forceful increases in smooth muscle tone after administration of PLS in vitro were obtained with umbilical arteries. The responses were less pronounced in veins from the lower extremities. The contraction could not be blocked with pentholamine.

It is not surprising to find a close resemblance when comparing the dose-response relationship of PLS and $PGE_2$ since the doses of PLS were determined with reference to $PGE_2$, the only difference being the target organ. We could, however, note specific characteristics regarding the kinetics of the response with shorter latency periods and faster contractions elicited by PLS which makes it reasonable to assume that PLS is not identical with $PGE_2$.

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## Some factors affecting Malpighian tubule fluid secretion and transepithelial potential in *Locusta migratoria* L.

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*Summary.* Both fluid secretion and transepithelial potential were stimulated by cAMP. Fluid secretion was unaffected by 5-HT over the concentration range $10^{-8}$–$10^{-4}$ M. The presence of ouabain in the bathing medium effected a decrease in transepithelial potential.

Ramsay[1] demonstrated that the transepithelial potentials across insect Malpighian tubules, irrespective of polarity, did not obey the Nernst equation for $K^+$. On this basis he proposed that active $K^+$ transport was taking place across the Malpighian tubules. Subsequent studies suggest that cation transport is the 'prime mover' in fluid secretion by insect Malpighian tubules[2]. The rate at which *Carausius* and *Rhodnius* tubules secrete fluid is considerably stimulated by the presence of 5-hydroxytryptamine (5-HT) in the bathing medium[3]. Similarly, cyclic 3′, 5′-adenosine monophosphate (cAMP) was found to stimulate secretion by the tubules of both species. It was concluded that 5-HT and the diuretic hormones may interact with the Malpighian tubule cells of *Rhodnius* and *Carausius* at specific sites, probably on the cell membrane facing the haemolymph. As a result of this, secretion is induced, possibly through the action of intracellular cAMP produced as a response to 5-HT. In contrast, *Schistocerca* tubules, whilst stimulated by cAMP, are insensitive to 5-HT[4]. The present study has been carried out to determine the effect of cAMP and 5-HT on fluid secretion and the transepithelial potential in *Locusta*. In addition, the effect of ouabain on the transepithelial potential has been examined to ascertain the role of $Na^+/K^+$-activated ATPase in maintaining ion transport.

*Materials and methods.* Mature adult *Locusta migratoria* L. were used. In vitro measurements of fluid secretion by Malpighian tubules were carried out as described previously[5]. The secretion rate for each tubule was determined by measuring the diameter of the secreted droplet at 5-min intervals over a period of 30 min. At the end of this time, the 'normal' Ringer solution was replaced by a fresh solution which had either the same (control) or a different composition (experimental). The rate of secretion was redetermined over the next 60 min.

The transepithelial potential (PD) of actively secreting tubules was measured with KCl/agar bridges connected via 3 M KCl to 2 Calomel half cells. A high input impedance amplifier with a gain of 10× was used. The PD was measured by placing the recording electrode in contact with the lumen and the reference electrode in contact with the outside of the tubule. The amplifier output was adjusted to zero with the KCl/agar bridges in the same Ringer pool. The PD was displayed and recorded on a flat-bed recorder (Servoscribe). Initially, the PD was measured with the tubules bathed in normal Ringer solution. Continuous recordings were taken for 15 min to ensure that the PD was stable. The normal Ringer solution was then changed for a fresh solution of the same (control) or different (experimental) composition. The PD was then recorded continuously over the next 50 min. The temperature was maintained at 30 °C throughout. The 'normal' Ringer solution had the following composition (mM): NaCl 129; KCl 8.6; $MgCl_2$ 8.5; $CaCl_2$ 2; $NaHCO_3$ 10.2; $NaH_2PO_4$ 4.3; glucose 34; pH 7. All solutions were made up in glass distilled, deionized water. All inorganic salts were AnalaR grade or the best commercially available. Ouabain, cAMP and 5-HT were obtained from the Sigma Chemical Co.

*Results.* The effects cAMP on fluid secretion are shown in table 1. Stimulation was most marked with $10^{-3}$ M cAMP in the bathing medium and the threshold was between $10^{-4}$ and $3\times10^{-4}$ M. Some stimulation was noted almost immediately following the addition of cAMP although maximum stimulation was observed some 10–20 min later. Thereafter, the rate of secretion returned to approximately its pre-stimulated level. In contrast to this, no significant stimulation of fluid secretion was observed by the inclusion of 5-HT in normal Ringer solution over the concentration range $10^{-8}$–$10^{-4}$ M.

The presence of $10^{-3}$ M cAMP in the normal Ringer solution effected an increase in lumen positivity from $+8.8\pm1.6$ mV to $+14.1\pm2.0$ mV ($n=14$). Application of a paired t-test indicates that this difference is significant ($p<0.01$). In contrast, control tubules, maintained in normal Ringer solution throughout, showed a slight decrease in lumen positivity from $+9.5\pm1.0$ to $+8.2\pm3.0$ mV ($n=7$). As was observed with studies on fluid secretion, the effect of cAMP on PD was gradual; approximately 16 min elapsing before a new stable potential was established. On return to normal Ringer solution, the PD showed a substantial return to the value observed prior to cAMP addition.

When normal Ringer solution was replaced by normal Ringer solution containing $10^{-3}$ M ouabain, the PD changed

from $+11.9\pm0.9$ to $-3.2\pm0.9$ ($n=9$) ($p<0.001$). The time taken for a new stable PD to be established was somewhat variable but was approximately 25 min. No corresponding change was observed in control experiments. The effect of ouabain was not reversible on return to normal Ringer solution.

*Discussion.* Application of the Nernst equation to the concentrations of $Na^+$ and $K^+$, measured by Anstee et al.[6] in the bathing medium and 'urine' of *Locusta* tubules are shown in table 2, where they are compared to those obtained by Ramsay[1]. For $K^+$, the PD was such that the lumen was considerably more positive than would be predicted on the basis of the Nernst equation, in both the present study and that of Ramsay[1]. It may be that this deviation from the Nernst equation indicates that $K^+$ is actively 'pumped', or alternatively, that the membranes are permeable to more than one ion. In the case of $Na^+$, the PD is less than that calculated by the Nernst equation. These deviations from the predicted values, together with the fact that ouabain was shown to effect a significant reduction in the PD of *Locusta* tubules, indicates that active ion transport is responsible for the potential and that a $Na^+/K^+$-activated ATPase is involved. This interpretation is consistent with the fact that fluid secretion by Malpighian tubules of *Locusta* is inhibited by ouabain, at 30 °C[5,6], that $Na^+/K^+$ ratios in secreted fluid are affected by ouabain[6], and the presence of a $Na^+/K^+$-activated ATPase in insect Malpighian tubules[5,7–9]. However, in *Carausius* and *Rhodnius* a 10-fold increase in $K^+$ concentration in the bathing medium effected a change in PD close to the value of 58 mV predicted by the Nernst equation[2].

Ouabain has been shown to decrease potentials across other tissues; for example, cockroach intestine[10,11], locust rectum[12], larval midgut of *Sarcophaga bullata*[13]. Berridge and Schlue[14] have shown that ouabain ($10^{-4}$ M) inhibits the membrane potential and affects internal $K^+$ levels in unstimulated salivary glands of *Calliphora*. However, glands stimulated by 5-HT were unaffected by ouabain. This effect is of particular interest because it may well offer an explanation for the lack of response to ouabain reported for the Malpighian tubules of certain insect species (see review by Anstee and Bowler[15]). However, Pilcher[16] reported that the PD of *Carausius* was unaffected by $10^{-4}$ M ouabain in the absence of any stimulant such as diuretic hormone or 5-HT.

Cyclic AMP stimulated fluid secretion by the Malpighian tubules of *Locusta;* the threshold for stimulation lying between $10^{-4}$ and $3\times10^{-4}$ M with maximal stimulation being observed in the presence of $10^{-3}$ M. This result is similar to that reported for *Schistocera* tubules[4] which were maximally stimulated by $5\times10^{-3}$ M cAMP. Maddrell et al.[3] found stimulation thresholds of $10^{-4}$ M and $4\times10^{-5}$ M cAMP with Malpighian tubules from *Carausius* and *Rhodnius* respectively. These concentrations of cAMP like those used with *Calliphora* salivary glands[17] are very high when compared to the intracellular levels which are thought to lie between $10^{-8}$ and $10^{-5}$ M[18]. Berridge[17] suggests that such relatively high concentrations are probably necessary due to the low permeability of the basal membrane for this negatively charged molecule. In the present study, there was a marked increase in lumen positivity when $10^{-3}$ M cAMP was included in the bathing medium, which was consistent with the effect of $10^{-3}$ M cAMP on fluid secretion. Similar increases in lumen positivity have been reported with *Calliphora* salivary glands[19,20]. Cyclic AMP is generally accepted as a 'second messenger' in hormone action and Mordue[21] has suggested that diuretic hormone acts by increasing the intracellular levels of cAMP. This has subsequently been shown to occur in *Rhodnius*[22]. In *Rhodnius* and *Carausius* the action of diuretic hormone can be mimicked by 5-HT in low concentrations[2]. In contrast, 5-HT had no effect on fluid secretion by the Malpighian tubules of *Locusta* in the present study nor did it stimulate secretion by Malpighian tubules from *Schistocera gregaria* or the pill millipede, *Glomeris marginata*[23]. It would seem, therefore, that 5-HT is not able to mimic the effect of the diuretic hormone in these species.

Table 1. The effect of cAMP on fluid secretion by the Malpighian tubules

| Concentration of cAMP (M) | n | Mean rate of secretion (% original rate ± 1 SE) | p |
|---|---|---|---|
| 0 | 11 | 98.3± 14.0 | n.s. |
| $10^{-5}$ | 19 | 119.0± 14.0 | n.s. |
| $3\times10^{-5}$ | 16 | 112.2± 20.0 | n.s. |
| $7\times10^{-5}$ | 6 | 117.5± 20.0 | n.s. |
| $10^{-4}$ | 14 | 153.3± 14.6 | n.s. |
| $3\times10^{-4}$ | 13 | 216.0± 40.0 | $<0.05$ |
| $10^{-3}$ | 14 | 273.1± 40.0 | $<0.001$ |
| $3\times10^{-3}$ | 13 | 151.4± 20.1 | $<0.05$ |
| $10^{-2}$ | 15 | 130.4± 17.8 | n.s. |

p-Values were obtained by comparing rate 1 and rate 2 by a paired t-test.

Table 2. Comparison of the observed transepithelial PD of locust Malpighian tubules with the values predicted by the Nernst equation for $Na^+$ and $K^+$

| Species | Reference | Recorded PD (mV) | Calculated PD (mV) | |
|---|---|---|---|---|
| | | | $K^+$ | $Na^+$ |
| *Locusta migratoria* | Present study | $+10.8\pm2.1$ ($n=74$) | $-73.2$ | $+38$ |
| *Locusta migratoria* | Ramsay[1] | $-16$ | $-46$ | $+5$ |

The polarity indicated is that of the Malpighian tubule lumen with respect to the bathing medium. Calculated PD is based on data from Anstee et al.[6].


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