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5-HYDROXYTRYPTAMINE IN THE SALIVARY GLANDS OF ADULT FEMALE *AEDES AEGYPTI* AND ITS ROLE IN REGULATION OF SALIVATION

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Summary

A dense plexus of axons, immunoreactive to antisera against 5-hydroxytryptamine (5-HT, serotonin) and surrounding the proximal medial lobe of the salivary gland of adult female *Aedes aegypti* mosquitoes, was demonstrated by means of whole-mount fluorescence immunocytochemistry. This innervation originates in the stomatogastric nervous system. 5-HT-immunoreactive innervation is absent in male salivary glands, suggesting that 5-HT is involved in blood-feeding. Furthermore, female mosquitoes treated with the 5-HT-depleting agent α-methyltryptophan (AMTP) and then allowed to feed on a rat exhibited a significantly longer mean probing period and a lower blood-feeding success rate than did control mosquitoes. When female mosquitoes were experimentally induced to salivate into mineral oil, AMTP-treated individuals secreted significantly less saliva than did control mosquitoes. These samples of saliva also contained significantly lower concentrations of apyrase, an enzyme important in blood-feeding. Injection of 5-HT into both AMTP-treated and control mosquitoes elicited significant increases in the volume of secreted saliva and/or its apyrase content. We conclude that 5-HT plays an important role in the control of salivation in adult female *A. aegypti*.

Key words: 5-hydroxytryptamine, serotonin, mosquito, saliva, salivary gland, salivation, apyrase, *Aedes aegypti*.

Introduction

Salivation in insects is controlled by neural or hormonal mechanisms or a combination of both. Many insects have salivary glands that are directly innervated, although the source of innervation is variable. In the sphinx moth *Manduca sexta*, the innervation of the salivary gland comes from the stomatogastric nervous system (Robertson, 1974), whereas in the locust *Schistocerca gregaria*, the source is the ventral nerve cord (Altman and Kien, 1979). The salivary gland of the cockroach *Periplaneta americana* is innervated by axons from both the stomatogastric system and the suboesophageal ganglion (Whitehead, 1971; Bowser-Riley, 1978). The salivary glands of the blowfly *Calliphora vicina* are not innervated, but salivation is activated by neurohormonal release of 5-hydroxytryptamine (5-HT, serotonin) from networks of fibres that lie in close proximity to the glands (Trimmer, 1985). Similarly, circulating 5-HT is associated with feeding activities of the triatomine bug *Rhodnius prolixus* (Lange *et al.* 1989). Recently, FMRFamide-like peptides that induce salivary secretion have been isolated from thoracic ganglia of *Calliphora vomitoria* (Duve *et al.* 1992).

Several neurotransmitter candidates have been detected in nerves that either innervate the glands or are intimately associated with them (and presumably affect salivation). Among these putative neuroeffectors are monoamines such as dopamine (Robertson, 1974; Baines *et al.* 1989), norepinephrine (Baines *et al.* 1989) and 5-HT (Lange *et al.* 1989; Peters *et al.* 1987) and peptides such as proctolin and YGGFMRFamide (Baines *et al.* 1989).

For mosquitoes, salivation is important in the location of a blood source and is correlated with duration of probing (time from insertion of stylets until blood appears in the gut; Ribeiro *et al.* 1984*a*). Because female mosquitoes ingest both blood and nectar, and because of the role of salivation in the transmission of disease, the control of salivation in mosquitoes is of particular interest. Nevertheless, regulation of salivation in these insects is not well understood.

Adult female mosquitoes have tri-lobed, acinar-type salivary glands that are situated around the ventrolateral aspect of the anterior midgut, dorsal to the cervical connectives and thoracic ganglia of the ventral nerve cord (Fig. 1). Innervation of the anterior portions of the salivary glands has been reported (Spielman *et al.* 1986), but neither the identity of the neurotransmitter(s) nor the functional significance of this innervation has been determined. Ultrastructural studies of

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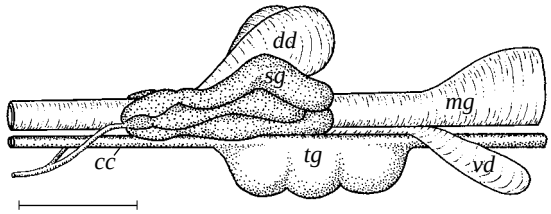

Fig. 1. Illustration of adult female salivary gland and surrounding structures. *cc*, cervical connectives; *dd*, dorsal diverticulum; *mg*, midgut; *sg*, salivary gland; *tg*, fused thoracic ganglia; *vd*, ventral diverticulum. Scale bar, 250 μm.

mosquito salivary glands have demonstrated morphologically distinct regions (Wright, 1969; Janzen and Wright, 1971), and it is also known that the synthesis and storage of secretory products are regionally differentiated (Rossignol *et al.* 1984; James *et al.* 1989; Ribeiro, 1992). It is not known, however, whether these distinct regions respond to the same signal(s) when salivation is elicited.

We report here that the salivary glands of adult female *Aedes aegypti* are innervated by 5-HT-immunoreactive nerve fibres and that 5-HT stimulates the salivation process of this species during blood-feeding.

Materials and methods

Mosquito rearing and maintenance

Aedes aegypti L. (Diptera: Culicidae) larvae were reared at 26–27 °C on a mixture of lactalbumin hydrolysate (US Biochem.), yeast hydrolysate (US Biochem.) and RMH3200 rat chow (A. Harlan Sprague Dawley Inc.). Adult female mosquitoes were maintained on 10 % sucrose solution at 26–27 °C and 70 % relative humidity. All stages were exposed to a long-day (16 h:8 h light:dark) photoperiod regimen.

Immunocytochemistry

The presence of 5-HT-immunoreactive innervation of adult salivary glands was demonstrated by the following procedure. The glands were dissected in *Aedes* saline solution (Hayes, 1953) and fixed in 4 % paraformaldehyde for 4–6 h at 4 °C. All dissections were performed during the first half of the photophase. After fixation, the specimens were rinsed overnight in 0.05 mol l^{-1} K^+/Na^+-phosphate-buffered saline containing 0.05 % Triton X-100 (PBST, pH 7.4). The tissue specimens were then washed in several changes of PBST and incubated for approximately 1 h in a blocking solution (0.05 mol l^{-1} Tris with 0.05 % Triton X-100, 3 % powdered milk, 0.25 % BSA, 0.2 % normal goat serum, pH 7.4) to prevent non-specific staining. Following this blocking step, the tissue samples were incubated overnight with rabbit anti-5-HT antiserum (Incstar Co.) diluted 1:1000 in blocking solution. On the following day, the specimens were washed several times in PBST and incubated overnight in donkey anti-rabbit IgG conjugated to Texas Red (Molecular Probes Inc.), diluted 1:200 in blocking solution containing 1 % normal donkey serum. The tissues were washed thoroughly on the following day and passed through 60 % glycerine before mounting in 80 % glycerine.

Female salivary glands, in whole mounts, were imaged by means of laser-scanning confocal fluorescence microscopy (BioRad MRC-600 on a Nikon Optiphot-2 microscope, krypton/argon laser, and BioRad YHS filter cube; excitation wavelength, 568 nm). Serial optical sections (at intervals of 2 μm) were imaged and saved as a Z series. Projection of the serial optical sections gave a two-dimensional reconstruction of the glands and their innervation.

Administration of 5-HT and α-methyltryptophan

The physiological effects of 5-HT on salivation were investigated by injecting 5-HT solutions into mosquitoes and by selectively depleting 5-HT stores with α-methyltryptophan (AMTP), an inhibitor of 5-HT biosynthesis. Both 5-HT (obtained as 5-HT–HCl) and AMTP (as AMTP–HCl) were acquired from Sigma Chemical Co.

Solutions of 5-HT at concentrations ranging from 5×10^{-8} to 5×10^{-4} mol l^{-1} were prepared in saline solution. By means of a capillary glass pipette, one of these solutions was injected (0.2 μl per insect) into the thorax of a mosquito immediately prior to induction of salivation (described below). The volume and apyrase activity of the saliva samples were determined after injection of 5-HT solution or *Aedes* saline solution (control).

Similar measurements were made on samples of saliva obtained from 5-HT-depleted mosquitoes. Levels of 5-HT in insects can be selectively depressed by oral treatment with AMTP (Sloley and Orikasa, 1988; Sloley, 1989). Previous studies showed no differences in feeding behaviour between mosquitoes that had received AMTP orally or by injection (Novak and Rowley, 1994). AMTP dissolved (2.5 mg ml^{-1}) in 10 % sucrose solution was fed, *ad libitum*, to starved mosquitoes on day 3 after emergence. The average ingested dosage of AMTP was approximately 5 μg per mosquito. After this treatment, mosquitoes had constant access to 10 % sucrose.

Blood-feeding behaviour

The blood-feeding behaviour of 5-HT-depleted (AMTP-treated) mosquitoes was investigated by permitting them access to the shaved abdomen of an anaesthetized rat or to blood through an artificial membrane of Parafilm. Probing and blood-feeding times of 5-HT-depleted and control mosquitoes were measured.

Collection and analysis of saliva

Samples of saliva from female mosquitoes were collected by oil-induced salivation (Hurlbut, 1966; Rossignol and Spielman, 1982; Ribeiro *et al.* 1984*b*). The rate of salivation and the activity of apyrase (ATP diphosphohydrolase, E.C. 3.6.1.5) of the salivary samples were measured after injection of 5-HT and also after the selective depletion of 5-HT by treatment with AMTP. The apyrase content of the saliva was evaluated because apyrase has an anti-platelet activity that aids in the location of blood vessels (Ribeiro *et al.* 1984*a*).

Mosquitoes used for oil-induced salivation were anaesthetized by placing them in a test tube chilled on crushed ice for approximately 10 s, removing their wings and legs, pulling the stylet sheath away from the mouthparts, and inserting the stylet bundle into a 1 cm piece of surgical polyethylene tubing (0.24 mm i.d.) containing water-saturated light mineral oil. Each mosquito was permitted to salivate into the mineral oil for 5 min; individuals that ingested the oil were discarded. With the aid of a dissecting microscope equipped with an ocular micrometer, we estimated the volume of the secreted saliva by measuring the diameter of the droplet of saliva under oil and calculating the volume of the droplet from its diameter. Occasionally, saliva was released as several droplets; in these cases, the droplets were manoeuvred with a fine glass needle so that they coalesced into a single droplet, which was then measured. After measurement, the samples of saliva (in mineral oil) were transferred into 20 μl of 0.05 mmol l^{-1} Tris–HCl buffer, pH 7.5, and stored at −80 °C until assayed for apyrase activity.

Salivary glands were also assayed for apyrase activity. Glands were dissected in 0.05 mmol l^{-1} Tris–HCl buffer, pH 7.5, and each pair was transferred to a microcentrifuge tube containing 20 μl of the same Tris buffer and stored at −80 °C prior to analysis.

Apyrase activity was assayed by measuring the release of inorganic phosphate from ATP (Fiske and SubbaRow, 1925). Glands that had been frozen in buffer were thawed and vortexed for 30 s. The resulting supernatant solutions (salivary gland extracts) were diluted to a final volume of 100 μl with Tris buffer. Samples of saliva (2 μl) or diluted salivary gland extract (1 μl) were transferred to individual wells in a plastic 96-well ELISA plate, and the apyrase reactions were started by adding a mixture containing 100 mmol l^{-1} NaCl, 50 mmol l^{-1} Tris–HCl (pH 8.95), 5 mmol l^{-1} $CaCl_2$ and 2 mmol l^{-1} ATP, to a final volume of 100 μl. After a 10 min incubation at 37 °C, the reaction was stopped by addition of 25 μl of acid molybdate solution (1.25 % ammonium molybdate in 2.5 mol l^{-1} H_2SO_4). Immediately after termination of the reaction, 2 μl of a reducing solution (0.11 mol l^{-1} $NaHSO_3$, 0.09 mol l^{-1} Na_2SO_3 and 8 mmol l^{-1} l-amino-2-naphthol-4-sulphonic acid) was added to each well. After 20 min, the optical density of the blue colour that developed was measured at 660 nm with an ELISA reader. Readings were quantified by comparison with an inorganic phosphate standard curve.

Data analysis

Errors are presented as standard errors of the means. Data from tests of feeding activity and collections of saliva typically were not normally distributed; therefore Mann–Whitney rank tests were used to determine statistical significance.

Results

A nerve associated with the anterior part of the salivary gland of female *A. aegypti* was strongly immunoreactive to anti-5-HT antiserum (Fig. 2A). This nerve branches extensively over the surface of the gland and surrounds the proximal medial lobe with a dense meshwork of fine processes. A smaller number of processes extend along the proximal lateral lobes, particularly along the inner (medial) side of these lobes. Occasionally, a few immunoreactive fibres were found to extend onto the distal portions of lobes. This 5-HT-immunoreactive plexus was not observed in glands from adult male or larval *A. aegypti* (Fig. 2B,C). The innervation of the female salivary glands comes from the ventricular nerves (Meola and Lea, 1972) of the stomatogastric nervous system (Figs 2D, 3).

In addition to this innervation, another 5-HT-immunoreactive fibre typically traverses the distal lobes (Fig. 2A,E), with minimal branching. The origin of this fibre is variable, but it appears to arise from a 5-HT-immunoreactive neurohaemal plexus that is associated with the cervical connectives, the thoracic ganglia and their nerves.

Because 5-HT-immunoreactive innervation of salivary glands was evident in adult females, but not in males or larvae, we hypothesized that 5-HT may be released to regulate some aspect of salivation during blood-feeding. We therefore treated female mosquitoes with AMTP to deplete their stores of 5-HT and subsequently tested their probing behaviour. Probing times and ingestion of blood were determined for 5-HT-depleted and untreated mosquitoes fed on a rat or offered blood through a Parafilm membrane.

Probing activity on a rat indicated that AMTP-treated mosquitoes had a probing success rate much lower than that of control mosquitoes (Fig. 4A). More than 90 % of control mosquitoes, but only 23 % of AMTP-treated mosquitoes, had acquired blood after 2 min of exposure. Only 45 % of the AMTP-treated mosquitoes had ingested blood by the end of the 5 min exposure period. In addition, the mean probing time was significantly longer in AMTP-treated mosquitoes (257±36 s *versus* 79±13 s for controls, $P<0.001$, $N=22$), apparently as a result of difficulty in the location of blood and/or the initiation of ingestion (Fig. 4B).

By contrast, when mosquitoes were offered a blood meal through a Parafilm membrane, the mean probing time of AMTP-treated mosquitoes was greatly reduced and not statistically different ($P>0.20$) from that of control mosquitoes feeding through the membrane (Fig. 4B). AMTP-treated mosquitoes had little difficulty acquiring blood through the membrane.

The duration of feeding of AMTP-treated mosquitoes was significantly longer than that of control mosquitoes in both the rat and membrane feeding trials (rat, AMTP=140.3±16.9 s, control=99.8±8.9 s, $0.02>P>0.01$; membrane, AMTP= 122.5±17.0 s, control 84.3±9.0 s, $0.005>P>0.002$).

To seek a possible explanation for the differences in probing time, we measured the salivation rate and apyrase activity of saliva in 5-HT-depleted and control mosquitoes. Similar measurements also were made with mosquitoes that had received injections of 5-HT. The volume of saliva secreted in 5 min and the total apyrase activity of the saliva were significantly lower for AMTP-treated mosquitoes than for non-treated controls (Fig. 5A,B). The apyrase activity per unit

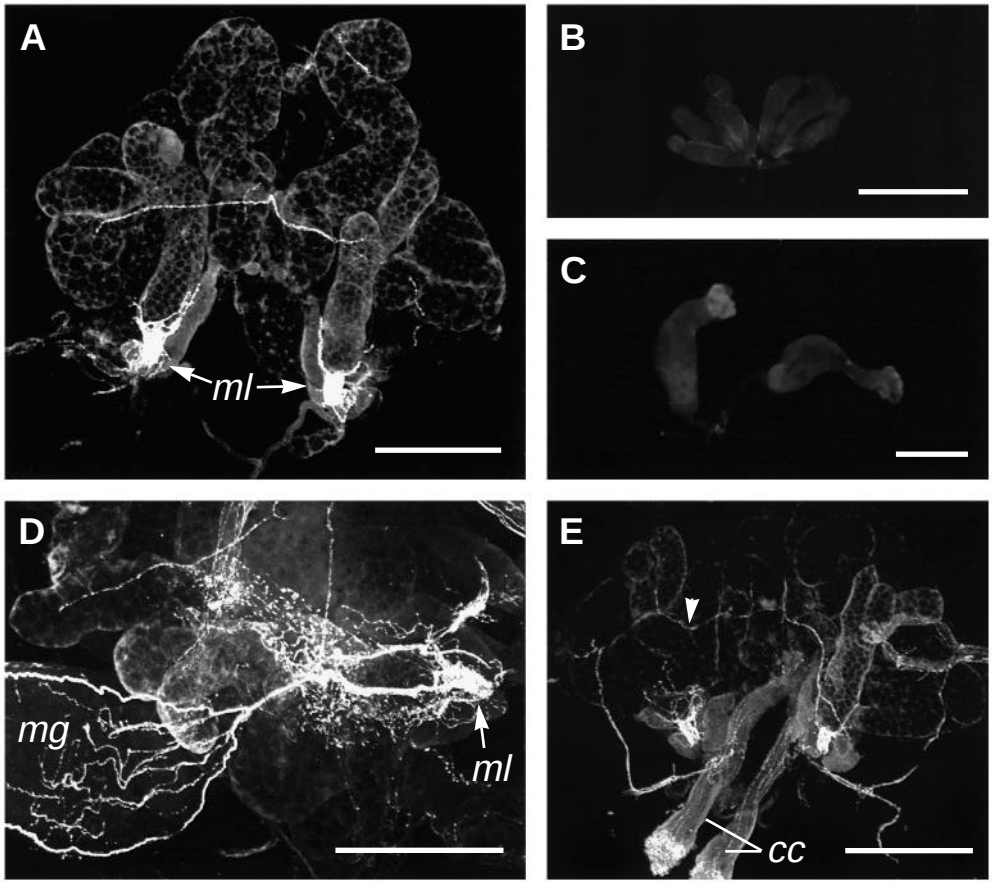

Fig. 2. Immunostaining of paraformaldehyde-fixed salivary glands of *Aedes aegypti*. (A) Neurones exhibiting 5-HT-like immunoreactivity form a dense plexus surrounding the proximal parts of the medial lobes (*ml*) of female glands. (B) Male and (C) larval glands have no associated 5-HT-like immunoreactivity. (D) Immunoreactive fibres branch from the ventricular nerves (Meola and Lea, 1972) of the stomatogastric system in the posterior foregut region; background fluorescence is due to 5-HT-like immunoreactivity in nerve processes at the base of the gut diverticula. *mg*, midgut. (E) 5-HT-immunoreactive fibres arise from a neurohaemal plexus associated with thoracic nerves and traverse the posterior lobes of female glands (arrowhead). *cc*, cervical connectives. A, D and E are laser-scanning confocal micrographs. Scale bars, 200 μm.

volume of saliva was also lower in AMTP-treated mosquitoes (Fig. 5C). The level of apyrase activity per salivary gland in AMTP-treated mosquitoes, however, was not significantly different from that of control mosquitoes (respectively 168±14 mU and 156±15 mU, P>0.2, N=27 per treatment, where enzyme activity is expressed in units, each one of which is the amount of apyrase activity that releases 1 μmole of orthophosphate per minute at 37 °C). These observations suggest that 5-HT depletion affects both the volume of saliva secreted and the salivary apyrase concentration, but not the synthesis of apyrase.

The effects of 5-HT were also examined by injecting the insect with 5-HT. There was a relationship between the amount of 5-HT injected and both the amount of saliva secreted and its apyrase activity (Fig. 6). Only the highest concentration (5×10^{-4} mol l^{-1}) of 5-HT, however, elicited statistically significantly increases.

Although the volume of saliva secreted by AMTP-treated, 5-HT-injected mosquitoes was higher than that of saline-

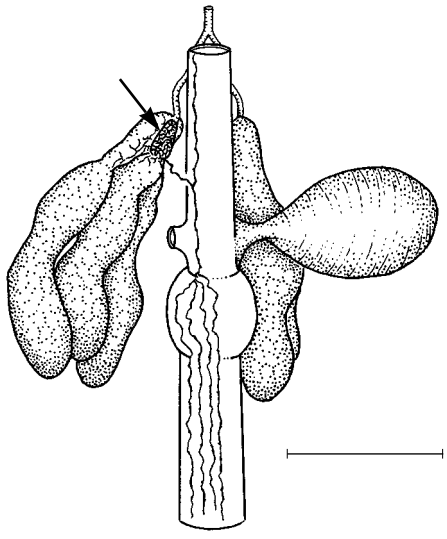

Fig. 3. Illustration of the alimentary canal and salivary glands, viewed dorsolaterally, in a female mosquito, showing 5-HT-like immunoreactivity. The dorsal diverticulum on the left side has been removed. The left salivary gland is lifted to show that the source of innervation of the proximal medial lobe (arrow) is the ventricular nerve of the stomatogastric system. Scale bar, 250 μm.

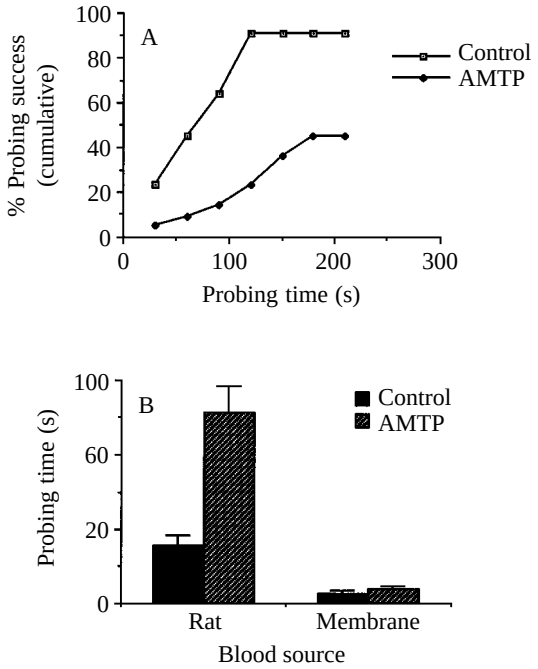

Fig. 4. Comparison of probing behaviour of AMTP-treated (5-HT-depleted) and control *Aedes aegypti* females. (A) The probing success of AMTP-treated mosquitoes was reduced by more than 50 % when blood-feeding on a rat (N=22 per treatment). (B) The probing time of AMTP-treated *A. aegypti* on a rat was significantly greater than that of control mosquitoes (P<0.001, N=22 per group), but no statistical difference was noted when the mosquitoes fed on blood through a Parafilm membrane (P>0.20, N=22 per group). Values are mean + S.E.M.

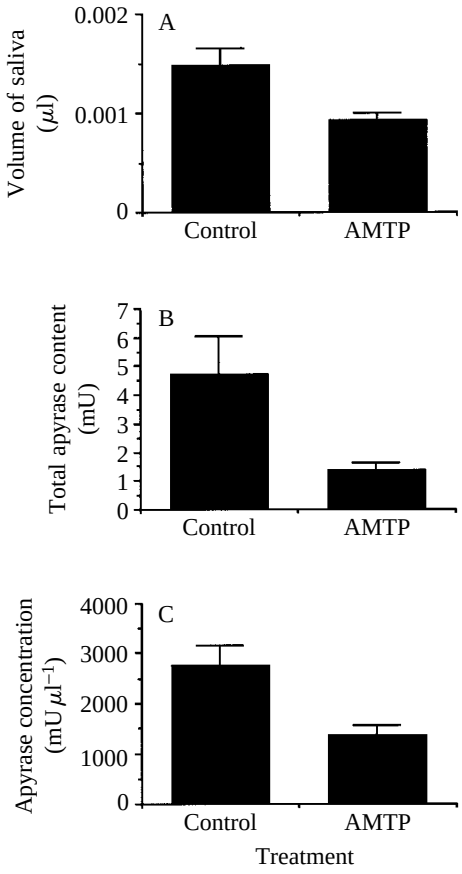

Fig. 5. Comparison of saliva from AMTP-treated and control *Aedes aegypti* females (N=24 per treatment). (A) The volume of saliva secreted by AMTP-treated mosquitoes was significantly lower than that of control mosquitoes (0.02>P>0.01). (B,C) The apyrase content and the apyrase concentration (apyrase activity per unit volume of saliva) of the saliva samples were also significantly reduced in AMTP-treated mosquitoes (0.002>P>0.001). Values are mean + S.E.M.

injected controls, the difference was not significant (Fig. 7A). Injection of 5-HT into AMTP-treated mosquitoes, however, did significantly increase the concentration of apyrase (Fig. 7B,C) in the saliva.

Discussion

5-HT has been shown to be an important neuroactive substance regulating a variety of physiological processes in diverse animal species, including insects. Studies have documented the presence of 5-HT in nerves associated with the mouthparts and digestive systems of insects (Tyrer *et al.* 1984; Davis, 1985) and have provided evidence of the importance of 5-HT in the feeding processes of insects such as *Rhodnius prolixus* (Lange *et al.* 1988, 1989) and *Calliphora vomitoria* (Berridge, 1972; Trimmer, 1985).

In *A. aegypti*, immunocytochemical methods revealed 5-HT-like innervation of salivary glands, which is largely restricted to the proximal part of the medial lobe. Spielman *et al.* (1986) observed that a neural plexus surrounding this region included axons located on the outer surface of the basal lamina of the gland and in the extracellular space beneath the basal lamina. Synaptic vesicles and 'omega profiles' were observed in the internal axons, suggesting neurotransmitter release. From the description of Spielman *et al.* (1986), it is not clear whether release of 5-HT from these axons would affect only the cells in the proximal medial lobe and adjacent lateral lobe cells, or if release could also elicit responses from secretory cells in the distal portions of the glands. Mosquito species of other genera (e.g. *Haemagogus*, *Culex*) exhibit more extensive ramifications of immunoreactive fibres (M. G. Novak, J. M. C. Ribeiro and J. G. Hildebrand, unpublished observations). This suggests that the more focal innervation of the gland in *A. aegypti* may represent not restricted release confined to the proximal lobes but regional release of 5-HT that diffuses to distal as well as to proximal parts of the lobes.

The innervated part of the medial lobe comprises epithelial cells without extracellular apical cavities and without canals through the wall of the cuticular duct (Janzen and Wright, 1971). These non-glandular cells are rich in mitochondria and microtubules and may be involved in hydration of the secretory material from the distal portions of the lobes (i.e. in water transport) (Janzen and Wright, 1971). It could be reasonably hypothesized that 5-HT released at this location stimulates water transport.

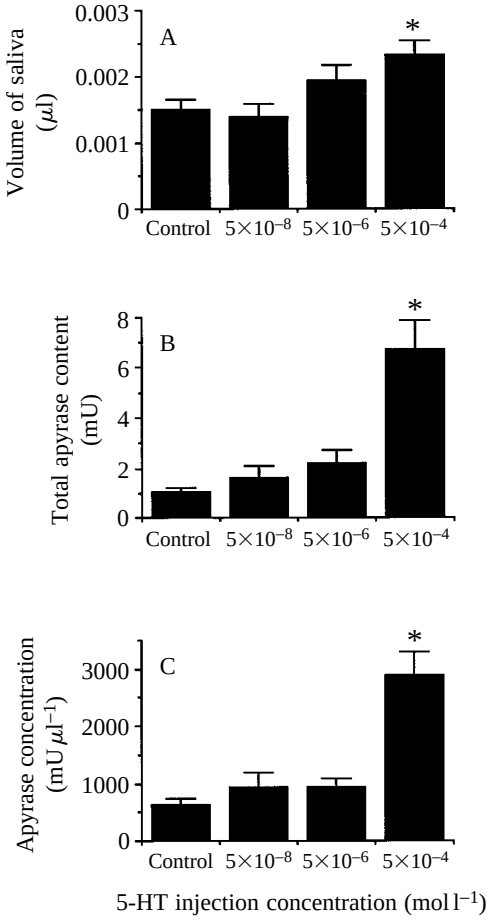

Fig. 6. Comparison of saliva from *Aedes aegypti* after injection of various concentrations of 5-HT with that from saline-injected controls (N=24 per treatment). (A) The volume of saliva secreted by 5-HT-injected mosquitoes depended on the dose of 5-HT. Only the highest concentration of 5-HT elicited a statistically greater response (*) compared with controls (P=0.05). (B,C) The highest concentration of 5-HT also elicited release of significantly larger (*) amounts of apyrase in comparison with controls (P<0.001). Values are mean + S.E.M.

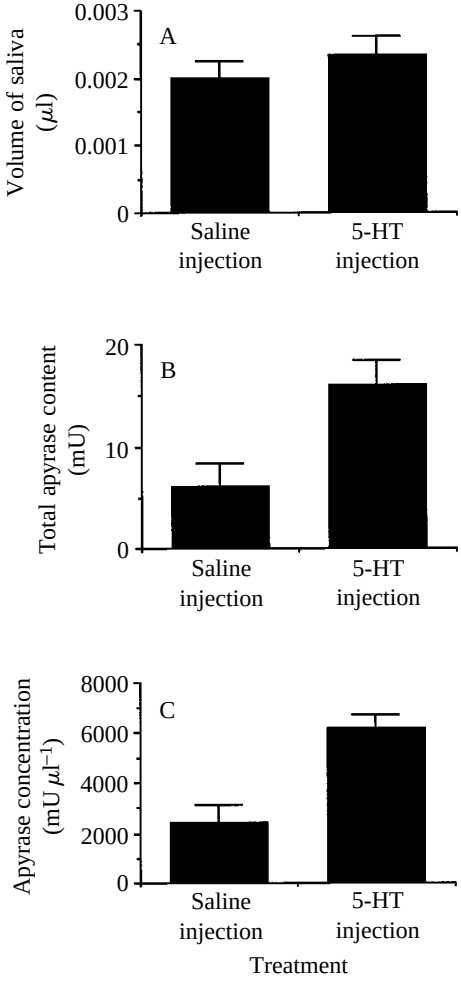

Fig. 7. Comparison of saliva from AMTP-treated and control *Aedes aegypti* following injection of 5-HT (0.2 ml of 5×10^{-4} mol l^{-1} 5-HT) (N=24 per treatment). (A) 5-HT-injected mosquitoes secreted a larger volume of saliva, but the difference was not statistically significant (P>0.2). (B,C) Injection of 5-HT elicited a significantly greater release of apyrase (P=0.002 and P<0.001 for B and C, respectively). Values are mean + S.E.M.

Some 5-HT-immunoreactive fibres also extend to the proximal portions of the lateral lobes, which comprise glandular epithelial cells and are principal storage sites for α-glucosidase, a sugar-digesting enzyme. The absence of 5-HT-immunoreactive innervation in male salivary glands, however, argues against a role for this innervation in sugar-feeding activities. Because the effect of 5-HT on salivation was not tested in male mosquitoes in this study, we cannot exclude the possibility that 5-HT, released elsewhere and carried by haemolymph, may influence male salivary glands.

Another 5-HT-immunoreactive fibre crosses the distal lateral lobes, but its association with the gland is less intimate. Nevertheless, the neurosecretory appearance of this fibre and of those that cover neighbouring thoracic nerves and ganglia, suggests a second site of 5-HT release that may affect salivation.

Because serotonergic innervation was evident, we examined more closely the effects of 5-HT-depletion on probing and blood-feeding behaviour. Oral administration of AMTP was used to reduce 5-HT levels because previous studies had indicated that this treatment induces a substantial, long-term and selective depletion of 5-HT in cockroaches (Sloley and Orikasa, 1988; Sloley, 1989) and in mosquitoes (Novak and Rowley, 1994). The most obvious difference between AMTP-treated and control *A. aegypti* was the prolonged mean probing time exhibited by females attempting to feed on an animal host. The increased probing time was not observed, however, when mosquitoes were fed blood through a Parafilm membrane. This difference suggests that there was little difficulty in determining the presence of blood and initiating uptake. Instead, the difficulty occurred in the initial location of a blood source. This suggests that 5-HT depletion interfered with salivation and its antihaemostatic functions.

Salivation is important to mosquitoes in locating blood.

Female mosquitoes feed on blood by inserting their stylets into a host and then probing for a source of blood. Blood flow from lacerations created by the probing actions of the stylets is maintained by the anti-platelet activity of the salivary enzyme apyrase (Ribeiro *et al.* 1984*b*). Consequently, a significant reduction of apyrase release into the saliva, or a reduction in the total volume of saliva released by a feeding mosquito, would presumably reduce the ability of the mosquito to locate a source of blood. The result would be an extension of the probing period or termination of feeding behaviour. The difficulty exhibited by AMTP-treated mosquitoes in finding a source of blood can be explained, in part, by the fact that AMTP-treated *A. aegypti* secrete less saliva and therefore presumably deliver a reduced amount of apyrase to the host. Although the reduction in salivation was significant (approximately 33 % in 5-HT-depleted mosquitoes), it was not clear whether this difference could account fully for the large disparity in probing success between treated and control mosquitoes. Indeed, the apyrase content of saliva samples was found to be even more profoundly affected by both AMTP treatment (5-HT depletion) and 5-HT injection. Although apyrase is sequestered in the distal portions of the lateral lobes, which have few associated 5-HT-immunoreactive fibres, it appears that apyrase release is also effected by 5-HT.

This induced change in the apyrase content of the saliva and the resulting changes in probing behaviour are reminiscent of the correlation between the natural apyrase content of salivary glands and the characteristic probing times observed in three species of anopheline mosquitoes (Ribeiro *et al.* 1985). The lower the apyrase content, the longer it took the mosquito to locate blood.

It should be noted that salivation is, in itself, not necessary for successful feeding. When salivary ducts are sectioned, non-salivating mosquitoes probe longer on an animal host than do mosquitoes with intact salivary ducts, but when blood is located they ingest it just as rapidly (Mellink and Van Den Bovenkamp, 1981). Probing time, however, does not increase when non-salivating mosquitoes feed on blood through a membrane. In this case, salivation is of little consequence because the mouthparts inevitably come into contact with blood as soon as the membrane is penetrated (Ribeiro *et al.* 1985). These observations correlate well with the findings of this study: probing time was greatly diminished when 5-HT-depleted mosquitoes were fed through a membrane.

The blood-feeding time of 5-HT-depleted mosquitoes was significantly longer than that of controls, both when fed on a rat and through a membrane. This extension of feeding time suggests that there are other effects of 5-HT depression on the feeding processes of *A. aegypti*.

In summary, this study has demonstrated the presence of 5-HT-immunoreactive processes in intimate contact with adult female salivary glands and the ability of 5-HT to affect the volume and apyrase content of released saliva. It cannot be concluded that the 5-HT-immunoreactive innervation of the proximal medial lobes of the salivary glands is the sole source of released 5-HT or the sole effector of salivation in *A. aegypti*.

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