

Electrophysiological Properties of Resting Secretory Membranes of Lamellibranch Mantles

Interaction between Calcium and Potassium

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ABSTRACT This study concerns the effects of ions on the shell-secreting membrane of clam mantles. The average resting potentials were -47 mV for freshwater mantles and -60 mV for marine mantles. Elevation of potassium in the absence of chloride gave a maximal slope of depolarization equivalent to 59 mV for a 10-fold change in the marine form but much less in the freshwater form. In normal potassium, a 10-fold reduction in calcium produced a hyperpolarization of 6 mV for the freshwater mantle. Neither reduction nor elevation of calcium affected the potential of marine mantles in the presence of normal potassium, but a hyperpolarization of 8 mV occurred when calcium was deleted in a low-potassium medium. Elevated calcium reduced the depolarization induced by raised potassium in both species and resulted in an increased effective membrane resistance in marine mantles. Lowered calcium enhanced the hyperpolarization caused by reduction in potassium in freshwater mantles but not in the marine species. Replacement of chloride by large anions produced transient depolarization in both freshwater and marine mantles and resulted in a maintained increased effective membrane resistance in marine mantles. The effects of sodium and magnesium on the membrane potential were not significant in normal potassium. We conclude that the secretory membrane of freshwater and marine clam mantles is permeable mainly to potassium and chloride, and that responses of the membrane potential to calcium are mediated through its effect on the permeability to potassium.

INTRODUCTION

In spite of the large number of studies on exocrine glands, much more information is available for basilar receptive membranes than for their apical

counterparts, the secretory membranes (Petersen, 1976). There is good reason for this disparity, because in most preparations the apical membrane is inaccessible. Thus it is difficult or impossible during electrophysiological studies either to control the ionic composition of the fluid bathing the apical membrane or to study directly its membrane characteristics. In recent studies of sweat and salivary glands (Imai, 1974; Berridge et al., 1975; Sato, 1977), attempts were made to estimate electrical characteristics of the apical membrane. In these cases it proved necessary either to calculate the apical membrane potential from the difference between the basilar and the trans-epithelial potentials or, more directly, to measure the apical membrane potential between an intracellular electrode and a reference electrode placed close to the duct orifice. In order to elucidate the events of stimulus- or excitation-secretion coupling, and especially the role of calcium, direct measurements from the secretory membrane in both resting and active states are essential. Our solution has been to use a morphologically simpler exocrine gland.

In lamellibranch mantles, the cells responsible for secretion of the proteins of the shell lie in an epithelial sheet with the secretory membranes exposed (Wilbur, 1972). In the first electrophysiological studies to take advantage of this tissue, the trans-mantle potential was found to be shell-side positive and calcium-dependent (Lund, 1947; Kirschner et al., 1960; Istin and Maetz, 1964). On the basis of intracellular recordings, Istin and Kirschner (1968) later reported that the effect of calcium was restricted to the apical secretory membrane. They also suggested that the secretory membrane was permselective for calcium, and that a calcium diffusion potential was responsible for the potential across the apical membrane surface.

The study of Istin and Kirschner (1968), however, was restricted to the effect of calcium on the resting potential. In a variety of tissues, calcium has been shown to affect the membrane potential indirectly by acting on the permeability to other ions (Koketsu, 1969). In the present study we have examined the effects of other ions in the presence of different calcium concentrations in order to define the electrophysiological role of calcium in the mantle. Our study deals primarily with the ionic dependence of the electrical potential across the apical membrane of mantles from marine and freshwater clams, supplemented with studies of the ionic dependence of the apical input resistance. We find that calcium is best described as a modulator of the resting permeability to potassium. Chloride is permeant and appears to be distributed passively across the apical membrane. The permeability to sodium is finite, but small, relative to that of potassium or chloride. Some of our results have been presented in preliminary form (Wood et al., 1972).

MATERIALS AND METHODS

Animals

Freshwater clams (*Anodonta* sp.) were bought from a Midwest supplier (Steinhilber, Oshkosh, Wis.) and stored at 12°C in dechlorinated tap water for 2 wk before use. Marine clams (*Anomalocardia brasiliensis*) were collected from Guanabara Bay near the

Federal University of Rio de Janeiro (Ilha do Fundão) and were used fresh or within a few days of storage in seawater. Experiments were performed at room temperature, 20–23°C for *Anodonta* and 24–28°C for *Anomalocardia*.

Clams selected for experiments were judged to be healthy if they pumped water actively while undisturbed and if they closed their valves quickly when handled. Mantles were obtained from clams that had a smooth and glistening inner shell surface. Mantles with a brittle plaque covering all or part of the epithelium and with a shell that was rough and chalky in appearance were not used since the animals were probably anaerobic and resorbing shell (Dugal, 1939).

Morphology and Histology

Lamellibranch mantles are double epithelia derived from lateral extensions of the body. The two epithelia are separated by an interstitial space containing hemolymph. One epithelium faces the mantle cavity and is bathed by the environmental medium. The other faces the shell and secretes the shell components into a thin layer of “extrapallial” fluid similar in composition to the hemolymph in the interstitial space (de Waele, 1930; Crenshaw, 1972).

The shell-facing epithelium is composed of two major portions: a folded peripheral strip responsible for increment in shell area and a large central region responsible for increasing the shell thickness. Our study was confined to the central mantle region. Histological (Beedham, 1958) and ultrastructural studies (Neff, 1972) of the central portion show a single layer of columnar cells as tall as 30 μm and as wide as 10 μm . These cells comprise the bulk of the central epithelium although a few mucous cells are present. The cells rest on a basal lamella and there are conspicuous convolutions of the basilar plasma membrane. *Zonulae occludentes* and *zonulae adhaerentes* appear on the lateral surfaces near the apical end and the apical membranes are comprised of microvilli. (Neff, 1972; Wood, 1973).

Solutions

The *Anodonta* saline was based on analyses of clam blood reported by Potts (1954). It contained (in millimolar): NaCl, 10.0; KCl, 0.5; CaCl₂, 6.0; and HEPES-Tris buffer (pH 7.2), 7.0. In experiments involving ion substitutions, Na was replaced with HEPES-Tris, Ca and K were replaced by equiosmolar Na, and Cl was replaced by propionate. The *Anomalocardia* saline was based on analyses of extrapallial fluid reported by Crenshaw (1972). It contained (in millimolar): NaCl, 410; KCl, 10; CaCl₂, 10; MgCl₂, 25; MgSO₄, 25; and Tris maleate buffer (pH 7.2), 5.0. Tris was used as a substitute for Na; Ca, K and Mg were replaced by equiosmolar Na. Cl was replaced by gluconate or propionate. Osmolarity was constant at the expense of ionic strength.

Dissection

Marine mantles ~2 cm in diameter were excised and placed shell-side up in a small plastic chamber on the stage of a compound microscope. The mantles were secured by a stainless steel spring clip on a small piece of peripheral mantle. In experiments that required intracellular recordings to be made during solution changes, the mantle was placed over a small plastic pedestal to reduce movement. The basilar membranes of marine mantles were shielded from the solutions by the second epithelium (see *Morphology and Histology*) and from diffusion from the edges by using cells in the center.

For freshwater mantles, the cavity-facing epithelium was removed by dissection and the shell-facing epithelium was clamped horizontally between two cylindrical plastic chambers with an exposed surface area of 2 cm².

Electrical Recording Apparatus

Micropipettes were pulled from acid-cleaned 0.8-mm (o.d.) pyrex capillary tubing. Microelectrodes for measuring voltage had between 8 and 20-M Ω resistance and were filled with 3 M KCl. For passing current, 5-M Ω resistance electrodes filled with 2.5 M K citrate were used. The measured tip potentials (Adrian, 1956) were <5 mV.

Micropipettes were connected by chlorided Ag wires to electrometer amplifiers for voltage measurements, or to a radio frequency isolation unit and a large resistor in series with a Tektronix 161 pulse generator (Tektronix, Inc., Beaverton, Ore.) for constant current stimulation. Membrane potentials were displayed on a Tektronix 564B oscilloscope for photography or on a Beckman RS chart recorder (Beckman Instruments, Inc., Fullerton, Calif.). Ground electrodes were Ag:AgCl wires connected to the bath directly or through 3 M KCl-agar bridges.

Membrane Potential Measurements

The micropipettes were held at an angle to the mantle surface, advanced by micromanipulators until a small voltage deflection appeared, and inserted by vibration. Successful electrode placements gave a rapid negative deflection to a steady level with complete reversibility when the electrode was withdrawn. After insertion of the electrode, an increase in the initially recorded potential sometimes occurred. If this later increase due to sealing was 5 mV or less and completed within 60 s, the final value was accepted. Recordings which showed either loss of potential or increments larger than 5 mV were rejected. For marine mantles, stable resting potentials could be recorded for at least 30 min after successful penetrations of the apical membrane. Penetrations of freshwater mantle cells were more difficult and less stable; we accepted recordings which were stable for 5 s or more. We also experienced difficulty in obtaining stable voltage measurements in solutions with lowered calcium or after exposure to increased K concentrations for both marine and freshwater mantles.

When the electrode was advanced further after a successful penetration, there was a rapid decrease in the recorded potential to about -5 mV. This indicated that the basilar membrane had been passed; it was rarely possible to regain the original resting potential during withdrawal of the electrode from a transepithelial position.

In experiments involving ion substitution, five or more penetrations of different cells were made in the control solution; the procedure was then repeated in the experimental solution and then for a second time in the original. The average results for both controls were taken for comparison with the experimental series. Measurements of membrane potential were started 3-5 min after solution changes and the series was usually completed within 10 min. Solution changes, as measured by dyes, were complete in 15 s.

Membrane Resistance Measurements

Current from one side of the isolation unit was injected from a second micropipette inserted within 100 μ m of the voltage electrode. The second lead from the isolation unit was connected to the oscilloscope for current measurement as an IR drop across the 1-M Ω input resistance. Because, in both marine and freshwater mantles, voltage deflections in response to the current were recorded at electrode separations, which exceeded the cell dimensions, the cells are probably electrically connected (Lowenstein et al., 1965; Bennett and Trinkaus, 1970).

The decay of potential with distance in cells connected in two dimensions is nonlinear (Noble, 1962; Jack et al., 1975), requiring multiple penetrations at different distances to determine specific membrane resistance (Shiba, 1971), and secondary ion

shifts might then occur. However, we found it possible to change solutions slowly with the electrodes in place and thereby were able to obtain an estimate of the effective membrane resistance for a given electrode separation in both experimental and control solutions. Owing to the difficulty in obtaining stable penetrations in the freshwater mantle, our experiments on membrane resistance were confined to the marine species.

Statistical Procedures

To determine the significance of the experimental results, we used each mantle for determination of both experimental and control measurements and used a two-tailed paired *t* test. Unless noted otherwise, a probability level of 0.05 was used as the criterion for statistical significance. Experimental variability was recorded as the standard deviation (SD) (Snedecor, 1956).

RESULTS

The first part of our report deals with the membrane potential, its response to ions, and to some interactions between those ions in marine and freshwater mantles. In the second part we report corroborative evidence obtained by investigation of the electrical resistance of the apical membrane of marine mantles.

Experiments on Membrane Potentials

NORMAL RESTING POTENTIALS The average (\pm SD) apical membrane potential for mantles from 14 freshwater clams was -47 ± 3 mV, cytoplasm negative relative to the extrapallial fluid. The apical membrane potential for 38 marine clams was significantly greater, averaging -60 ± 5 mV. Records of typical penetrations are shown in Fig. 1.

Our value for the apical membrane potential in the freshwater mantle is significantly greater than the value of -15 mV reported by Istin and Kirshner (1968). In our hands, the method used in that study, in which the potential was measured after retracting the electrode back into the cell, gave low and variable membrane potentials (-7 to -42 mV, see Methods). These could be the result of lack of a satisfactory seal around the micropipette tip.

As seen in Fig. 1, there does not appear to be more than one population of cells in either freshwater or marine mantles. A single mode of apical membrane potentials would be expected from histological and ultrastructural studies which indicate uniformity of cell structure in the central portion of the mantle (Beedham, 1958; Neff, 1972).

EFFECT OF POTASSIUM The apical membrane potential is similar in sign and magnitude to that recorded for numerous animal cells and would be consistent with a K diffusion potential (Grundfest, 1966).

Fig. 2 shows the results of our experiments on the effect of K in the absence of Cl. Elevated K depolarized the apical membrane in both freshwater and marine mantles, but in the former, an increase in K from 0.5 to 10 mM produced only a 26 mV depolarization, whereas in marine mantles the maximum depolarization was equivalent to a 59 mV change for a 10-fold change. Reduction of K was less effective: a reduction in K from 0.5 to 0.1

mM hyperpolarized the apical membrane by only 7.5 mV in freshwater mantles, and in marine mantles a reduction of K from 10 to 1.0 mM produced a hyperpolarization of only 3.5 mV.

The response to K in the physiological range cannot be described as that of a "good" K electrode for either marine or freshwater mantles, although the response of marine mantles approaches the theoretical in the case of very elevated K. Departure of the apical membrane from that expected for a K diffusion potential could be due to permeability to other cations.

EFFECT OF CALCIUM In five freshwater mantles, reduction of Ca from 6.0 to 0.6 mM resulted in a significant hyperpolarization from -46 ± 1.2 mV to -52 ± 3.3 mV. This was a small effect in spite of the increased activity of calcium expected from the 20% reduction in ionic strength, but qualitatively the change was in the same direction as that found by Istin and Kirschner

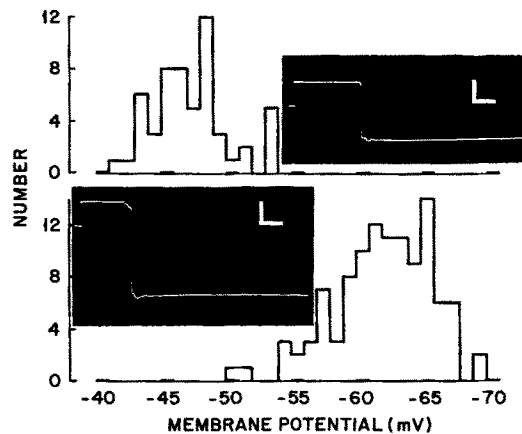


FIGURE 1. Records of impalements and histograms of membrane potentials of freshwater (above) and marine (below) mantle cells. For the histograms, six freshwater (47 penetrations) and four marine (106 penetrations) mantles were used. Calibrations for the impalement records, 20 mV and 10 s.

(1968), and in judging only by its effect on the membrane potential, we observe that a small resting permeability to Ca might be present. In three marine mantles, however, even complete deletion of Ca resulted only in a minor depolarization, from -65 ± 7.9 mV to -62 ± 10 mV. Three additional mantles gave no response to elevation of external Ca to 100 mM (-56 ± 2.5 mV in normal Ca and -55 ± 1.7 mV in elevated Ca). Finally, two more mantles tested at 0.1, 1.0, 10, and 100 mM Ca also showed only minor changes in the resting potential. Inasmuch as it was unlikely that a different mechanism of action of Ca existed in the two types of mantles, the effect of Ca was investigated further in the marine form.

EFFECT OF CALCIUM IN LOWERED POTASSIUM An important difference between the saline solutions for the two types of mantles lies in the Ca:K ratio, and because examples of interaction between the two ions are well known (Höber, 1945), we undertook a series of experiments on marine mantles

with 1 mM K. Five mantles examined for the effect of Ca withdrawal showed a significant hyperpolarization of 7.8 ± 2.7 mV. This result is qualitatively similar to that found for freshwater mantles, but since in both cases the degree of hyperpolarization was small, it became possible that the action of Ca might be indirect.

EFFECT OF CALCIUM ON THE RESPONSE TO POTASSIUM Were Ca to act by altering the permeability to another ion, a likely candidate would be K (see Fig. 2). Ca significantly altered the response of both marine and freshwater mantles to K (Fig. 3).

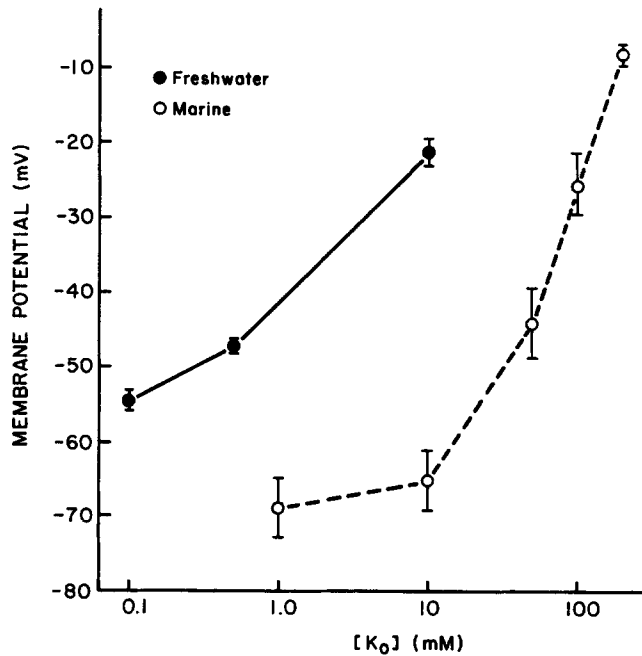


FIGURE 2. Relation between apical membrane potential and concentration of external potassium. Average results for three marine (○) and five freshwater (●) mantles. These experiments were done in the absence of chloride. Length of bar represents 1 SD unit.

Experiments on freshwater mantles were performed in normal (6 mM) and reduced (0.6 mM) Ca. Hyperpolarization in lowered K was enhanced by reduced Ca. The changes effected by Ca in the depolarization caused by elevated K were not statistically significant but were in the same direction as those found for marine mantles.

Larger changes in Ca were used for the experiment on marine mantles: one was placed in saline without added Ca and the other was placed in saline with 100 mM Ca in the absence of Na. Included in Fig. 3 are the average results from three mantles in normal Ca. The effect of elevated Ca was marked: an increase in K to 100 mM produced only a 6.5 mV depolarization; a further increase in K to 210 mM was necessary to effect a slope approaching

that of the mantles in normal or lowered Ca. The response to reduced K was also modified by elevated Ca: instead of hyperpolarization, depolarization occurred. This latter finding was confirmed in three other mantles, also in the absence of Na, where in lowered K (1 mM), depolarization from -65 ± 8.5 mV to -56 ± 4.5 mV was produced by raising Ca to 100 mM.

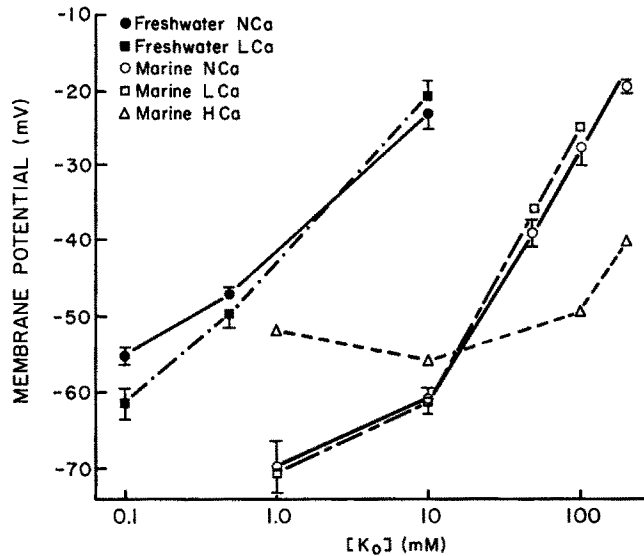


FIGURE 3. Effect of calcium on the relation of apical membrane potential to the concentration of potassium. Results are shown from five freshwater mantles and five marine mantles. For the marine mantles, empty circles (○) represent average results from three mantles with normal calcium (N Ca, 10 mM). The results from two additional mantles in low calcium (L Ca, no Ca added, □) and high Ca (H Ca, 100 mM, △) are presented. For the experiments on freshwater mantles each of the five mantles was subjected to both normal calcium (6 mM, ●) and lowered calcium (0.6 mM, ■) for three levels of potassium. These experiments were done in the presence of normal constant chloride concentrations for marine mantles and 0 chloride for the freshwater mantles. The maximum slope (normal Ca) in high potassium was 34 mV for marine and 18.5 mV for freshwater mantles for a 10-fold change. Length of bar represents 1 SD unit.

The effect of elevated Ca in lowered K could be interpreted either as reflecting a small permeability to Ca, as a depression of the permeability to K, or both. The effect of high Ca in elevated K, however, is not consistent with the first possibility and is most simply explained as an inhibition of the resting K permeability.

EFFECT OF CHLORIDE Cl withdrawal resulted in transient depolarization in both freshwater and marine mantles (Fig. 4). In these experiments the marine mantles were placed in saline solutions with 1 mM K. Results similar to those in Fig. 4 were obtained for two additional freshwater and three

marine mantles. Two of the experiments with marine mantles were performed in normal K. The depolarization recorded indicates that Cl is permeant; that it is transient indicates that Cl is redistributed across the cell membrane (Hodgkin and Horowicz, 1959). The apparent steady hyperpolarization for the marine mantle was also found in the second mantle (2 mV) and in two mantles in normal K (3 and 5.5 mV), but two other mantles in normal K showed 2-mV depolarizations, so further experiments would be necessary to determine whether the distribution of Cl is not completely passive.

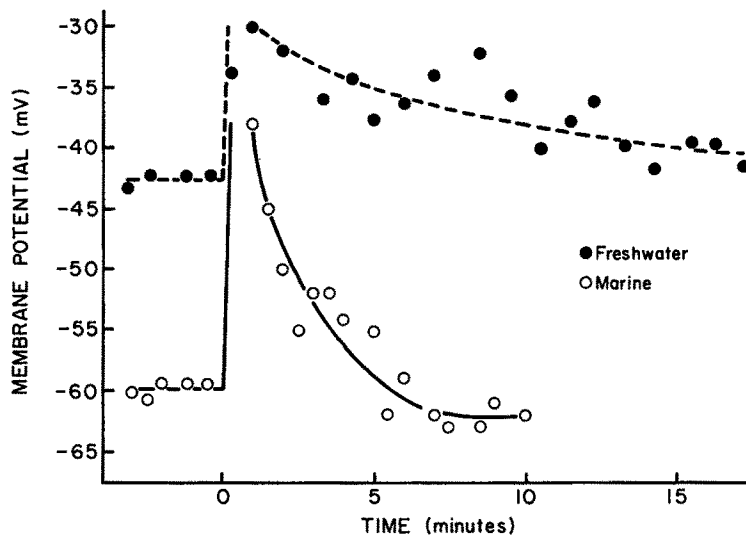


FIGURE 4. Effect of chloride withdrawal on apical membrane potential. Results for one freshwater (●) and one marine (○) mantle are shown. The freshwater experiment was done in normal potassium (0.5 mM); the marine experiment was performed in lowered potassium (1.0 mM).

EFFECT OF SODIUM We were unable to demonstrate any significant effect of Na replacement on the membrane potential in otherwise normal saline solutions. Three freshwater mantles gave apical membrane potentials of -46 ± 0.5 mV with and -46 ± 0.3 mV without Na. Two marine mantles gave -59 ± 7.3 mV with Na and -62 ± 6.4 mV without Na. This latter difference, although not significant, was in the proper direction to indicate a small permeability to Na, so we performed Na withdrawal experiments also in marine mantles bathed in solutions containing 1 mM K. Five mantles were studied. An average hyperpolarization of 8.8 ± 6.2 mV was found to be significant ($P = 0.10$).

EFFECT OF MAGNESIUM Mg was not tested in freshwater mantles inasmuch as we were not able to demonstrate significant effects of Mg withdrawal in the marine form in either normal K (-61 ± 3.9 mV with Mg, -61 ± 4.8 mV without Mg; 13 mantles) or in the presence of 1 mM K (-77 ± 5.9 mV with Mg, -78 ± 5.5 mV without Mg; 3 mantles).

Studies on Apical Input Resistance

These experiments were undertaken to provide added evidence concerning the permeability of the apical membranes of marine mantles to Ca and to Cl.

EXPERIMENTS IN NORMAL SALINE In our first experiments we found that currents injected at a distance from the voltage electrode caused a smoothly rising change in membrane potential to a steady final level. This is evidence that the cells are electrically connected. The relation between current and voltage was linear for small potential changes in either the hyperpolarizing or the depolarizing direction (Fig. 5). Thus the membrane behaves in an ohmic manner and small perturbations can safely be used to estimate membrane resistance.

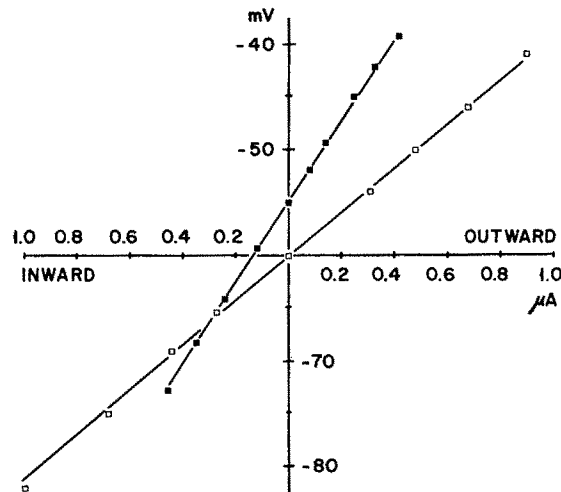


FIGURE 5. Current-voltage relationship in two marine mantles. Electrode separation, 22 μm for one mantle (■) and 62 μm (□) for the second mantle. The responses of the two mantles can not be compared directly because of the different electrode separations.

Larger hyperpolarizing currents elicited no marked deviations from this linearity but larger depolarizing currents elicited time-variant potential changes in response to constant currents (Fig. 6). In the marine mantle, these responses were found to be all-or-none, propagated, and not dependent on the presence of Na or Cl (Sorenson, 1978). They are also cardiac-like in appearance in that there is an initial upstroke and overshoot followed by a rapid repolarization to a plateau which is terminated by a second rapid repolarization (Cranefield, 1975). Preliminary experiments on freshwater mantles showed them to be electrically excitable in the sense that time-variant potential changes were present (Grundfest, 1966) but no action potentials per se were found.

EFFECT OF CHLORIDE ON MEMBRANE INPUT RESISTANCE If Cl is permeant, as suggested by the results in Fig. 4, its replacement by an impermeant anion

should result in an increased membrane input resistance. Fig. 7 shows one such experiment in which the solution was changed with the electrodes in place while recording the response to repeated hyperpolarizing currents. The resistance rapidly increased and then remained steady as expected. Five determinations on three marine mantles gave an average increase in membrane input resistance to $176 \pm 58\%$.

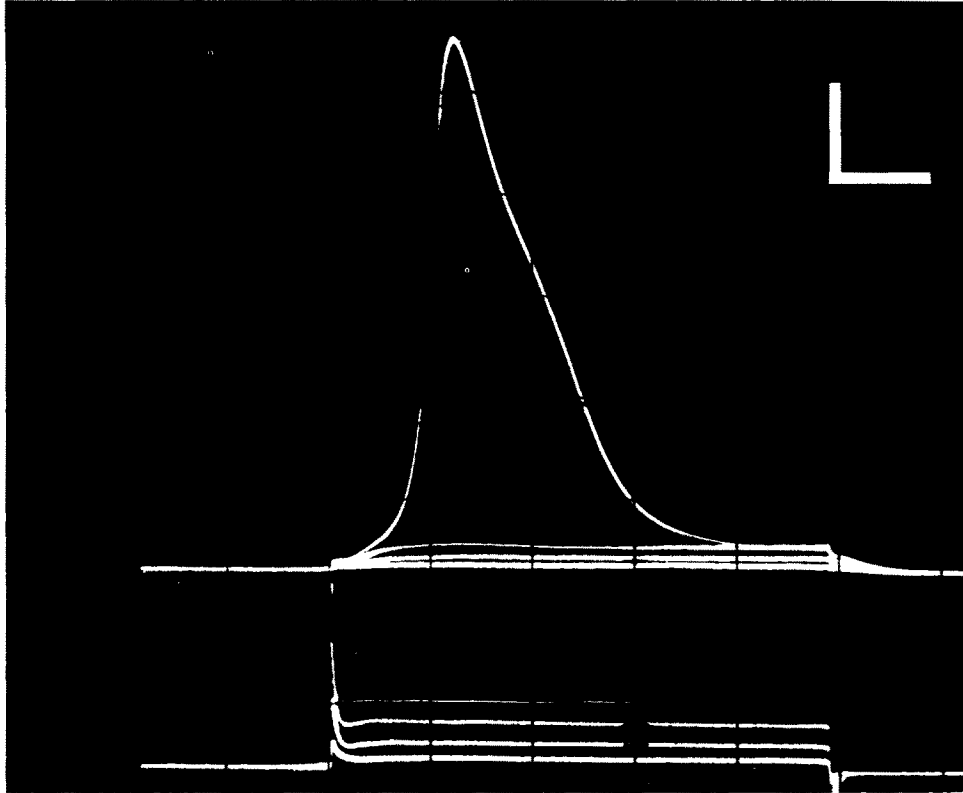


FIGURE 6. Action potential in apical membrane of a marine mantle. Electrotonic responses (upper traces) to three subthreshold currents (lower traces), followed by the active response. Calibrations: 20 mV/cm, 10 μ A/cm, and 10 ms/cm. Resting potential 60 mV.

EFFECT OF CALCIUM ON MEMBRANE INPUT RESISTANCE The possibility that Ca acts primarily by modification of the permeability to K rather than by directly contributing to the resting potential was tested by measuring its effect on membrane resistance. Neglecting possible voltage-dependent changes in conductance (see Fig. 5), added Ca should cause an increased membrane resistance if it modifies the permeability to K. If Ca itself is significantly permeant, added Ca should result in a decreased membrane resistance as it increases the concentration of ions that can carry current. Elevated, rather than lowered, Ca was used because decreased input resistance in low Ca could

result from either an increased shunt around the electrode because of poor sealing or from a true decrease in input resistance. That elevated Ca might also increase sealing resulting in a spurious increase in input resistance is less likely, for this effect would be reflected in greater recorded membrane potentials as well and this was not found (see EFFECT OF CALCIUM). Fig. 8 shows the results from one such trial in a marine mantle. The effective membrane resistance clearly increased in response to added Ca. Three determinations on this mantle showed that Ca caused an average increase in input resistance to

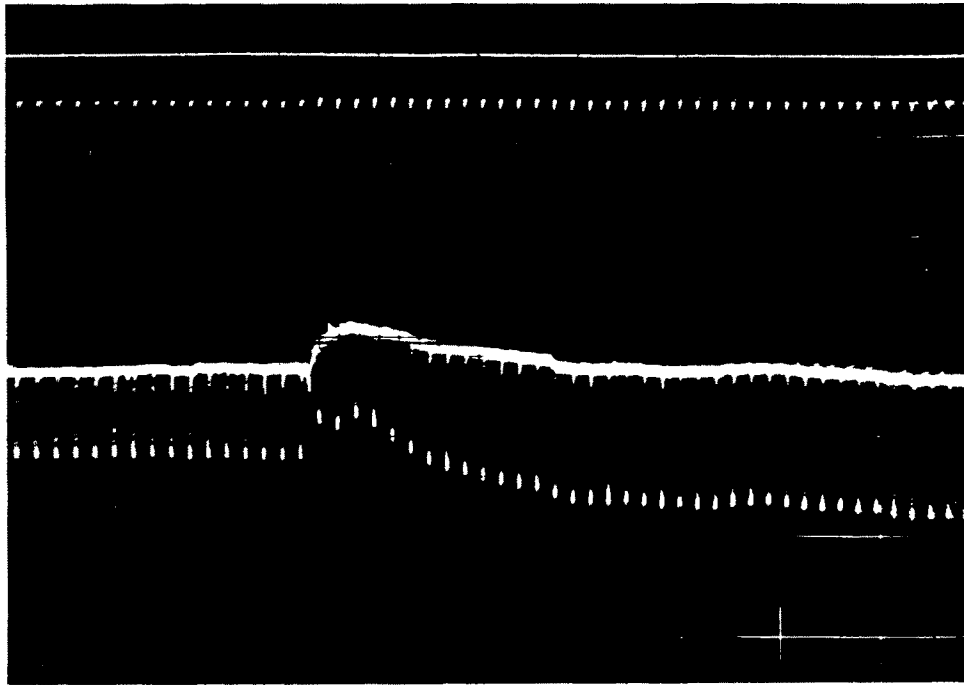


FIGURE 7. Effect of chloride withdrawal on membrane input resistance. Continuous recording of current (upper trace) and voltage responses (lower trace). Pulse frequency 2/s, voltage calibration 10 mV/cm, current 2.0 μ A/cm. Record from marine mantle.

$159 \pm 9\%$. Further experiments would be necessary to show that Ca is acting on the permeability to K alone, but our results are clearly counter to the proposition that Ca is relatively highly permeant.

DISCUSSION

Our main conclusion is that the secretory membrane of mantle cells is permeable mainly to K and to Cl and less so to Na. Judging by our electrophysiological findings, Ca is relatively impermeant but exerts a strong influence on the permeability to K.

Permeability to Chloride

Depolarization following Cl withdrawal (Fig. 4) and an associated increase in input resistance (Fig. 7) constitute good evidence that the apical membranes of the mantle are permeable to Cl (Hodgkin and Horowicz, 1959). The decreased slope of K depolarization in constant Cl concentration (compare Figs. 2 and 3) can also be explained by the presence of an emf for Cl acting to offset the effect of elevated K if the redistribution of KCl in these experiments is slower than in our experiments involving Cl withdrawal. This appears likely, for the electrochemical gradient for KCl entry is much less in the experiments at constant Cl than for KCl loss after Cl replacement. This repolarization following Cl withdrawal indicates that Cl is lost from the cell across the apical membrane (Hodgkin and Horowicz, 1959), and to the extent

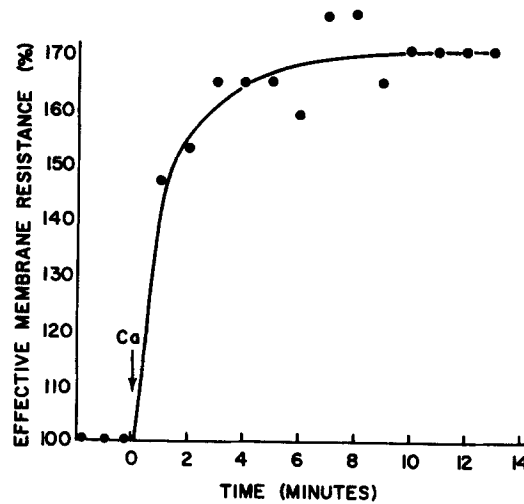


FIGURE 8. Effect of calcium on membrane input resistance. Experiment done in the same manner as for Fig. 7, but presented directly in terms of percent change in membrane resistance. Data from a marine mantle. At the arrow, the concentration of calcium was raised from 10 to 100 mM.

that the original membrane potential is recovered, that the distribution of Cl can be considered to be in accordance with the membrane potential.

Permeability to Sodium

Our observation that the marine mantle cells hyperpolarize upon removal of Na (in low K salines) is consistent with a finite resting permeability to that ion. Williams (1970) has ascribed the departure of the membrane potential from that predicted by the Nernst relation in secretory tissues to an increased permeability of Na relative to that of K.

Permeability to Potassium

Our evidence suggests that the resting potential across marine and freshwater apical membranes is established by a diffusion potential for K (Fig. 2). Marine

mantles, however, are more responsive to K than those of the freshwater species; a possible explanation for this will be discussed in a later section on the effect of Ca on the permeability to K.

Relative Impermeability to Calcium

The results of three types of experiments led us to conclude that Ca is relatively impermeant. First, the response of the membrane potential to Ca withdrawal in marine mantles was minor depolarization when the bathing solution contained normal levels of K, and even in low K solutions (c.f. Fig. 3), the response to lowered Ca (freshwater mantles) and to Ca deletion (marine mantles) was small. Second, the effect of Ca on the membrane potential in solutions of elevated K (Fig. 3) gave results of a sign inappropriate for a permeant Ca in both freshwater and marine mantles. Finally, in solutions with normal levels of K, elevating Ca had no effect on the membrane potential in marine mantles, while with elevated Ca the input resistance was significantly increased (Fig. 8).

Hyperpolarization caused by lowered Ca is unusual but not unprecedented. Hoffman and Suckling (1955) observed the same effect in dog ventricular and Purkinje tissue, and Cuthbert (1966) reported such an effect in smooth muscle of mammalian mesenteric vein. The former authors interpreted their results as representing an influence of Ca on the permeability to K; we believe that Ca has a similar influence in mantle apical membranes.

Effect of Calcium on the Permeability to Potassium

In mantle secretory membranes, Ca seems to act through an increase in membrane resistance. Two kinds of evidence suggest that Ca acts primarily on the permeability to K. First, added Ca increased the membrane input resistance to current flow and the ion with the greatest resting permeability appears to be K (Fig. 2). Second, added Ca decreased the response of the apical membrane potential to K (Fig. 3).

Ca could act by screening or binding fixed negative surface charges (Frankenhaeuser and Hodgkin, 1957; Huxley, 1959; McLaughlin et al., 1971). Since Ca acted even in the presence of high Mg and since Mg itself was without significant effect, some specificity for Ca appears to exist. The differences we observed between freshwater and marine mantles could be related to this action. Freshwater mantles had lower membrane potentials, smaller responses to K, and greater responses to Ca than marine mantles. The lower ionic strength of the freshwater saline, and the further reduction when Ca was replaced, would result in greater effective surface charge seen by Ca and thus a lower permeability to K. However, Ca may also influence the permeability to other ions.

Effect of Calcium on the Permeability to Sodium and Chloride

In other tissues, Ca appears to act primarily by controlling the permeability to Na (Koketsu, 1969); lowered Ca could not produce hyperpolarization by increasing the conductance to Na, but it is possible that alterations in Ca might influence an electrogenic extrusion of Na. This would be consistent

with Ca-induced depolarization and with hyperpolarization induced by Na withdrawal in low K. Without experiments using pump inhibitors, it is difficult to predict whether it is also consistent with results on input resistance (Fig. 8).

Hyperpolarization in lowered Ca also seems unlikely to be caused by an increase in the conductance to Cl, for our results suggest that Cl distribution is largely determined by the membrane potential (Fig. 4).

Electrically Excitable Responses

Direct evidence for electrical excitability of apical membranes was shown in Fig. 6. Indirect evidence is suggested by the shift produced by Ca of the relation between external K and the membrane potential (Fig. 3). A propagated spike constitutes strong evidence that the muscle cells are electrically coupled (Bennett, 1966). A study of its physiological role is in progress. Electrically excitable responses in tadpole skin have been regarded as sensory (Roberts, 1971). On the other hand, the responses reported by Mackie (1976) for a coelenterate gland were considered by him to be involved in secretion. The ionic basis of the action potential in clam mantle secretory membranes may bear on this issue (Hagiwara, 1974).

Transport of Calcium to the Shell

From the first studies of the mantle transepithelial potential, it was inferred that Ca played a direct role in the genesis of the potential and that this potential thus reflected the active transport of Ca to the shell (Kirschner et al., 1960). Neither inference has been borne out. The transepithelial potential is in fact sensitive to Ca, but our data indicate that it acts indirectly by influencing the permeability of the apical membrane to K.

Two studies using radioactive Ca indicated that the movement of Ca across the mantle toward the shell was passive (Kirschner and Sorenson, 1964; Istin and Maetz, 1964), but in these studies intact mantles with both epithelial cell layers were used. Istin and Kirschner (1968) suggested that movement of Ca from the shell-facing cells to the extrapallial fluid could be passive and provide a basis for the membrane potential, if the mantle cells exhibited a high resting permeability to Ca. Our present results do not support this suggestion.

If these mantle cells share with numerous other cells the property of a low internal Ca, the electrochemical gradient strongly opposes Ca extrusion from the cell, and active transport thus appears necessary if the route of flux is transcellular. Measurements of Ca efflux from the cell to the extrapallial fluid are now needed.

We wish to thank Dr. John P. Reuben for a valuable critical review of an earlier version of this article.

This work was supported in part by grants GM-04254 from the National Institutes of Health, TC 16-841 and TC 17-456 from the Conselho Nacional de Pesquisas in Brazil, and 7447 and 7454 from the Westchester Heart Association.

Received for publication 16 February 1979.

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