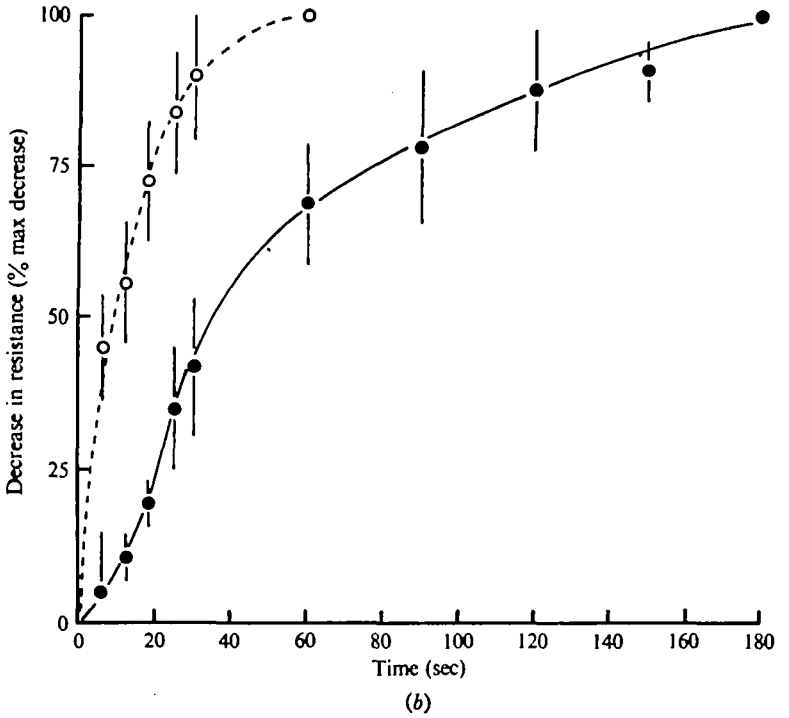
drift back towards the resting potential. In the absence of calcium, the transepithelial potential remained more positive than in the presence of calcium.

The vertical bars in Fig. 4 represent potential deflexions produced by current pulses of 0·1 μA. In the absence of calcium, there was little change in the magnitude of these deflexions, indicating only very small changes in resistance. In the presence of calcium, however, the potential deflexions decreased, suggesting a marked decrease in resistance.

A comparison of the effect of 120 mM potassium with 1×10^{-8} M 5-HT showed that the decrease in resistance during the action of potassium occurred more slowly than during 5-HT (Fig. 5*a*). A large number of such traces have been summarized in



(*a*)



(*b*)

Fig. 5. (*a*) Transepithelial potential (mV) responses to salines containing 120 mM potassium or 10^{-5} M 5-HT (applied for the duration of the solid bars). Potential deflexions were caused by 250 msec current pulses of 0·2 μA. (*b*) A summary of experiments comparing resistance changes caused by 10^{-5} M 5-HT (○) and 120 mM potassium (●). Each point is the average of six experiments (±2 × S.E.M.).

Fig. 5(*b*) which clearly illustrates the difference in the time course of these resistance changes induced by potassium or 5-HT.

(*c*) *Cyclic AMP concentrations*

In a resting gland the cyclic AMP level was $0{\cdot}121 \pm 0{\cdot}01$ pmole/gland and this increased significantly during stimulation with 1×10^{-8} M 5-HT, to $0{\cdot}225 \pm 0{\cdot}03$ p-mole/gland. Although there was a small increase in the cyclic AMP level during the action of 120 mM potassium (to $0{\cdot}16 \pm 0{\cdot}02$ pmoles/gland) this was not significant.

DISCUSSION

Previously we proposed that both cyclic AMP and calcium mediated the intracellular effects of 5-HT (Berridge & Prince, 1972*b*). Calcium increases anion permeability whereas cyclic AMP has two possible actions. First, it may augment the calcium signal by causing a release of calcium from some intracellular reservoir, and secondly, it may activate a potassium pump on the apical plasma membrane. The net increase in potassium and chloride transport into the lumen is responsible for the increased flow of saliva. Since high potassium solutions, like 5-HT, could stimulate fluid secretion, it is of interest to examine the intracellular basis of this stimulation. It is also of interest to consider whether similar intracellular control mechanisms are activated when potassium stimulates a wide range of other cellular activities as outlined in the Introduction.

Potassium stimulation of fluid secretion by salivary glands is apparently mediated by calcium. As in other tissues where high potassium stimulates cell activation there is an absolute requirement for extracellular calcium (Fig. 2). In addition to the salivary gland system described here, other examples include contraction of smooth muscle (Robertson, 1960; Durbin & Jenkinson, 1961), release of vasopressin from the neurohypophysis (Douglas & Poisner, 1964) and release of hormones from the anterior pituitary (Vale *et al.* 1971; Samli & Geschwind, 1968). Evidence of potassium stimulated calcium entry in the salivary gland is provided by the electrical responses (Figs. 4 and 5). High potassium causes a decrease in resistance (Fig. 5) which is calcium dependent (Fig. 4). The time course of the potassium-induced resistance change is much slower than the change in resistance induced by 5-HT (Fig. 5) but is the same order as the time course for the onset of potassium-induced fluid secretion. Previously it was suggested that 5-HT-induced resistance changes are caused by an increase in the intracellular concentration of calcium (Berridge *et al.* 1975). Also, in gastropod nerve cells injection of calcium causes changes in membrane resistance (Meech, 1972). By analogy with both the nerve cell and the effect of 5-HT on the resistance of salivary glands, the potassium-induced resistance changes are probably caused by the entry of calcium.

Whether or not cyclic AMP is involved in potassium-induced fluid secretion by salivary glands is open to discussion. Unfortunately, the cyclic AMP measurements were inconclusive because, although potassium did stimulate an increase in the level of cyclic AMP, the rise was not significant. There is no evidence for cyclic AMP mediation in potassium-contractures in muscle (Andersson, 1972) or in potassium-induced release of hormones from the anterior pituitary (Zor *et al.* 1970). The calcium ionophore A 23187 can stimulate fluid secretion in isolated salivary glands

without elevating the intracellular level of cyclic AMP (Prince *et al.* 1973). However, we must be cautious about relating cyclic AMP levels to cell activity because in the adrenal cortex maximal steroidogenesis is observed after very small changes in the cyclic AMP level (Beall & Sayers, 1972). If there is an increase in the intracellular level of cyclic AMP during the action of high potassium, the rise is apparently insufficient to make the glands independent of external calcium. It was shown previously that, in the absence of external calcium, 5-HT was capable of stimulating secretion by isolated salivary glands for a considerable period of time (Prince & Berridge, 1973). This temporary independence of external calcium is evident because cyclic AMP can release calcium from intracellular reservoirs (Prince *et al.* 1972; Berridge, Lindley & Prince, 1974). In contrast, the stimulatory effect of potassium was totally dependent on external calcium. An analogous situation exists in the anterior pituitary where the ability of high potassium to release hormones is completely abolished by removing calcium (Katsumi, Kamberi & McCann, 1969). However, removal of calcium only partially inhibited the effect of the normal releasing factor. In fact, the lack of calcium could be completely overcome by increasing the concentration of the releasing factors which are known to elevate the cyclic AMP levels in the anterior pituitary (Zor *et al.* 1970; Peake, Steiner & Daughaday, 1972). These observations suggest that cyclic AMP may function in the anterior pituitary, as in the insect salivary gland, by mediating a release of intracellular calcium. In this respect it is interesting to note that there is an additive effect between high potassium and the hypothalamic releasing factors (Samli & Geschwind, 1968; Macleod & Leymeyer, 1970). A similar effect occurs in salivary glands where a subthreshold concentration of 5-HT was found to potentiate the action of 120 mM potassium (Fig. 3).

The central importance of calcium as an intracellular regulator is highlighted by these studies on potassium stimulation of cellular activity. The exact role of cyclic AMP is not so clear but there are many reasons for supposing that, under normal conditions, it may function to modulate the intracellular level of calcium. In many cases cyclic AMP may lead to cell activation by facilitating the release of calcium from intracellular reservoirs.

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