At the last stage of postembryonic development (figure 1), the glial cell processes interpose between the developing nervous cell processes and the basement lamina, forming a complete peripheral sheet around the nerve. Glial cell processes are also seen in the interior of the nerve, where they separate the nervous cell processes into several distinct groups. Unlike nervous cell processes, glial processes are tightly packed with glycogen granules; one of the prominent morphological features of glial processes is the large number of electron-opaque granules of variable size i.e. gliosomes. Mitochondria are seen in both glial and nervous cell processes.

During the maturation of the nervous system, the overall diameter of the nerve increases. The percentage of the cross-sectional area of glial cells gradually decreases, while that of nervous cell processes gradually increases. The number of gliosomes diminishes. These modifications lead to the following relations between glial cells and nervous cells in mature worms (figure 2): glial cells form a fairly uniform peripheral layer only 1 or 2 cells thick; a patch of glial processes is occasionally found in the interior of the nerve; the gliosomes are fewer than in earlier stages of development, and are less electron-opaque. The cross-sectional area of the whole nerve is occupied almost completely by nervous cell processes. For the calculation of the cross-sectional area of glial and nervous cells in the nerve, we used a grid of regularly intersecting lines[7].

In the postembryonic nerve, the mean over-all cross-sectional area of the nerve is 60 $\mu m^2 \pm 0.8\ \mu m^2$, of which: 41.56% ($\pm$ 0.26%) glial cells, 17% ($\pm$ 0.6%) gliosomes, 58.44% nervous cells. In the mature worm nerve, the mean overall cross-sectional area of the nerve is 213 μm^2, of which: 11.8% ($\pm$ 0.31%) glial cells, 2.1% ($\pm$ 0.31%) gliosomes, 88.2% nervous cells.

The present data reveal an orderly sequence of events in the postembryonic development and during the maturation of the nervous system in Tubifex. During the maturation of the nervous system, the percentage of the cross-sectional area of glial cells diminishes parallel to a drastic fall in the number of gliosomes, and it may be reasonably concluded that the gliosomes accumulate at a biologically significant period to carry out a specific biological function. This period may be considered as a transition from the stage of formation of the nerve to its maturation. It is, then, reasenable to conclude that gliosomes in Tubifex are involved in the maturation of the nerve cells.

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The effect of prostaglandin E_1 on cyclic AMP production in the salivary glands of *Calliphora erythrocephala*

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Summary. Prostaglandin E_1 noncompetitively inhibits 5-hydroxytryptamine- and theophylline-stimulated cyclic AMP production in the salivary glands of Calliphora erythrocephala by an inhibitory effect on adenyl cyclase. Phosphodiesterase is not affected.

Prostaglandins have been implicated in the regulation of the action of a variety of hormones particularly those whose effects are thought to be mediated by the secondary messenger cyclic adenosine monophosphate (cyclic AMP)[1]. Depending upon the tissue involved prostaglandins either increase or decrease the synthesis of cyclic AMP by adenyl cyclase or its degradation by phosphodiesterase. Prostaglandins appear to inhibit adenyl cyclase in adipose tissue where they inhibit the lipolytic effect of several hormones[2] and in toad bladder inhibit an adenyl cyclase associated with osmotic water flow (although stimulating an adenyl cyclase associated with sodium transport) where they noncompetitively inhibit vasopressin- and theophylline- but not cyclic AMP-induced osmotic water flow[3-5]. In most other tissues studied prostaglandins effect an increase in cyclic AMP and hence mimic many of the hormonal responses. Fluid secretion by the isolated salivary glands of Calliphora erythrocephala is stimulated by 5-hydroxytryptamine (5-HT) and analogues[6,7] the action of which appears to be mediated by cyclic AMP by activation of adenyl cyclase. Intracellular levels of cyclic AMP are elevated by the application of 5-HT[8] and the application of exogenous cyclic AMP and theophylline (an inhibitor of phosphodiesterase) mimic the effects of 5-HT on fluid secretion[9]. Pharmacological studies[7] have shown that prostaglandin E_1 (PGE_1) is an inhibitor of stimulated fluid secretion by isolated salivary glands acting via a different receptor to 5-HT and analogues but one that is functionally connected with cyclic AMP production. In this study an investigation was made into the modulation of cyclic AMP levels by PGE_1 – specifically into whether PGE_1 acts directly on adenyl cyclase, phosphodiesterase, both or neither by comparing the effects of PGE_1 on the response of salivary glands to theophylline-cyclic AMP combinations (response independent of adenyl cyclase activity but dependent upon phosphodiesterase) with the response to theophylline-5-HT combinations (adenyl cyclase dependent response).

Materials and methods. The salivary glands of 3-day-old Calliphora erythrocephala were set up for the measurement of fluid secretion using the method of Berridge and Patel[10]. Each gland was isolated in a 10 µl droplet of

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Ringer solution under liquid paraffin; the cephalic end of the gland was drawn out of the Ringer droplet and attached, under the liquid paraffin, to a small glass rod. The rate of fluid secretion was measured by removal of the secreted droplet at the cephalic end at standard time intervals and its volume calculated from the measured diameter. The Ringer solution had the composition (mM): NaCl 120; KCl 20; NaH_2PO_4 8; $CaCl_2$ 2; $MgCl_2$ 2; trehalose 5; glucose 5; glutamine 2; sodium glutamate 2; proline 2; alanine 2; glycine 2; malic acid 2; citric acid 2; fumaric acid 2; pH adjusted to 7.2 using KOH.

All compounds tested were dissolved in Ringer solution and freshly prepared prior to use since it has been shown that 5-HT rapidly loses its activity when kept in solution[11]. Standard concentrations of theophylline (10^{-2} M) and PGE_1 (3×10^{-7} M) were used throughout the study since they have been shown to have maximal effects at these concentrations[7]. 5-HT and dibutyryl cyclic AMP were used at 2 concentrations: 10^{-8} M 5-HT and 3×10^{-3} M cyclic AMP eliciting maximal stimulation of fluid secretion and 3×10^{-11} M 5-HT and 10^{-3} M cyclic AMP eliciting sub-maximal stimulation of fluid secretion[6,7].

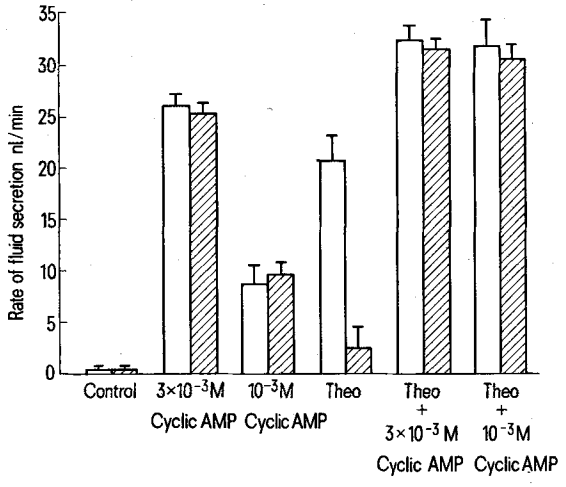

Fig. 1. The effect of PGE_1 on the response of isolated salivary glands to the addition of theophylline-cyclic AMP combinations. The histograms represent the rate of fluid secretion in the presence of the various agonists in the absence (open histograms) and in the presence of 3×10^{-7} M PGE_1 (cross-hatched histograms). Each histogram represents the mean ± SEM of 12 experiments.

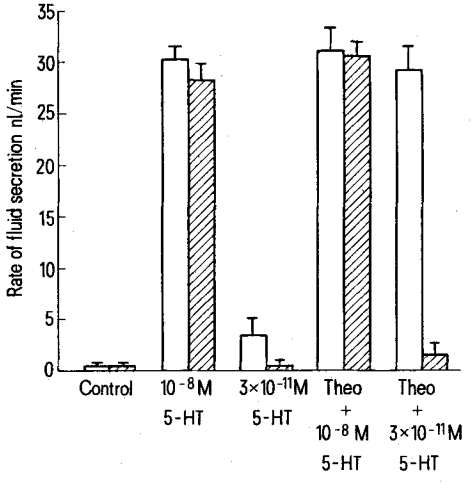

Fig. 2. The effect of PGE_1 on the response of isolated salivary glands to the addition of theophylline-5-HT combinations. The results are expressed as depicted in figure 1 and represent the mean ± SEM of 12 experiments.

Results and discussion. As shown in figure 1 PGE_1 does not affect the basal rate of fluid secretion by isolated salivary glands nor the response to the application of exogenous cyclic AMP but does inhibit the response to theophylline. Theophylline and cyclic AMP act synergistically in stimulating fluid secretion and this synergistic effect is not affected by PGE_1. Figure 2 shows that whilst the response to high concentrations of 5-HT is unaffected by PGE_1 the response to low concentrations is inhibited by PGE_1. Low doses of 5-HT and theophylline act synergistically in stimulating fluid secretion, an effect which is abolished in the presence of PGE_1. High concentrations of 5-HT and theophylline do not act synergistically and the response is unaffected by PGE_1.

The PGE_1 inhibition of the theophylline response could be explained by either of 4 mechanisms: a) PGE_1 could compete with theophylline for the site on phosphodiesterase and block its inhibitory effect as has been suggested for the action of PGE_1 on adipose tissue[12]; such an effect however would be expected to diminish the tissue response to a cyclic AMP + theophylline combination – an effect which is not observed. b) PGE_1 could stimulate phosphodiesterase by an amount sufficient to overcome the theophylline inhibition; c) PGE_1 could bind to and inhibit adenyl cyclase while at the same time stimulating phosphodiesterase activity as in b; however the observation that PGE_1 has no effect on the cyclic AMP response (either submaximal or maximal response) negates both these possibilities. d) PGE_1 could bind to, and inhibit, adenyl cyclase; this idea is substantiated by the observed actions of PGE_1 on 5-HT stimulated (adenyl cyclase-dependent) responses. The lack of effect of PGE_1 on basal secretion rate suggests that cyclic AMP does not play a major role in controlling unstimulated fluid secretion. The response to high concentrations of 5-HT is unaffected by PGE_1 whereas the response to low concentrations is inhibited an observation which suggests that the PGE_1 inhibition may be competitive. A similar observation of PGE_1 on the vasopressin-stimulated water flow across toad bladder has been interpreted by Lipson and Sharp[4] in terms of PGE_1 noncompetitively binding to a different receptor site on adenyl cyclase which then effects a decrease in the affinity of the vasopressin receptor site. The 5-HT response can be explained in terms of a receptor reserve for 5-HT in the salivary glands[7] whereby high doses of 5-HT produce levels of cyclic AMP far in excess of those required for the maximal physiological response; thus a decrease in the activity of adenyl cyclase under these conditions – by (noncompetitive) PGE_1 inhibition – will not reduce the intracellular levels of cyclic AMP sufficiently to affect the physiological response. Conversely, increasing the level of cyclic AMP further by the addition of theophylline with high doses of 5-HT will not induce any further increase in physiological response. At low concentrations of 5-HT an increase in intracellular cyclic AMP levels is accompanied by a corresponding increase in fluid secretion, thus theophylline acts synergistically with 5-HT at these concentrations and inhibition of adenyl cyclase by PGE_1 results in an inhibition of response. It is concluded therefore that PGE_1 noncompetitively inhibits adenyl cyclase and has no effect on phosphodiesterase.

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