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CALCIUM-DEPENDENT ACTION POTENTIALS IN LEECH GIANT SALIVARY CELLS

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SUMMARY

Two pairs of discrete salivary glands are located at the base of the muscular proboscis of the sanguivorous Glossiphoniid leeches *Haementeria ghilianii* and *Haementeria officinalis*. Each anterior gland is 0·8 cm to 2 cm in length, and comprises over 200 giant salivary cell bodies ranging from 150 μm to over 1000 μm in diameter, depending on the size of the animal. The salivary cells are neither electrically nor dye coupled, and there is no acinar structure or common duct, but instead each cell extends an individual ductule.

The cells fire action potentials of 100–200 ms duration and 70–100 mV amplitude in response to depolarizing pulses, or at the cessation of a hyperpolarizing pulse. The impulse is abolished by procedures known to antagonize calcium currents, and persists in sodium-free solution, or when calcium is replaced with strontium or barium. Our results support the hypothesis of a purely calcium-dependent impulse.

INTRODUCTION

To understand fully the phenomena involved in stimulus-secretion coupling (e.g. Ginsborg & House, 1980), it will be necessary both to describe the membrane properties which lead to changes in membrane potential, and to elucidate the relationship between electrical and secretory events. Gland cell electrophysiology has often been technically difficult, due to the relatively small size of secretory cells which have been studied, and the electrotonic intercoupling within acinar structures (Petersen, 1980). Invertebrate animals have often provided useful preparations for the electrophysiological study of cellular phenomena of general interest, (Hodgkin & Huxley, 1952*a*; Bullock & Hagiwara, 1957; Hagiwara & Naka, 1964) because of experimentally favourable size, structure or specialization. Kater (1977) has noted the possible advantages of studying large molluscan exocrine cells, although these too are coupled, and pose problems in the interpretation of the electrical activity of single cells.

We report here that the giant salivary cells in Glossiphoniid leeches are exceptionally large, electrically and morphologically independent, and fire calcium-dependent action potentials.

Key words: Salivary gland cells, electrophysiology, calcium.

MATERIALS AND METHODS

Haementeria ghilianii were obtained from a breeding colony, and *Haementeria officinalis* collected from Lake Patzcuaro, Mexico, *via* Mr Joel Glover and the laboratory of Dr W. B. Kristan, University of California, San Diego. The animals were maintained at room temperature, 19–25 °C, in a holding solution of the following composition (mmol l^{-1}): NaCl, 4; K_2SO_4, 0·5; $CaCl_2$, 0·4; $MgCl_2$, 1; $NaHCO_3$, 2·4; Na_2CO_3, 0·3, at a pH between 8·75 and 9·0, approximating conditions in the lake from which they were obtained. No more than ten specimens were held in each 2-litre aquarium, and the solution was changed daily.

Dissection

The leech was pinned to a wax block through its anterior and posterior suckers, and stretched to approximately its normal extended length. A dorsal midline incision was made through the external muscle layers of the anterior third of the animal, taking care not to cut too deeply. Reflecting the skin flaps and underlying connective tissue layers reveals two pairs of salivary glands attached to the base of the muscular proboscis (Fig. 1).

The anterior gland is isolated by freeing the distal end from the body wall by cutting the adhering connective tissue strands, and removing a small piece of the base of the proboscis with the proximal end attached. Removal of the proximal end is aided by cutting through the retractor muscle fibres attached to the smaller posterior glands, thus allowing the proboscis to move anteriorly and to straighten. An incision can then be made from the crop through the bulbous base of the proboscis, and a convenient small slip of muscle left attached to the proximal end of the anterior gland.

Dissection and electrophysiological recording were carried out in a Ringer's solution (pH 7·4) of the following composition (mmol l^{-1}): NaCl, 126; KCl, 4; $CaCl_2$, 2; Tris (Sigma Chemical), 10. All experiments were performed at ambient temperatures between 21 and 24 °C.

Electrophysiology

The isolated anterior gland was pinned out using 0·15 mm *minuten nadeln* through the muscle slip at the proximal end and through either adhering strands of connective tissue or the gland itself at the distal end. Careful pinning is essential for obtaining stable recordings, and it is particularly important to stretch the gland to its original length, thereby re-applying tension to the investing connective tissue sheath. Despite the large size of the cell bodies, it sometimes proves difficult to insert a second microelectrode, as the cells are inclined to move within the gland when impalement is attempted.

The recording chamber was fashioned from a 7·5 × 2·5 × 0·8 cm Perspex block glued to a microscope slide, with a 3·5 × 1 cm slot drilled out and lined with Sylgard (Dow-Corning) to accommodate the gland. The bathing solution was exchanged by gravity feed and vacuum aspiration, and required 45 s to replace each chamber volume of 1·5 ml.

Cells were impaled with glass microelectrodes filled with 3 mol l^{-1} KCl (resistance

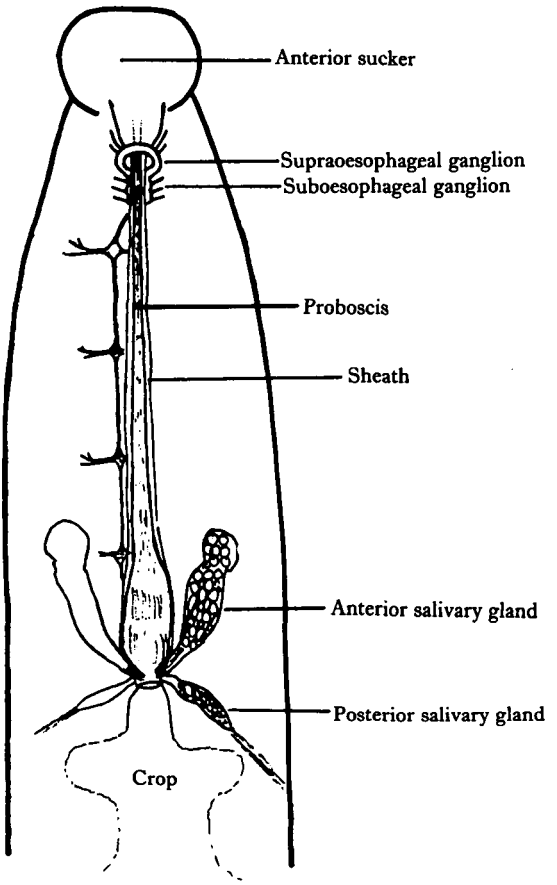

Fig. 1. A stylized representation of the anterior dorsal view of the leech *Haementeria ghilianii* after dissection. The proboscis is here shown extended, but when retracted may lie in any orientation. Four ganglia in the segmental nervous system have been displaced to the left for illustrative purposes.

30–45 MΩ), connected to a high input impedance amplifier. Cells were impaled under visual control using a Wild dissection microscope and illuminated by a substage darkfield condenser. Current was injected through the recording electrode using a bridge circuit, or *via* a second electrode inserted into the cell. All quantitative I/V measurements were made using separate current and voltage electrodes. The amplifier output was displayed on a Tektronix D-13 analogue storage oscilloscope, and permanent records obtained on Polaroid film or with an ink-writing oscillograph (Hewlett-Packard 7404A). Some records were stored on magnetic discs in a Nicolet 2090-III digital storage oscilloscope, and traced directly onto paper using an X-Y plotter.

Dye injection

Micropipettes were filled with Lucifer Yellow CH (gift of W. W. Stewart) dissolved in distilled water (5–10 mg ml^{-1}), and connected *via* a silver wire to the input stage of the amplifier. Dye was iontophoretically injected into cells by passing hyperpolarizing current pulses, 500 ms, 10 nA, once per second for 20–45 min. Successful dye

injection produced a very pale yellow tint in the gland cell body, and was confirmed under the fluorescence microscope before processing the tissue.

The gland was fixed in 10% formalin for 30 min following overnight diffusion, dehydrated, cleared in xylene and mounted whole in light paraffin oil. The preparation was viewed using a Leitz MPV-2 microscope, and photographed using the attached Vario-Orthomat camera.

RESULTS

Gland morphology

Inspection of the anterior gland under the dissection or compound microscope shows it to be composed of around 200 spherical or ovoid bodies (Fig. 2A). Each cell extends a single process, or ductule, anteriorly to the proboscis. The cytoplasm has obvious granular inclusions, and often appears more opaque at the side further from the ductule, probably because the secretory material displaces the nucleus to that side. The processes are narrow and grouped together where they enter the base of the proboscis (Fig. 2B). Some of them can be traced along their length following vital staining with Neutral Red dye, and terminate at various points along the proboscis, extending several centimetres from their respective cell bodies. In the related Rhynchobdellid leech *Glossiphonia complanata*, Damas (1962) has shown that the ductule terminals are distributed along the length of the proboscis, and exit variously into the cavity of the proboscis sheath, along the length of the proboscis lumen, and at the proboscis tip.

It is quite clear that each body is a single salivary gland cell. There is no acinar structure of any kind, nor any common duct: each cell has an individual ductule. These observations are strikingly confirmed by intracellular injection of the fluorescent dye molecule Lucifer Yellow CH into individual gland cell bodies (cf. Iwatsuki & Petersen, 1979). Fluorescence can be seen to be completely localized to the single cell body into which dye was injected (Fig. 3A), and this lack of dye-coupling excludes the possibility of cytoplasmic continuity between cells by means of the usual type of gap junction (Stewart, 1981). A single stained ductule may be followed along the length of the gland and into the proboscis in whole mount (Fig. 3B,C). Background illumination with white light allows the fluorescent process to be seen clearly among unstained ductules, and confirms there is no dye coupling where the ductules run in parallel at the proboscis base. In a recent histological investigation of these salivary cells, Sawyer, Damas & Tomic (1982) have made sections along the proboscis, and show that fluorescent dye is confined to single ductules at this level, and that there is no transport between adjacent processes.

Electrophysiology

Passive properties

Intracellular recording shows that cells in the isolated anterior gland are not spontaneously active, and a sample of 25 cells impaled with one microelectrode gives an average resting potential of −61 mV (S.D. 8 mV). A sample of 150 cells impaled with two microelectrodes over the course of many separate experiments gives an average

2A

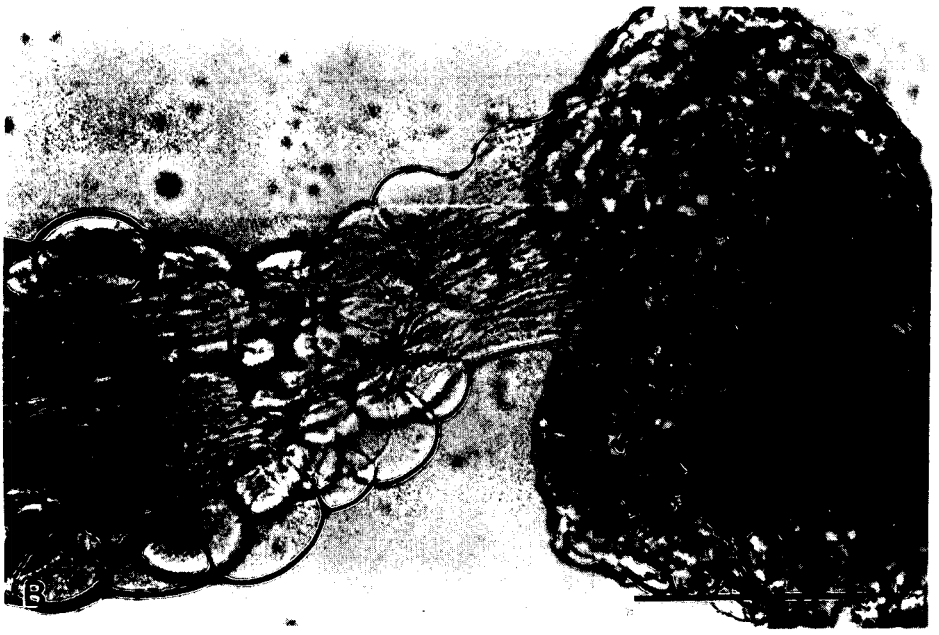

Fig. 2. Microscopical appearance of the unstained anterior salivary gland of *Haementeria*. (A) Living isolated gland prepared for electrophysiological recording. The distal end is on the left, and the proximal end attached to the proboscis is on the right. Apparent variation in density is due to varying thickness of the gland and the conditions of illumination. (B) Higher magnification view of the proximal end. Gland cell ductules are clearly seen grouped together entering the proboscis base. The connective tissue sheath investing the gland is visible between adjacent cells at the bottom left of the micrograph. Calibration bar is 1 mm.

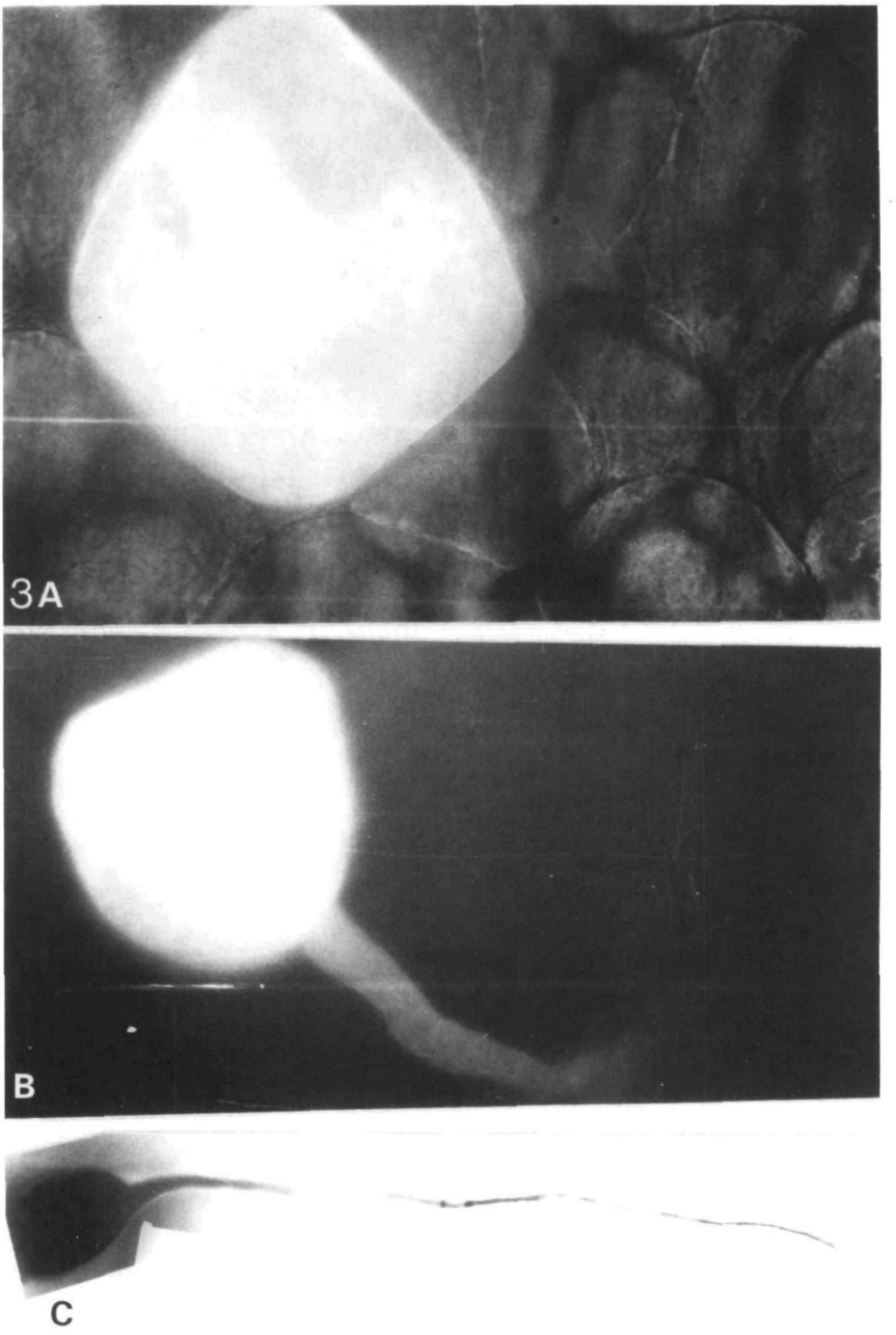

Fig. 3. Fluorescent micrographs of a single cell in the anterior salivary gland of *Haementeria* injected with the dye Lucifer Yellow CH. The cell body is approximately 400 μm in diameter. (A) Fluorescence is clearly localized to the injected cell body: adjacent cells show no sign of dye-coupling. (B) A single cell process (ductule) can be seen leaving the cell body. (C) The ductule can be traced along the length of the gland and into the proboscis. This micrograph was printed from a colour slide. The intense fluorescence around the cell body was masked to allow greater contrast.

resting potential of −50 mV (S.D. 11·6 mV). The more negative values tend to correspond to those impalements qualitatively judged to be better. Namely, a rapid negative-going deflection is obtained on the first attempt to impale the cell, a single action potential occurs, and the resting potential stabilizes within 1 min to a value which does not decay during the course of prolonged experimental manipulation.

At high external K^+ concentrations, the values for resting potential conform closely to the slope predicted by the Nernst equation for a potassium equilibrium potential (Fig. 4). There is a clear deviation from the predicted slope at physiological K^+ concentrations. Zero sodium solution does not alter either the resting potential or cell input resistance. The effect of varying external chloride has not been studied.

Cell input resistance varies with cell size: lower values for input resistance correlate with cells of larger diameter. We have found values of 5–16 MΩ corresponding to cell diameters in the range 400 to 80 μm. There is no correlation between input resistance and membrane potential, besides the observation that low input resistances of less than 1 MΩ are measured in cells where the resting potential has decayed to a low

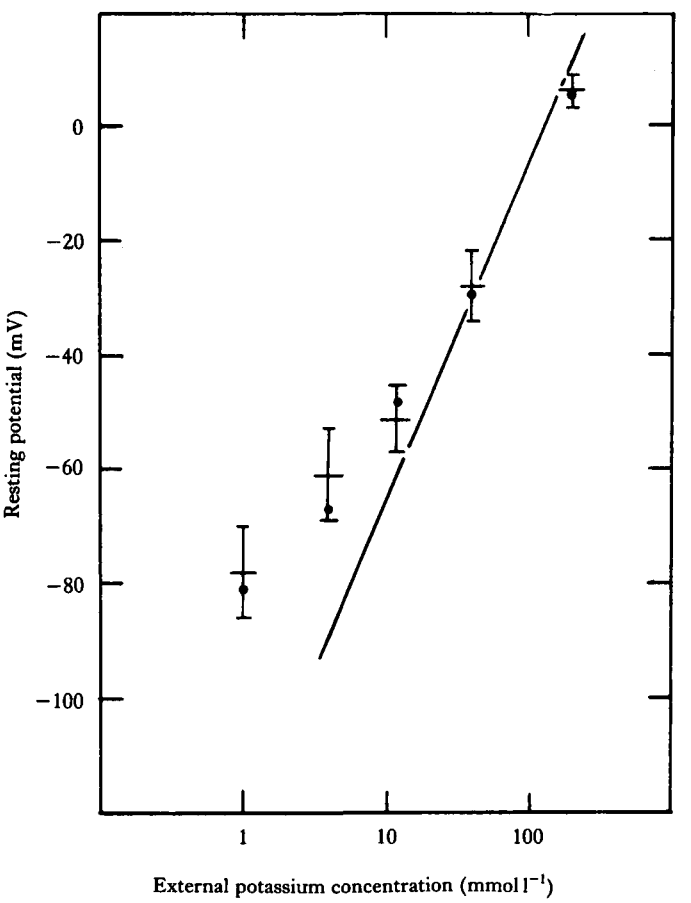

Fig. 4. Variation of gland cell resting membrane potential with external potassium ion concentration. Horizontal bars represent sample mean and vertical bars standard deviation. Filled circles represent values from a single cell which was impaled and held while external potassium concentration was varied. Solid line indicates best fit of the Nernst equation for a potassium electrode.

value, both resulting from the cell membrane being made leaky around the site of impalement.

Calculations based on a spherical cell body of diameter 300 μm and input resistance 6 MΩ yield an area-specific membrane resistance, Rm, of 17 kΩcm^2. Since this figure neglects the cell process and is a conservative estimate of cell surface area, it is an underestimate of Rm. Area-specific membrane capacitance, Cm, is generally regarded as a constant in animal cell membranes, having a value of approximately 1 μF cm^{-2}. This predicts a membrane time constant of 17 ms, whereas a measured value of 250 ms is obtained. Although this discrepancy might largely be accounted for

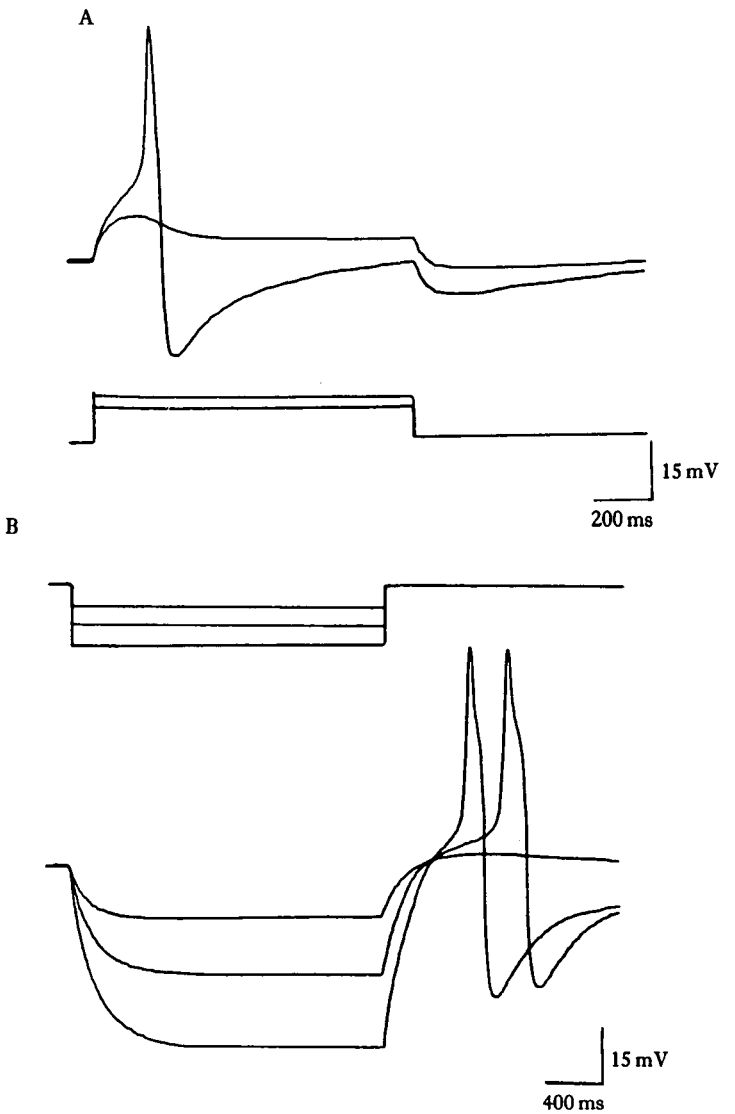

Fig. 5. Action potentials in leech giant salivary cells. Current was passed *via* a second electrode inserted into the cell. (A) Resting potential −49 mV, depolarizing current pulses of 0·6 and 0·8 nA. The peak overshoot is +24 mV and peak undershoot −74 mV. (B) Resting potential −42 mV, hyperpolarizing current pulses of 2, 4 and 7 nA.

by the underestimate of Rm, it is likely that the presence of the ductule influences the time course of membrane charging.

Recording from many pairs of adjacent and distant salivary cells clearly shows that they are not electrically coupled, that is, passing current pulses into one cell has no effect on the membrane potential of any other cell, consistent with the preceding anatomical observations.

Active properties

We noted that the salivary cells often fire a single action potential in response to the depolarization resulting from impalement, but are subsequently silent. Depolarizing current pulses also elicit overshooting all-or-none action potentials, which last 100–200 ms (Fig. 5A). The maximum peak-to-peak amplitude is 100 mV, and the maximum rate of rise 5 V s^{-1}. The mean overshoot from 30 cells impaled with two microelectrodes is +17 mV, and both the rate of rise and the overshoot decrease as a cell becomes depolarized. Repolarization clearly occurs in two phases, followed by a pronounced after-hyperpolarization of long duration. Subthreshold current pulses can produce a voltage response which decreases with time, which is indicative of some form of delayed rectification (Hodgkin, Huxley & Katz, 1952).

Action potentials may also be elicited at the end of a hyperpolarizing pulse, so-called

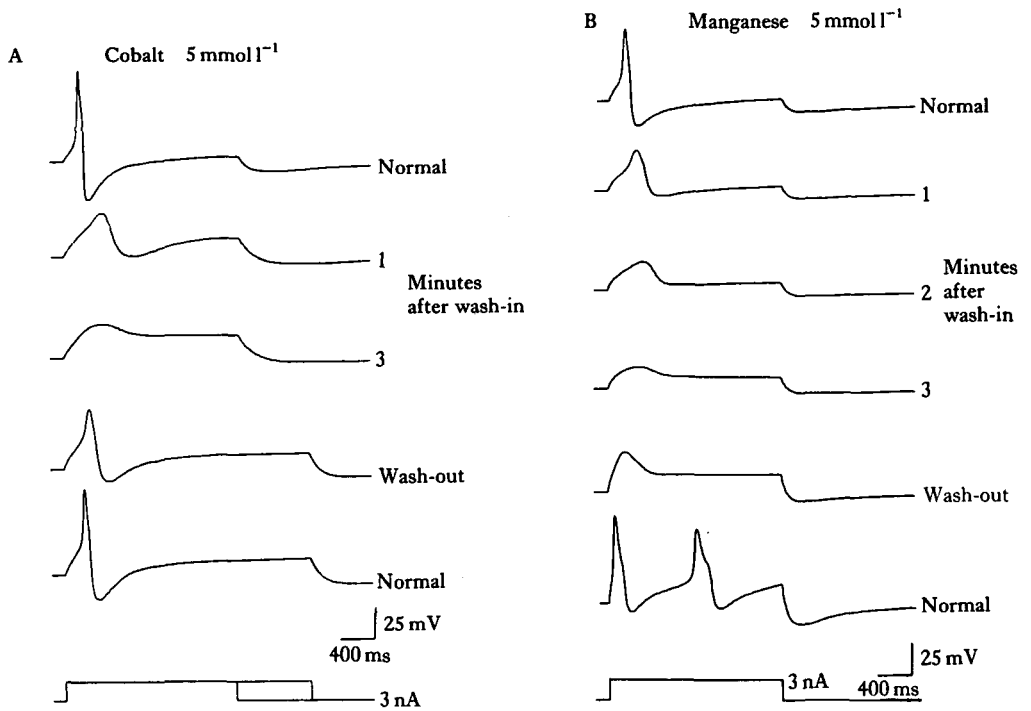

Fig. 6. (A) The effect of 5 mmol l^{-1} cobalt. Resting potential −47 mV, overshoot +21 mV, undershoot −75 mV (top trace only). (B) The effect of 5 mmol l^{-1} manganese. Resting potential −42 mV, overshoot +12 mV, undershoot −63 mV (top trace only). A and B from different cells. External calcium was maintained at 2 mmol l^{-1}. Successive traces represent control, and 1, 2 (B only) and 3 min after wash-in, and 3 and 15 min after wash-out. The cells were stimulated with 3 nA depolarizing current pulses in all cases.

anode break excitation (Fig. 5B). An identical impulse is then seen, showing overshoot, shoulder on the repolarizing phase, and after hyperpolarization. This type of excitation has been rationalized on the basis of removal of voltage-sensitive sodium inactivation by the hyperpolarizing pulse in squid axon (Hodgkin & Huxley, 1952*b*), and may indicate the presence of voltage-dependent inactivation of calcium channels in the salivary cell membrane. However, measurement of ionic currents, such as Fox (1981) has demonstrated in eggs of the polychaete *Neanthes*, is clearly required before a definite statement can be made. No exogenous current is being applied during the impulse, and thus anode break is a convenient method of evoking action potentials when dual microelectrode impalement proves difficult, or when the voltage artefact caused by passing depolarizing current down a single recording electrode is unacceptably large.

Ionic basis of the action potential

The overshooting action potential is abolished when either 5 mmol l^{-1} cobalt or 5 mmol l^{-1} manganese is added to the Ringer's solution (Fig. 6). As these solutions are washed in, the overshoot is reduced and the action potential is prolonged. A delayed rectification is seen when depolarizing current is applied once full blockade is achieved. Neither increased depolarizing current nor large hyperpolarizing currents result in a regenerative response under these conditions. The effect is completely reversible, and the same prolonged impulse of reduced amplitude may be seen before full recovery in normal Ringer's. Lanthanum (La^{3+}) is similarly effective in blocking the action potential at a lower concentration of 1 mmol l^{-1}; however, its action is not reversible.

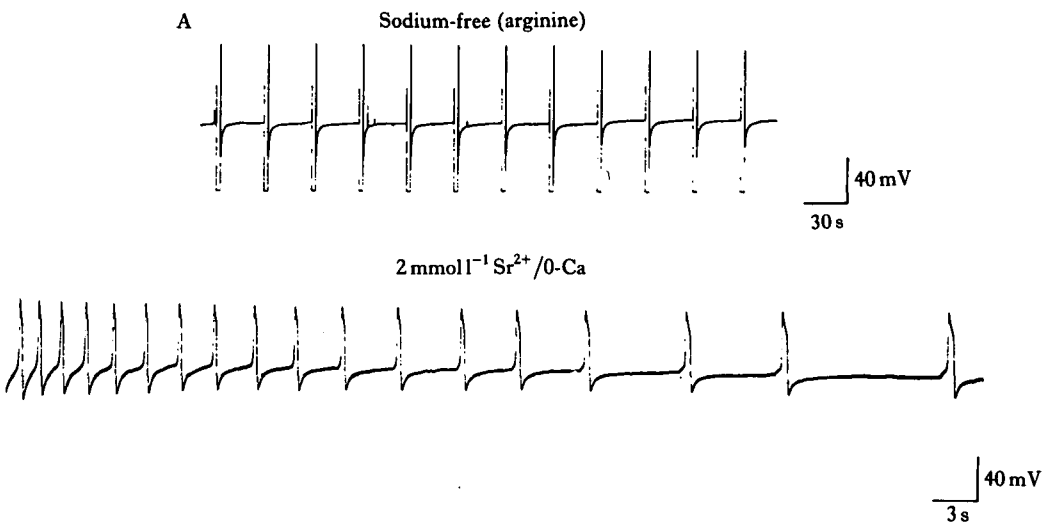

Fig. 7. (A) Cell impaled with a single microelectrode. Resting potential −50 mV. The cell was hyperpolarized with a 10 nA pulse of 2 s every 30 s. The action potential persists when external sodium is completely replaced with isosmolar arginine. Wash-in of sodium-free Ringer's solution began 1 min before the first anode-break impulse shown. The bath volume is exchanged in approximately 45 s. (B) Strontium-supported activity in response to a brief anodal current given before the first recorded impulse. 2 mmol l^{-1} strontium replaced 2 mmol l^{-1} calcium in the Ringer's solution. A and B are from different cells. Both were recorded on an ink-writing oscillograph.

Fig. 8. The amplitude of the gland cell action potential is dependent on external calcium. Cell resting potential is −63 mV. An increase in overshoot of 24 mV, from +24 mV to +48 mV, resulted when calcium was raised from 2 to 20 mmol l^{-1}. External sodium was reduced by 27 mmol l^{-1} to maintain constant osmolarity.

There is no change in either the rate of rise or the overshoot when a preparation is exposed to sodium-free (arginine) Ringer's solution (Fig. 7A). These impulse properties are not affected by tetrodotoxin (TTX) at a concentration of 2×10^{-5} mol l^{-1}. These data provide substantive evidence that sodium current is not required for regenerative activity in these cells.

Action potentials persist when the external calcium is replaced by either strontium or barium (Fig. 7B). There is some prolongation of the action potential, and excitability is increased. Increased excitability as a result of lowering external calcium is well documented (e.g. Junge, 1981). This may be a result of a shift in the voltage sensitivity of inward current due to decreased screening of negative surface charges in zero-calcium solution. It may also be explained by a decreased calcium-sensitive potassium conductance, since there is no longer calcium influx during the action potential. Trains of impulses are seen in response to a single brief stimulus, whereas only a single action potential is elicited in normal solution.

When 2 mmol l^{-1} barium is added to normal Ringer's (containing 2 mmol l^{-1} calcium) a prolongation of the impulse is also seen, but trains of impulses are not. The ability of barium to prolong the action potential as well as substituting for calcium as a current carrier has been observed in many preparations, and has been related to a blocking action on potassium channels (Sperelakis, Schneider & Harris, 1967).

Given that calcium is the current carrier in the salivary cell action potential, it is predicted that the overshoot should vary with external calcium concentration, and that removal of calcium in normal sodium will abolish the impulse. Fig. 8 shows that the overshoot increases when external calcium is increased from 2 to 20 mmol l^{-1}, by

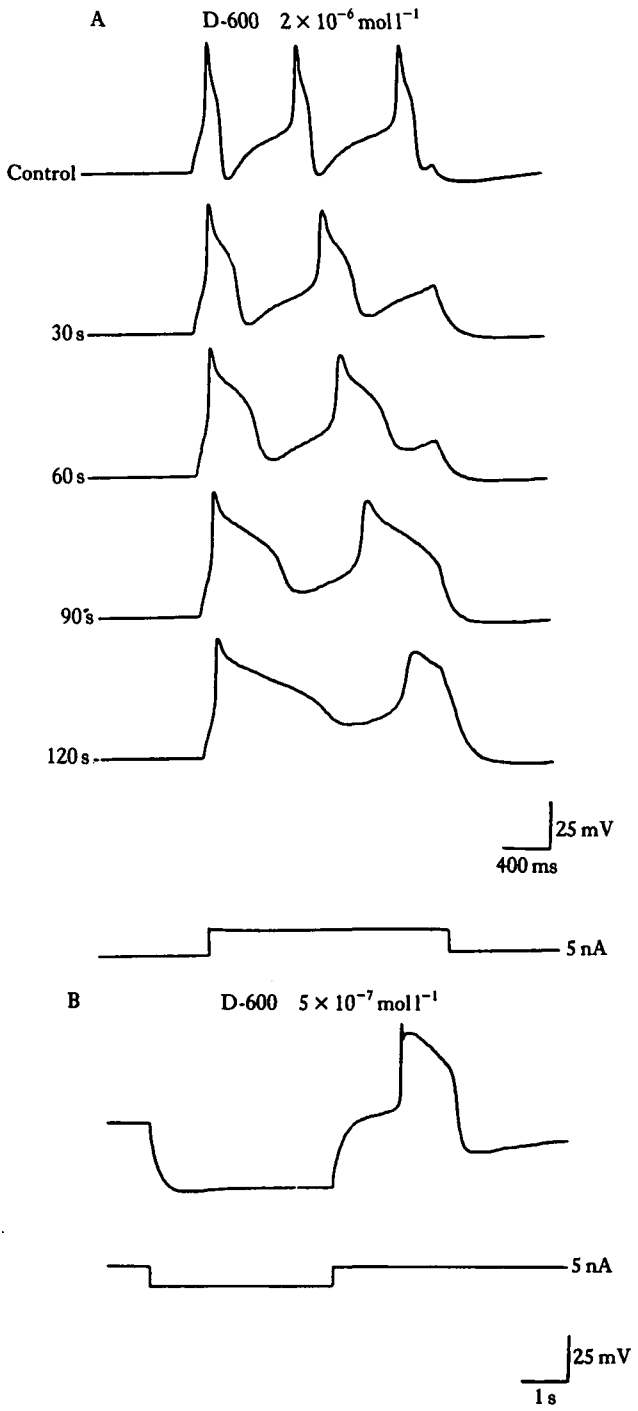

Fig. 9. The effect of D-600 (methoxyverapamil) on the gland cell action potential. (A) 2×10^{-6} mol l^{-1} D-600. Resting potential −54 mV. Successive traces show control, and impulses evoked at 30-s intervals after wash-in had begun. The impulse is rapidly prolonged, and blocked after 5 min. (B) 5×10^{-7} mol l^{-1} D-600. Resting potential −46 mV. Anode break excitation is an effective stimulus, and results in a prolonged impulse. Current is 5 nA in both traces. A and B are from cells in different preparations.

an amount which averages 25 mV/log concentration change. Furthermore, no active response can be elicited in zero-calcium solution. Studying the effect of reduced calcium is hampered by the tendency of the proboscis musculature to then undergo spontaneous contractions and dislodge the electrodes.

D-600 (methoxyverapamil) acts as a calcium channel antagonist in a variety of tissues. When 2×10^{-6} mol l^{-1} D-600 (Gallopamil-HCl, Knoll AG) is washed into the bath, a progressive prolongation of the action potential is seen, (Fig. 9A), although the time course and peak value of the upstroke are unchanged, as is the resting potential. Five minutes after wash-in, a depolarizing pulse no longer evokes an overshooting response, nor is anode-break excitation effective. However, several seconds of large hyperpolarizing current followed by the same depolarizing current evokes a single impulse with a prolonged repolarizing phase. The lower concentration of 5×10^{-7} mol l^{-1} D-600 also prolongs, then abolishes, the impulse in response to depolarizing pulses, but anode-break excitation now results in a prolonged impulse (Fig. 9B). The phases of repolarization are accentuated, and there is an obvious 'rebound' after the initial fast phase. An interaction between membrane potential, action potential frequency and the Ca^{2+} current blocking properties of the organic calcium antagonists is well documented (Triggle, 1981). Our data suggest that the antagonism may be partially relieved by hyperpolarization of the membrane: we have not studied frequency dependence of D-600 effects.

External application of 10 mmol l^{-1} tetraethylammonium (TEA) reversibly prolongs the action potential (Fig. 10). The slower phase of repolarization is delayed, and impulses of 8–10 s duration are seen. TEA always inhibits calcium-induced potassium fluxes when applied at the inner membrane surface, but also in some cases (skeletal muscle, molluscan neurones) when applied to the outer surface (Schwarz & Passow, 1983). TEA is also known to block voltage-sensitive potassium channels, and so this prolongation of the impulse does not allow us to draw a firm conclusion about the type of K^+ channels present. The pronounced after hyperpolarization is markedly reduced in the presence of TEA, suggesting that this undershoot is due to elevated potassium permeability.

DISCUSSION

Exocrine tissues in a wide range of species are composed of cells which are in cytoplasmic continuity *via* gap junctions, detected by electrical or dye coupling between adjacent groups of cells (Petersen, 1980). Many exocrine glands are inexcitable, in that they do not produce any kind of action potential, and these are exemplified by mammalian salivary and lacrimal glands, exocrine pancreas and insect salivary glands (Petersen, 1976; House, 1980). However, electrical excitability has been demonstrated in mammalian endocrine gland cells, and in the salivary glands of nine genera of gastropod molluscs, whose action potentials propagate from cell to cell along the glandular epithelium (Kater, Rued & Murphy, 1978). Like previously studied exocrine glands, these molluscan salivary glands are composed of cells which are electrically inter-coupled *via* low-resistance junctions.

The leech salivary gland described in this report is novel, for it is composed of unicellular, exocrine gland cells which are excitable. The tissue is properly termed a

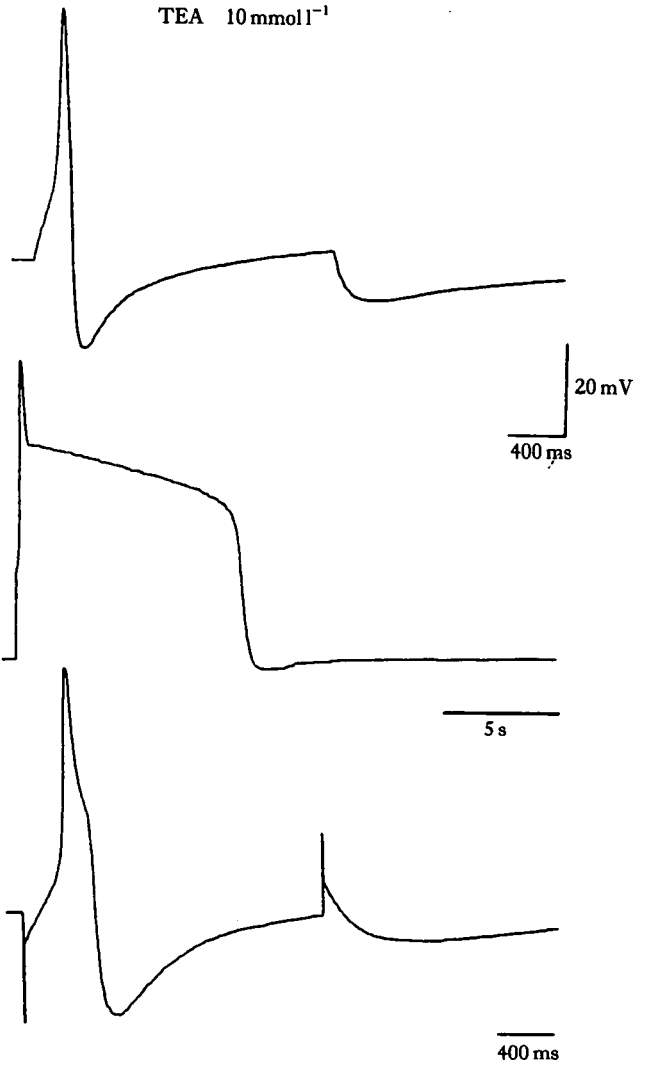

Fig. 10. Action potential prolongation by 10 mmol l^{-1} tetraethylammonium (TEA). Cell resting potential is −49 mV, peak overshoot is +10 mV, peak undershoot is −67 mV. A brief depolarizing current pulse is now effective, and the rising phase of the impulse continues after the pulse. Note change in time calibration of centre trace: the prolonged impulse is approximately 10 s in duration. The lower trace, recorded after return to normal Ringer's solution, was obtained by injecting current down the recording electrode *via* a bridge circuit, which is slightly overbalanced.

salivary gland; cells release their contents into the anterior portion of the digestive tract, and electrophoretic analysis has shown that gland protein extracts contain anticoagulant and fibrinolytic activity, which is correlated with the animal's blood diet (Budzynski *et al.* 1981*a*; Budzynski, Olexa & Sawyer, 1981*b*). The cells cannot be considered neurosecretory, for their site of release is not associated with either neural or haemal elements.

The cortical arrangement of the salivary cell bodies and medullary location of the ductules has led Sawyer *et al.* (1982) to comment that the anterior gland is 'closely

reminiscent of a (leech) central nervous system'. Despite our finding of electrical excitability, this seems not to be a useful analogy, for the cells are clearly not neuronal, and the medullary processes are not part of an integrative neuropile, but are totally independent of one another. On the basis of histology and histochemistry, they concluded that the anterior gland was composed of sub-populations of both serous and mucous cells. They do not indicate whether there is any stereotyped arrangement of the cell bodies of a particular class when the whole gland is viewed. Our studies have not revealed any sub-populations on the basis of electrophysiology.

The importance of calcium in secretion is by now well known (Douglas, 1968, 1974; Rubin, 1974, 1982), and it is generally agreed that an increase in cytosolic calcium ion concentration leads to secretion by exocytosis. Similarly, calcium has been documented as a current carrier in excitable cells (Reuter, 1973; Hagiwara & Byerly, 1981; Tsien, 1983), and so it is not surprising to find a calcium component to the action potential of a secretory cell. Several properties of leech giant salivary cells make them unusually amenable to electrophysiological studies, and suggest that the preparation will prove useful for investigation of both the properties of ion channels and the process of secretion.

Firstly, the salivary cells are exceptionally large, many over 200 μm in diameter, and the largest exceeding 1 mm in larger animals. Secondly, the cell bodies are relatively stable within the gland, and it is routinely possible to impale them with one or two microelectrodes under visual control. Thirdly, the absence of coupling in leech giant salivary cells enables them to be studied singly *in situ*. In other glands, where electrical coupling is present, recordings from one cell are affected by the activity of communicating neighbouring cells. This problem led Goldring, Kater & Kater (1983) to develop enzymatic dissociation techniques to study salivary gland cells from the terrestrial slug *Ariolimax*, the largest of which exceed 100 μm. Fourthly, the results show that we have been able to satisfy most of the tests suggested by Hagiwara & Byerly (1981) to identify calcium-dependent action potentials. Namely, the overshoot varies with external calcium, and does not change in the absence of sodium. The impulse is blocked by polyvalent cations, is unaffected by high concentrations of TTX, and persists when barium or strontium substitute for calcium.

The prolongation of the action potential both by procedures which are known to interfere with calcium currents, and by TEA, suggests that there may be a calcium-sensitive potassium conductance mechanism in the salivary cell membrane. Thus, reduced inward calcium current would delay the activation of a repolarizing potassium current. However, prolongation might also result from reduced inactivation of calcium channels whose inactivation is sensitive to intracellular calcium levels. The presence of sodium channels in the membrane has not been excluded, although the low rate of rise of the action potential and lack of change in overshoot when sodium is removed indicate that any inward sodium current is of low density. Successful application of a voltage clamp technique to allow measurement of ionic currents could determine if any sodium currents were detectable. If, as our present results suggest, sodium currents are absent, these cells might provide a preparation for studying both calcium channels and cellular secretion.

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REFERENCES

BUDZYNSKI, A. Z., OLEXA, S. A., BRIZUELA, B. S., SAWYER, R. T. & STENT, G. S. (1981*a*). Anticoagulant and fibrinolytic properties of salivary proteins from the leech *Haementeria ghilianii*. *Proc. Soc. exp. Biol. Med.* **168**, 266–275.

BUDZYNSKI, A. Z., OLEXA, S. A. & SAWYER, R. T. (1981*b*). Composition of salivary gland extracts from the leech, *Haementaria ghilianii*. *Proc. Soc. exp. Biol. Med.* **168**, 259–265.

BULLOCK, T. H. & HAGIWARA, S. (1957). Intracellular recording from the giant synapse of the squid. *J. gen. Physiol.* **20**, 565–577.

DAMAS, D. (1962). Histologie, cytologie, histochimie du tube digestif de *Glossiphonia complanata* L. *Archs Zool. exp. gén.* **101**, 41–72.

DOUGLAS, W. W. (1968). Stimulus-secretion coupling. The concept, and clues from chromaffin and other cells. *Br. J. Pharmac.* **34**, 451–474.

DOUGLAS, W. W. (1974). Involvement of calcium in exocytosis and the exocytosis-vesiculation sequence. *Biochem. Soc. Symp.* **39**, 1–28.

FOX, A. P. (1981). Voltage dependent inactivation of a calcium channel. *Proc. natn. Acad. Sci. U.S.A.* **78**, 953–956.

GINSBORG, B. L. & HOUSE, C. R. (1980). Stimulus – response coupling in gland cells. *A. Rev. Biophys. Bioeng.* **9**, 55–80.

GOLDRING, J. W., KATER, J. W. & KATER, S. B. (1983). Electrophysiological and morphological identification of action potential generating secretory cell types isolated from the salivary gland of *Ariolimax*. *J. exp. Biol.* **102**, 13–23.

HAGIWARA, S. & BYERLY, L. (1981). Calcium channel. *A. Rev. Neurosci.* **4**, 69–125.

HAGIWARA, S. & NAKA, K. (1964). The initiation of spike potential in barnacle muscle fibers under low intracellular Ca^{++}. *J. gen. Physiol.* **48**, 141–162.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol., Lond.* **116**, 449–472.

HODGKIN, A. L. & HUXLEY, A. F. (1952*b*). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol., Lond.* **117**, 500–544.

HODGKIN, A. L., HUXLEY, A. F. & KATZ, B. (1952). Measurement of current voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol., Lond.* **116**, 424–448.

HOUSE, C. R. (1980). Physiology of invertebrate salivary glands. *Biol. Rev.* **55**, 417–473.

IWATSUKI, N. & PETERSEN, O. H. (1979). Direct visualization of cell to cell coupling: transfer of fluorescent probes in living mammalian pancreatic acini. *Pflügers Arch. ges. Physiol.* **380**, 277–281.

JUNGE, D. (1981). *Nerve and Muscle Excitation*, Second Edition, Chapter 8. Sunderland, Massachusetts: Sinauer Associates, Inc.

KATER, S. B. (1977). Calcium electroresponsiveness and its relationship to secretion in molluscan exocrine gland cells. *Neurosci. Symposia*. **II**, 195–214.

KATER, S. B., RUED, J. R. & MURPHY, A. D. (1978). Propagation of action potentials through electrotonic junctions in the salivary glands of the pulmonate mollusc, *Helisoma trivolvis*. *J. exp. Biol.* **72**, 77–90.

PETERSEN, O. H. (1976). Electrophysiology of mammalian gland cells. *Physiol. Rev.* **56**, 535–577.

PETERSEN, O. H. (1980). *The Electrophysiology of Gland Cells*. Monographs of the Physiological Society, No. 36. London: Academic Press.

REUTER, H. (1973). Divalent cations as charge carriers in excitable membranes. *Prog. Biophys. mol. Biol.* **26**, 1–43.

RUBIN, R. P. (1974). *Calcium and the Secretory Process*. New York: Plenum Press.

RUBIN, R. P. (1982). *Calcium and Cellular Secretion*. New York: Plenum Press.

SAWYER, R. T., DAMAS, D. & TOMIC, M. T. (1982). Anatomy and histochemistry of the salivary complex of the giant leech *Haementeria ghilianii* (Hirudinea: Rhyncobdellida). *Archs Zool. exp. gén.* **122**, 411–425.

SCHWARZ, W. & PASSOW, H. (1983). Ca^{++}-activated K^+ channels in erythrocytes and excitable cells. *A. Rev. Physiol.* **45**, 359–374.

SPERELAKIS, N., SCHNEIDER, M. F. & HARRIS, E. J. (1967). Decreased K^+ conductance produced by Ba^{++} in frog sartorius fibers. *J. gen. Physiol.* **50**, 1565–1583.

STEWART, W. W. (1981). Lucifer dyes – highly fluorescent dyes for biological tracing. *Nature, Lond.* **292**, 17–21.

TRIGGLE, D. J. (1981). Calcium antagonists: basic chemical and pharmacological aspects. In *New Perspectives on Calcium Antagonists*, (ed. George B. Weiss). Bethesda, Maryland: American Physiological Society.

TSIEN, R. W. (1983). Calcium channels in excitable cell membranes. *A. Rev. Physiol.* **45**, 341–358.