
REVIEW

THE AMINERGIC AND PEPTIDERGIC INNERVATION OF INSECT SALIVARY GLANDS

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Summary

Insect salivary glands are glands associated with nutrient intake whose secretions are generally involved in the digestion and lubrication of food. They are under the control of neuroactive substances and may be innervated from several sources including the suboesophageal ganglion, the stomatogastric nervous system and the unpaired median nerves. Both amines and peptides have been suggested to play roles in the control of insect salivation, as indicated by their association with terminals on salivary glands, their effects in salivary gland bioassays and their ability to alter second messenger levels and ion channel conformations. Serotonin and dopamine appear to be the most prominent amines associated with insect salivary glands. Either one or both of these amines are found associated with the salivary glands of the locust, stick insect, cockroach, cricket, dragonfly, mosquito, adult moth and kissing bug. Their roles, although not fully elucidated,

appear to be in the control of salivary secretion. Several peptides, including members of the FMRFamide-related family of peptides, are also found associated with insect salivary glands. Sources of peptidergic innervation are as varied as those for aminergic innervation, but information regarding the physiological role of these peptides is lacking.

The relevance of the different levels of complexity of salivary gland innervation, which range from the absence of innervation in some species (blowfly) to the presence of several distinct sources in others (locust, cockroach), is not well understood. This review serves to consolidate what is known of the phenotype of salivary neurones in relation to the control of salivation.

Key words: innervation, salivary gland, insect, serotonin, dopamine, FMRFamide, amine, peptide, salivation.

Introduction

Insect salivary (labial) glands are of two main types: tubular, as in the blowfly and Lepidoptera, and acinar, as in locusts and cockroaches (Fig. 1). Tubular glands are long coiled tubes in which the epithelial layer is one cell thick and is covered by a basement membrane (House and Ginsborg, 1985). These glands may extend into the thorax and abdomen and open at their proximal end into the preoral cavity. Acinar glands have a greater degree of structural complexity. The fluid-secreting regions or acini are grape-like structures, usually composed of two or three main cell types (Just and Walz, 1994; House and Ginsborg, 1985; Kendall, 1969). The acini produce and secrete saliva into a series of ducts that gradually coalesce to form the main salivary ducts, which open into the preoral cavity (House and Ginsborg, 1985) (Fig. 1A,B). As a general rule, salivary secretions are digestive, but they may perform additional functions in some insects. For instance, the saliva of locusts and cockroaches contains digestive enzymes (Gardiner, 1972; Kendall, 1969), the saliva of mosquitoes contains irritants and anticoagulants

(Ribeiro, 1992; Gardiner, 1972) and the labial glands of larval Lepidoptera produce silk (Kafatos, 1968).

The control of salivation is achieved either by direct nervous innervation or *via* neurohormones. Salivary glands may be innervated from several different sources. Many insects have a salivary nerve which projects from the suboesophageal ganglion (SOG), but other species (*Periplaneta americana* and *Rhodnius prolixus*) are also equipped with a salivary nerve which projects from the stomatogastric nervous system (Tsang and Orchard, 1991; Davis, 1985; Baptist, 1941). In addition, innervation from the median-transverse nervous system may also exist, as it does in the locust *Locusta migratoria* (Fusé *et al.* 1996; Myers and Evans, 1985). The nature of the innervation is variable such that the terminals of salivary neurones may either be intimately associated with the cells of the fluid- and enzyme-secreting regions of the glands (Ali *et al.* 1993; Peters *et al.* 1987) or they may appear to end blindly on the acini (Fusé *et al.* 1996). Furthermore, the projections of other neurones have a neurohaemal appearance and are

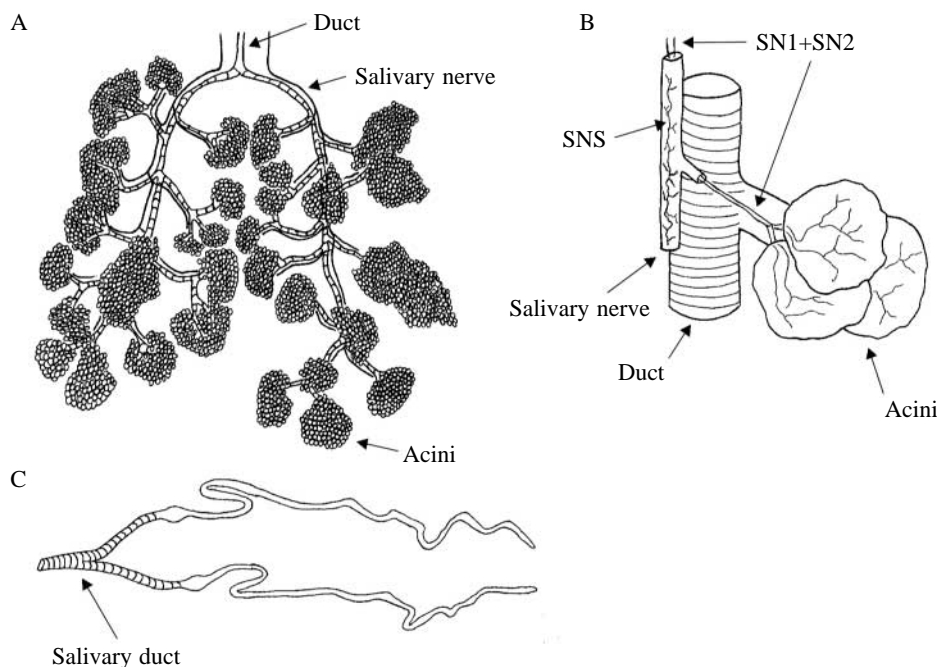


Fig. 1. Schematic drawing of salivary glands of two insect species. (A) Salivary glands of *Locusta migratoria*; the salivary duct, nerve and acini are indicated. (B) Innervation of salivary acini in *Locusta*. Axons of SN1 and SN2 (SN1+SN2) are found within the salivary nerve, while the satellite nervous system (SNS) is associated with the surface of the nerve and does not directly innervate the acini. (C) Uninnervated salivary glands of *Calliphora vomitoria* (the extent of coiling is reduced here for clarity), not drawn to scale.

associated with the salivary nerve (Lange *et al.* 1988; Bräunig, 1987) or the salivary duct (Ali and Orchard, 1996a; Tsang and Orchard, 1991; Lange *et al.* 1988). Tubular glands, such as those of the moth *Manduca sexta* and the blowfly *Calliphora vomitoria*, are not innervated to the same extent as acinar glands. Adult *Manduca sexta* labial glands are only innervated from the stomatogastric system, while the salivary glands of *Calliphora vomitoria* are devoid of innervation (Oschman and Berridge, 1970). In those cases where the glands are not innervated, they appear to be under neurohormonal control. For instance, serotonin acts neurohormonally to increase salivation in *Calliphora* via cyclic AMP and inositol trisphosphate (InsP₃) second messenger systems (Berridge and Heslop, 1981; Berridge, 1970; Berridge and Patel, 1968).

Even though salivary gland innervation patterns vary widely within the insect class (Table 1), certain neuroactive substances, such as serotonin and dopamine, appear to be favoured salivary secretagogues. Either one or both biogenic amines have been identified in nerve fibres associated with the salivary glands of locusts, stick insects, cockroaches, bugs and dragonflies (Ali and Orchard, 1996a; Elia *et al.* 1994; Ali *et al.* 1993; Lange *et al.* 1988; D. W. Ali, unpublished observations). Here, particular attention is paid to those glands that are innervated in contrast to glands that are solely under neurohormonal control. The extent of salivary gland innervation is discussed, and the literature on the aminergic and peptidergic control of insect salivation is briefly reviewed.

Aminergic innervation of salivary glands

Serotonin (5-hydroxytryptamine)

The indolalkylamine serotonin has been mapped throughout

the nervous system of a variety of insects using immunohistochemical techniques (Hörner *et al.* 1995; Lange *et al.* 1988; Longley and Longley, 1986; Tyrer *et al.* 1984). Several of these studies have revealed serotonergic efferent neurones innervating the salivary glands. Locust (*Locusta migratoria*) salivary glands are innervated via a serotonergic motoneurone (salivary neurone 2, SN2) whose cell body is located in the SOG (Fig. 2). The soma of SN2 is ventral and posterior and the axon projects ipsilaterally through nerve 7 to the salivary nerve, nerve 7b. SN2 projects to the salivary glands and ramifies throughout the acini (Ali *et al.* 1993) (Fig. 3A). There is evidence to suggest a classical neurotransmitter role for serotonin at the salivary glands since serotonin fulfils many of the neurotransmitter criteria in this preparation (Ali and Orchard, 1996b). Additional serotonergic projections to the locust salivary glands come from a small group of three pairs of neurones whose somata are located in the anterior SOG (Bräunig, 1987, 1988) and whose axons give rise to a serotonergic neurohaemal system termed the satellite nervous system (SNS; Bräunig, 1987) (Figs 1, 2). Axons from these cells form neurohaemal networks on the surface of several suboesophageal nerves including the salivary nerve where they project to, but terminate just short of, the acini (Bräunig, 1987). The role of the serotonergic neurohaemal system may be to release serotonin into the haemolymph bathing the mouthparts and feeding apparatus, including the salivary ducts. Locust mandibular closer muscles are known to be receptive to serotonin (Baines *et al.* 1990) and isolated salivary ducts experience elevations in cyclic AMP levels in response to serotonin (D. W. Ali, unpublished observations).

Suboesophageal serotonergic salivary neurones have also been found in two close relatives of the locust: the stick insect *Carausius morosus* (Ali and Orchard, 1996a) (Fig. 3C) and the

Table 1. Source of salivary gland innervation in various insects

Insect	Source of salivary innervation				Reference
	SOG	SNS	STS	MTNS	
<i>Locusta migratoria</i>	+	+	–	+	Altman and Kien (1979), Myers and Evans (1985), Bräunig (1987), Bräunig <i>et al.</i> (1994)
<i>Gryllus bimaculatus</i>	+	+	+	–	Helle <i>et al.</i> (1995), Hörner <i>et al.</i> (1995)
<i>Periplaneta americana</i>	+	+	+	–	Davis (1985)
<i>Carausius morosus</i>	+	–	–	–	Ali and Orchard (1996a)
<i>Rhodnius prolixus</i>	+	+	+	–	Baptist (1941), Lange <i>et al.</i> (1988), Tsang and Orchard (1991)
<i>Aedes aegypti</i>	–	?	+	?	Novak <i>et al.</i> (1995)
<i>Manduca sexta</i>	–	–	+	–	Robertson (1974)
<i>Sympetrum obtrusum</i>	+	+	?	?	D. W. Ali (unpublished observations)
Perlidae, <i>Agnetina</i> sp.	+	–	?	+	D. W. Ali (unpublished observations)
<i>Leptinotarsa decemlineata</i>	–	–	+	–	van Haeften and Schooneveld (1993)

+, innervation is present; –, innervation is absent; ?, innervation is unknown.

SOG, suboesophageal ganglion; SNS, satellite nervous system; STS, stomatogastric nervous system; MTNS, median-transverse nervous system.

cricket *Gryllus bimaculatus* (Hörner *et al.* 1995). In both species, the salivary neurones (referred to here as SN2), project ipsilaterally through the salivary nerve to the glands. A serotonergic neurohaemal system is associated with the salivary glands of the cricket (Helle *et al.* 1995), but not with those of the stick insect.

Serotonergic innervation of cockroach (*Periplaneta americana*) salivary glands exhibits a different pattern from

that in *Locusta*, *Carausius* and *Gryllus*. Even though the glands are innervated *via* an SN2 with a similar morphology to those of *Locusta*, *Carausius* and *Gryllus*, this salivary neurone is not serotonergic (Gifford *et al.* 1991; Davis, 1987). However, the glands are innervated by serotonergic efferents from two separate sources: the stomatogastric nervous system and several small serotonergic processes found within the salivary nerve (Davis, 1985). It is quite probable that these smaller processes, which are neurohaemal in appearance, belong to a system homologous to the satellite nervous system in *Locusta*.

The salivary glands of the blood-sucking hemipteran *Rhodnius prolixus* are innervated *via* the suboesophageal and stomatogastric nerves and are covered with a plexus of serotonergic immunoreactive fibres (Lange *et al.* 1988). The neurohaemal nature of the staining together with its close association with the duct and suboesophageal nerve (Fig. 3E) suggest a satellite-like system. The salivary glands of another bloodfeeder, the mosquito *Aedes aegypti*, also appear to use serotonin as a salivary neurotransmitter. The salivary glands of the adult female receive serotonergic neurohaemal-like elements which originate from two sources, the ventricular nerve of the stomatogastric nervous system and a neurohaemal plexus associated with the thoracic ganglia (Novak *et al.* 1995). Interestingly, serotonin-like

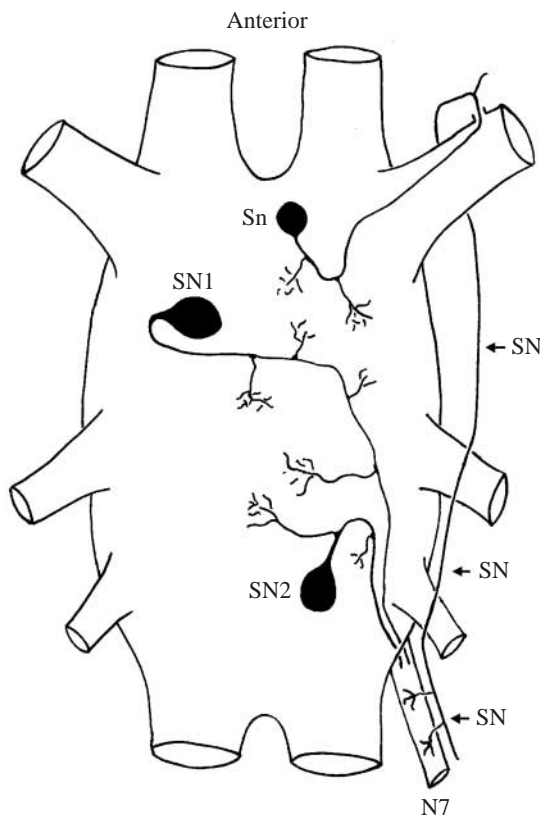


Fig. 2. Schematic drawing of neurones in the suboesophageal ganglion of *Locusta migratoria* which innervate the salivary glands. Only one member of each pair of salivary neurones is shown. In addition, only one of the three paired satellite neurones is shown. The soma of salivary neurone 1 (SN1) is dorsal and anterior; the soma of salivary neurone 2 (SN2) is ventral and posterior, and the soma of the satellite neurone (Sn) is dorsal. The Sn axons exit the mandibular nerve *via* the satellite nerve (SN) and project to several suboesophageal nerve roots (not shown), including nerve 7 (N7). Axons of SN1 and SN2 project to the salivary glands through nerve 7, then nerve 7b (not shown), not drawn to scale.

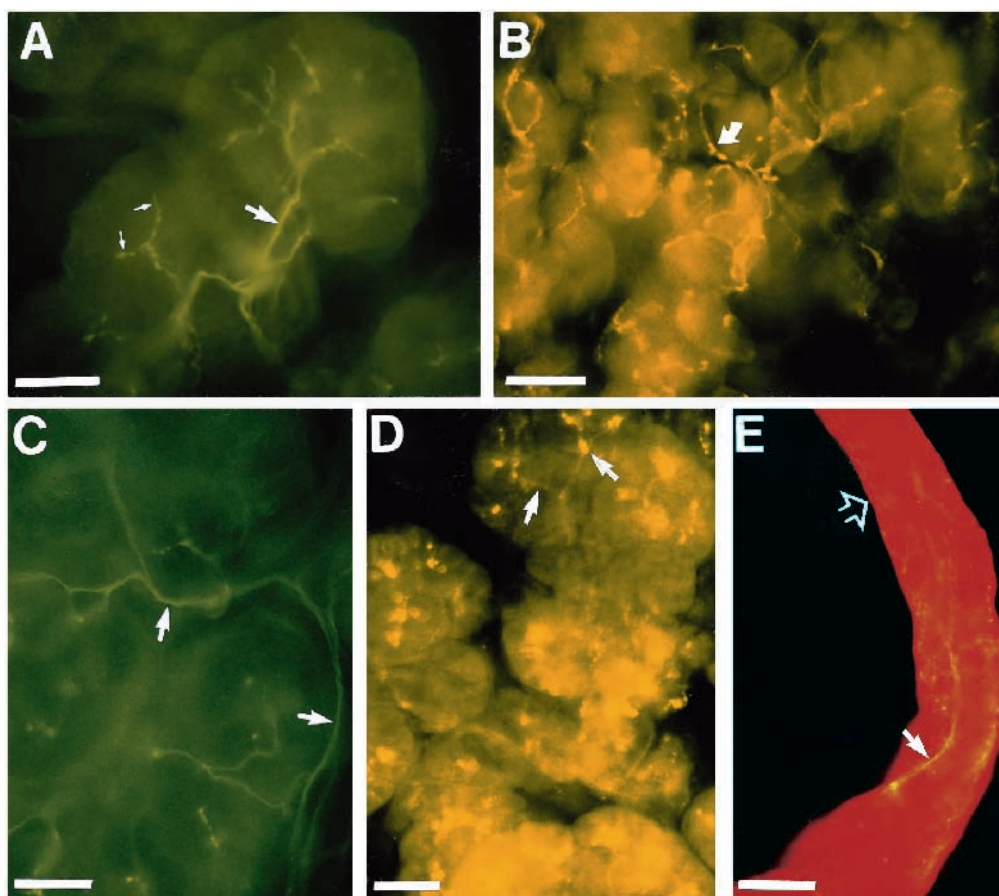


Fig. 3. Serotonin-like immunoreactivity (arrows) in the salivary gland acini of (A) *Locusta migratoria* (Ali *et al.* 1993), (B) *Sympetrum obtrusum*, (C) *Carausius morosus* (Ali and Orchard, 1996a) and (D) *Acheta domestica*. (E) Serotonin-like immunoreactivity (filled arrow) along the salivary duct (open arrow) of *Rhodnius prolixus*. Scale bars, 50 μ m. A,C: FITC-conjugated secondary antibody; B,D,E: CY-3-conjugated secondary antibody.

immunoreactive fibres are not found associated with the salivary glands of the adult males, which are not blood-feeders, suggesting that serotonin plays a specific role in blood-feeding (Novak *et al.* 1995). Additional immunohistochemical studies in other species reveal the presence of serotonergic-like processes associated with the salivary glands of *Acheta domestica* (Fig. 3D) and the dragonfly *Sympetrum obtrusum* (Fig. 3B), the serotonergic nature of which is confirmed by an HPLC analysis of *Sympetrum* salivary gland homogenates (D. W. Ali, unpublished observations). The origin of these processes is yet to be fully determined, but they are believed to arise from a serotonergic satellite-like system.

Catecholamines

Of the three members of the catecholamine family (dopamine, noradrenaline, adrenaline), dopamine is the major amine found in insects (Evans, 1980). There is little information regarding the precise physiological function of dopamine, but we believe that one role is to act as a neurotransmitter in the salivary glands of locusts (Ali and Orchard, 1996b). In *Locusta*, dopamine is present within salivary neurone 1 (SN1; Gifford *et al.* 1991; Ali *et al.* 1993), which is situated within the SOG (Altman and Kien, 1979). The SN1s project contralaterally through nerve 7 to the salivary gland *via* nerve 7b (Ali *et al.* 1993) (Figs 2, 4C). They

have a very characteristic morphology that is also found in *Carausius morosus* (Fig. 4A), *Periplaneta americana* (Fig. 4B), *Acheta domestica*, *Sympetrum obtrusum* (Fig. 4E), the Plecoptera, *Agnetina* sp. (Fig. 4D) and *Gryllus bimaculatus* (Hörner *et al.* 1995). Indeed, in *Locusta*, *Carausius*, *Periplaneta* and *Gryllus*, the SN1s are the most heavily stained and the only efferent tyrosine-hydroxylase-like immunoreactive neurones in the ventral nerve cord. An interesting feature in *Carausius* is the presence of small, neurohaemal, tyrosine-hydroxylase-like processes associated with the salivary ducts, a pattern not previously seen in either *Locusta* or *Periplaneta*. The role of these processes may be to supply the duct cells with dopamine, which may serve to activate the cells to reabsorb water and ions in order to concentrate the saliva and prevent dehydration. This hypothesis is yet to be tested.

The tubular salivary glands of adult *Manduca sexta* are innervated from only one source, the oesophageal nerve of the stomatogastric nervous system (Robertson, 1974). Falck–Hillarp histochemical techniques indicate a catecholaminergic content of these nerves, while enzymatic-isotopic assays confirm a dopaminergic, but not a noradrenergic, presence (Robertson, 1975). Only the fluid-secreting regions of the glands are innervated, and it is likely that the protein-secreting region of the gland is acted upon in a hormonal fashion (Robertson, 1974).

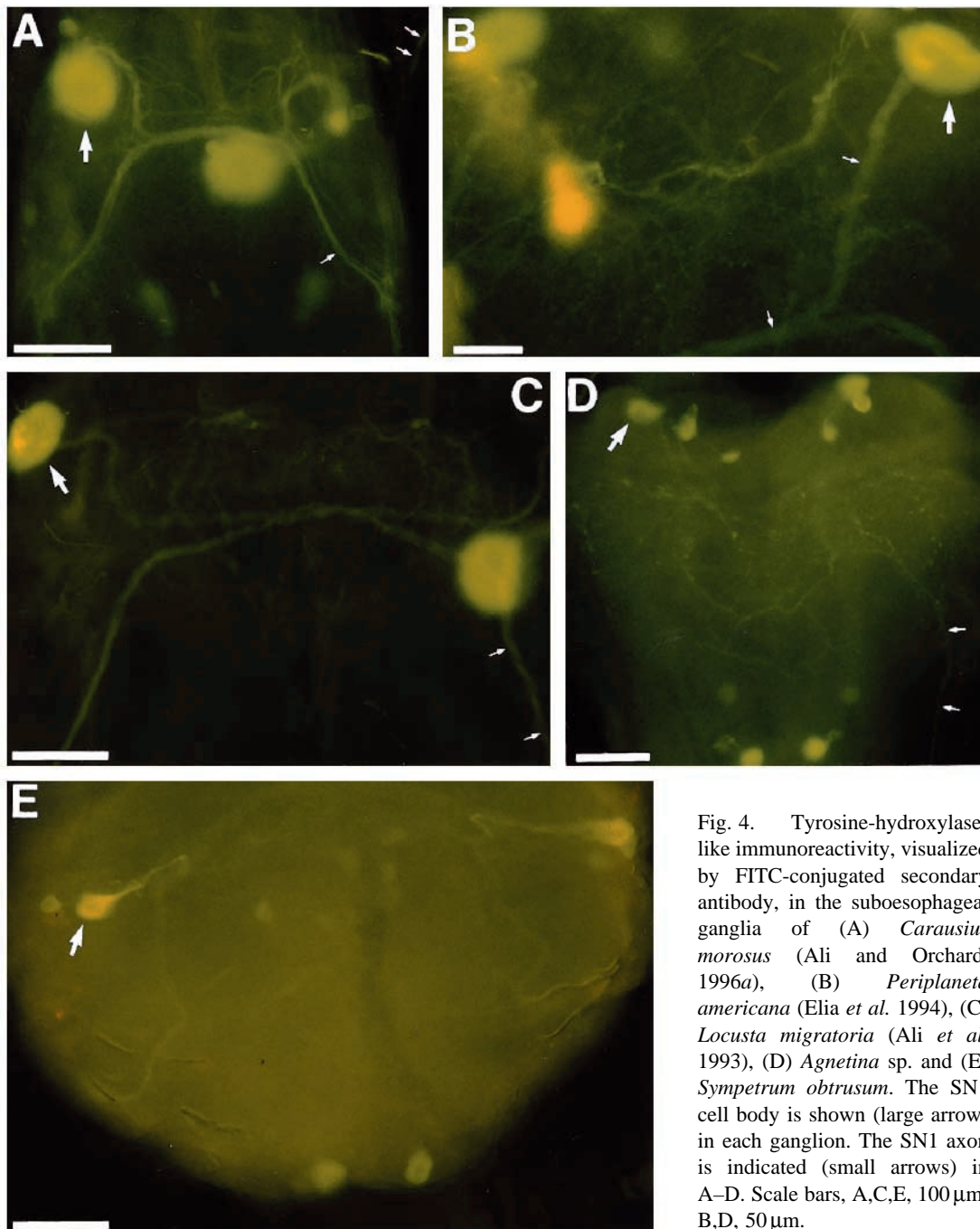


Fig. 4. Tyrosine-hydroxylase-like immunoreactivity, visualized by FITC-conjugated secondary antibody, in the subesophageal ganglia of (A) *Carausius morosus* (Ali and Orchard, 1996a), (B) *Periplaneta americana* (Elia *et al.* 1994), (C) *Locusta migratoria* (Ali *et al.* 1993), (D) *Agneta* sp. and (E) *Sympetrum obtrusum*. The SN1 cell body is shown (large arrow) in each ganglion. The SN1 axon is indicated (small arrows) in A–D. Scale bars, A,C,E, 100 μ m; B,D, 50 μ m.

Since tyrosine-hydroxylase-like immunoreactivity is indicative of the presence of catecholamines, and not solely dopamine, it is possible that noradrenaline is also present in tyrosine-hydroxylase-positive salivary neurones. Noradrenaline is found in insects to a much lesser extent than is dopamine (Evans, 1980), and there is no clear evidence for the presence of noradrenaline associated with insect salivary glands. Radioenzymatic assays for dopamine on the SN1 in *Locusta* and *Periplaneta*, and positive dopaminergic-like immunoreactivity in *Gryllus*, confirm the dopaminergic content of the SN1. An HPLC analysis of locust salivary glands suggested the presence of noradrenaline (Baines and Tyrer,

1989), but this has not been confirmed, and noradrenaline does not affect salivation at physiologically relevant concentrations (Baines *et al.* 1989).

Octopamine

Octopaminergic innervation of locust salivary glands arises from a dorsal unpaired medial neurone (DUM1b) in the metathoracic ganglion that projects through nerve 1 and sends collaterals to the transverse nerves, which join the subesophageal salivary nerve (Bräunig *et al.* 1994). The octopaminergic projections of DUM1b travel to the acini, but are not extensive or diffuse.

Aminergic control of salivation

Serotonin (5-hydroxytryptamine)

Serotonin increases the salivation rate of isolated locust salivary glands at a threshold concentration of $10^{-10} \text{ mol l}^{-1}$ and is maximally effective at $10^{-6} \text{ mol l}^{-1}$ (Baines and Tyrer, 1989). These effects are paralleled by the ability of serotonin to induce increases in cyclic AMP levels in salivary glands with a threshold of $5 \times 10^{-8} \text{ mol l}^{-1}$ and a maximum effectiveness at $10^{-5} \text{ mol l}^{-1}$ (Ali *et al.* 1993). SN2s release serotonin in response to stimulation of the salivary nerve (Ali and Orchard, 1994, 1996b). In addition, the neurones that give rise to the satellite nervous system are also active during feeding (Schachtner and Bräunig, 1993) and probably release serotonin into the haemolymph that bathes the salivary ducts and glands. Thus, the ducts and glands probably receive adequate supplies of serotonin. Serotonin increases the secretion of proteinaceous saliva in isolated cockroach salivary glands in a dose-dependent manner (Just and Walz, 1996), but little is known of the transduction mechanisms that mediate these effects. Preliminary studies in *Aedes aegypti* show that serotonin is important for probing success and the duration of feeding (Novak *et al.* 1995), probably because it is necessary for salivation and for the release of the appropriate concentration of salivary apyrase (Novak *et al.* 1995).

Catecholamines

Dopamine is capable of inducing salivation in the locust at a threshold of $10^{-9} \text{ mol l}^{-1}$ and is maximally active at $10^{-5} \text{ mol l}^{-1}$ (Baines and Tyrer, 1989). Furthermore, it induces dose-dependent elevations of cyclic AMP levels in locust salivary glands (Ali *et al.* 1993). It is intriguing to observe that dopamine and serotonin appear to perform similar functions in this preparation, but some evidence suggests that the roles of these amines may not be identical. One such piece of evidence comes from the activity of SN1 and SN2. The salivary neurones in *Locusta migratoria* are active at different rates during feeding: SN1 is active at approximately 8 Hz and SN2 at 5 Hz, and between feeding bouts the SN2s are completely inactive but the SN1s are periodically active (Schachtner and Bräunig, 1995). These differences suggest distinct roles for the salivary neurones. The acini of the desert locust are composed of two main cell types, the zymogenic and parietal cells (Kendall, 1969), which perform slightly different functions in terms of fluid and enzymatic secretion. It is tempting to speculate that serotonin and dopamine affect the zymogenic and parietal cells separately to induce salivary secretions that have different proteinaceous compositions (Ali and Orchard, 1994; Schachtner and Bräunig, 1995). Separate roles for these amines have been found in *Periplaneta americana*, where serotonin and dopamine induce the secretion of proteinaceous and non-proteinaceous saliva, respectively (Just and Walz, 1996).

The effects of dopamine on cockroach salivary glands have been reviewed in detail (House and Ginsborg, 1985), but a brief overview is in order. Application of dopamine to isolated

salivary glands leads to an increase in the salivation rate (Just and Walz, 1996; House and Smith, 1978). The effect of salivary nerve stimulation on salivation rate can be mimicked by applying dopamine externally to isolated salivary glands (Ginsborg *et al.* 1974; Grewe and Kebabian, 1982). Dopamine appears to activate two types of receptors in the glands, DA₁ and DA₂ receptors (Evans and Green, 1990, 1991). Activation of DA₁ receptors leads to a rise in cyclic AMP level and an increase in ion and water transport from peripheral cells, while activation of DA₂ receptors leads to a Ca²⁺-dependent hyperpolarizing response in salivary gland acinar cells (Evans and Green, 1991, 1990; Gray *et al.* 1984; Ginsborg *et al.* 1980). More recently, dopamine has been shown to induce a watery, non-proteinaceous salivary secretion in *Periplaneta americana*, suggesting that activation of both receptors is necessary for the secretion of a watery saliva, while activation of other (serotonergic) receptors is necessary for the secretion of proteins and enzymes (Just and Walz, 1996).

Noradrenaline has been implicated in the activity of the salivary glands of the locust (Baines and Tyrer, 1989), but it has such a high threshold of activity ($10^{-6} \text{ mol l}^{-1}$) compared with that of serotonin ($10^{-10} \text{ mol l}^{-1}$) and dopamine ($10^{-9} \text{ mol l}^{-1}$) that a neurotransmitter or neurohormonal role for this catecholamine seems unlikely. In addition, noradrenaline has no significant effect on cyclic AMP levels in locust salivary glands even when applied at $10^{-5} \text{ mol l}^{-1}$ (Ali and Orchard, 1994). Similar effects were obtained in cockroach salivary glands in which the potency of noradrenaline (to induce salivation) was approximately 2% of that of dopamine (House and Ginsborg, 1985). The presence of noradrenaline in the salivary glands of *Locusta* needs to be confirmed.

Octopamine

Octopamine has an insignificant effect on cyclic AMP levels in locust salivary glands (Ali and Orchard, 1994), nor is it capable of increasing salivation (Baines and Tyrer, 1989). The association of octopamine with locust salivary glands is intriguing. Bowser-Riley and House (1976) showed that it is a weak salivary secretagogue in *Nauphoeta cinerea* and that it enhances neurotransmitter release in cockroach salivary glands. Might octopamine perform a similar function in the locust? This remains to be seen, but its scant presence in the glands (23 ng g⁻¹ wet tissue mass in *Schistocerca gregaria*; Robertson and Juorio, 1976) hints at a more indirect role than that of serotonin or dopamine, which are highly concentrated throughout the tissue.

Peptidergic innervation and control

Locust salivary glands are innervated from the median-transverse nervous system *via* peptidergic neurones that stain positively for bovine pancreatic polypeptide (BPP)- and FMRFamide-like immunoreactivity (Myers and Evans, 1985; Fusé *et al.* 1996). Two of these peptides have been tentatively identified as being similar to AFIRFamide and GQERNFLRFamide *via* coelution profiles on HPLC (Fusé *et*

al. 1996). Although a previous study reported the presence of YGGFMRFamide associated with locust salivary glands (Baines *et al.* 1989), this has not been confirmed. Various studies have also been unable to confirm the presence of SchistoFLRFamide associated with either the salivary neurones or the salivary glands (Swales and Evans, 1995; Fusé *et al.* 1996), even though it had been suggested to be found within the SN2 of *Locusta* (Schoofs *et al.* 1993). The roles played by AFIRFamide and GQERNFLRFamide are still unknown, but we do know that these peptides are incapable of directly elevating cyclic AMP or cyclic GMP levels in isolated salivary glands (Fusé *et al.* 1996). It appears that the transverse nerve may influence the firing of the suboesophageal salivary neurones (Baines *et al.* 1989) since activation of the transverse system is correlated with an increase in activity of SN1 but a suppression of firing of SN2. Thus, AFIRFamide and GQERNFLRFamide may affect fluid secretion indirectly or they may have a function that is altogether separate from salivation.

It appears that the amino acid γ -aminobutyric acid (GABA) is present within the SN2 (Watkins and Burrows, 1989); however, its role is unknown. More recently, a 15-amino-acid peptide (Lom-SG-SASP: $\text{NH}_2\text{-EVGDLFKEWLQGNMN-COOH}$) has been isolated from the salivary glands of *Locusta migratoria* (Veelaert *et al.* 1995); it induces increases in both the salivation rate and cyclic AMP levels in salivary gland preparations. The direct mode of action of Lom-SG-SASP and its site of origin are unknown. FMRFamide-like immunoreactivity in *Rhodnius prolixus* salivary glands originates from both the SOG and the stomatogastric system (Tsang and Orchard, 1991). This supply from both sources is neurohaemal in nature, but since the co-localization of serotonergic and FMRFamide-like elements was not observed, it is unlikely that the FMRFamide-related peptides in this system arise from the serotonergic satellite-like system. Unfortunately, there have been no studies to test the physiological roles of FMRFamide or its related peptides in *Rhodnius* salivary glands.

Conclusion

Serotonin and dopamine are neurotransmitters in the salivary glands of several insects. They induce salivation, at least partially, through cyclic AMP and InsP_3 second messenger system. Serotonin and dopamine presumably act on different cell types to induce the secretion of proteinaceous or non-proteinaceous saliva. The exact mode of action and transduction mechanisms for the effects of serotonin and dopamine are still under investigation and, in its most parsimonious form, the effects of these two amines can account for salivation in *Locusta migratoria*. However, the additional presence of octopamine, GABA, AFIRFamide-like, GQERNFLRFamide-like, LOM-SG-SASP and possibly other peptides suggests that the control of salivation is more complicated than was originally assumed. This situation also appears to be true for *Periplaneta americana*. In this animal,

the SN1s are dopaminergic, the innervation from the stomatogastric system is serotonergic, but the neurotransmitter content of the SN2s is unknown. A final example is the innervation of *Rhodnius prolixus* salivary glands; even though serotonin and FMRFamide-related peptides are associated with the salivary glands, the phenotype of the suboesophageal salivary neurones is unknown. They (assuming more than one neurone) are not serotonergic, catecholaminergic or FMRFamide-like in nature. Clearly, salivary gland control is not a simple matter. Much has yet to be learned of the physiology and control of salivation in insects. Indeed, insect salivary glands may provide us with several unique opportunities. For instance, they may prove to be ideal systems for the study of amine-amine and amine-peptide interactions. Since individual salivary neurones have been identified in the locust, cockroach and stick insect, one can preferentially stimulate single or multiple salivary neurones, either simultaneously or in sequence, while studying glandular activity at a cellular level. In addition, the presence of serotonergic and dopaminergic receptors (Ali and Orchard, 1994) and the recent finding of a dopaminergic, Na^+ -independent uptake transporter in locust salivary glands (Ali and Orchard, 1996b) make this system an excellent one for the isolation, cloning and full characterization of locust aminergic receptors and uptake transporters. The identification of salivary neurones, neurotransmitters and putative salivary secretagogues is a necessary first step towards understanding the physiology of salivation. These first steps have been taken with the recent identification of some of the salivary neurones and salivary neurotransmitters in *Locusta*, *Carausius*, *Rhodnius*, *Periplaneta*, *Aedes* and *Gryllus*.

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