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EXCITABILITY AND SECRETORY ACTIVITY IN THE SALIVARY GLAND CELLS OF JAWED LEECHES (HIRUDINEA: GNATHOBDELLIDA)

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Summary

Thousands of salivary cells fill the interstices throughout the anterior ends of jawed leeches. The somata are large (30–200 μm in diameter). They project single processes (ductules) into the three jaws, and were found to fire overshooting action potentials of 50–85 mV amplitude and 100–200 ms duration at low spontaneous rates. The action potentials were not detected in the presence of cobalt (10 mmol l^{-1}), but could be recorded when sodium was absent from the Ringer, so they appear to be calcium-dependent.

Salivary material is transported by the long processes of these unicellular glands and secreted into ducts which alternate with paired teeth on the jaws. Secretion is activated reliably by 10^{-6} mol l^{-1} serotonin, but not by other neurotransmitters found in the leech nervous system. Each jaw secretes at an average rate of 230 nl min^{-1} in the presence of serotonin, and secretion is completely abolished by cobalt.

Perfusion with serotonin excites the salivary gland cells into impulse activity, and often evokes bursting. Impulse activity of the peripherally projecting, serotonergic Retzius cells evokes both depolarizations and action potentials in the salivary gland cells. In jawed leeches, central neurones appear to control salivation by a peripheral release of serotonin. This neurotransmitter evokes calcium-dependent action potentials and calcium, in turn, stimulates secretion.

Introduction

Microscopic studies of leech salivary glands date from over a century ago, when Haycraft (1884) observed that bloodsucking leeches produce a powerful anticoagulant from simple salivary sacs that invaginate into the muscle fibres surrounding the oral cavity. The practice of medicinal leeching gradually fell into disrepute, and interest in the anticoagulant properties of the hirudin molecule (Markwardt,

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1957) waned after the synthesis of heparin. Since then, the leech has become a focus of neurobiological studies, and much has been learned of the synaptic physiology, development and regeneration of its nervous system (for a review see Muller *et al.* 1981). Recently, medical interest has been renewed in the practice of leeching, which may be beneficial in aiding blood flow in tissue grafts (Mutimer *et al.* 1987), and agents in leech salivary glands may have therapeutic potential (Lent, 1986). Although the histology of salivary cells of jawed leeches has been examined by many workers, little information is currently available on their physiology.

We report here that salivary gland cells in jawed leeches are electrically excitable and that the action potential is supported by calcium ions. Our results suggest that secretion is controlled from the central nervous system by the peripheral release of serotonin.

Materials and methods

European medicinal leeches, *Hirudo medicinalis*, were shipped from the suppliers (Ricarimpex, Audenge, France, and Biopharm, Swansea, UK) by air. American medicinal and horse leeches, *Macrobdella decora* and *Haemopis marmorata*, respectively, were obtained from commercial suppliers in the United States (St Croix Biological Supply, Stillwater, MN, and Wholesale Bait Company, Hamilton, OH). All three species were maintained in artificial pond water in a cold room at 12°C on a 12 h:12 h light:dark cycle. Dissection and experiments were performed in a Ringer's solution containing (in mmol l^{-1}): NaCl, 116; KCl, 4; $CaCl_2$, 2; Tris-maleate, 10, titrated to pH 7·4. Sodium-free solution was prepared by isosmotic replacement with sucrose. All experiments were performed at room temperature (19–23°C).

Dissection and anatomy

The leech was pinned by its suckers to a wax board and, following a dorsal midline incision, the body wall was reflected to reveal the internal organs. As much crop tissue was removed as possible, and the areas around the salivary cell bodies were cleared of overlying muscle and connective tissue. Best results were obtained in preparations re-pinned in a Petri dish lined with Sylgard resin (Dow Chemical, Midland, MI), with the skin stretched to form a flat sheet. For experiments where secretion was studied, the jaws were separated from one another by cutting through the buccal cavity epithelium. These dissections proceeded posteriorly, removing the longitudinal jaw muscles together with the ductules and as many salivary cells as possible. Where simultaneous recording from cells in the central nervous system (CNS) was attempted, animals were dissected from dorsum and the ventral nerve cord was left attached to the body wall by the lateral nerve roots.

Electrophysiology

Salivary cells were viewed using a stereomicroscope, and were illuminated by

both a sub-stage darkfield condenser and a low-intensity light from a fibre-optic cable positioned at the side of the recording chamber. Impalements were made under visual control using conventional glass microelectrodes (30–50 MΩ resistance when filled with 3 mol l^{-1} KCl solution). An electrometer of high input impedance (Getting model 5) was connected to the electrode *via* a non-polarizable Ag/AgCl electrode. Since it has not been possible to insert a second microelectrode into a single salivary cell, experimental displacement of the membrane potential was achieved by injecting current through the recording electrode. Compensation for the voltage drop across the electrode was made using a circuit in the electrometer (see Purves, 1981). Electrometer output was displayed on a Tektronix D-13 analogue storage oscilloscope, and permanent records made on a Brush 220 rectilinear ink-writing oscillograph.

Secretion

Jaw and salivary gland preparations were dissected as described above, and pinned out through the tufts of muscle and connective tissue attached to the jaw. It was most convenient to arrange each jaw with its lateral surface uppermost, so that secretion was across the plane of view, as in Fig. 6. Preparations were photographed using a Leitz MPV-2 microscope equipped with a Vario-Orthomat camera. Secretion was routinely viewed upon a Zeiss (Jena) Ergaval microscope equipped with Hoffman modulation contrast optics, and a video camera was mounted on its trinocular head. Secretion was observed both directly and *via* a high-resolution television monitor (Koyo, model TMC-17M), and permanent records were made using a commercial VHS format video cassette recorder.

Quantitative estimates of volumes of saliva secreted were made using a modified haemocytometer slide (Improved Neubauer Levy chamber, number 500). Small squares of wax were fitted in the depressions around the edge of the counting area. The jaw was pinned to the wax using fine pins, and arranged so as to secrete laterally onto the calibrated area. The salivary cells were bathed in Ringer or test solutions, and the volume secreted was estimated by counting the number of squares covered by secreted product in the cavity of known thickness formed by the haemocytometer slide and its overlying coverslip.

Results

Anatomy

The cell bodies of the salivary gland cells of the gnathobdellid leeches *Hirudo medicinalis* and *Macrobdella decora* are located approximately from segments V to XI according to the numbering system of Mann (1962); that is, from the base of the jaws posteriorly to the anterior end of the crop, around the level of the third segmental ganglion of the nervous system. Somata are generally spherical or ovoid and range in diameter between 30 and 200 μm. Each soma extends a single process, or ductule, anteriorly to the base of one of the three jaws (Fig. 1). The ductules narrow to a width of around 2 μm within a few millimetres of the cell body, and the

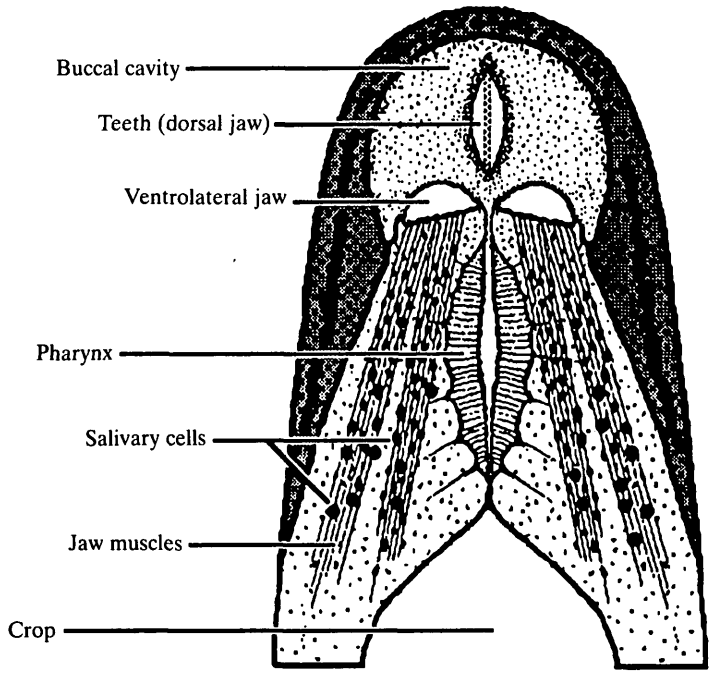

Fig. 1. A diagram of the head of a Gnathobdellid leech after dissection from the ventral side. The three jaws reside within the buccal cavity, and two longitudinal bands of muscles from each jaw terminate near the crop. The dark spheres among the jaw muscles represent the numerous salivary gland cell bodies. The lines radiating from the pharynx are the extrinsic dilator muscles.

longest of them, from the salivary cell bodies located most posteriorly, reach 2 cm in length. As the ductules run anteriorly, they coalesce into fascicles and are intimately associated with the bands of longitudinal muscle fibres emanating from the jaws. Fig. 2 shows a fresh, unfixed jaw, with hundreds of gland cell bodies which *in situ* fill all the interstices between the various muscles of the jaws, pharynx and body wall. The salivary cells clearly do not form a discrete gland, but are dispersed widely and enmeshed in connective and vasofibrous tissues.

When freshly dissected salivary cells were illuminated using an ultraviolet source, a small proportion (approx. 5 %) exhibited a marked, yellow autofluorescence (Fig. 3A). The fluorescence was most intense around the nucleus in the salivary cell soma, and was clearly discernible in other cytoplasmic regions. The fluorescent ductule of a single salivary cell could be followed along its trajectory and into the base of a jaw in favourably positioned preparations. In such cases, there was no evidence of fusion of any of the secretory ductules, or of transcellular transport of fluorescent material into ductules of non-fluorescent cell bodies (Fig. 3B). On the basis of these and many previous histological observations (Lankester, 1880; Haycraft, 1884; Bhatia, 1941; Harant & Grasse, 1959; Van der Lande, 1968; Dev & Mishra, 1972), it is clear that the salivary cells of these jawed leeches are unicellular glands. While performing electrophysiological studies, it

Fig. 2. A dark-field photomicrograph of a freshly dissected jaw (*j*) showing the two muscle bands with many of the salivary gland cell bodies adhering (arrowheads). A 'halo' of saliva (*s*) can be seen along the cutting edge of this jaw. The jaw is from *Macrobdella decora* and has 51 pairs of teeth which appear dark. Scale bar, 500 μm.

became obvious that individual salivary cell bodies could be pulled aside from neighbouring cells by means of the electrode tip, and there was no evidence of clustering or anatomical connection. The histological and ultrastructural studies of Damas (1972, 1974) show clearly that the individuality of the salivary cell ductules is maintained throughout their trajectory, and within the musculature of the jaw

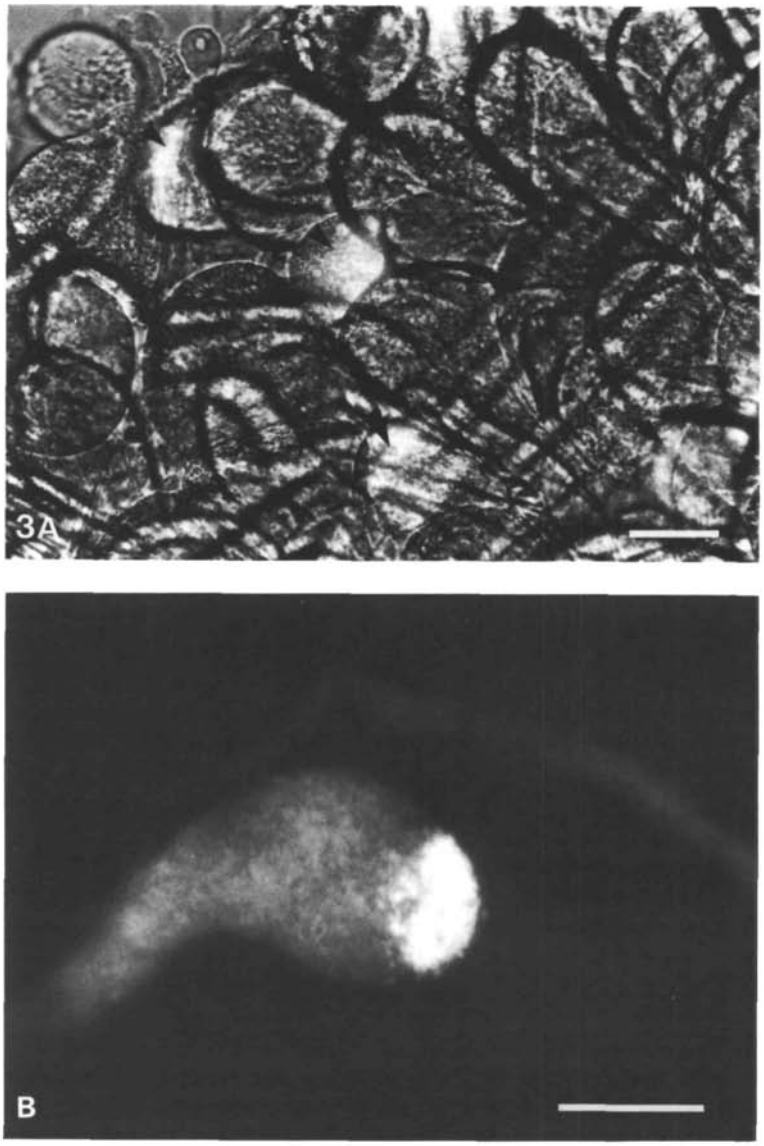

Fig. 3. Photomicrographs of the salivary cells of *Macrobdella decora*. (A) Cell bodies viewed with simultaneous light and epifluorescent illumination. Four autofluorescent somata (arrowheads) are visible in this field which also contains more than 50 non-fluorescent salivary gland cell bodies. The diagonal lines in the lower right corner are jaw muscle fibres, slightly out of the plane of focus. (B) A single autofluorescent salivary gland cell body of *Macrobdella decora*. The fluorescent material is granulated and this intense apical fluorescence is associated with the nucleus. The fluorescent process seen above the cell body is a ductule from another gland cell. Scale bars, 50 μm.

itself. Groups of ductules discharge their secretion in parallel at the base of the teeth. We therefore state unequivocally that there are no anatomical connections, no acinar structure and no common ducts, and that the salivary cells discharge individually to the buccal cavity.

Gland cell excitability

Upon impaling a gnathobdellid salivary cell, an initial rapid deflection of variable size between −15 and −70 mV was observed. In the majority of such impalements (>90 %), a discharge of action potentials occurred, accompanied by a progressive depolarization (Fig. 4A). As the cell depolarized, the rate of rise, amplitude and overshoot of the impulses decreased. In some cases, a stable resting

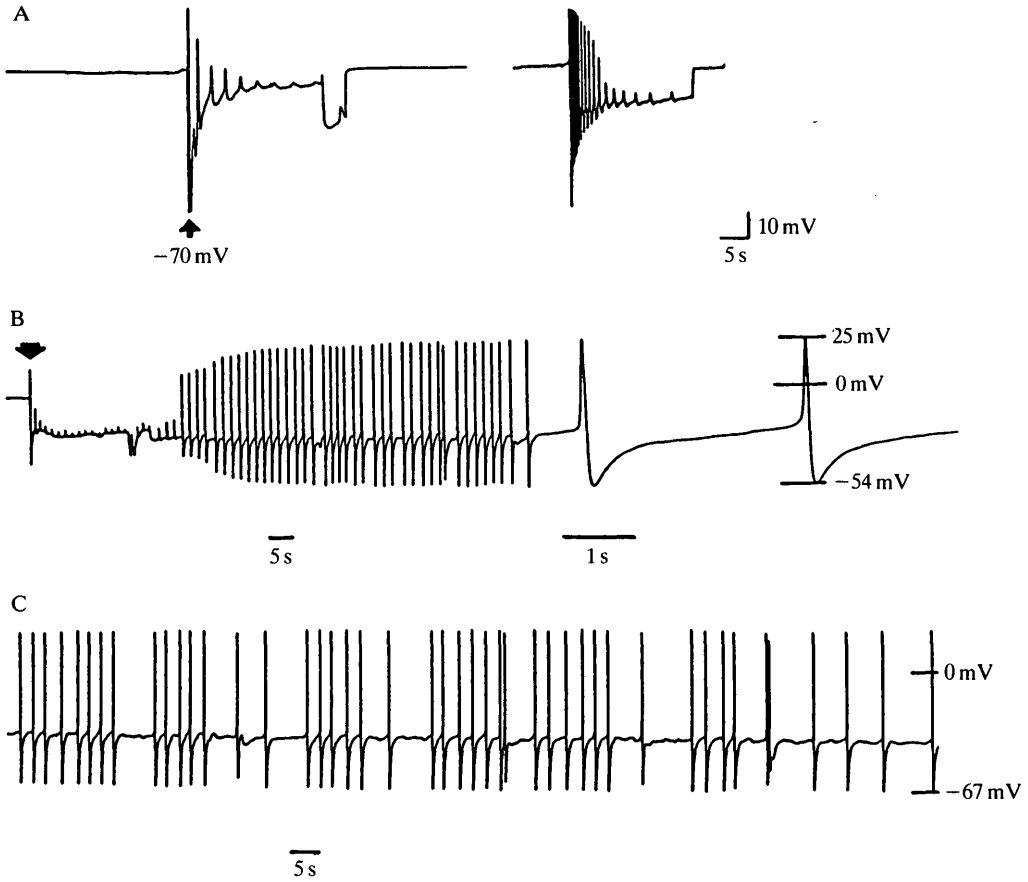

Fig. 4. Intracellular recording from salivary gland cell bodies in the head of *Macrobdella*. (A) Typical impalements of salivary cells. In the first trace, the potential returns to zero when a body wall contraction dislodges the electrode. In the second trace, the electrode was withdrawn indicating a resting membrane potential of −12 mV. (Traces from different cells.) (B) Impalement is followed by sealing, and the appearance of spontaneous overshooting impulses. Resting potential −29 mV. (C) Recording from same cell as B, 1 min later. Resting membrane potential has increased to −37 mV.

potential of −15 to −20 mV persisted, but most frequently the potential fell to zero, indicating that the electrode had slipped from the cell. In approximately 1 in 30 cells successfully impaled, the membrane potential increased over a time course of 20–180 s, presumably due to sealing of the leak caused by impalement with the microelectrode. We have recorded from salivary cells for up to 20 min, but only rarely, as most impalements were stable for less than 3 min.

Spontaneous overshooting action potentials were recorded from the salivary gland cells in the three species studied: *Hirudo medicinalis*, *Macrobdella decora* and *Haemopis marmorata*. No inter-species variation in electrical activity was noted, nor was any electrophysiological evidence for subpopulations of salivary cells adduced. Depending on the stability of the resting potential, the overshoot ranged between +5 and +30 mV, and there was a pronounced afterhyperpolarization, or undershoot, of 300–1000 ms. Fig. 4B shows an impalement of a salivary cell in *Macrobdella*, with a period of sealing followed by spontaneous overshooting action potentials. Impulses arose from a membrane potential of −29 mV, had an overshoot of +25 mV and an undershoot of −54 mV and, hence, a peak-to-peak amplitude of 79 mV. The duration of this action potential between half amplitudes was 140 ms. Fig. 4C was recorded from the same cell 1 min later. As a result of further improvement in membrane potential, the impulse amplitude had increased to 96 mV, and the firing frequency had decreased. The impulse appeared to occur in a series of bursts. These long-duration action potentials had a plateau which resulted from two phases of repolarization. This distinctive shape in these jawed leeches is similar to that of the salivary cell action potentials in proboscis-bearing (glossiphoniid) leeches (Marshall & Lent, 1984).

In many cases, a stable resting potential was obtained with virtually no spontaneous activity. Fig. 5 shows recordings from a salivary cell in *Hirudo* which fired a single impulse 2 or 3 times per minute. Injection of depolarizing current caused repetitive firing which was marked by a progressive decrease in amplitude, and an increase in impulse duration. When a depolarizing current of smaller amplitude was used, repetitive firing at a lower frequency was evoked and maintained with no change in amplitude for 90 s, as is seen in spontaneously active salivary cells. Action potentials could also be elicited by anodal break at the termination of a hyperpolarizing current pulse (Fig. 5B). Attempts to measure the input resistance and time constant of the salivary cells have been frustrated by the rectification which occurs when passing the large current pulses required to produce a measurable displacement of the membrane potential in the hyperpolarizing direction. The electronic subtraction of stimulus artefact described in Materials and methods is satisfactory only when the electrode has a linear current–voltage relationship, which is also constant over the duration of individual current pulses. In the present case, we were unable to separate the responses of electrode and cell with any degree of confidence.

The difficulty in obtaining stable impalements of these cells has made it impossible to exchange the bathing solution while recording intracellularly. However, we have obtained data which suggest that the ionic basis of the action

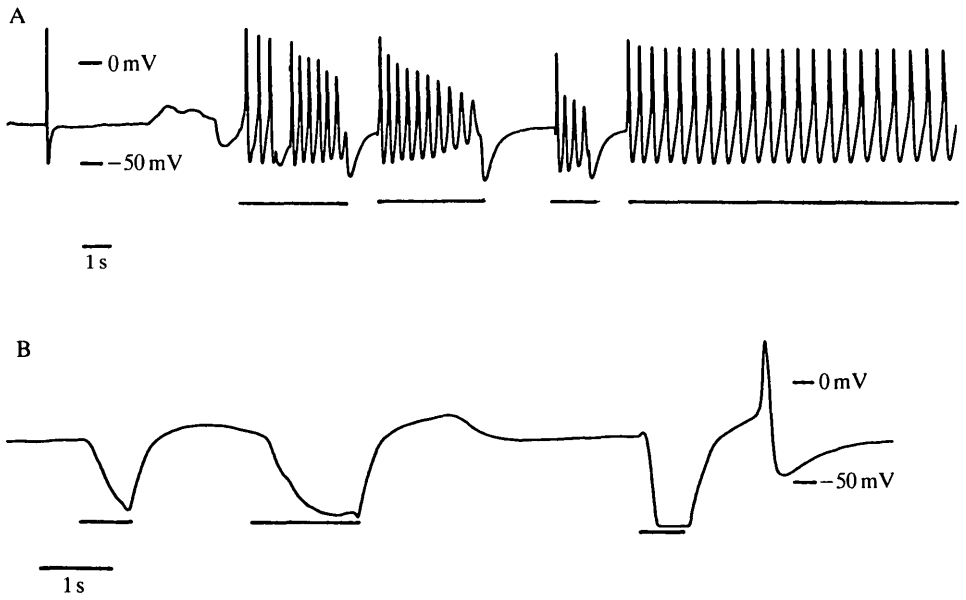

Fig. 5. Action potentials evoked by current injection in a salivary cell of *Hirudo medicinalis*. (A) Depolarizing current was passed *via* the recording microelectrode where bars appear beneath the trace. Current pulses of 6 nA were applied for the first three bars and 2 nA during the longer bar at the end of the record. Resting potential −32 mV. (B) Hyperpolarizing current was passed where indicated by bars. A current of 6 nA was passed at the two left-hand traces and 10 nA at the right-hand one, resulting in an anode-break action potential. Resting potential −29 mV. The apparent change in time constant in B is due to electrode rectification, and cannot be taken as a cellular response.

potential may be similar to that described in detail for the giant salivary cells of glossiphoniid leeches (Marshall & Lent, 1984), where calcium is the principal charge carrier. Reduction of external calcium level proved unfeasible because it increased the spontaneous contractions of the muscle fibres among the gland cells. Conversely, decreased excitability as a result of increased external calcium level made it difficult to evoke impulses. As an alternative, 10 mmol l^{-1} cobalt chloride was added to the bathing solution, after checking for normal bursts of impulses on impalement. In the presence of cobalt, impulses were never observed on impalement and neither depolarization nor anode-break excitation was effective ($N = 150$ in three experiments). After a 30 min wash with normal Ringer's solution (45 bath volumes), impulses were again observed. Preparations were also washed with sodium-free Ringer for 30 min, and cells then impaled. Impulses of normal appearance persisted in sodium-free solution ($N = 40$ in two experiments), indicating that other ions can support the action potential. As described previously, there was a considerable range of stable membrane potential from cell to cell. We believe that this is due to varying degrees of leak on impalement, and the corresponding variability in rate of rise and overshoot of the action potential

has made it impossible to determine whether these parameters are affected by sodium removal.

Secretion

The salivary glands of these gnathobdellid leeches secrete from three jaws, one dorsomedial and two ventrolateral, within the buccal cavity (Fig. 1). Each jaw is semiluniform with pairs of conical, recurvant teeth along the cutting edge. The number and morphology of the teeth reflect the feeding habits of each species and constitute important taxonomic characteristics (Sawyer, 1972; Klemm, 1972). Within the jaws, salivary cell ductules run in groups which terminate in secretory ducts at the base of the teeth (Damas, 1974). Duct orifices alternate with pairs of teeth, and so the number of ducts approximates the number of pairs of teeth. We estimate a minimum of 2000 salivary cells in *Hirudo*. Since there are 65–75 teeth on each of three jaws, this suggests that approximately 100 salivary cell ductules release their secretions into each duct. During examination of a living, isolated jaw–salivary gland cell preparations under the compound microscope, we were surprised to observe active secretion. Saliva is secreted into the ducts, apparently by exocytosis, even though this cellular process cannot itself be observed. Rather, the secreted material from many ductules causes a flow of saliva through the ducts onto the cutting edge of the jaw and granular material within the saliva is visible. The accumulation of a 'halo' of secreted material (Fig. 2) is particularly obvious after a jaw has been first irrigated with Ringer to clear its surface of residual saliva. No baseline secretion was observed in half the preparations studied. In the others, average spontaneous rates of secretion varied considerably, probably because of variations in cell damage on dissection.

Several biologically active substances were tested for an effect on secretion. Acetylcholine at 10^{-6} mol l^{-1} did not lead to secretion, but caused contraction of the muscle fibres interspersed among the salivary cell ductules. No effect was noted on addition of 10^{-5} mol l^{-1} gamma-aminobutyric acid, dopamine or noradrenaline. Peptides were not applied. However, serotonin, at concentrations of 10^{-7}–10^{-6} mol l^{-1}, reliably activated secretion and increased the rate in preparations with an observable baseline level. An increased secretory rate was observed for up to 1 h, although the rate generally declined gradually after a period of 20 min in the continued presence of serotonin. Fig. 6 is a series of photomicrographs showing the halo of secreted material at the edge of a *Hirudo* jaw 10 and 20 min after the preparation had been exposed to 10^{-6} mol l^{-1} serotonin. The rate of secretion declined over a period of 3–5 min after serotonin had been washed out with normal Ringer, and usually stopped completely. Secretion recommenced if serotonin was reapplied. Fig. 7 shows the variation in rate of secretion in response to three serotonin concentrations. The jaw was irrigated clean, then the volume secreted in a 20-min period was estimated on the haemocytometer slide. An average rate of 230 nl min^{-1} was obtained for each jaw. The maximum rate of secretion may be somewhat higher, although inspection of taped records of secretion did not reveal any apparent reduction in rate over the

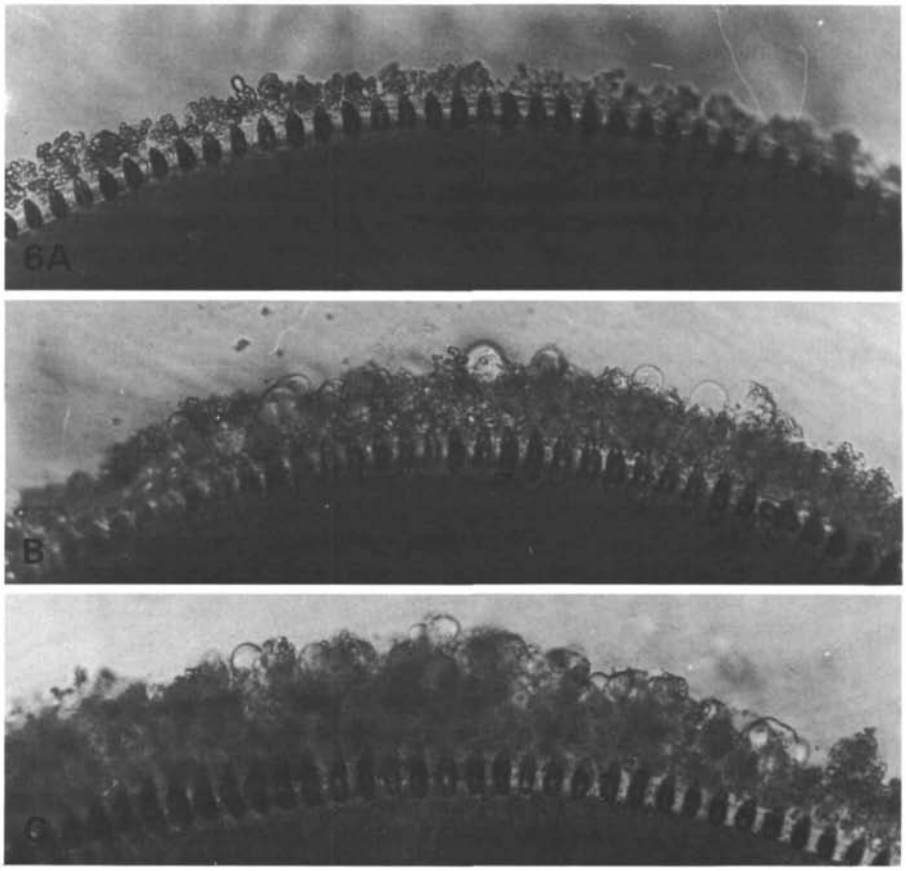

Fig. 6. Secretion along the cutting edge of a jaw and salivary cell preparation from *Hirudo medicinalis*. (A) A control jaw was irrigated with Ringer to clear the surface, then bathed in normal Ringer and photographed 30 min later. (B,C) Secretion after 10 and 20 min of exposure to 10^{-6} mol l^{-1} serotonin. Thirty-five of the teeth are visible, a field of view of approximately 1 mm.

20-min period of measurement. The estimated rate may be compared to maximum rates of 60 nl min^{-1} for blowfly and 350 nl min^{-1} for cockroach salivary glands (House, 1980).

Application of 10 mmol l^{-1} cobalt chloride to a preparation secreting in response to serotonin caused complete abolition of secretion within 2–4 min. The rate of discharge between the teeth clearly slowed as secretion stopped, and the blockage was completely reversible. Bathing preparations in calcium-free Ringer decreased the rate of serotonin-induced secretion, but did not abolish it fully. However, it is likely that calcium was not entirely washed out of the glandular interstices by perfusion and, furthermore, calcium is probably released in small quantities from

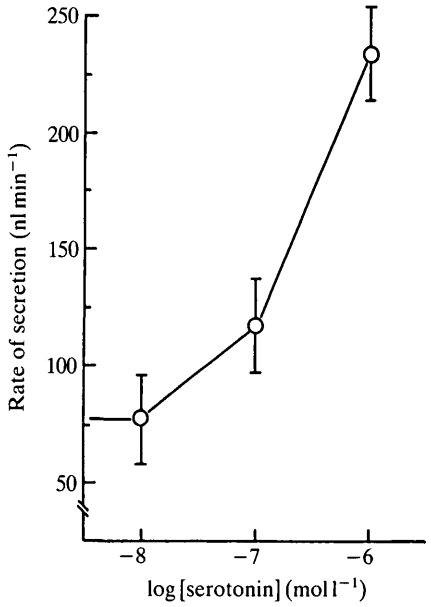

Fig. 7. Secretion increases with serotonin concentration. Saliva volume was estimated on a haemocytometer slide after a 20-min period at each of three serotonin concentrations, and expressed as an average rate per minute. The average rate at 10^{-8} mol l^{-1} was not measurably higher than in normal Ringer. Measurements were made on three separate jaw preparations. Circles represent mean values and are shown with ±1 S.D.

tissues damaged during dissection. Our observations suggest that salivary secretion is dependent on external calcium, because cobalt is well-known as an antagonist of calcium-dependent events.

Control of secretion

Since serotonin has been shown to be potent in enhancing secretion, it was of interest to look for an effect of serotonin on the gland cells themselves. These experiments were often without success for the technical reasons described earlier. Fig. 8 shows an experiment where a clear effect was noted. A stable membrane potential of −32 mV was recorded, and injection of depolarizing current verified that the cell was excitable. A volume of 10^{-6} mol l^{-1} serotonin in Ringer was added to give a final concentration of 10^{-7} mol l^{-1}. A rapid depolarization to a plateau of −8 mV was seen, followed by oscillations in membrane potential of increasing amplitude. The cell slowly hyperpolarized and the oscillations developed into impulses which occurred at a rate of 4 s^{-1} for 150 s. This initial tonic firing response was followed by a long period of bursts of impulses at approximately 20-s intervals. In the example shown in Fig. 8C, impulses occurred at the peak of slow depolarizing waves, and the interval between successive impulses increased during a single burst. This bursting activity continued for 16 min, at which point the impalement was lost. Observations in two such experiments suggest that serotonin

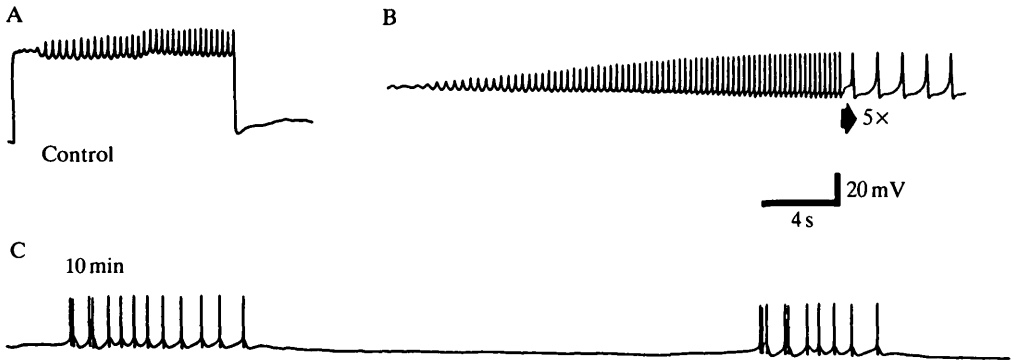

Fig. 8. Effect of serotonin on the membrane potential of a salivary gland cell in *Hirudo*. (A) Depolarizing current is passed through the bridge circuit of the intracellular amplifier. The bridge could not be completely balanced and so a voltage artefact is seen. Membrane potential −32 mV. (B) 10^{-6} mol l^{-1} serotonin is added to the bath Ringer. After 3 s, the membrane depolarizes to −8 mV after which it hyperpolarizes slowly and begins tonically firing action potentials of increasing amplitude. The recording speed of the oscillograph was increased five-fold at the end of the trace. (C) 10 min after wash-in of serotonin, the gland cell is firing bursts of impulses which ride upon slow membrane potential oscillations. Membrane potential −30 mV, overshoot +4 mV. Note that serotonin altered the shape and increased the amplitude of the gland cell action potentials.

directly alters membrane conductance of the leech gland cell, but no further reliable information on the nature of the response was obtained.

Of the population of serotonin-containing cells in the segmented central nervous system of the leech, paired Retzius cells (RZ) have peripherally projecting axons. Since these cells are the only neuronal source of peripheral serotonin in the somatic segments, the effect of impulse activity in the RZ on the salivary cell membrane potential was investigated. Fig. 9A shows a simultaneous intracellular recording from the RZ in the first segmental ganglion of *Macrobdella* and from a salivary cell body at that level. A stable resting potential of −27 mV was obtained in the gland cell. Single action potentials in the RZ had no effect on the gland cell membrane potential. However, high-frequency impulse activity in the RZ (by sustained depolarizing current) evoked depolarizations of the salivary gland cell (Fig. 9B). These 3–5 mV events usually continued for several seconds following the RZ impulse burst. The oscillations in membrane potential did not give rise to action potentials in this and one other experiment. In a similar experiment, in the second suboesophageal ganglion in *Hirudo* (Fig. 9D), single action potentials of RZ had no effect; however, a high-frequency burst of RZ impulses, induced by current injection, elicited action potentials in a previously silent gland cell. Once initiated, the gland cell impulses continued for more than 2 min after the RZ burst ended. These two experiments suggest that rapid firing by the RZ in the central nervous system releases sufficient serotonin at the level of the peripheral gland

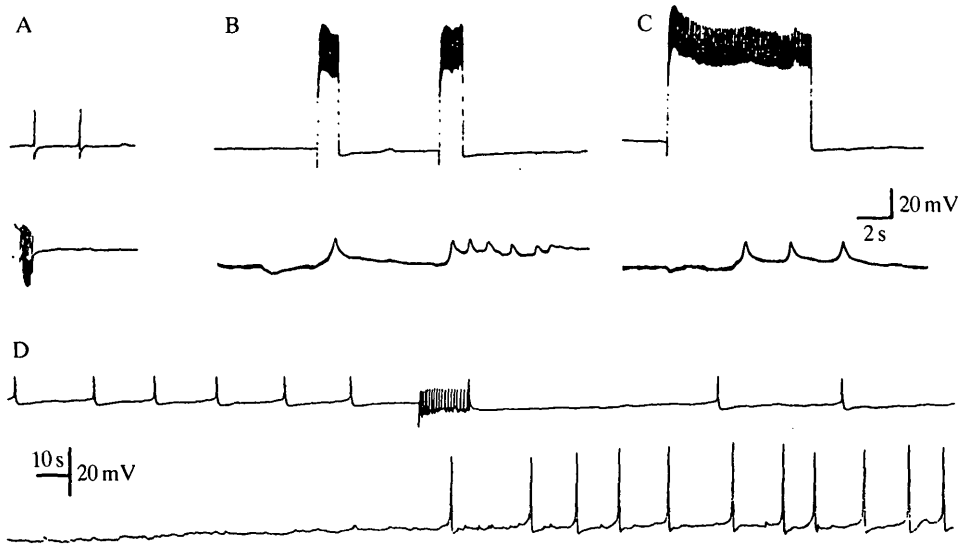

Fig. 9. Effect of Retzius cell (RZ) activity on salivary gland cell membrane potential. Simultaneous intracellular recording from RZ cells and salivary gland cell bodies. (A) Upper trace from RZ cell in the first segmental ganglion, lower trace shows salivary cell impulses on impalement. Salivary cell membrane potential −27 mV. (B) The RZ is stimulated to fire impulses at a high frequency by passing a large depolarizing current which evokes a 5 mV depolarization in the gland cell. The voltage drop across the resistance of the recording electrode caused by current injection could not be completely balanced with the bridge circuit. A subsequent RZ burst evokes six depolarizations of 3–5 mV. (C) A subsequent trial several minutes later in the same experiment. A–C were recorded from *Macrobdella*. (D) A similar experiment in *Hirudo*. Action potentials of the RZ in the second suboesophageal ganglion are spontaneous at about 5 min^{-1} and do not affect the gland cell. A burst of RZ impulses evokes a train of overshooting action potentials in the salivary gland cell. The salivary cell impulses continue for 2 min after the RZ burst which initiated them.

cells to cause depolarizations, which in the unperturbed cell appear to be sufficient to result in repetitive gland cell action potentials.

Discussion

Within the phylum Annelida, the structure of salivary glands in leeches has more in common with that in oligochaetes than that in polychaetes, as might be expected, since the Hirudinea are generally held to have evolved from an ancestral oligochaete (Clark, 1980). The buccal cavity of most oligochaetes has an attached dorsal complex, or pharyngeal bulb, composed of small unicellular gland cells which are interdigitated between muscle fibres and separated from the digestive lumen by cylindrical epithelial cells. An electron microscopic study of *Enchtraeus albidus* by Reger (1967) revealed clearly that elongate processes extend from individual gland cells to the pharyngeal lumen, where they are located between epithelial cells. That is, secretions are released from individual cell processes and

not transported through compound multicellular ducts. A similar structure has been described for cells in the pharyngeal gland of *Eisenia foetida*. Gland cells in the pharyngeal bulb of *Lumbricus terrestris* (Keilin, 1920) have been shown, by intracellular recording, to be capable of firing action potentials (C. G. Marshall, unpublished results), further strengthening the similarity to leech salivary cells. In contrast, the digestive apparatus of the polychaetes does not usually include individual salivary glands as such. In those polychaete species such as *Nereis virens*, where some form of digestive gland is present, its cells have an acinar structure (Michel & Devillez, 1980).

The results now available for gnathobdellid and rhynchobdellid (Marshall & Lent, 1984) leeches allow some comparisons to be made on the physiology of salivary glands in the three major protostomatous invertebrate phyla. Salivary glands are present in the Mollusca and the Arthropoda: they are well-developed in insects, but they are absent from most crustaceans. An acinar structure is found in both insect and molluscan salivary glands. The pedal gland of terrestrial slugs most closely resembles leech salivary glands in being composed of excitable cells which are morphologically and electrically independent (Kater, 1977). Salivary glands in insects are inexcitable, whereas those of gastropods support action potentials. No information is available on the electrophysiology of the large, distinctive salivary glands in the cephalopods. Thus, on the basis of electrical activity, the salivary glands of leeches bear a closer resemblance to those in gastropod molluscs than those in insects, but differ from both in their cellular structure.

All mammalian salivary glands are acinar in structure and inexcitable, but ductless glands are found in other locations, and hence unicellular glands as such cannot be regarded as a primitive feature. Electrical coupling between secretory cells is generally regarded as a means of synchronizing activity, enabling groups of cells to function as a unit. The existence here (and elsewhere) of secretory cells which are not coupled, but which function in concert, suggests that such coupling is not an absolute requirement for coordinated secretory activity. Virtually all salivary glands examined receive direct innervation from the central nervous system and do not rely on circulating hormones for their activation (House, 1980). An exception appears to be the blowfly, whose neurosecretory terminals release serotonin into the blood to activate the salivary gland hormonally (Trimmer, 1985). Salivary cells of jawed leeches respond to serotonin and to the impulse activity of serotonin-containing cells in the CNS. These findings support the report (Lent & Dickinson, 1984) that salivary secretion from the jaws of *Hirudo* is increased by the impulse activity of Retzius cells. Even though serotonin has been detected in *Hirudo* salivary glands (Lent, 1984), their serotonergic innervation has yet to be demonstrated anatomically in gnathobdellids. In glossiphoniids, serotonin-containing axons are present in the proboscis sheath in the vicinity of salivary cells (Marshall & Lent, 1985). Serotonin could be released from axonal varicosities near the salivary cells, at either the level of their somata or their ductule terminals. We do not think it likely that serotonin causes local release of another salivary transmitter, since serotonin is clearly effective on isolated

preparations, and there is no anatomical evidence for a peripheral synapse at the level of the salivary cells. However, the possibility of a novel local action has not been rigorously excluded.

An increase in intracellular calcium concentration is widely accepted as the prerequisite to the secretory process (Rubin, 1982), but there is a fundamental difference in how this is achieved in excitable and non-excitable cells. In non-excitable secretory cells, agonist binding to receptor sites leads to intracellular reactions which increase membrane calcium permeability, and changes in membrane potential are usually thought to result from secretion rather than to cause it. In excitable secretory cells, agonist binding leads to regenerative electrical activity, and calcium enters as a current carrier during the impulses. Our results support the hypothesis that secretion from the salivary glands of leeches is controlled by calcium entry during impulse activity, which is regulated in turn by serotonergic neurones in the central nervous system. This conclusion has found support in some unpublished findings, in which we observed that calcium ionophore alone will activate secretion from the jaws of leeches. The discovery of excitability in leech salivary cells strengthens the observation made by Kater (1977) that the production of regenerative action potentials in non-neural secretory cells may be a more general phenomenon than has been supposed.

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