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IONIC BASES OF RESTING AND ACTION POTENTIALS IN SALIVARY GLAND ACINAR CELLS OF THE SNAIL *HELISOMA*

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SUMMARY

Values for resting and action potentials of *Helisoma* salivary gland cells are much the same as in most neurones and muscle cells. The resting potential is primarily due to the distribution of potassium ions across the membrane, with a small contribution by an electrogenic sodium pump. Estimated values for intracellular potassium concentration and the relative membrane permeabilities to sodium and potassium ions correspond to similar estimates in other excitable tissues. The inward current of the salivary gland action potential is carried predominantly by calcium ions and possibly serves as a mechanism of calcium entry for stimulus-secretion coupling.

INTRODUCTION

The ionic bases of secretory processes have been investigated in all of the major classes of secretory tissues including neural (e.g. Katz, 1969; Llinas, Blinks & Nicholson, 1972), endocrine (e.g. Douglas, 1975), and exocrine cell types (e.g. Peterson, 1976; Peterson & Ueda, 1976; Kanno & Nishimura, 1976). Until recently regenerative transient voltage changes have been associated primarily with neurones and muscle cells. However, careful studies of pancreatic islet cells (Matthews & Sakamoto, 1975), adrenocortical cells (Matthews & Saffran, 1973) and chromaffin cells (Brandt *et al.* 1976) have revealed action potentials in these endocrine cell types. The continuity of the general mechanism of transient regenerative responses underlying secretion (Douglas, 1968) in widely disparate cell types has been extended further by the discovery of action potentials in exocrine glands of various molluscs including salivary glands (Kater, Rued & Murphy, 1978*b*) and mucous secreting pedal glands (Kater, 1977).

The salivary gland neuroeffector system of *Helisoma* has been previously characterized in detail. The secretory acinar cells of the salivary gland are extensively electrically coupled (Kater *et al.* 1978*b*). They receive excitatory input from identified neurones in the buccal ganglia, resulting in excitatory postsynaptic potentials (EPSP's) which can give rise to overshooting action potentials (Kater, Murphy & Rued, 1978*a*). Salivary gland cells also serve as the postsynaptic element for studies of

specific regeneration of the buccal ganglion secretoeffector neurones (Murphy & Kater, 1978, 1979). The present study examines the ionic bases of resting and action potentials in the acinar secretory cells of the salivary glands of *Helisoma*.

MATERIALS AND METHODS

Animals were obtained from our laboratory stocks of *Helisoma trivolvis* (Red_1) which were maintained at 18–20 °C. Dissection procedures were as described by Kater & Kaneko (1972) and Kater *et al.* (1978*a*). Salivary glands were pinned to a Sylgard (Dow-Corning) pad by muscle fragments attached to the salivary ducts. The Sylgard pad was placed in a Lucite chamber (volume: ~ 0·5 ml) through which there was a continuous flow of saline (Thomas, 1972). Salines were delivered by gravity flow through a rotary tap (Partridge & Thomas, 1974) and were removed by aspiration. Complete saline changes could be accomplished in approximately 5 s while maintaining stable intracellular recordings from secretory cells (see Fig. 1).

Helisoma normal saline consisted of (mM): NaCl, 51·3; KCl, 1·7; $CaCl_2$, 4·1; $MgCl_2$, 1·5; Tris Cl, 5·0 (pH 7·3). Concentrations of K were varied by substituting for Na. In low Ca salines, Mg substituted for Ca. Zero Na salines employed either Tris HCl or sucrose as a substitute for NaCl. Ouabain (1 mM Strophanthin G; Sigma) was employed as a specific blocker of the Na/K exchange pump. Tetrodotoxin (10 μM; Sigma) was used to block voltage dependent Na ion channels. Cobaltous ions (10 mM-$CoCl_2$) or manganous ions (10 mM-$MnCl_2$) were used to block Ca ion channels.

Conventional electrophysiological recording, stimulation and display techniques were used. Glass, fibre-filled microelectrodes filled with 4 M potassium acetate (20–50 MΩ resistance) were connected via a Ag/AgCl wire to a high input impedance, unity gain amplifier equipped with a bridge circuit for current injection. The reference electrode was a Ag/AgCl wire at virtual ground and was connected to the recording chamber through an agar bridge. Signals were displayed and recorded either from the face of a storage oscilloscope or from a Brush 220 chart recorder modified for a chart speed of 5 mm/min.

For the estimation of $[K]_{in}$ and P_{Na}/P_K, Moreton's (1968) modification of the Goldman equation (Goldman, 1943; Hodgkin & Katz, 1949) was used.

$$e^{VF/RT} = \frac{[K]_{out}}{[K]_{in}} + \frac{P_{Na}[Na]_{out}}{P_K[K]_{in}}. \qquad (1)$$

In equation (1) e = base of Napierian logarithms, V = membrane resting potential, F = Faraday's constant, R = universal gas constant, T = absolute temperature, P_{Na} and P_K = permeabilities of Na ions and K ions, respectively.

This equation specifies a linear relationship between $e^{VF/RT}$ and $[K]_{out}$ – providing that certain assumptions hold (see Moreton, 1968; Gorman & Marmor, 1970*a*). From a plot of $e^{VF/RT}$ *v.* $[K]_{out}$ one can estimate $[K]_{in}$ (slope = $1/[K]_{in}$) and the ratio of P_{Na} to P_K (intercept = $P_{Na}/P_K([Na]_{out}/[K]_{in})$).

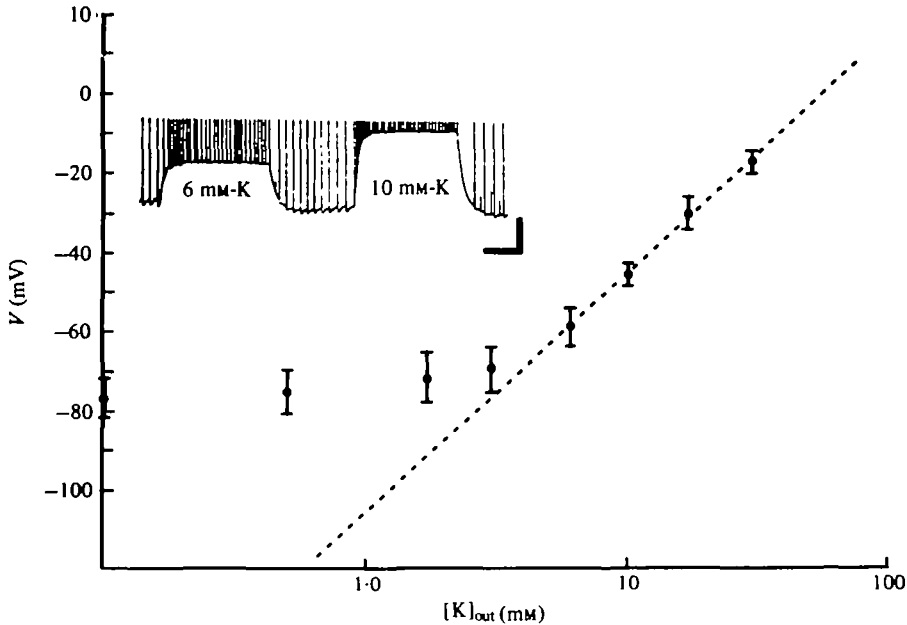

Fig. 1. The potassium dependence of the resting potential in salivary gland secretory cells of *Helisoma*. The resting potential (ordinate) is plotted as a function of the log of the external potassium concentration ($[K]_{out}$). Each point represents the mean (±S.D., n = 9) from observations on nine animals. The dashed line shows the slope predicted by the Nernst equation. Each series of K changes was performed during continuous recording from a single acinar cell. The inset shows part of a representative series (action potentials clipped) starting at resting potential in normal saline (1·7 mM-K) changed to 6 mM-K and back, then changed to 10 mM-K and back. Calibrations for inset are 5 min and 10 mV.

RESULTS

Resting potential

Upon impalement, stable resting potentials were reached after 3–4 min of recording, or else the recording was rejected. Recordings could be maintained from impaled cells for as long as 2 h during saline changes. Electrical activity included action potentials, EPSP's and miniature excitatory potentials (Kater *et al.* 1978*b*; Kater *et al.* 1978*a*). This type of activity persisted throughout experiments (see inset, Fig. 1) and the activity of a given cell after a set of experimental saline changes closely resembled activity before altering the saline.

Nernst predictions. The mean resting potential of the salivary gland cells from the nine different animals in Fig. 1, in normal saline, was −71·9 mV (S.D. = 6·1; n = 9). When the external K concentration was changed there was an immediate shift in membrane potential and a new apparent steady-state membrane potential was reached in 3–4 min (see inset of Fig. 1). Membrane potential as a function of external K concentration ($[K]_{out}$) is presented in Fig. 1. Each cell represented was subjected to a complete series of K changes from 0 mM-K to 30 mM-K and returned to normal saline between test salines (inset). At higher values of $[K]_{out}$ the function closely approximates the slope of 58 mV/decade predicted by the Nernst equation for a pure K electrode at 18 °C (dashed line). At lower values of $[K]_{out}$ the function deviates such that the membrane potential is smaller than predicted. This function is remark-

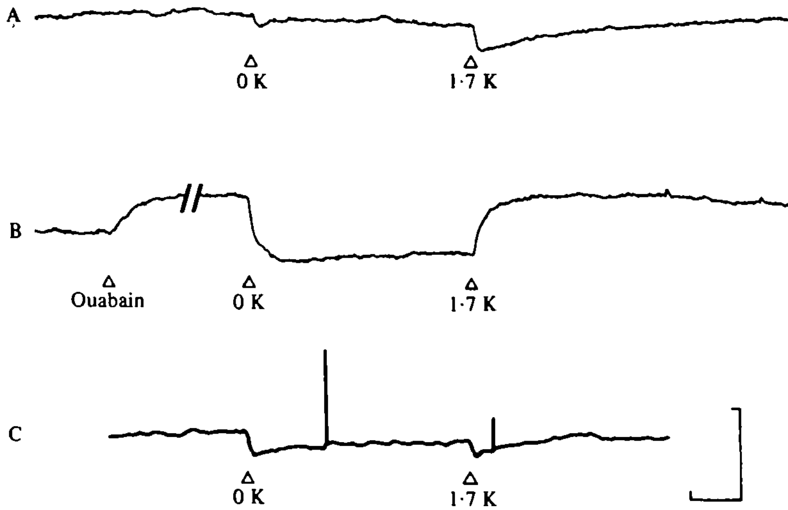

Fig. 2. The effects of active sodium pumping on the resting potential. (A) In normal saline solution, removal of potassium (0 K) results in a small transient hyperpolarization with another transient, larger hyperpolarization upon reintroduction of normal saline (1·7 K). The magnitude and time course of this second membrane response is somewhat variable but always occurs. (B) Addition of 1 mM ouabain (Strophanthin G) in normal saline solution results in a depolarizing shift of approximately 3 mV which remains steady (9·0 min break in record). Removal of K in the presence of ouabain results in a steady hyperpolarization, while reintroduction to 1·7 mM-K results in a repolarization with no transient hyperpolarizing response. (C) After removal of ouabain and washing in 1·7 mM-K saline for 10 min the sodium pump is again functioning as in (A). Calibrations are 30 s and 10 mV.

ably similar to the ones derived for neurones, including snail neurones (e.g. Kerkut & Meech, 1967). The deviation at lower $[K]_{out}$ might be due to membrane permeability to ions other than K ions as predicted by the Goldman equation (Hodgkin & Katz, 1949). However, application of Moreton's (1968) modification of the Goldman equation does not produce a linear relationship between $e^{VF/RT}$ and $[K]_{out}$ (Fig. 3). This deviation suggests the possibility of a contribution of metabolic pumps to the resting potential.

Presence of Na pump. The relationship of the membrane potential to low K concentrations deviates from what is expected from purely Nernst equation considerations in two respects (Fig. 2A). (1) A *transient* hyperpolarization is observed upon changing to 0 mM-K saline rather than a steady hyperpolarization predicted by the Nernst equation. (2) A transient hyperpolarization (usually 3–7 mV) is observed upon reintroduction of normal $[K]_{out}$. The Nernst equation predicts a steady depolarizing response to increased $[K]_{out}$. Such behaviour could be due to a reactivation of an electrogenic Na/K exchange pump after inhibition by removal of external K ions (Kerkut & Thomas, 1965; Gorman & Marmor, 1970*a*; Merickel & Kater, 1974).

Several series of K changes were performed in the presence of ouabain – a specific inhibitor of the Na/K exchange pump. Application of 1 mM ouabain in normal saline produces a depolarization of approximately 3 mV which reaches a steady state within 1 min. In addition, ouabain completely abolishes the hyperpolarizing response to reintroduction of external K ions (Fig. 2B). The effect of ouabain on the hyperpolarizing response is reversed after 10 min of washing the preparation in ouabain-free saline (Fig. 2C).

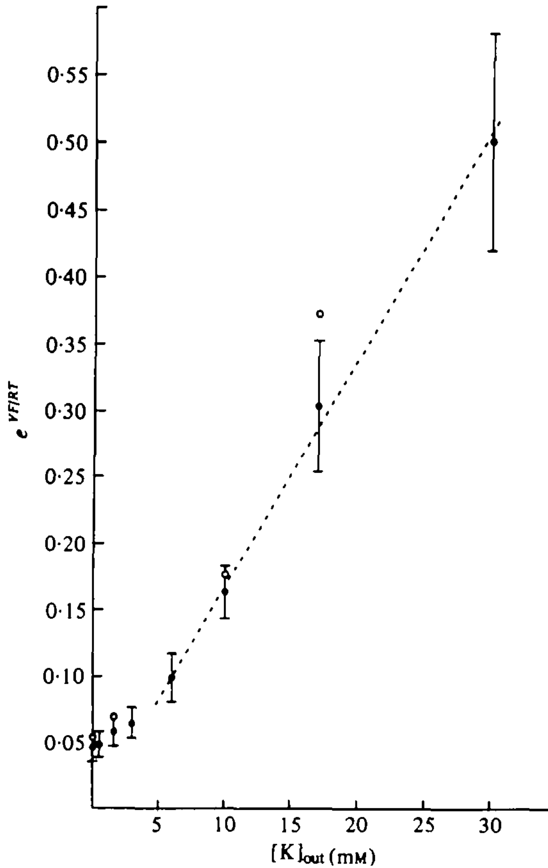

Fig. 3. Membrane potential, expressed as $e^{VF/RT}$, as a function of $[K]_{out}$. Data from Fig. 1 (solid circles with error bars) are compared with the mean values for three K changes in the presence of 1 mM ouabain (open circles). The nonlinearity of the function indicates a deviation from behaviour predicted by the Goldman equation. Inhibition of the sodium pump failed to produce a linear function. The slope of the linear portion of the function in the absence of ouabain (dashed line), $1/[K]_{in}$, indicates an internal potassium concentration of 64 mM and the intercept ($0{\cdot}047 = P_{Na}[Na]_{out}/P_K[K]_{in}$) provides the estimate of relative permeabilities of Na and K to be $P_{Na}/P_K = 0{\cdot}04$.

Estimation of $[K]_{in}$ *and* P_{Na}/P_K. When the data from Fig. 1 are replotted in the form $e^{VF/RT}$ *v.* $[K]_{out}$ (Fig. 3), it is possible to derive additional quantitative information on the ionic basis of the resting potential. (1) The slope of the linear portion of the plot is $1/[K]_{in}$ (Moreton, 1968). (2) The intercept of the plot of $e^{VF/RT}$ *v.* $[K]_{out}$ is (P_{Na}/P_K) $([Na]_{out}/[K]_{in})$. Regression analysis of the function at the higher $[K]_{out}$ values (6 mM and greater, dashed line, Fig. 3) yields an estimated linear slope of 1/64, indicating an internal K concentration of 64 mM. The intercept provides an estimate of the relative permeabilities of Na and K ions. Using the derived estimate for $[K]_{in}$ and knowing $[Na]_{out}$, the ratio $P_{Na}/P_K = 0{\cdot}04$. Under conditions which inhibit the Na/K exchange pump one would expect to see a decline in $[K]_{in}$ as in molluscan neurones (Moreton, 1969). The data for ouabain inhibition of the Na pump (Fig. 3, open circles, averaged data for three experiments) exhibit a slope that appears to be greater than that in the absence of inhibitors. A greater slope would indicate a smaller $[K]_{in}$ although our data are insufficient for rigorous estimations

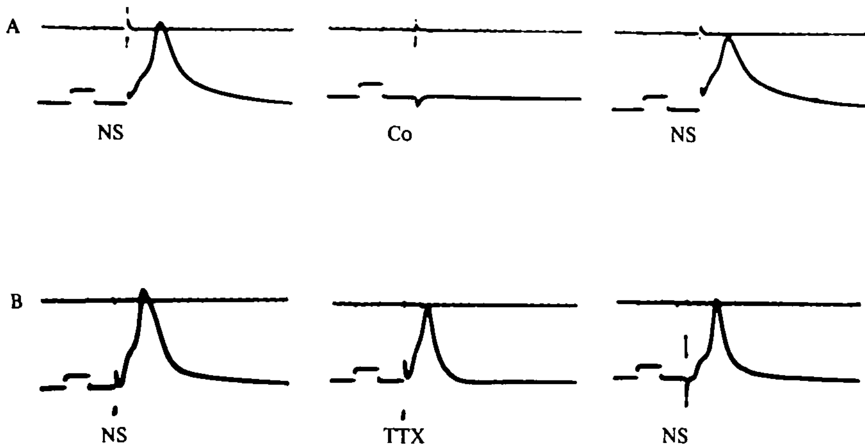

Fig. 4. The effects of ionic blockers on the salivary-gland action potential. Action potentials were elicited by extracellular field stimulation of the glandular surface adjacent to the cell from which activity was being recorded. The top trace in each case is a current monitor and indicates zero potential. (A) 10 mM-$CoCl_2$ completely blocked the action potential even at higher stimulus intensities. Recovery was rapid (right-hand record) although incomplete. (B) In a different preparation, tetrodotoxin (TTX) at 10 μM does not abolish the action potential although it is slightly diminished and recovers partially within 5 min (right-hand record). Square calibration pulses on the voltage traces are 50 ms and 10 mV.

to be made. The relevance of these estimated values for $[K]_{in}$ and P_{Na}/P_K will be discussed later.

In some systems abolition of Na pump activity results in a relationship between resting membrane potential and $[K]_{out}$ that is described by the Goldman equation (e.g. Moreton, 1968; Gorman & Marmor, 1970*a*). In the salivary gland cells this is not the case (Fig. 3). Averaged data from three series of K changes in ouabain (open circles, Fig. 3) do not display a more nearly linear relationship than the data without ouabain. Additional experiments in which chloride ions were replaced with the impermeant sulphate anion (data not shown) indicate that chloride ions do not affect the nonlinear aspects of this function. These results raise the possibility that additional phenomena are making a small contribution to the resting potential of *Helisoma* salivary gland acinar cells (see Discussion).

Action potential

Salivary gland action potentials propagate throughout the gland via low-resistance electrical junctions between the acinar cells. Consequently, the input resistance of most cells is so low that it is impossible to evoke action potentials by direct intracellular depolarization in all but a few cells (Kater *et al.* 1978*b*). Action potentials can be readily evoked through the use of extracellular field stimulation by electrodes placed near the surface of the gland. All subsequently described experiments were performed using both direct intracellular depolarization and extracellular field stimulation to elicit action potentials. Results from intracellular stimulation were in all cases the same as results from extracellular stimulation.

Effects of cobaltous ions and TTX. The application of 10 mM-$CoCl_2$ in normal saline immediately blocks salivary gland action potentials (Fig. 4A). Increasing

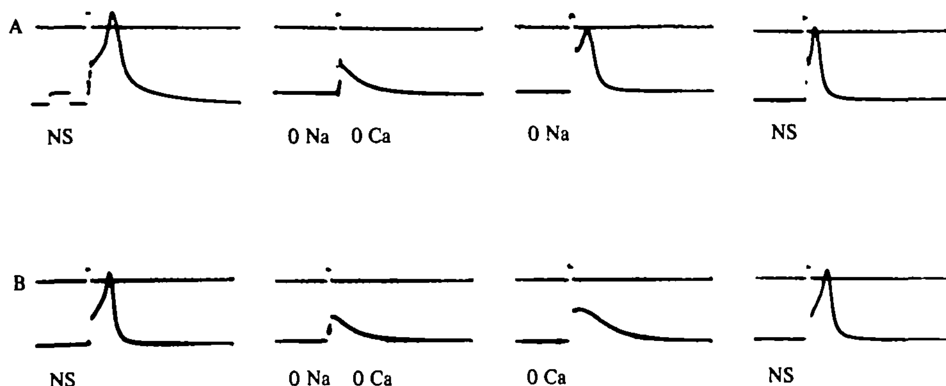

Fig. 5. The effects of ionic substitutions on the salivary gland action potential. All records are from a single cell stimulated by intracellular current injection. Top traces are a current monitor and indicate zero potential. In both (A) and (B), Na and Ca ions were removed by switching to 0 Na 0 Ca saline and then either Ca ions (A) or Na ions (B) were reintroduced. 0 Na 0 Ca completely abolished the action potential so that only a passive membrane response was observed. (A) In the presence of external Ca (third record) a nearly full-size action potential was evoked. (B) When Na was reintroduced (third record) a small depolarization followed the current injection. In both A and B a return to normal saline solution (NS – –, right-hand records) restores the cell's ability to generate action potentials. The effects of 0 Na and 0 Ca salines are the same regardless of the order in which they are introduced. Calibrations are as on the square calibration pulse on the first voltage trace, 50 ms and 10 mV.

stimulus strength has no effect in overcoming this blockage. Full recovery of action potential waveform was not observed during the time course of this experiment (*ca.* 30 min). Manganous ions (10 mM) have the same effect.

Tetrodotoxin (TTX) at a concentration of 10 μM causes a small reduction in the amplitude of evoked action potentials (Fig. 4B) that is partially reversible. The inflexion on the rising phase of the waveform in Fig. 4B (middle record) is due to depolarization of neighbouring acinar cells caused by the extracellular field stimulation (Kater *et al.* 1978*b*). The small effect of TTX and the dramatic effect of cobaltous ions make it probable that salivary gland action potentials have a predominating calcium current.

Effects of ion substitutions. In the absence of Na and Ca ions (0 Na, 0 Ca), no regenerative potentials can be evoked experimentally nor are any observed as spontaneous events in salivary gland acinar cells (Fig. 5). When saline is changed from 0 Na 0 Ca to 0 Na (i.e. restoring Ca concentration) a nearly full-sized action potential can be evoked (Fig. 5A). It is unlikely that the action potential is caused by a pure Ca ion current (i.e. that the reduced amplitude in 0 Na is due to non-specific effects of sucrose substituted for Na ions). Tris HCl, when substituted for NaCl, also slightly reduces the amplitude of evoked action potentials (data not shown). In the presence of Na but not Ca ions we observe a small regenerative response (Fig. 5B) that is greater than the passive response seen in 0 Na 0 Ca saline. This response is probably not dependent on residual Ca ions (no chelating buffer was used) because we were unable to evoke any sort of regenerative response in 0 Na 0 Ca saline.

A small (3–4 mV) change in resting potential is observed on changing from normal saline solution to 0 Na 0 Ca saline (Fig. 5A, NS and 0 Na 0 Ca) that never entirely reverses. This is probably due to deleterious effects of removing Ca ions, causing

changes in membrane stability, input impedance, and resting potential. Due to extensive electrical coupling between the salivary gland cell (precluding accurate input impedance measurements), the effects of 0 Ca saline on input impedance have not been measured.

DISCUSSION

Resting potential

The resting potential of cells from a wide variety of animals and tissues has been shown to be primarily dependent on potassium. This Nernst dependence has been demonstrated in squid and cuttlefish giant axons (Curtis & Cole, 1942; Hodgkin & Keynes, 1955), frog muscle (Adrian, 1956; Conway, 1957) and glial cells (Kuffler, Nicholls & Orkand, 1966). For the most part, resting potentials in molluscan neurones are also dependent on the electrogenic Na pump (Kerkut & Meech, 1967; Moreton, 1968; Gorman & Marmor, 1970*a*, *b*). Both dependencies have been demonstrated for *Helisoma* salivary gland acinar cells. However, there are additional factors affecting the resting membrane potential, as shown by the effects of ouabain (Figs. 2 and 3).

The observed deviations in linearity of the resting potential function in the presence of ouabain (Fig. 3) might be explained if P_K depended on membrane potential such that P_K is lower at more negative potentials. This possibility has been suggested for the frog (Stämpfli, 1959) and the squid (Cole & Baker, 1941; Baker, Hodgkin & Shaw, 1962). Alternatively, the salivary gland cell membrane might contain metabolic pumps other than the Na pump which could have electrogenic effects. (Unpublished experiments with 2,4-dinitrophenol and cyanide proved inconclusive, probably due to secondary effects of these inhibitors.) The observed nonlinear behaviour of the resting potential could be a result of the general morphology of the salivary glands. The primary lumen of the gland and the interior spaces of each acinus may not be freely accessible to externally applied solutions. This idea is supported by recent data from D. Senseman (personal communication): changing K salines by internal perfusion of the ducts of the gland results in a relationship that is more nearly linear, but a straight line fit still cannot be made.

Although all the phenomena contributing to the resting potential of *Helisoma* salivary glands are not completely understood, two conclusions can be reached. (1) The major determinant of the resting potential is the transmembrane distribution of K ions, as in muscle, neural and glial cells. (2) The cell membrane contains an electrogenic Na pump which behaves exactly as described in previous studies on other tissues. Other factors also contribute to the resting potential but in a way which is minor compared to the effect of K ions.

Two conditions must be met if internal potassium concentrations and relative permeabilities of Na and K ions are to be estimated from Moreton's (1968) modification of the Goldman equation (Goldman, 1943; Hodgkin & Katz, 1949). (1) Cl ions must not significantly affect membrane potential and (2) $[K]_{in}$ must remain constant. When $[K]_{out}$ is altered, membrane potential rapidly shifts and reaches a new value which remains steady for many minutes. The redistribution of Cl ions after a change is either passive and rapid (redistributes by the time the plateau is reached) or is so slow as to become negligible (Cl ion nearly impermanent, plateau looks flat because Cl distribution is too slow to be detected). Direct experiments in which Cl ions were

replaced with sulphate ions show that the K dependence of the resting potential is the same as when Cl ions are present. $[K]_{in}$ may be assumed to be very close to constant given that $[K]_{in}$ would change by only a few mM for the entire range of $[K]_{out}$ employed (Boyle & Conway, 1941). Thus in our experiments the conditions are met: chloride ions do not contribute to the resting potential and $[K]_{in}$ remains nearly constant.

Our estimated value for $[K]_{in}$ is 64 mM. This is somewhat different from the value of 93 mM estimated for *Helix* by Moreton (1968). However, the relationship to blood osmolality is similar in the two species (93/220 = 0·42 for *Helix*, 64/130 = 0·49 for *Helisoma*).

The value of P_{Na}/P_K (= 0·04) in *Helisoma* closely agrees with values published for other tissues and animals: squid axons = 0·01 (Hodgkin, 1958); skeletal muscle = 0·01 (Hodgkin & Horowicz, 1959); *Helix* neurones = 0·10 (Moreton, 1968); *Anisidoris* neurones = 0·03 (Gorman & Marmor, 1970*b*).

$[K]_{in}$ during blockage of the Na pump by ouabain appears to be lower than in the absence of the blockage. This is indicated by the apparently greater slope of the function $e^{VF/RT}$ *v.* $[K]_{out}$ (slope = $1/[K]_{in}$). A similar observation was made by Moreton (1969) in *Helix* neurones. Meaningful estimates of the value for $[K]_{in}$ in the presence of ouabain cannot be made with the data available although P_{Na}/P_K in the presence of ouabain is similar to P_{Na}/P_K in its absence.

Action potential

Inward currents of action potentials of various nerve and muscle cells are carried by Na ions (cf. Hodgkin & Katz, 1949), Ca ions (cf. Fatt & Ginsborg, 1958) or by both Ca and Na ions (cf. Junge, 1967). A correlation between ionic currents and cell function has been established. Ca dependent action potentials in some secretory cell types (adrenocortical cells – Matthews & Saffran, 1973; pancreatic islet cells – Matthews & Sakamoto, 1975; chromaffin cells – Brandt *et al.* 1976) appear to ensure that Ca ions are available intracellularly at the site of release, analogous to Ca entry into the presynaptic element of the squid giant synapse (Katz & Miledi, 1969; Llinas, 1977). In some other secretory systems Ca entry is not by way of action currents but is induced by increasing Ca ion permeability in inexcitable cells (Douglas, 1968; Peterson, 1976).

Throughout our studies on exocrine tissues we have confirmed that cells which display action potentials are truly secretory in nature. Several lines of evidence indicate that this is so. Morphological examinations of *Helisoma* salivary glands reveal large secretory cells arranged as an acinus with cilia rapidly propelling secretions down the salivary duct (Kater *et al.* 1978*b*). This general morphology, coupled with our method of impaling cells (only surface cells are recorded from) virtually guarantees the secretory nature of the impaled cells. Furthermore, intracellular dye injection with Fast Green and Lucifer Yellow shows that cells which display action potentials also display the morphology characteristic of secretory acinar cells (Kater, 1977). Most recently, individual secretory cells have been isolated by enzymatic dissociation of *Ariolimax* salivary glands and pedal glands (Kater, 1977) and shown to exhibit action potentials. Such isolated cells, which can be viewed, identified and impaled under

Fig. 6. Amplitude differences in spontaneous and intracellularly evoked salivary-gland potentials. The left-hand action potential was evoked by intracellular depolarization (lower trace is current monitor) and the right-hand action potential occurred spontaneously and propagated to the recording site. The amplitude of the evoked action potential is smaller due to shunting currents into neighbouring cells (see text). Calibrations are 500 ms and 20 mV or 20 nA.

Nomarski optics have been examined individually in the electron microscope and their secretory nature has been unequivocally confirmed (J. G. Goldring, J. W. Kater & S. B. Kater, in preparation).

Helisoma salivary gland acinar cells exhibit action potentials with a major dependence on external Ca ions and an apparently small contribution by an inward Na current. These dependencies were deduced by examining the effects of TTX and cobaltous ions (Fig. 4) and of ionic substitutions (Fig. 5). The more quantitative experiments of varying $[Ca]_{out}$ and measuring action potential overshoot and maximum rate of rise were not performed. The extensive electrical coupling between salivary gland acinar cells make it impossible to clearly ascertain whether any electrical activity observed in a cell originates from that cell or from neighbouring cells. Additionally, electrical coupling affects the waveform of an intracellularly evoked action potential. This is illustrated in Fig. 6. The left-hand action potential on the upper trace was evoked by intracellular depolarization (lower trace is a current monitor) and the action potential on the right occurred spontaneously. Note that the amplitude of the evoked action potential is smaller than that of the spontaneous one. Although this effect is variable, it is always observable in some degree when one evokes action potentials by intracellular depolarization. We interpret these observations as evidence that the evoked action potential 'sees' a greater current sink due to surrounding electrically coupled cells which must be depolarized by current flowing from the impaled cell. Spontaneous spikes originate elsewhere in the gland and propagate through the gland to the recording site. When the cells in a discrete area of the gland depolarize more or less synchronously, as in invasion by a propagating action potential, the cells become functionally electrically uncoupled (cf. Getting, 1974; Kater *et al.* 1978*b*) because equipotential cells do not act as a significant current sink for each other. Any set of experiments in which quantitative measurements of the action potential are to be made would assume that electrical events originate in the impaled cell and that

electrical coupling does not affect the waveform of electrical events. These assumptions cannot be safely made. Because of these complicating factors, quantitative measurements of action potential characteristics could not be performed reliably.

The action potentials of *Helisoma* salivary glands differ from those of the only other excitable exocrine tissue studied, the pedal gland of *Ariolimax californicus*. In this tissue, action potentials can be evoked in either 0 Ca or 0 Na solutions (Kater, 1977). This is in contrast to *Helisoma* whose salivary gland action potentials are nearly completely blocked by cobaltous ions or 0 Ca saline. The small regenerative event evoked in 0 Ca saline (Fig. 5 B, third record) is likely to be a local event that does not propagate. Thus, there seems to be a fundamental difference between the action currents of two closely related pulmonate molluscan exocrine tissues.

What difference in function could underlie this difference in inward current? Perhaps in the *Ariolimax* pedal gland there is an additional source of increased $[Ca]_{in}$, e.g. release of Ca ions from intracellular stores, as proposed for pancreatic acinar cells (Case & Clausen, 1973; Chandler & Williams, 1974, 1977). Depolarization by an inward Na current in concert with a Ca current might release additional Ca ions from intracellular stores. This possibility has not yet been tested in either *Helisoma* or *Ariolimax*, although there is evidence that external Ca ions are necessary for secretion in some *Ariolimax* cell types (J. G. Goldring, J. W. Kater & S. B. Kater, unpublished observations).

There remains an intriguing speculation on the functions of the compound action currents deduced from this and other studies. Various studies have shown that there is a spatial localization of specific ion channels in neurones. The elegant voltage clamp work of Kado (1973) showed that inward currents in the soma of the *Aplysia* left giant neurone are carried primarily by Ca ions while axonal inward currents are carried by Na ions. Hodgkin & Katz (1949) demonstrated that Na ions carry the inward current in the squid giant axon, whereas mixed Na and Ca ionic currents are present in the presynaptic element of the squid giant synapse (Llinas, Steinberg & Walton, 1976). Such voltage-clamp studies lacked the spatial resolution to define whether the currents in the *immediate* region of vesicle fusion are mixed Na/Ca or pure Ca. At membrane surfaces where vesicular fusion and secretion take place, a pure Ca ion current could exist. Thus, in a neurone, Na currents in axonal excitability would ensure reliable propagation of action potentials, while at the axon terminal, depolarization would be brought about by Na or Na/Ca mixed ionic currents, and at the site of release a pure Ca ionic current would ensure reliable exocytosis. In secretory cells arranged around a lumen, mixed currents could be interpreted similarly. One could envisage a spatial restriction of ion-specific channels such that Ca fluxes occur across the luminal membrane and Na fluxes occur across the basal membrane. Such spatial localization of currents in secretory tissues may account for observations of action potentials dependent on mixed ionic currents.

Taken together, the data show that *Helisoma* salivary glands exhibit action potentials that are largely Ca dependent with minor contributions by Na currents. The importance of Ca ions for secretory processes has been reviewed elsewhere (Katz, 1969; Douglas, 1976) and is probably a major factor in the evolution of the ability of snail salivary glands to exhibit action potentials with these ionic dependencies.

Ionic regulation

It is likely that the salivary glands of *Helisoma* are involved in ionic regulation of the animal as are the salivary glands of some insects (e.g. Berridge & Prince, 1972); for the glands comprise a secretory epithelium which is placed at the interface of the blood (haemocoel) and the external environment (buccal cavity). Action potentials might serve as a means of carrying ions which are to be transported across the epithelium. A basal ionic transport rate of charged species could affect resting membrane potential and might constitute an electrogenic pump not inhibited by ouabain.

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