

Calcium-Activated Conductance in Skate Electrorceptors

Voltage Clamp Experiments

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ABSTRACT Voltage clamp experiments allow further characterization of the calcium-dependent repolarizing process in skate electroreceptor epithelium. Four current components are described: a prolonged capacity current, a leakage current, an early active current which flows inward across the luminal membranes of the receptor cells, and a late current which flows outward. The leakage and capacity currents are linear and may be subtracted from the total current, giving net active currents. The early active current is carried by calcium and does not undergo inactivation for at least several seconds. When large stimuli exceed the reversal potential for the early calcium current, the late current is suppressed. Reduction of the ionized calcium concentration in the lumen lowers the reversal potential for the early current and the suppression potential for the late current by the same amount. We conclude that the late current is initiated by a calcium influx into the cytoplasm. During pulses of moderate duration, activation of the late current does not begin until a fixed amount of calcium has entered the receptor cells. The required amount of calcium is reduced if a recent calcium influx has occurred. We suggest that the calcium-activated outward current is mediated by a distinct macromolecule that is insensitive to voltage. Such macromolecules are likely to have an important role in the regulation of electrical activity in excitable cells.

INTRODUCTION

The preceding paper (Clusin and Bennett, 1977) presented evidence that excitability of skate electroreceptors results from an active inward calcium current across the luminal membranes of the receptor cells and that repolarization of the action potential does not occur when there is no calcium influx. In this paper we confirm and extend these conclusions by use of the voltage clamp technique. By changing the epithelial voltage faster than the response of the active conductances, we are able to measure instantaneous epithelial resistance, to obtain temporal separation of the active currents, and to determine their reversal potentials. We find that measurements of reversal potential are little affected by the series resistance of the basal membrane, even though the luminal membrane voltage is poorly controlled during changes in the active currents.

Voltage clamp measurements during perfusion of the lumen with EGTA show that inward calcium current initiates a late outward current which is presumably responsible for repolarization of the action potential. Activation of this late current is facilitated during closely spaced stimuli. The late current is not activated by depolarization in the absence of calcium influx, and is suppressed during voltage stimuli that equal or exceed the reversal potential for the early current. We infer that the underlying conductance increase is mediated by a special calcium-activated macromolecule. In various nerve cells injection of calcium into the cytoplasm produces a hyperpolarizing conductance increase (Meech, 1974). Calcium-activated conductance macromolecules are probably widespread and may be important in the regulation of electrical excitability, particularly if they exhibit the facilitated activation described here.

MATERIALS AND METHODS

The dissection technique, salines, and method of internal perfusion are described in the preceding paper (Clusin and Bennett, 1977). The voltage clamp consisted of an operational amplifier (Analog Devices 40J, Analog Devices, Inc., Norwood, Mass.) connected as shown in Fig. 1. This voltage clamp was tested on a model circuit in which the reference electrode was represented by a 100- Ω resistor and the preparation by a 0.4-M Ω resistor in parallel with a 0.25 μ F capacitor. Command voltage steps were developed across the model circuit within 100 μ s and no capacity current was discernible after 200 μ s.

In experiments involving perfusion of the lumen of the ampulla the perfusion cannula was used to measure voltage, as indicated in Fig. 1. In most experiments not requiring internal perfusion, the ampullary potential was measured by using a microelectrode thrust through the canal wall as shown in Fig. 3 of Clusin and Bennett (1977). In a few experiments no microelectrode was used and voltage was measured at the opening of the canal with a chlorided silver wire. In these cases positive series feedback (Hodgkin and Huxley, 1952*a*) was used to compensate for the series resistance of the canal. Ionic currents recorded in this manner were indistinguishable from those obtained when the potential across the ampulla was measured directly. Furthermore, when a transmural microelectrode and a separate amplifier were used to obtain an independent measurement of the ampullary potential, voltage displacements across the ampulla produced by using compensated feedback were found to occur within 200 μ s.

RESULTS

Capacity Currents and Their Origin

When the voltage across the ampullary epithelium is stepped to a new value within the linear range, there is a transient current that relaxes, leaving a maintained current (Fig. 2 A). After a brief initial surge of current while the voltage displacement is completed, the transient current declines exponentially (Fig. 2 B) and may be perceptible for up to 15 ms. This decline is brief compared with the duration of the action potential, but is much slower than the voltage clamp circuitry. The prolonged transient is symmetrical for equal and opposite voltage displacements when there are no active currents (Fig. 2 A) and varies linearly with the magnitude of the voltage step. As shown below, the transient current is independent of absolute epithelial potential and is not affected by the presence of maintained active currents. We infer that the prolonged transient

arises from a capacity that is in series with a fixed resistance and not from a time-dependent resistance change.

Integration of the current in Fig. 2 shows that the capacitance responsible for the prolonged transient is $0.16 \mu\text{F}$. Since the prolonged transient decays much faster than the time constant of the resting epithelium in current clamp experiments (about 130 ms), the capacitance responsible for the prolonged transients is included in the total ampullary capacitance determined by passing constant current. Subtraction of the capacitance responsible for the prolonged transient from the total ampullary capacitance of $0.34 \mu\text{F}$ (calculated from Fig. 5 A of the

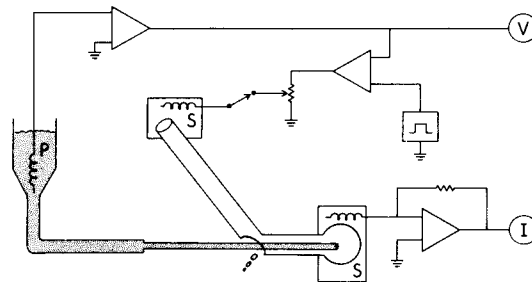


FIGURE 1. A diagram of the experimental arrangement for voltage clamping the ampullary epithelium during perfusion of the lumen. A more detailed diagram of the perfusion chamber is shown in Fig. 4 of Clusin and Bennett (1977). The voltage electrode was a chlorided silver wire in the perfusate reservoir (*P*). This electrode was connected to a Bioelectric NF1 single-ended preamplifier with output *V*. Current was measured with a current-voltage transducer made from an Analog Devices 40J operational amplifier with output *I*. The reference electrode was a chlorided silver plate (resistance less than 100Ω) in the saline pool (*S*) containing the ampulla. A second 40J amplifier served as the voltage clamp. The feedback was adjusted with a $10 \text{ k}\Omega$ potentiometer. The command pulse was produced by a Bioelectric voltage source, and the output was connected to a chlorided silver wire in the saline pool (*S*) containing the distal end of the canal. As in the current clamp experiments, treatment of the external surface of the canal with an ion-free solution insured that over 99% of the total current flowed down the axis of the canal and across the ampullary epithelium.

preceding article) shows that $0.18 \mu\text{F}$ of the total capacitance charges rapidly during voltage clamp experiments.

One possible source of the fixed series resistance responsible for the prolonged capacity transient is the axial resistance of the lobulations or alveoli of the ampulla. This possibility can be excluded by measurements of the alveoli and determination of their approximate space constant. The total surface area of an electroreceptor of standard size is estimated to be 0.036 cm^2 . If the input resistance of an inactive ampulla with this surface area were $0.3 \text{ M}\Omega$, then the resistivity of the inactive epithelium would be $11 \text{ k}\Omega/\text{cm}^2$. Likewise, if the resistance of the ampulla when both active currents were flowing (see below) were $60 \text{ k}\Omega$, the epithelial resistivity would be $2.2 \text{ k}\Omega/\text{cm}^2$. Typical alveoli of *R. ocellata* look like those drawn in Fig. 1 of the previous paper (Clusin and

Bennett, 1977). If such an alveolus were approximated as a cylinder 150 μm across, and if the resistivity of the ampullary jelly were 25 Ω/cm , then the space constant of such a cylinder would be 6 mm when the epithelium was active. Almost all the alveoli are less than 300 μm long or 0.05 of the minimum space

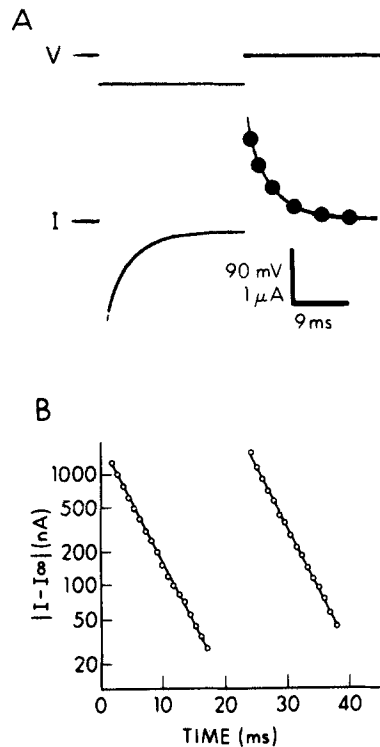


FIGURE 2. Capacity currents after a displacement of the epithelial voltage. In A, the lower trace shows the epithelial current required to produce the 49-mV, 24-ms lumen-positive voltage displacement shown in the upper trace. The initial current transient decays leaving a maintained leakage current. When the epithelium is returned to the holding potential, an oppositely directed transient occurs. The dots represent superposition of the first transient on the second, which was accomplished by subtraction of the total current from the leakage current at each point. The fact that all of the dots lie on the second transient indicates that the two transients are symmetrical. In B, the logarithm of the absolute value of the transient current is plotted against time. The origin of the plot in B is aligned with the onset of the stimulus in A, the time scale being the same in both figures. Both current transients appear to decay as a simple exponential with a time constant of 4 ms. Integration of this exponential between zero and infinity gives a charge of 7.7 nC. The proportion of the total capacitance responsible for the prolonged transient is therefore 0.16 μF . The holding potential is 20 mV lumen positive.

constant. Moreover, attenuation along a short closed cylinder is much less rapid than exponential. Therefore the ampulla is electrically equivalent to an isopotential sheet of epithelium.

The receptor cells may be discounted as a possible source of the prolonged

capacity currents because their total membrane area is too small. As shown below, over 96% of the voltage drop across inactive receptor cells occurs in the luminal membranes. Measurements of electron micrographs (Waltman, 1966) show that the luminal membrane area of each receptor cell is about $8 \mu\text{m}^2$. By use of Nomarski optics, the size and density of receptor cells can be determined in the living ampulla. A standard-size ampulla with an alveolar surface area of 0.036 cm^2 has about 10^4 receptor cells with a total luminal membrane area of 0.0008 cm^2 . Assuming a specific capacitance of $1 \mu\text{F}/\text{cm}^2$, one obtains a total luminal membrane capacitance of $0.0008 \mu\text{F}$. This value is insignificant compared to the capacitance of the standard-size ampulla, which is $0.34 \mu\text{F}$ (calculated from Fig. 5 A of the preceding article). Even when the low-resistance basal faces are considered, there is not enough receptor cell membrane to account for the ampullary capacitance. Each receptor cell is approximately spherical with a diameter of $13 \mu\text{m}$ and a basal membrane area of about $500 \mu\text{m}^2$. If one assumes 10^4 receptor cells and a specific membrane capacitance of $1 \mu\text{F}/\text{cm}^2$, the total basal membrane capacitance is only $0.052 \mu\text{F}$.

Another possible source of the prolonged capacity current is the canal wall near the ampulla, where insulation by the sucrose treatment might not be adequate to prevent flow of current along the external surface. The total capacity of the canal wall between the cannula insertion point and the ampulla is $0.22 \mu\text{F}$, which may be large enough to account for the observed transients. However, since each patch of canal wall is separated from ground by a different series resistance, the total capacity current should not be exponential. The fact that the capacity currents in Fig. 2 A are well fit by a single exponential suggests that they do not arise from the distributed capacity of the canal.

The last possible source of capacity currents to be considered is the supporting cells. The luminal membranes of the supporting cells have a total surface area approximately equal to the alveolar surface area— 0.036 cm^2 in a standard-size ampulla. This value is also too small to account for the total ampullary capacitance of $0.34 \mu\text{F}$ (see previous article, Fig. 5 A). However, the basal membranes of the supporting cells have a much greater surface area than the luminal membranes (see Fig. 2 of the preceding article). This difference is especially pronounced in the marginal zone, where the basal membranes are highly involuted. Thus, the basal membranes of the supporting and marginal zone cells are likely to be the source of most of the ampullary capacitance.

Presumably, the basal membranes of cells responsible for the prolonged component of the capacity current charge slowly through the series resistance of their luminal membranes, while the basal membranes of cells responsible for the fast component charge rapidly. Rapid charging of a cell's basal membrane requires that the product of the luminal membrane resistance and the basal membrane capacitance be small. Since the basal membranes of the supporting and marginal zone cells have a large capacity and a significant resistance compared with the luminal membranes, the luminal membrane resistivity must be comparatively low. The required difference in resistivity would be smaller if the rapidly charging capacitance resided in the ordinary supporting cells, where there is a smaller difference in membrane areas. The slowly charging capacitance would then reside in the marginal zone cells. This arrangement would

allow the resistivities of corresponding faces in the two cell types to be the same. However, the assignment is tentative, since the membrane resistances are unknown.

The foregoing considerations are important because they suggest that capacity currents flow through cells that are electrically in parallel with the receptor cells. As will be seen below, the form of the capacity currents is independent of epithelial conductance and absolute epithelial voltage. The prolonged capacity transient therefore sums algebraically with active currents arising from the receptor cells.

Active and Leakage Currents and Their Origins

Between 0 and 100 mV lumen positive, the current following the capacitative transient is constant and linearly related to voltage. The leakage resistance calculated from this current agrees with the resistance of the inactive epithelium obtained in current clamp experiments (Clusin and Bennett, 1977). When the epithelium is stepped from a lumen-positive holding potential to a lumen-negative voltage, active currents occur. The active currents are about 100 times slower than those observed in squid axons. Currents flowing inward across the luminal surface of cells in the epithelium are defined as inward and are shown in the figures as downward. In Fig. 3 A there are multiple peaks of inward current between 0 and 30 mV lumen negative. Between about 30 and 88 mV there is a single peak of inward current followed by a maintained outward current. Above 88 mV, the transepithelial current is entirely outward.

The multiple peaks of inward current are more pronounced in some experiments than in others. In Fig. 3 A only a small second peak of inward current occurs at 25 mV. In Fig. 3 B, however, trains of sinusoidal oscillations are evoked by small excitatory stimuli in the range of -2 to $+28$ mV. With large stimuli, these oscillations become increasingly damped until only a single peak of inward current occurs. As in barnacle muscle fibers (Keynes et al., 1973) the oscillations are attributed to inadequate clamping. As discussed below, active currents generated by the luminal membrane can cause significant changes in voltage across the basal membrane, which means that the voltage across the luminal membrane also changes. Thus, receptor cells can escape from the clamp and furthermore they may escape independently of one another. The large number of oscillatory peaks in Fig. 3 B indicates that the cells are fairly well synchronized, owing in part to the common stimulus onset. However, it is also possible that individual receptor cells interact through as yet unidentified gap junctions in the epithelium or through very small changes in the ampullary potential not prevented by the clamp. In Fig. 3, both A and B, multiple inward current peaks are confined to the negative slope region of the current voltage relation (see Fig. 3 B). With larger stimuli, which maximally activate the early conductance (see below), there are no oscillations; the onset of the late outward current is smooth.

Fig. 3 C shows a current voltage relation obtained from the data in Fig. 3 A. A similar plot obtained from the experiment in Fig. 3 B is not shown. Excitatory (lumen negative) stimuli are shown to the right, in conformity with the usual conventions for excitable membranes. Inward current is shown downward. The holding potential is -12 mV, which is the resting potential recorded from this

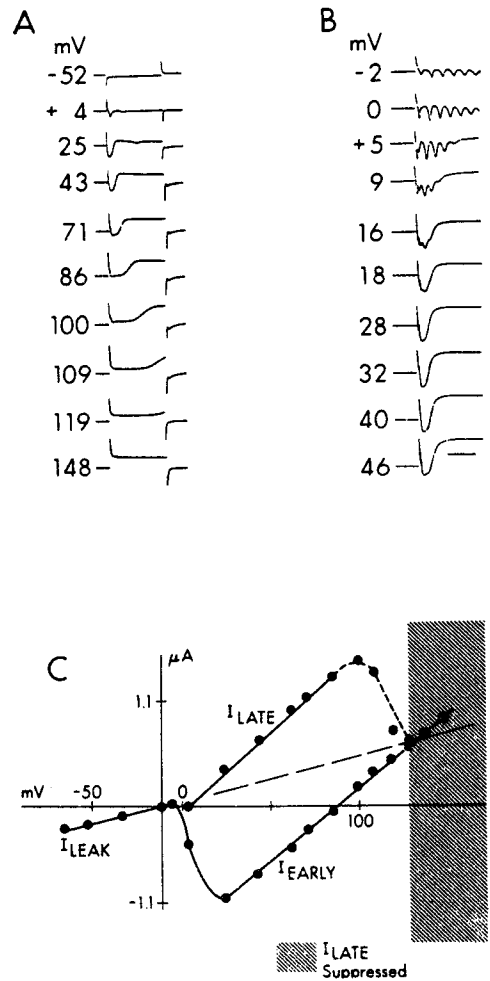


FIGURE 3. Active currents of skate electroreceptor epithelium. A shows a family of currents obtained when constant voltage displacements are imposed across an ampulla of standard size. Current flowing inward across the luminal membranes is defined as inward current and shown downward. Voltage displacements that depolarize the luminal membranes (lumen negative) are defined as positive. The epithelium is held at its resting potential of -12 mV. Lumen-positive stimuli evoke maintained leakage currents. Lumen-negative stimuli between 0 and $+88$ mV evoke an early inward current followed by a late outward current. With larger excitatory stimuli, onset of the late outward current is progressively slowed and delayed. At $+128$ mV, activation of the late outward current is suppressed. The currents obtained at $+4$ and $+25$ mV show a secondary peak of inward current. A current voltage relation of the data in A is plotted in C. The vertical axis is placed at the holding potential. The leakage current (I_{LEAK}), the peak early current (I_{EARLY}), and the late outward current (I_{LATE}) are linearly related to voltage over a broad range. The slope resistance during the early current is 64 k Ω within the linear range, while that of the late outward current is 58 k Ω . The leakage resistance is 203 k Ω . The current traces in B are from an electroreceptor whose current voltage relation (not shown) is similar to that in C. However, the traces in B show several inward current peaks during small stimuli. These inward current peaks blend together with progressively larger stimuli. Above 28 mV, only one peak occurs. The holding potential in B is -18 mV. The vertical calibration represents 1 μA in B and 2.4 μA in A. The horizontal calibration represents 100 ms in B and 220 ms in A.

ampulla when electrically isolated in the absence of applied current (Clusin and Bennett, 1977). For inhibitory (lumen positive) stimuli, the leakage current is plotted as a function of voltage. For excitatory stimuli, the peak early current and the late current at the end of the stimulus are plotted as functions of voltage. The peak early current is linearly related to voltage above about 30 mV. The late current is linearly related to voltage between 0 and about 100 mV. It will be shown below that the early current represents the sum of the leakage current and an early active transepithelial current. This early active current does not inactivate. The late current therefore represents the sum of three currents: the leakage current, the early active current, and a late active current which flows outward across the luminal membranes of the receptor cells.

In spite of the voltage clamp, onset of the late outward current will tend to repolarize the luminal membrane because of the series resistance of the basal membrane. It is therefore conceivable that a voltage-dependent reduction in the inward calcium current contributes to the transition to late outward current. Several lines of argument to be detailed below indicate that this is at most a minor effect.

In Fig. 4A the transepithelial current and the postsynaptic potential are shown during an excitatory voltage displacement. Tetrodotoxin is present in the saline bathing the afferent nerve fibers to block postsynaptic action potentials. Each of the inward current peaks is associated with a depolarizing peak in the PSP. Moreover, the falling phase of the PSP corresponds to the onset of the sustained late outward current across the receptor epithelium. This correspondence suggests that the early inward current flows outward across the secretory (basal) faces of the receptor cells depolarizing them, and that the late outward current repolarizes the secretory membranes. When progressively larger lumen-negative stimuli are applied, the postsynaptic potential becomes smaller and is eventually blocked. Fig. 4B shows data obtained from an ampulla with a current voltage relation similar to that in Fig. 3C. Synaptic transmission is blocked above 115 mV lumen negative. Presumably, the early inward current flowing through the luminal faces of the receptor cells does not sufficiently depolarize the secretory membranes to initiate transmitter release (Obara and Bennett, 1972).

In Fig. 4B the total early current is outward beyond +82 mV. This early current is the sum of the early active current flowing through the receptor cells and the leakage current. There are three possible sources of leakage conductance. Leakage current could flow through the receptor cells like the active currents. It could flow through the supporting cells, or it could traverse the tight junctions between cells. Since transmitter release is not suppressed between +82 and +115 mV, the direction of current flowing across the receptor cells during the PSP must be inward across the luminal membranes and outward across the secretory membranes. However, the total early current at these voltages is in the opposite direction. This indicates that some of the leakage current flows through pathways other than the receptor cells. These pathways may include the supporting cells and the intercellular clefts.

An important difference between the active currents in skate electroreceptors and those recorded in axons is seen when large stimuli are applied. In axons, onset of both early and late currents is hastened during large stimuli (Hodgkin

and Huxley, 1952*c*). In skate electroreceptors, however, onset of the late outward current during increasingly large excitatory stimuli becomes slowed and delayed. In Fig. 3 A the late outward current is incompletely activated by the 470-ms stimuli between 100 mV and 119 mV. Above 128 mV, activation of the late outward current is completely suppressed (Fig. 3 C); nor does any activation of the late outward current occur when the pulse is lengthened to more than 1 s.

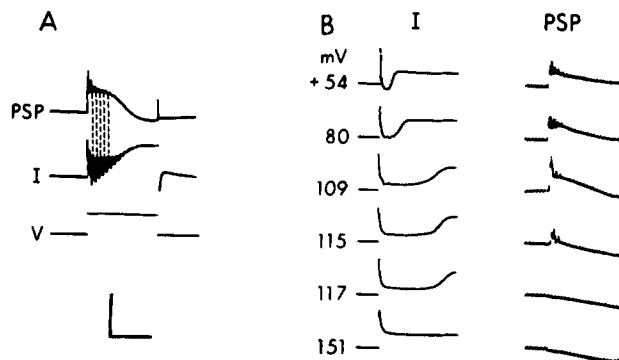


FIGURE 4. Relation of the epithelial currents to synaptic transmission. In A, an 11-mV excitatory (lumen negative) stimulus (lower trace) is applied to an ampullary epithelium held at -6 mV. Multiple peaks of inward current are followed by a sustained outward current (middle trace). A simultaneous DC-coupled extracellular recording of the postsynaptic potential is shown in the upper trace. Depolarization of the postsynaptic membrane (which produces a positive potential in the postsynaptic electrode farthest from the ampulla) is shown upward. The sodium-dependent action potentials in the nerve are abolished by addition of 10^{-8} M tetrodotoxin to the saline bathing the basal surface of the ampulla (Steinbach, 1974). The early inward current is associated with an excitatory postsynaptic potential, which is terminated by onset of the late outward current. B shows the effects of large excitatory stimuli. As the active inward current (total early current minus leakage) is progressively reduced, the postsynaptic potential becomes smaller and is suppressed between $+115$ and $+117$ mV. The holding potential in B is -12 mV. Tetrodotoxin is not present and several fast sodium spikes are superimposed on the PSP. There are two recording artifacts in the postsynaptic trace. There is a low level of 60 Hz interference throughout the sweep, and a progressive downward deflection of the trace during strong stimuli, which results from electrode polarization. The vertical calibration represents an epithelial current of $0.2 \mu\text{A}$ in A and $2.25 \mu\text{A}$ in B; a PSP amplitude of $400 \mu\text{V}$ in A and $225 \mu\text{V}$ in B, and an epithelial voltage of 20 mV in A. The horizontal calibration represents 200 ms in A and 225 ms in B.

Absence of Inactivation of the Early Current

Internal perfusion of the lumen during current clamp experiments (see Clusin and Bennett, 1977) suggested that the early inward current across the luminal membranes is carried by calcium. A common feature of voltage-dependent calcium currents is the absence of inactivation over periods of at least several seconds (Keynes et al., 1973; Katz and Miledi, 1971; Baker et al., 1973). However, other calcium currents appear to undergo fairly rapid inactivation (Standen, 1974; Reuter, 1973).

Three observations suggest that the early current in skate electroreceptors does not undergo inactivation, at least for many seconds. First, when large voltage stimuli are applied, which approach or exceed the suppression potential for the late current, the early current is maintained at a constant level for hundreds of milliseconds. Second, current clamp experiments in low calcium demonstrate prolonged plateau-shaped action potentials that do not spontaneously repolarize within 10 s. Third, experiments with prepulses (Fig. 5) fail to demonstrate inactivation. In squid giant axons, Hodgkin and Huxley (1952*b*) showed that depolarizing prepulses which produce no detectable sodium current reduce the size of the sodium current evoked by a subsequent test pulse. Hyperpolarizing prepulses increase the sodium current. In skate electrorecep-

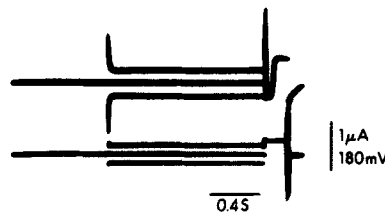


FIGURE 5. Absence of inactivation of the early inward current. The epithelium is held at -50 mV and three stimulus configurations are applied 10 s apart. The three sweeps are superimposed. During the first sweep, the voltage (lower trace) is stepped to -13 mV for 1.5 s (the prepulse) and then to $+10$ mV for 0.2 s (the test pulse). The prepulse evokes no active current. The test pulse evokes an early inward current followed by a late outward current (upper trace). During the second sweep, the epithelium is stepped to -87 mV for 1.5 s and then to $+10$ mV for 0.2 s. Again, there are no active currents during the prepulse, but an early inward and a late outward current occur during the test pulse. During the third sweep there is no prepulse. The test pulse, $+10$ mV for 0.2 s, again evokes early and late currents. The superimposed current traces show that there is no variation in the size or time course of the early and late currents evoked by the test pulse during the three sweeps.

tors, the size of the inward current evoked by a test pulse is not affected by prepulses spanning a 70-mV range, which do not themselves activate the early current.

Measurement of Epithelial Conductance

In order to study the conductance changes responsible for the two active currents, it is necessary to make measurements of epithelial resistance more rapidly than the active conductances develop. Measurement of the "instantaneous" epithelial resistance is possible only if the prolonged capacity currents described above can be subtracted out. These prolonged capacity currents are attributed to the supporting or marginal zone cells on morphological grounds. If so, they flow in parallel with the active currents generated by the receptor cells. If the supporting cells are electrically linear, the time course of the prolonged capacity current will be independent of the transepithelial potential. Moreover, it will not be affected by conductance changes occurring in the receptor cells.

Fig. 6 shows that the time course of the prolonged transient current in skate electroreceptors is independent of a steady transepithelial voltage and is unaffected by the active currents. An identical lumen-positive voltage step is applied across the epithelium from three different base-line potentials. In Fig. 6 A the epithelium is held at its resting potential. The steady-state current after the prolonged transient is the leakage current. In Fig. 6 B, the epithelium is stepped

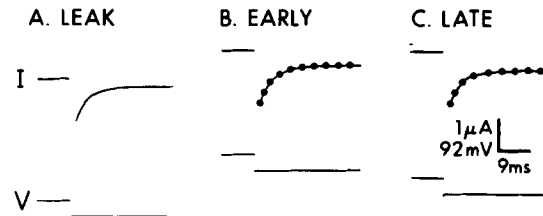


FIGURE 6. The capacity transient is unaffected by the presence of active currents. In A, B, and C a 45-mV lumen-positive test stimulus (lower trace) is applied under three different conditions and the resulting capacity currents are displayed at a fast sweep speed. In A, the epithelium is held at its resting potential of -19 mV. A capacity transient and a maintained leakage current occur. In B, the epithelium is depolarized to $+115$ mV 75 ms before the onset of the test stimulus. The early current is fully activated when the test stimulus occurs, but the late current, whose onset is considerably delayed at $+115$ mV, has not yet begun to activate. The total current would therefore be constant during the 37-ms sweep if there were no test stimulus. The test stimulus brings the epithelium to $+70$ mV, and produces a capacity transient which decays to a maintained level. In C, the epithelium is depolarized to $+55$ mV 400 ms before the onset of the test stimulus. By 400 ms, the early inward and late outward currents are fully activated. Current would therefore remain constant if there were no test stimulus. The test stimulus brings the epithelial potential to $+10$ mV and produces a capacity transient, followed by a maintained current. A current voltage relation of the ampulla used in this experiment (Fig. 7 C) shows that the slope resistance of the early current is constant between $+8$ and $+130$ mV, while the slope resistance of the late current is constant between $+1$ and $+80$ mV. Thus, the brief lumen-positive test stimulus in B and C would not change the slope resistance of the epithelium. The filled circles represent superimposition of the capacity transient in A on the capacity transients in B and C. For each pair of traces, the difference between the final currents is subtracted from the total current at various times. The fact that all of the filled circles fall on the current transients actually recorded indicates that all three transients are identical.

to $+115$ mV about 75 ms before the beginning of the trace. This large excitatory voltage displacement initiates an early active current. The total current remains constant for about 100 ms until the onset of the late active current (see Fig. 3 A). The lumen-positive voltage step is superimposed on the initial steady current before the onset of the late active current. In Fig. 6 C the voltage is stepped to $+55$ mV about 400 ms before the beginning of the trace. Both the early and late conductances activate before the superimposed lumen-positive voltage step which occurs during the maintained late outward current. Although the lumen-positive voltage displacement in Fig. 6 is applied from three different potentials, when the epithelium is in three different conductance states, the amplitude and

shape of the prolonged transient currents are identical in all three traces. The superimposability of these current transients supports the inference that they arise from a membrane capacitance which charges through a fixed resistance. Moreover, the fact that the prolonged capacity transients are unaffected by the presence of steady-state active currents suggests that the active currents and prolonged capacity currents flow through anatomical structures that are electrically in parallel. It therefore appears legitimate to subtract the prolonged capacity transients from the total current during onset of the active currents. For this purpose, symmetric voltage displacements of opposite polarity can be used. This technique was employed by Armstrong and Bezanilla (1974) and by Keynes and Rojas (1974) to discern the sodium channel gating current in squid axons.

Granted that the prolonged transient is capacitative in origin, the instantaneous ionic current produced by a lumen-positive stimulus will be equal to the leakage current measured at the end of the pulse. Then, since the leakage current measured by using long stimuli is linearly related to voltage, the "instantaneous" ionic current is also linear for stimuli which do not evoke active currents. If there is no rectification of the leakage resistance during strong lumen-negative stimuli, then the current immediately after the voltage displacement will be equal and opposite to the current produced by an equal lumen-positive stimulus.

Fig. 7 A shows the current evoked by two identical voltage pulses of opposite polarity. Subtraction of these two currents gives the current indicated by the open circles. The fact that the net current at 0.9 ms is zero indicates that the leakage resistance is the same for both stimuli. The net current is therefore the early inward current evoked by the excitatory voltage stimulus. Like other voltage-dependent currents, the early active current in skate electroreceptors has a sigmoidal onset. However, the sigmoidal shape has no simple interpretation, since individual receptor cells probably escape from the clamp and do so at somewhat different latencies (see Discussion).

Fig. 7 B shows a family of early active currents obtained by subtraction of the capacity and leakage currents using symmetric pulses. As with other voltage-dependent currents, the onset of the early active current is more abrupt during strong excitatory stimuli. As in Fig. 7 A, none of these traces shows a difference in the leakage current at 0.9 ms. Thus the leakage resistance remains constant over a broad voltage range. The invariance of the leakage resistance is indicated by the open triangles of Fig. 7 C. Each point is determined for a pair of equal and opposite stimuli that give equal transients at 0.9 ms. Leakage current for both stimuli is therefore taken as the maintained current at the end of the lumen-positive stimulus. (With very large voltage displacements, prolonged transient currents become so large that accurate subtraction is not possible.) Since the leakage resistance does not rectify up to 60 mV lumen negative (80 mV from the resting potential), it appears justified to determine the reversal potential for the early current by subtraction of the calculated leakage. In Fig. 7 C, this reversal potential is the point where the leakage line intersects with the early current. Since the early current results from an active conductance change in the luminal membranes, the reversal potential must be the transepithelial voltage at which the luminal membranes reach the reversal potential for the early active

current. This reversal potential is also the suppression potential for the late outward current. The suppression potential in voltage clamp experiments corresponds to the potential at which the repolarizing process is suppressed in current clamp experiments (Clusin and Bennett, 1977). The fact that the late outward current is suppressed very near the reversal potential for the early current suggests that the late current is initiated by a calcium influx across the luminal membranes.

Instantaneous current voltage relations can also be constructed for the two active currents by applying symmetrical voltage stimuli and subtracting the prolonged transient currents. For this purpose voltage steps of different amplitudes are superimposed on a prolonged excitatory stimulus. The capacitative component of the current produced by each voltage step is determined by applying an equivalent lumen-positive step to the inactive epithelium and taking capacitative current as total current minus maintained current. Capacitative current is then subtracted from the current produced by each superimposed stimulus leaving the ionic current. In Fig. 7 C, the points indicated by open squares are the ionic currents 0.9 ms after voltage steps that occurred during the maintained early current. The points indicated by open circles are the ionic currents 0.9 ms after voltage steps that occurred during the late outward current. The figure shows that the instantaneous current voltage relations obtained during the early and late current are identical with the current voltage relations obtained by using long pulses. These results confirm the tentative conclusions drawn from slope resistance measurements: (a) that the onset of the early active current is associated with a real increase in the transepithelial conductance; and (b) that the onset of the late active current is associated with a further increase in the transepithelial conductance.

Effects of Perfusing the Lumen with EGTA

Perfusion of the lumen of a voltage-clamped ampulla with a calcium-free solution containing 20 mM EGTA confirms earlier evidence that the inward current is carried by calcium. Removal of calcium from the ampulla, together with inward diffusion of free EGTA, would eventually reduce the concentration of calcium at the receptor cells to a very low level. Fig. 8 B shows the currents obtained after 30 min of perfusion. Both the early inward and late outward currents seen in the control records (Fig. 8 A) are absent. (A rather high concentration of EGTA has been used because of diffusion limitations. Reversibility of the EGTA effect has not been investigated for the calcium-free solution but is demonstrated for the calcium-EGTA solution as described below.) These observations support the inferences that the inward current is carried entirely by calcium, and that the initiation of the late outward current requires a calcium influx.

Further confirmation is obtained from currents recorded as the concentration of ionized calcium is gradually reduced. When the lumen is perfused with 10 mM calcium and 20 mM EGTA the decline in ionized calcium is slower than during perfusion with zero calcium and 20 mM EGTA. Fig. 9 B shows currents obtained after 15 min of perfusion with the solution containing calcium and EGTA. Data are plotted with the leakage current subtracted. (The leakage

resistance is unchanged from the control records shown in Figs. 9 A, 3 A, and 3 C). Since ionic equilibration is undoubtedly incomplete, it is not possible to determine the ionized calcium concentration at the receptor cells. However, the reversal potential for the early current and the magnitude of the early current at all voltages are reduced. Significantly, the suppression potential for the late outward current is still the same as the reversal potential for the early current. Fig. 9 C shows data obtained after 50 min of perfusion with the control solution (10 mM Ca and no EGTA). Although the leakage resistance has fallen, subtraction of the leakage current shows that considerable recovery of the early inward current occurs. Again, the suppression potential for the late outward current is the same as the reversal potential for the early current. The identity of these potentials at different calcium concentrations suggests that the late current is suppressed when the luminal membranes reach the calcium equilibrium potential.

Repolarization Tail Currents

When the epithelium is repolarized before onset of the late outward current

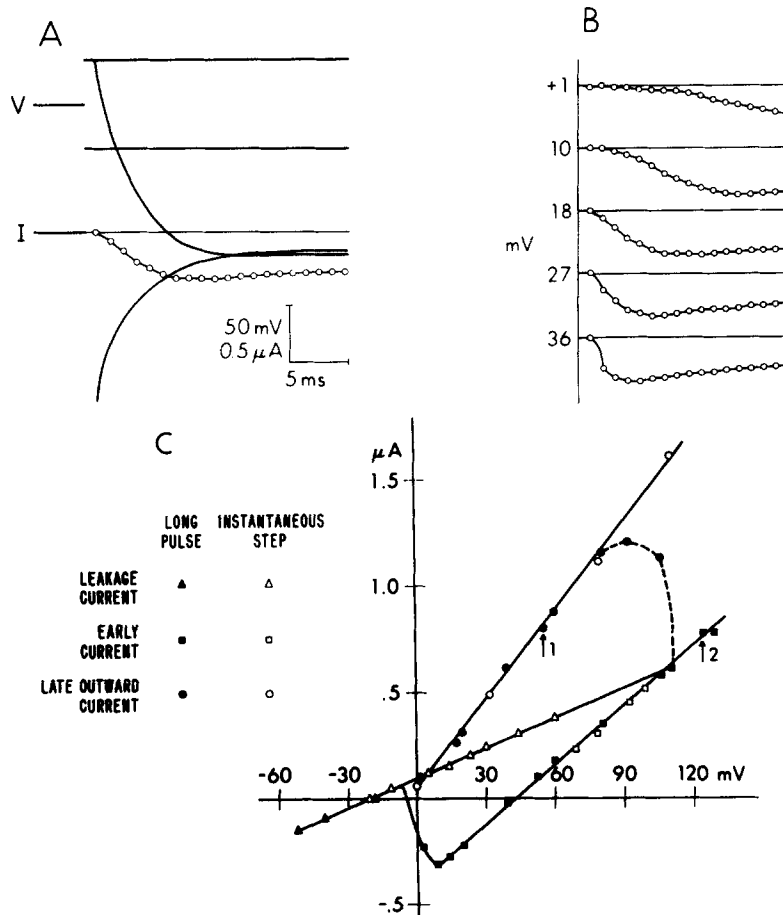


FIGURE 7

(Fig. 10 C and D, trace 1) there is only a brief tail current buried in the capacity transient. This brief tail current is attributable to the early calcium conductance. However, when the epithelium is repolarized after activation of the late outward

FIGURE 7. A, Subtraction of prolonged transient currents to obtain net active current. Two sweeps are superimposed. During the first sweep, a 36-mV lumen-positive stimulus hyperpolarizes the epithelium from its resting potential of -18 mV to -54 mV. Current during the stimulus is the sum of a leakage current and a capacity transient. During the second sweep a 36-mV lumen-negative stimulus depolarizes the epithelium to $+18$ mV. Current produced by this stimulus represents the sum of an oppositely directed capacity transient, a leakage current, and any active current that develops during the 21-ms stimulus. Open circles represent the difference between the currents produced by these stimuli at 1-ms intervals. The fact that there is no difference in total current at 0.9 ms indicates that the linearity of the leakage resistance extends into the lumen-negative region of the current voltage relation. To improve resolution, activation of the early current is slowed by cooling to 5°C . B, Kinetics of the early inward current, as demonstrated by subtraction of prolonged transient currents. Differences between currents evoked by pairs of equal and opposite stimuli are plotted at 1-ms intervals. Integers to the left represent the epithelial voltage during the excitatory stimulus. The holding potential is -18 mV and the temperature is 5°C . Onset of the early current is sigmoidal at all voltages, but is much faster for large excitatory stimuli. At $+36$ mV, complete activation of the early current occurs within 3 ms. Oscillations like those in Fig. 3 B were not seen in this preparation, even for stimuli in the negative slope region. C, Comparison of slope resistances with "instantaneous" resistances obtained by subtraction of the prolonged current transients. Filled symbols represent currents obtained during 500-ms current pulses as in Fig. 3 C. Open symbols represent instantaneous changes in ionic current obtained by subtraction of the prolonged capacity transients. *Leakage current*: The capacitive component of the transient current evoked by a lumen-positive stimulus has been computed by subtraction of the current at the end of the stimulus from the total current. The capacitive current at 0.9 ms is then subtracted from the total current evoked by an equal lumen-negative stimulus. *Active currents*: For the early current, a long pulse has been applied which brings the epithelium to the point indicated by arrow no. 2 on the current voltage relation. Lumen-positive stimuli of varying size are then superimposed on the long pulse during the early current. For each lumen-positive stimulus, the capacitive current is determined by applying the same stimulus from the resting potential. Subtraction of the capacitive component of the current at 0.9 ms gives the "instantaneous" change in ionic current produced by the superimposed stimulus. A similar technique is used for the late current with a long pulse which brings the epithelium to the point indicated by arrow no. 1 on the current voltage relation. The open symbols show that the "instantaneous" resistance of the epithelium is equal to the slope resistance in all of the linear regions of the current voltage relation. The instantaneous resistance remains constant even when the test pulse is large enough eventually to change the active conductance. The epithelial resistance is 109 k Ω during the early current and 75 k Ω during the late outward current. The leakage resistance is 218 k Ω . The intersection of the leakage line with the early current gives the equilibrium potential for the early current. This equilibrium potential at 5°C is $+112$ mV, which is also the suppression potential for the late current. The equilibrium potential for the early current is always somewhat lower at 5°C than at 10°C .

current, tail currents lasting about 600 ms are observed (Fig. 10 A, trace 1; Fig. 10 C and D, trace 4). These tail currents are always inward when the epithelium is repolarized to its resting potential. However, with lesser degrees of repolarization, prolonged outward tail currents are observed as shown in Fig. 10 B (see also

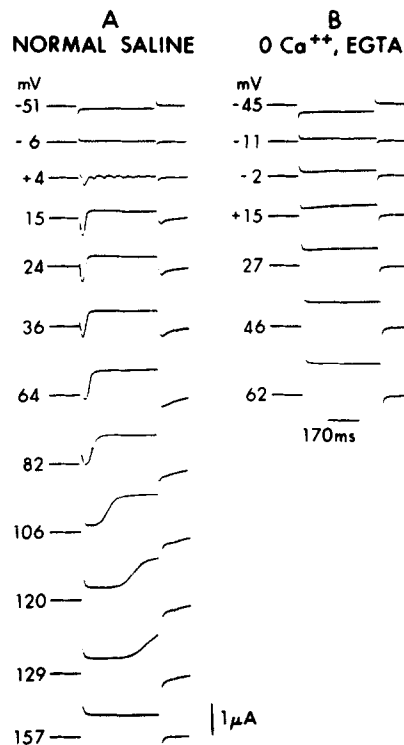


FIGURE 8. Blockage of active currents during perfusion of the lumen with a calcium-free solution containing EGTA. In A the lumen is perfused with the "control solution" whose composition is given in the previous paper (Clusin and Bennett, 1977). The epithelium is held at its resting potential of -20 mV, the leakage resistance is 278 k Ω , and the temperature is 10°C . The reversal potential for the early current and the suppression potential for the late current are both $+145$ mV. Currents in B have been obtained after 30 min of perfusion with a calcium-free solution containing 20 mM EGTA. The early inward and late outward currents are nearly abolished, 3.5 h have elapsed since dissection, and the leakage resistance has fallen to 113 k Ω . A fall in leakage resistance is common after 3 h, but the active currents ordinarily remain stable.

Fig. 4 A). The reversal potential for the prolonged tail current in Fig. 10 B is about -7 mV.

These prolonged tail currents are attributed to the late active conductance for two reasons:

- (a) When the epithelium is repolarized during onset of the late current, prolonged tail currents with a similar time course are observed, but the magni-

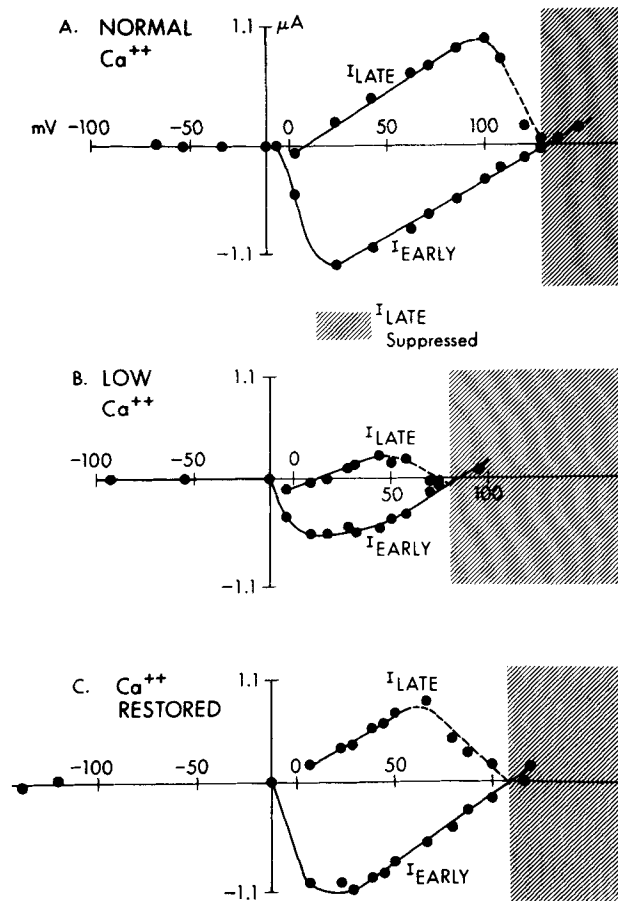


FIGURE 9. Correspondence between the reversal potential for the early current and the suppression potential for the late current at different ionized calcium concentrations. In A the current voltage relation in Fig. 3 C is replotted after subtraction of the leakage current calculated by extrapolation of the line I_{LEAK} . The leakage resistance is 203 $\text{k}\Omega$, the holding potential is -12 mV, the temperature is 10°C , and the lumen is perfused with the control solution. B shows data after 15 min of perfusion with a solution containing 10 mM Ca and 20 mM EGTA. The leakage resistance is unchanged but the maximum early current is reduced to 49% of its original value, and the reversal potential for the early current has fallen from +128 mV to +87 mV, with a corresponding reduction in the suppression potential for the late outward current. The perfusate is then switched to the control solution and after 50 min the data in C are obtained. 4 h have elapsed since dissection and the leakage resistance is 40 $\text{k}\Omega$. However, subtraction of the leakage current shows that considerable recovery of the active currents has occurred. The maximum inward current has increased to 82% of the control value. The equilibrium potential for the early current and the suppression potential for the late outward current have risen to 109 mV.

tude of the tail current depends on the degree to which the late outward current has become active (Fig. 10 C and D, traces 2 and 3).

(b) Prolonged tail currents are not observed when the stimulus voltage exceeds the suppression potential of the late current (Fig. 10 A).

Large stimuli presumably fail to activate the late current because they exceed

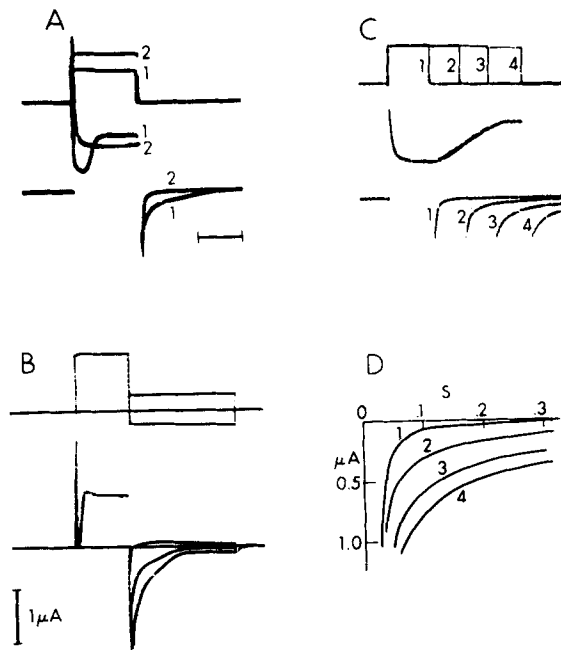


FIGURE 10. Repolarization tail currents recorded from skate electroreceptor epithelium. In A, two sweeps with voltage steps of different amplitude are superimposed. The smaller stimulus, 1, evokes a late outward current and a repolarization tail current lasting 600 ms. The larger stimulus, 2, exceeds the reversal potential for the early current, and evokes no late current and no prolonged repolarization tail. In B, four sweeps are superimposed. During three sweeps, the epithelium is depolarized from the holding potential of -29 mV to $+60$ mV, evoking an early inward and late outward current. The epithelium is then repolarized to a different voltage in each sweep, producing a different repolarization tail current whose magnitude and direction depend on voltage. When the epithelium is repolarized to -4 mV, a very small outward tail current is observed, but at more negative voltages, -29 and -52 mV, the tail current becomes inward. The stimulus is omitted during the fourth sweep to show the base line. In C, sweeps with voltage steps of identical amplitude but different duration are superimposed. The initial portion of the tail currents after repolarization are displayed on a common time scale in D. When the stimulus ends before onset of the late outward current (1), only a brief repolarization tail current occurs. When the stimulus occurs after the late current is fully activated (4), a large prolonged tail current occurs. Interruption of the stimulus during activation of the late current (2 and 3) produces tail currents similar in time course to the prolonged tail current, but of smaller amplitude. The vertical calibration represents 200 mV in A and C and 80 mV in B. The horizontal calibration represents 0.35 s in A and B and 0.17 s in C. All superimposed sweeps occurred more than 10 s apart.

the calcium equilibrium potential. Under current clamp conditions, delayed repolarization occurs after a large stimulus because the active calcium conductance keeps the luminal membranes depolarized until the repolarizing process is initiated (Clusin and Bennett, 1977). However, in voltage clamp experiments, repolarization of the epithelium occurs immediately, and the calcium conductance apparently falls before there is sufficient inward calcium current to activate the late outward current. The brevity of the calcium tail currents observed under conditions where there is no late outward current also suggests that the prolonged tail currents do not include calcium current.

The Relation Between the Inward Current and the Latency of the Late Outward Current

Voltage clamp records show that the onset of the late outward current is progressively delayed and slowed as the epithelial voltage approaches the suppression potential for the early current. Near the suppression potential there is no detectable onset of late outward current for several hundred milliseconds. As in respect to onset of inward currents, the rise in outward currents is distorted compared to what would be obtained with well-clamped membrane. Because of the resistance of the basal membranes, (see Discussion) the luminal membranes are partially repolarized by the late current, and the driving force for calcium is increased during onset of the late current. The resulting increase in calcium influx will allow the late current to turn on regeneratively. This escape from the clamp will show some dispersion in time among different cells. Despite these difficulties, we have attempted to investigate the relation between the rate of calcium influx and the latency of the late current. The analysis is simpler because the calcium current does not inactivate. To minimize problems arising from inadequate clamping we measure the time required for 10% activation of the late outward current, $T_{0.1 \text{ ACT}}$, as illustrated in Fig. 11 B (and a smaller degree of activation could have been used equally well). In Fig. 11 A the reciprocal of the latency $1/T_{0.1 \text{ ACT}}$ is plotted as a function of voltage for currents in the experiment of Fig. 8 A. The plot is linear and intersects the voltage axis near E_{Ca} .

A simple interpretation of the foregoing result is that a constant amount of calcium must enter before the outward current begins to turn on. The calcium current should be given by the driving force for calcium (the membrane potential minus calcium equilibrium potential) times the calcium conductance. Since the calcium conductance does not inactivate, calcium current is constant after a brief period for activation and before onset of the late current. Thus, if a constant amount of calcium influx is required before the outward current turns on, the latency of onset will be inversely proportional to the driving force for calcium, as is seen in Fig. 11 A. This constancy is measured over a transepithelial potential difference of some 90 mV and for times between ~5 and 200 ms. What these observations suggest about intracellular calcium movements is discussed below.

Comparison of Current and Voltage Clamp Experiments

In the preceding paper (Clusin and Bennett, 1977) current voltage relations were obtained by passing constant current across the ampullary epithelium. The

peak voltage of the action potential, the steady-state voltage after the action potential, and the voltage produced by a subthreshold stimulus were found to be linearly related to current. To determine whether an identical current voltage relation is obtained under voltage clamp conditions, a single ampulla can be studied by both techniques. In current clamp experiments the points obtained

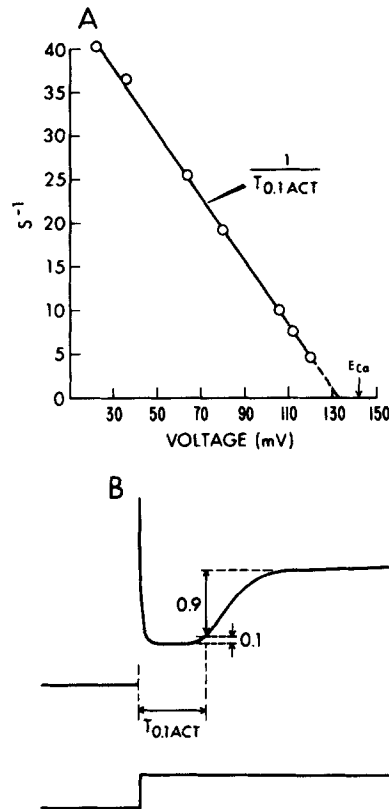


FIGURE 11. The latency of the late current is inversely related to voltage. The time required for activation of 10% of the late outward current, $T_{0.1ACT}$, is defined in B. The reciprocal of $T_{0.1ACT}$, determined for each of the records in Fig. 8 A between +24 and +120 mV, is plotted in A, and is linearly related to voltage with an intercept near E_{Ca} .

by using subthreshold stimuli fall along the leakage line obtained with voltage clamp. Similarly, the steady-state current voltage relation after the action potential coincides with the late outward current line. However, the peak of the action potential does not lie along the line determined by the peak early current. The action potential evoked by a given stimulus current is smaller than the voltage required to produce a corresponding early current.

In voltage clamp experiments, the epithelial potential can be stepped to a command voltage in 100 μ s. For excitatory stimuli outside the negative resistance region, the early current reaches half its peak value in less than 3 ms (Fig. 7), but

the late current requires more than 42 ms for half-activation (Figs. 3, 8). This difference in latencies suggests that the peak inward current arises solely from the early conductance, which is maximal. Near the suppression potential, the temporal separation of early and late currents is obvious from inspection of the current traces (Fig. 3). The early currents obtained near the suppression potential are colinear with the peak inward currents obtained at lower voltages (Fig. 7). This colinearity confirms that the peak inward current at moderate voltages is a pure early current.

Under current clamp conditions, epithelial voltage changes so slowly that some activation of the late current occurs during the rising phase of the action potential. In Fig. 7 of the preceding paper (Clusin and Bennett, 1977), the peak voltage of the action potential is never reached in less than 90 ms. Thus, the peak voltage of the action potential, unlike the peak early current, reflects both the early and late conductances. Even when the suppression potential is exceeded, some activation of the late current may occur during the rising phase. These considerations account for the discrepancy between the I-V relations obtained with current and voltage stimuli. Although the suppression potential for the late current can be determined from the current clamp data, the reversal potential for the early current cannot.

Facilitated Onset of the Late Outward Current

Onset of the late outward current in skate electroreceptors can be facilitated by prior stimulation. In Fig. 12 A, two identical voltage pulses are applied. The second pulse occurs 2 s after the first. The conductance of the epithelium returns to normal between pulses, as can be demonstrated by interposing a lumen-positive pulse; and the size of the early current evoked by the two lumen-negative stimuli is uniform. However, onset of the late outward current occurs considerably earlier during the second stimulus (trace 2). Up to 10 s between stimuli are required for the epithelium to return to its initial condition.

Facilitation of the late outward current by the conditioning stimuli could result either from the change in the transepithelial voltage or from a change in the inward calcium current during the conditioning stimulus. Fig. 12 D and E shows that facilitation occurs only if there is a net inward current during the conditioning stimulus. In Fig. 12 D, two stimuli of the same amplitude and duration have been applied 2 s apart. The onset of the late outward current in trace 2 is clearly earlier than in trace 1. In Fig. 12 E the conditioning stimulus to the same ampulla (trace 1) is very large. It evokes no late current because it exceeds the reversal potential for the early current. 2 s later, a stimulus identical to that used in Fig. 12 D is applied (trace 2). The preparation is then rested for 1 min and the same stimulus is repeated (trace C). Fig. 12 E shows that the conditioned stimulus and the unconditioned stimulus applied 1 min later evoked currents that are superimposable. Thus, the large excitatory stimulus, which does not cause a net inward current, does not facilitate onset of the late outward current. The facilitation is therefore due to inward current, presumably a calcium influx, rather than to a change in voltage. (The small inward tail current that the large excitatory stimulus produces is evidently too small to cause any detectable effect.)

Fig. 12 B and C shows that the degree to which onset of the late outward current is facilitated can depend on the duration of the conditioning stimulus. In Fig. 12 B the two superimposed records are taken 2 s apart. In neither record is there significant activation of the late outward current, although the late current has just begun to activate at the end of the second stimulus. Presumably, a small

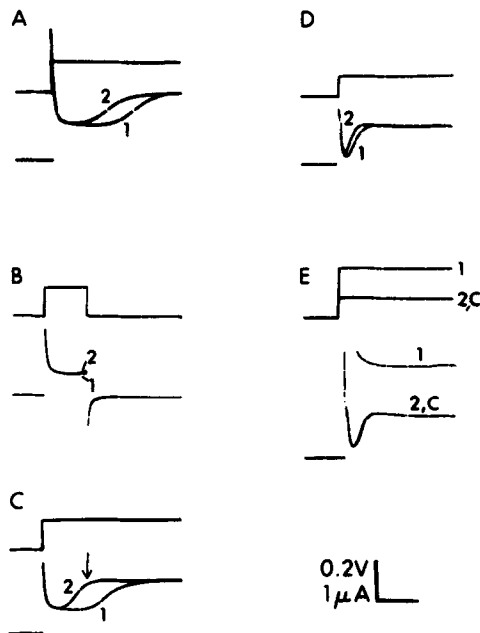


FIGURE 12. Onset of the late outward current is facilitated by a conditioning stimulus. In A, two uniform voltage steps are applied 2 s apart and the currents are superimposed. The early current is identical for the two stimuli, but the late outward current evoked by the test stimulus, 2, occurs at much shorter latency than that evoked by the conditioning stimulus, 1. In B, two stimuli 0.21 s in duration and 136 mV in amplitude are applied 2 s apart, and the resulting currents are superimposed. The holding potential is -25 mV. Little activation of the late outward current occurs during either sweep, but there is a small beginning in 2. In C, two 136-mV stimuli 0.7 s in duration are applied to the same preparation 2 s apart. During the test stimulus (2), activation of the late outward current is complete within 0.21 s (arrow). Thus, the 0.7-s conditioning stimulus in C produces much greater facilitation of the late outward current than the 0.21-s conditioning stimulus of equal amplitude in B. In D, two 100-mV stimuli 0.55 s in duration are applied 2 s apart. The holding potential is -60 mV. There is considerable facilitation of the late outward current evoked by the test stimulus (2). Data in E were obtained by using the same preparation. The conditioning stimulus (1) of 240 mV and 0.55 s exceeds the reversal potential for the early current and evokes no late outward current. The conditioned stimulus (2) is 0.55 s in duration and 100 mV in amplitude. A control stimulus (C) identical to the test stimulus is applied 1 min later. Currents evoked by the conditioned stimulus and the control stimulus are identical. The horizontal calibration represents 0.1 s in A and 0.2 s in B-E.

amount of calcium has entered during the first stimulus, for if the pulse is lengthened (Fig. 12 C), the late current is activated. In Fig. 12 C, two stimuli of the same amplitude as in Fig. 12 B, but of longer duration, have been applied to the same preparation 2 s apart. Both stimuli evoke late outward currents, indicating that they cause large calcium influxes. Onset of the late current evoked by the second stimulus (trace 2) is considerably earlier and more abrupt than in trace 1. In fact, onset of the late current in trace 2 occurs in less time than the total duration of the stimulus in Fig. 12 B (indicated by an arrow in Fig. 12 C). Thus, the facilitation of the late current by the long conditioning stimulus in Fig. 12 C is greater than that produced by a short conditioning stimulus of the same amplitude. Facilitated onset of the late outward current probably contributes to shortening and reduction in amplitude of the ampullary action potential during repetitive stimulation (Clusin and Bennett, 1977), since rapid inactivation of the early current, which commonly is a factor in relative refractoriness, does not occur in this tissue.

DISCUSSION

Electrical Model of the Ampullary Epithelium

EQUIVALENT CIRCUIT The results of the voltage clamp experiments lead to the equivalent circuit in Fig. 13, which is discussed in detail in subsequent sections. The ampullary epithelium is represented by several parallel elements. Leakage pathways through the supporting cells, marginal zone cells, and intercellular clefts are represented by a shunt resistor, R_{SH} . The effective internal potential of these pathways is represented by E_{SH} . (Batteries in Fig. 13 are unlabeled.) The ampullary capacitance is attributed to the basal membranes of the supporting and marginal zone cells. The exponential time course of the prolonged capacitive transient in voltage clamp experiments suggests that some of the ampullary capacitance is in series with a fixed resistance. The remainder of the ampullary capacitance charges rapidly and is represented by a capacitor without a series resistance.

Active currents arise in the receptor cells, one of which is shown in parallel with the passive elements. Capacities of the receptor cell membranes have been omitted because these represent only a small fraction of the total capacity of the ampulla. The luminal membrane is represented as a fixed resistance, r_{LUM} , in parallel with two active elements. One of these, r_{Ca} , gives rise to the voltage-sensitive early current. Its battery is presumably close to the calcium equilibrium potential, since chelation of calcium in the lumen abolishes the inward current. Voltage clamp experiments indicate that for excitatory voltage stimuli beyond the negative slope region, the early conductance becomes maximal within a few milliseconds and does not significantly inactivate in seconds. The other active element, r_{LATE} , is activated by the calcium influx and gives rise to the late outward current. Its battery is not identified. During sufficiently long-lasting stimuli the late conductance also becomes maximally active, except near or beyond the calcium equilibrium potential. The basal membrane is represented by a fixed resistance, r_{BAS} , in series with the luminal membrane. The series

resistance provided by the basal membranes limits the degree of voltage control in the presence of active currents.

By using the circuit diagram in Fig. 13 it is possible to relate the epithelial voltage and resistance measurements to receptor cell membrane properties. Let E_{EARLY}^* be the epithelial potential at which activation of the early conductance produces no change in current flowing across the receptor cells. In Figs. 3 C and 7 C, E_{EARLY}^* is the voltage at which the total early current (early active current plus leakage) intersects with the leakage line. Let E_{TOTAL}^* be the epithelial

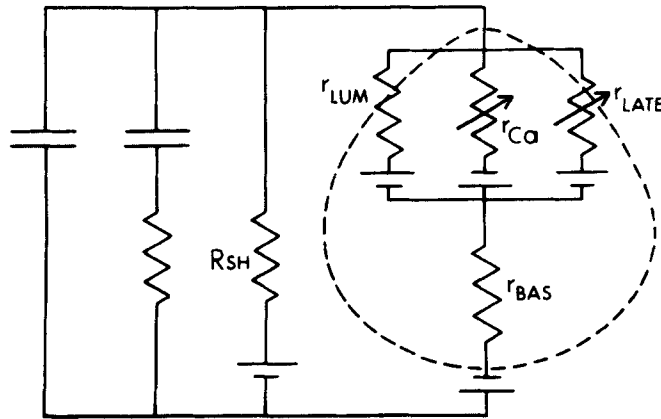


FIGURE 13. Equivalent circuit of the ampullary epithelium. Active currents arise in the luminal membranes of the receptor cells. These luminal membranes are represented by a fixed resistor, r_{LUM} , in parallel with two variable resistors. The early calcium current flows through r_{Ca} and the late current through r_{LATE} . The basal faces of the receptor cells are represented by a fixed resistance r_{BAS} . Most of the leakage current flows through the supporting cells, the marginal zone cells, or the intercellular clefts. These pathways are represented by a single resistor R_{SH} . The batteries E_{Ca} , E_{LUM} , E_{LATE} , E_{BAS} , and E_{SH} are drawn but not labeled. The ampullary capacity is attributed to cells that are electrically in parallel with the receptor cells, namely the supporting cells and marginal zone cells, whose basal membrane area is large. Some of the ampullary capacitance is in series with a significant fixed resistance.

potential at which the current flowing across the receptor cells is unchanged by maximal activation of both the early and late conductances. In Figs. 3 C and 7 C, E_{TOTAL}^* is the voltage at which the total late current (early active current plus late active current plus leakage) intersects the leakage line. Let E_{LATE}^* be the epithelial potential at which the prolonged repolarization tail current reverses direction. Since the early current channels close quickly, the prolonged tail current consists of late active current plus leakage. Let E_{LEAK}^* be the potential across the inactive epithelium in the absence of applied current. For the luminal membrane resistances r_{LUM} , r_{Ca} , and r_{LATE} , there are corresponding conductances g_{LUM} , g_{Ca} , and g_{LATE} . n identical receptor cells are assumed. If V_{LUM} is the potential across the luminal membranes when there is no net epithelial current, then the epithelial potentials E_{LEAK}^* , E_{EARLY}^* , E_{LATE}^* , and E_{TOTAL}^* are given by:

$$E_{\text{LEAK}}^* = \frac{E_{\text{SH}} (r_{\text{LUM}} + r_{\text{BAS}})/n + (E_{\text{LUM}} - E_{\text{BAS}}) R_{\text{SH}}}{(r_{\text{LUM}} + r_{\text{BAS}})/n + R_{\text{SH}}}, \quad (1)$$

$$E_{\text{EARLY}}^* = \frac{r_{\text{LUM}} + r_{\text{BAS}}}{r_{\text{LUM}}} (E_{\text{Ca}} - V_{\text{LUM}}) + E_{\text{LEAK}}^*, \quad (2)$$

$$E_{\text{LATE}}^* = \frac{r_{\text{LUM}} + r_{\text{BAS}}}{r_{\text{LUM}}} (E_{\text{LATE}} - V_{\text{LUM}}) + E_{\text{LEAK}}^*, \quad (3)$$

$$E_{\text{TOTAL}}^* = \frac{r_{\text{LUM}} + r_{\text{BAS}}}{r_{\text{LUM}}} \left(\frac{g_{\text{Ca}} E_{\text{Ca}} + g_{\text{LATE}} E_{\text{LATE}}}{g_{\text{Ca}} + g_{\text{LATE}}} - V_{\text{LUM}} \right) + E_{\text{LEAK}}^*. \quad (4)$$

V_{LUM} is affected by E_{SH} and E_{BAS} , as well as by the luminal membrane leakage battery, E_{LUM} :

$$V_{\text{LUM}} = E_{\text{LUM}} - (E_{\text{LUM}} - E_{\text{SH}} - E_{\text{BAS}}) \left(\frac{r_{\text{LUM}}/n}{(r_{\text{LUM}} + r_{\text{BAS}})/n + R_{\text{SH}}} \right). \quad (5)$$

Similarly, the epithelial resistances can be expressed in terms of receptor cell membrane resistances. Let R_{LEAK} be the resistance of the inactive epithelium, let R_{EARLY} be the resistance of the epithelium during maximal activation of the early current, and let R_{TOTAL} be the resistance of the epithelium when both early and late currents are maximally activated. Then,

$$\frac{1}{R_{\text{LEAK}}} = \frac{1}{R_{\text{SH}}} + \frac{n}{r_{\text{LUM}} + r_{\text{BAS}}}, \quad (6)$$

$$\frac{1}{R_{\text{EARLY}}} = \frac{1}{R_{\text{SH}}} + \frac{n}{\frac{1}{g_{\text{LUM}} + g_{\text{Ca}}} + r_{\text{BAS}}}, \quad (7)$$

$$\frac{1}{R_{\text{TOTAL}}} = \frac{1}{R_{\text{SH}}} + \frac{n}{\frac{1}{g_{\text{LUM}} + g_{\text{Ca}} + g_{\text{LATE}}} + r_{\text{BAS}}}. \quad (8)$$

Values for each of the circuit elements will be derived with the exception of absolute intracellular potentials which are unknown.

ELECTRICAL REPRESENTATION OF THE BASAL MEMBRANES OF THE RECEPTOR CELLS Perfusion experiments described in the previous paper (Clusin and Bennett, 1977) indicate that the basal membranes can be represented as a fixed resistance. This approximation is supported by the voltage clamp data. Figs. 3 and 7 show that lumen-positive stimuli which directly depolarize the basal membranes produce no change in epithelial resistance. When appropriate lumen-negative stimuli are applied, the basal membranes are indirectly depolarized by the active inward current across the luminal membranes. The magnitude of this active current varies linearly with voltage above 25 mV, and at 130 mV its direction reverses. However, the resistance of the epithelium during the early current is constant beyond 25 mV. Moreover, during the late outward current, when the basal membranes are hyperpolarized, the epithelial resistance

remains low. Thus, epithelial resistance is not greatly affected by changes in the voltage across the basal membranes of the receptor cells.

The basal membranes cannot be completely passive, since release of transmitter requires an inward calcium current across them, which can be demonstrated under certain circumstances (Clusin and Bennett, manuscript in preparation). However, the current voltage relation of the epithelium is little affected by treatment of the basal membranes with cobalt which abolishes synaptic transmission by blocking the basal membrane calcium current. It follows that representation of the basal membranes by a fixed resistor is a reasonable approximation.

Because excitation of the luminal membranes produces a fourfold increase in epithelial slope conductance under current clamp conditions, it was concluded that the basal membrane resistance must be at least three times smaller than the resting resistance of the luminal membranes. Calculations based on voltage clamp measurements show that the difference in resting resistance of the two receptor cell faces is actually much greater.

In the experiment shown in Fig. 4 B, synaptic transmission persists during excitatory (lumen-negative) voltage stimuli up to 115 mV. At this voltage, the total early current (early active current plus leakage) is $0.327 \mu\text{A}$ in the outward direction. In order for synaptic transmission to occur, the current flowing across the receptor cells must be inward across the luminal membranes and outward across the basal membranes. It follows that at 115 mV, $0.327 \mu\text{A}$ (plus a small inward current across the receptor cells) is flowing in the outward direction through R_{SH} . R_{SH} is therefore at least $352 \text{ k}\Omega$. The minimum value according to Eq. (6) is R_{LEAK} , which was found to be $322 \text{ k}\Omega$ in the experiment described in Fig. 4 B.

By substituting the maximum value of R_{SH} into Eq. (6) (a worst case assumption), it can be shown that the resistance across a single inactive receptor cell, $(r_{\text{LUM}} + r_{\text{BAS}})/n$, is greater than $n \times 3.8 \text{ M}\Omega$. Thus

$$\frac{(r_{\text{LUM}} + r_{\text{BAS}})/n}{R_{\text{SH}}} \geq 11. \quad (9)$$

In the experiment described above, the resistance of the epithelium during the early current, R_{EARLY} , is $110 \text{ k}\Omega$ and the resistance after activation of the late current, R_{TOTAL} , is $88 \text{ k}\Omega$. Substituting these values, and the minimum value of R_{SH} (a worst case assumption) into Eq. (7) and (8), one can calculate that the maximum resistance across a single receptor cell is $n \times 167 \text{ k}\Omega$ after activation of the early current and $n \times 121 \text{ k}\Omega$ after maximal activation of both currents. The resistance across the single receptor cell is therefore reduced at least 23-fold by activation of the early current and at least 31-fold by activation of both currents.

If one assumes that the resistance of the luminal membranes becomes negligible after activation of the early and late currents, it follows from the above that

$$\frac{r_{\text{LUM}} + r_{\text{BAS}}}{r_{\text{BAS}}} \geq 31; \quad (10)$$

giving

$$\frac{r_{\text{LUM}}}{r_{\text{BAS}}} \geq 30. \quad (10 a)$$

If one then assumes that the resistance of the basal membranes is negligible, it follows that

$$\frac{g_{LUM} + g_{Ca}}{g_{LUM}} \geq 23, \quad (11)$$

giving

$$\frac{g_{Ca}}{g_{LUM}} \geq 22. \quad (11 a)$$

Both of the above ratios are increased if the neglected membrane resistances are greater than zero.

Four conclusions from the foregoing discussion are necessary for the subsequent analysis:

- (a) The total conductance across the inactive receptor cells is much smaller than the conductance of the inactive epithelium. As a result, almost all of the leakage current flows through shunt pathways which are in parallel with the receptor cells.
- (b) Because r_{LUM} is much greater than r_{BAS} , over 96% of the voltage drop across the inactive epithelium occurs in the luminal membranes of the receptor cells.
- (c) Because r_{LUM} is much greater than r_{BAS} , less than 4% of the net active current generated by the luminal membranes flows back across r_{LUM} . The remaining 96% or more flows outward across the basal membranes.
- (d) For most purposes the resting conductance of the luminal membranes g_{LUM} can be neglected when g_{Ca} and $g_{Ca} + g_{LATE}$ are activated.

ELECTRICAL REPRESENTATION OF THE SHUNT PATHWAYS In the absence of applied current, the potential across the electrically isolated ampullary epithelium is typically 10–30 mV lumen positive. Since the conductance through the inactive receptor cells is at least 11-fold smaller than the conductance of the shunt pathways (Eq. [9]), it is unlikely that the receptor cells contribute significantly to the epithelial resting potential. Eq. (1) then reduces to

$$E_{LEAK}^* = E_{SH}. \quad (12)$$

Eq. (12) states that the epithelial resting potential is dominated by the potentials of the shunt pathway which include extracellular junction potentials and differences in the resting potentials of luminal and basal faces of the supporting cells.

Consideration of the very high luminal membrane resting resistance (neglecting R_{SH} and r_{BAS} as per Eq. [9] and [10 a]) also allows simplification of Eq. (5), giving:

$$V_{LUM} = E_{SH} + E_{BAS}. \quad (13)$$

Thus, the potential across the resting luminal membranes is determined almost entirely by the basal membrane leakage battery, plus whatever potential is imposed across the epithelium by sources external to the receptor cells, which include the supporting cells and stimuli of natural or experimental origin. The leakage battery of the luminal membrane, E_{LUM} , makes little contribution because r_{LUM} is high.

In the preceding paper (Clusin and Bennett, 1977), excitability of the ampullary epithelium was ascribed to the receptor cells because excitation evoked transmitter release from the receptor cell basal membranes. However, the possibility that the receptor and supporting cells had similar electrophysiological properties was not excluded. We can now conclude that there are important physiological differences between these cell types. As discussed above, only the basal membranes of the supporting and marginal zone cells have enough surface to account for the ampullary capacitance. Therefore, a substantial fraction of the transepithelial voltage drop must occur in these basal membranes, which means that the luminal membrane resistance must be low, especially in the cells responsible for the rapidly charging component of the ampullary capacity. In contrast, the excitable cells have a basal membrane resistance that is almost insignificant compared to the resistance of their resting luminal membranes, as indicated by the conductance increase during excitation. Thus, the active currents cannot be attributed to the supporting or marginal zone cells. The uniform time course of the capacity currents at different voltages indicates that the supporting and marginal zone cells are electrically linear. If the resistance of tight junctions is as high in the ampulla as in the canal, then the leakage current must flow mainly through the supporting and marginal zone cells. Moreover, E_{SH} , which dominates the epithelial resting potential, E_{LEAK}^* , would reflect differences in the potentials of the luminal and basal membranes of these cells.

We previously thought that the epithelial resting potential E_{LEAK}^* was set by the receptor cells. Of course, if all the luminal and basal membranes have the same ionic selectivities, the difference in resting potentials will be the same across all the cells in the epithelium.

SOLUTION OF THE EQUIVALENT CIRCUIT Because g_{LUM} is at least 22-fold smaller than g_{Ca} , g_{LUM} can be ignored in Eq. (7) and (8). Similarly, since r_{LUM} is at least 30-fold greater than r_{BAS} , r_{BAS} can be ignored in Eq. (2-4) and (6-8). Using these approximations together with Eq. (12) and (13), one obtains:

$$E_{EARLY}^* = E_{Ca} - E_{BAS}, \quad (14)$$

$$E_{LATE}^* = E_{LATE} - E_{BAS}, \quad (15)$$

$$E_{TOTAL}^* = \frac{g_{Ca}E_{Ca} + g_{LATE}E_{LATE}}{g_{Ca} + g_{LATE}} - E_{BAS}, \quad (16)$$

$$\frac{1}{R_{EARLY}} = \frac{1}{R_{SH}} + \frac{n}{\frac{1}{g_{Ca}} + r_{BAS}}, \quad (17)$$

$$\frac{1}{R_{TOTAL}} = \frac{1}{R_{SH}} + \frac{n}{\frac{1}{g_{Ca} + g_{LATE}} + r_{BAS}}. \quad (18)$$

By substituting Eq. (14) and (15) into Eq. (16) one obtains

$$\frac{g_{LATE}}{g_{Ca}} = \frac{E_{EARLY}^* - E_{TOTAL}^*}{E_{TOTAL}^* - E_{LATE}^*}. \quad (19)$$

Eq. (17-19) now contain three unknowns, r_{BAS} , g_{Ca} , and g_{LATE} , and can therefore be solved. In the experiment of Fig. 4 A, E_{EARLY}^* is +126 mV and E_{TOTAL}^* is +8 mV (at 5°C). E_{LATE}^* , although not precisely determined, lies between 0 and -12 mV. The values for R_{EARLY} , R_{TOTAL} , and R_{SH} are as above.

The resistance values listed in Table I were obtained by solution of the simultaneous Eq. (20-22) assuming $E_{\text{LATE}}^* = -7$ mV, and $R_{\text{SH}} = 352$ k Ω . The values of g_{Ca} and g_{LATE} are shown as the reciprocals, r_{Ca} and r_{LATE} . The calculations were repeated four times assuming -12 mV $\leq E_{\text{LATE}}^* \leq 0$, with $322 \leq R_{\text{SH}} \leq 352$ k Ω . The error shown for each resistance in Table I is the maximum deviation of the listed resistance value from one of the values calculated with extreme assumptions. Variation of R_{SH} between the two extreme values produces less than a 10% change in the calculated resistances. Changes in the assumed value of E_{LATE}^* produce less than a 10% change in the calculated values of r_{BAS} and r_{Ca} ; however, the calculated value of r_{LATE} varies over a threefold

TABLE I
MEMBRANE RESISTANCE VALUES

	Whole ampulla, r/n	1 receptor cell, r	1 cm ² of membrane, $r \times \text{area}$
	K Ω	M Ω	Ωcm^2
r_{LUM}	$\geq 3,800$	$\geq 38,000$	$\geq 3,000$
r_{BAS}	112 ± 6	$1,120 \pm 60$	$5,500 \pm 300$
r_{Ca}	48 ± 6	480 ± 60	38 ± 5
r_{LATE}	6.1 ± 3.0	61 ± 30	4.9 ± 2.4
$1/(G_{\text{Ca}} + G_{\text{LATE}})$	5.4 ± 2.5	54 ± 25	4.3 ± 2.0

The values of r_{Ca} and r_{BAS} are based on the assumption that $E_{\text{LATE}}^* = -7$ mV and $R_{\text{SH}} = 352$ k Ω . The errors represent maximum deviations of the listed value from values calculated with the extreme assumptions $E_{\text{LATE}}^* = 0$ and -12 mV, and $R_{\text{SH}} = 322$ and 352 k Ω . The calculated value of r_{LUM} does not depend on E_{LATE}^* . Values for a single cell (second column) assume 10^4 cells. Values in the third column assume the receptor cells to have a luminal membrane area of $8 \mu\text{m}^2$ and a basal membrane area of $500 \mu\text{m}^2$.

range. None of these variations affect the theoretical inferences that follow. Values for the single receptor cell are calculated on the assumption that the ampulla under discussion has 10^4 receptor cells. The specific resistivities are calculated by assuming a luminal membrane area of $8 \mu\text{m}^2$ per receptor cell and a basal membrane area of $500 \mu\text{m}^2$. These specific resistivities are comparable to values obtained in other excitable membranes.

From the data in Table I it can be shown that the luminal membrane resistance falls at least 70-fold during activation of the early conductance, and at least 413-fold after activation of both conductances. After activation of the early conductance about 30% of the resistance across the receptor cell is in the luminal membranes, and after activation of both conductances, this value falls to about 5%.

EFFECTS OF THE BASAL MEMBRANE RESISTANCE The value of the series resistance r_{BAS} in Table I is large enough that the luminal membrane voltage will vary significantly during changes in the active current across the voltage-clamped epithelium. In the experiment used for Table I, the net inward current produced by a 32-mV depolarization from the epithelial resting potential of -12

mV is $0.65/\mu\text{A}$. If the calculated value of τ_{BAS} is correct, this current will produce a 73-mV drop across the basal faces, bringing the luminal membrane from 32 to 105 mV positive to the resting potential. With onset of the late current, the net active current becomes $0.06 \mu\text{A}$ in the outward direction, repolarizing the luminal membrane from 105 to 25 mV positive to the resting potential. (The upper limit of the negative resistance region in this experiment is 22 mV positive to the resting potential.) These changes in membrane potential have three important effects on the current measurements:

(a) The kinetics of both the early calcium current and the calcium-activated late current are distorted. The on kinetics of the early current are distorted because the luminal membrane depolarizes regeneratively, tending to narrow the negative slope region. The on kinetics of the late current are distorted because repolarization of the luminal membrane increases the driving force for calcium, causing the calcium-activated late conductance to turn on regeneratively.

(b) Spatial inhomogeneities can arise, with different receptor cells escaping from the clamp at different times. These inhomogeneities can occur in the repolarizing as well as the depolarizing processes, since both are regenerative. Because of these inhomogeneities, it is likely that conductance changes in single cells occur over a narrower voltage range than the epithelial currents would indicate. Spatial inhomogeneities will be less important under current clamp and under voltage clamp during large excitatory stimuli, when the individual receptor cells are well synchronized.

(c) For small stimuli, repolarization of the luminal membrane produced by the late outward current may be sufficient to turn off calcium channels. Such an interaction between inward and outward currents was postulated to explain the oscillations in barnacle muscle fibers under conditions of inadequate clamping (Keynes et al., 1973). This explanation does not adequately explain the oscillations produced by skate electroreceptor under voltage clamp, which are much faster than the off kinetics of the late current. Further studies of these oscillations are therefore necessary.

For larger stimuli, our mathematical analysis of the late current and our conclusions about its latency assume that repolarization of the luminal membranes is not sufficient to effect the voltage-sensitive calcium conductance. Three observations support this assumption. First, during large stimuli the late outward current and the epithelial resistance, R_{TOTAL} , are constant, even when the stimuli last much longer than the repolarization tail currents. If calcium channels were turned off by repolarization of the luminal face, the outward current should decline and the epithelial resistance R_{TOTAL} increase. In fact, a small droop in outward current can be observed for clamping pulses just above the range in which oscillations occur (Fig. 3, 43 mV; Fig. 4 B, 54 mV, Fig. 8, 15 and 24 mV), but this droop is not observed for larger stimuli. Second, current produced by superimposing a second excitatory stimulus after onset of the late current does not vary with time and is identical to the late current obtained with a single stimulus of the same total amplitude. Nonlinearities would be expected if calcium channels were turned off by the late current. Third, in the above example, an 80-mV repolarization produced by the late outward current fails to

bring the luminal membrane into the negative resistance region. To be sure, the true extent of the negative resistance region cannot accurately be inferred from epithelial measurements. Because these arguments are somewhat indirect, intracellular recordings are necessary to confirm the inference that reduction in g_{Ca} does not contribute significantly to the shift towards late outward current.

ISOPOTENTIALITY OF THE LUMENAL MEMBRANE Each receptor cell bears an apical cilium with a diameter of $0.5 \mu\text{M}$ and a length of up to $4 \mu\text{M}$ (Waltman, 1966). By using the above dimensions it can be shown that the cilium accounts for about 80% of the luminal membrane area. Since active conductance channels may be located on the cilium it is necessary to determine whether it is isopotential. From a reasonable value of $75 \Omega \text{ cm}$ for the resistivity of the ciliary cytoplasm, the axial resistance of a cilium $4 \mu\text{M}$ long is calculated to be $15 \text{ M}\Omega$. Table I shows that the resistance of a single luminal membrane is over $38,000 \text{ M}\Omega$ when the epithelium is inactive, and about $54 \text{ M}\Omega$ when both currents are maximally active. The luminal membrane is therefore regarded as isopotential.

Characterization of the Active Currents

IONIC BASIS OF THE EARLY CURRENT The voltage clamp experiments have confirmed that the early current arises from the calcium gradient across the luminal membranes. Perfusion of the lumen with an EGTA solution containing zero calcium completely abolished the inward current. Perfusion with an EGTA solution containing 10 mM calcium reversibly lowers the equilibrium potential for the early current and reduces the size of the early current at all voltages.

Although the luminal membranes are poorly clamped when there are active currents, the series resistance has almost no effect on the measurement of the equilibrium potentials E_{Ca} and E_{LATE} . When the luminal membrane is at E_{Ca} , activation of the calcium channels produces no change in the current flowing across the receptor cell, and therefore no change in membrane voltages. Because the ratio of $r_{LUM}:r_{BAS}$ is large, E_{EARLY}^* , the reversal potential of the early current across the epithelium approximates E_{Ca} , the true calcium equilibrium potential of the luminal membranes, minus a constant voltage E_{BAS} , which is the resting potential of the basal membranes (Eq. [2] and [14]). The stability of the clamp currents near the suppression potential indicates that there is little nonuniformity among the cells in respect to this parameter.

Attempts to determine the intracellular calcium concentration from the voltage clamp data are limited by two large uncertainties. First, the value of E_{BAS} is unknown. Second, although the calcium concentration of the ampullary jelly has been measured, no account was taken of the filamentous protein found in the jelly. As in serum, binding of calcium to protein may significantly reduce the extracellular concentration of ionized calcium.

In two experiments performed at 10°C , the reversal potential for the early current E_{EARLY}^* was 145 mV and 128 mV . (The reversal potential was somewhat lower if the ampulla was cooled to 5°C or below, but recovery occurred on warming, and the reversal potential always corresponded to the suppression potential for the late current.) If one takes E_{EARLY}^* as 145 mV , and assumes that E_{BAS} has the rather low value of 30 mV inside negative, and that the ionized

calcium concentration in the lumen is 2 mM, one obtains an intracellular ionized calcium concentration of 2.0×10^{-7} M. This value is in the range cited for other cells (see Meech and Standen, 1975; Baker et al., 1971; Hagiwara and Nakajima, 1966).

IONIC BASIS OF THE LATE CURRENT The calcium-activated outward current in skate electroreceptors is presumably carried by potassium or chloride. However, neither of these ions has yet been implicated as the exclusive charge carrier. Preliminary attempts to abolish the late outward current by perfusion of the lumen with tetraethylammonium have not been successful (Clusin and Bennett, unpublished observations). In these experiments it was not possible to determine the concentration of TEA that reached the luminal membranes because of diffusion delays. Meech and Standen (1975) found that 50 mM of extracellular TEA produces a 75% reduction in the calcium-activated potassium current in *Helix* neurons. However, in squid giant axons, Armstrong and Binstock (1965) found that TEA blocks the voltage-sensitive potassium channels only when applied on the cytoplasmic side of the membrane.

Preliminary experiments show that the prolonged perfusion of the lumen with low chloride solution reduces the late outward current without affecting the leakage resistance or the early current. However, Parnas and Strumwasser (1974) have reported that low chloride solutions abolish a calcium-activated potassium permeability in *Aplysia* neurons, which is also blocked by injection of TEA into the cytoplasm. Thus, the reduction of a calcium-activated outward current by low chloride solutions does not show that the outward current is carried by chloride.

ABSENCE OF INACTIVATION OF THE LATE CURRENT Like the early current, the late outward current fails to undergo inactivation for many seconds. At voltages below the suppression potential, the outward current increases with time until a steady state is reached. There is no subsequent decline. The late conductance is probably maximally activated during the steady state because the slope conductance, which is equal to the instantaneous conductance, is constant over a wide voltage range. Superimposed pulses affect the rapidity with which the maximum conductance is achieved in a manner consistent with the predicted changes in calcium influx, but do not affect the magnitude of the maximum conductance. Moreover, the late conductance is unaffected by prepulses that do not themselves produce active currents (Fig. 5).

In other excitable membranes potassium inactivation may lead to prolonged action potentials similar in appearance to those evoked in skate electroreceptors by strong stimuli. Nakajima and Kusano (1966), and Nakajima (1966) showed that the delayed outward current in supramedullary cells of puffer declines during a long-lasting depolarization. The degree of this inactivation increases with increasing depolarization. Recovery occurs over several hundred milliseconds and is faster at more negative potentials. Hyperpolarizing prepulses increase the size of the outward current during a test pulse. Potassium inactivation with similar characteristics was described in voltage clamped skeletal muscle fibers by Adrian et al., (1971). However, in skate electroreceptors these voltage effects are absent.

ACTIVATION OF THE LATE CURRENT BY A CALCIUM INFLUX The experiments described in this and the preceding paper (Clusin and Bennett, 1977) demonstrate that the late outward current is initiated by a calcium influx. Any experimental manipulation which blocks this influx suppresses the late outward current. The calcium influx is prevented by depolarization of the luminal membrane beyond the calcium equilibrium potential (Fig. 3), reduction of the concentration of ionized calcium in the lumen by EGTA perfusion (Fig. 8), or a combination of the two (Fig. 9). Blockage of delayed rectification by cobalt (Clusin and Bennett, 1977) is ascribable to a suppression of the calcium influx without a change in the ionized calcium gradient. The intracellular free calcium concentration presumably is increased by liberation of calcium from mitochondria after DNP treatment, or by interference with extrusion of cytoplasmic calcium by removal of extracellular sodium. Both these treatments activate a luminal membrane conductance that is unaffected by hyperpolarizing the luminal membrane with applied current (Clusin and Bennett, 1977). The fact that the late outward current does not occur in the absence of a calcium influx also demonstrates that the late conductance is insensitive to voltage.

INFERENCES ABOUT INTRACELLULAR CALCIUM AND OUTWARD CURRENT ACTIVATION By examining onset of the late outward current during a small sustained calcium current, two rather surprising properties of the calcium-activated conductance were noted:

(a) For stimulus durations up to several hundred milliseconds, onset of the calcium-activated current does not begin until a fixed amount of calcium has entered the receptor cells (Fig. 11 A).

(b) The amount of calcium required to activate the late outward current is reduced by a calcium influx occurring up to 10 s earlier (Fig. 12). Previous voltage stimuli which produce no calcium influx do not affect onset of the late outward current. Thus there appears to be a long-lasting residual calcium effect, even though the late conductance decays within 600 ms after repolarization.

There is no discrepancy in these two kinds of observation, for if complete restoration of Ca to resting levels requires 10 s, no appreciable removal need occur over several hundred milliseconds after onset of a stimulus. Granted that there is a finite rate of calcium removal, it should be possible to sustain a very small calcium influx indefinitely with no activation of the late current. This phenomenon was not looked for carefully in voltage clamp experiments with normal solutions, because the large voltage displacement required could not be sustained for more than 2 s without damage to the epithelium (irreversible lowering of the leakage resistance). However, in current clamp experiments (Clusin and Bennett, 1977, Fig. 10 D), reduction of E_{Ca} by prolonged perfusion of the lumen with 10 mM Ca and 20 mM EGTA produced a prolonged plateau-shaped action potential with no evidence of repolarization for many seconds. During these responses there must have been a maintained calcium current flowing inward across the luminal membranes that equalled the sum of the currents flowing through r_{LUM} and R_{SH} . Apparently this inward calcium current under conditions of reduced E_{Ca} was too small to activate the late outward current. When an adequate calcium gradient was restored (Clusin and Bennett, 1977, Fig. 10 B) repolarization occurred normally.

The requirement for a minimum calcium current to activate the late conductance indicates that the reversal potential for the early current should exceed the suppression potential for the late current to some degree. Such a discrepancy is obvious when short voltage pulses are used (Fig. 10 C), but was undetectable with pulses longer than 400 ms.

A number of simple mechanisms could underlie the observation of a fixed calcium influx before outward current onset. Activation of outward current channels may be sigmoidally dependent on Ca concentration, and this relation would be steepened by regenerative onset of the late current due to escape from the clamp. Alternatively, a high affinity but saturable binding mechanism may have to be filled before calcium becomes available for activation of the outward current channels. In either case slow removal of calcium remaining in the cytoplasm or in the saturable storage site would be responsible for the time course of facilitation. Katz and Miledi (1968) hypothesized that residual Ca underlies short-term facilitation of transmitter release at the frog neuromuscular junction.

While slow calcium removal is suggested by the long time course of facilitation, removal may be much more rapid when the rate of calcium influx is high. Rapid removal during larger calcium influxes is suggested by relatively short duration of the tail currents and the fact that these currents are not significantly prolonged when an excitatory voltage stimulus is lengthened.

Physiology of Calcium-Activated Conductances

COMPARISON WITH OTHER CELLS Activation of a conductance in nerve cells by intracellular calcium was first described by Meech and Strumwasser (1970). They pressure-injected calcium chloride into *Aplysia* neurons and observed a hyperpolarization with an increase in membrane slope conductance. The increased conductance was found to reflect an increased potassium permeability. Experiments supporting this conclusion have now been reviewed. Similar evidence for a calcium-activated potassium conductance has been adduced in a variety of other cells including leech neurons (Jansen and Nicholls, 1973), cat spinal motoneurons (Krnjevič and Lisiewicz, 1972), frog spinal motoneurons, (Barrett and Barrett, 1976), cardiac Purkinje fibers (Isenberg, 1975), and human erythrocytes (Whittam, 1968; Lew, 1971, 1973).

Meech and Standen (1975) recently studied the potassium currents in *Helix* neurons under voltage clamp conditions. These potassium currents are reduced by removal of extracellular calcium or by treatment with blockers of calcium permeability. By subtracting the outward current recorded in zero calcium from that recorded in normal calcium salines, Meech and Standen were able to measure the calcium-dependent component. This current increases with voltage up to 100 mV and declines linearly with larger stimuli. In some cases there is a null point at about 150 mV, where there is no calcium-activated outward current. This null point appears to vary with external calcium concentration according to the Nernst relation. Meech and Standen therefore suggested that the null point is the calcium equilibrium potential. The null point corresponds to what is called the suppression potential in the present study.

After a stimulus of 100 mV, the repolarization tail current in *Helix* neurons was found to have a fast and a slow component. The slow component has the same properties as the prolonged tail currents in skate electroreceptors. It does not occur in calcium-free saline or when the preceding voltage pulse exceeds 150 mV. Meech and Standen concluded that, as in skate electroreceptors, the calcium channels close so rapidly after repolarization from 150 mV that there is insufficient calcium influx to activate the calcium-dependent current.

Differences between the observations in skate electroreceptors and those in *Helix* neurons are due to the greater number of current components in the latter. In *Helix* neurons there are at least two populations of calcium channels with different kinetics so that the calcium influx has a different time course at every voltage. The onset of the calcium-activated late current has not been determined during a measured sustained calcium influx, nor has the calcium equilibrium potential been determined in voltage clamp measurements.

THE ROLE OF THE CALCIUM-ACTIVATED LATE CONDUCTANCE IN ELECTRORECEPTION Obara and Bennett (1972) proposed that the luminal membranes of individual electroreceptor cells produce action potentials and are spontaneously active *in situ*. This hypothesis is supported by the inference that the basal membranes constitute a significant series resistance. Furthermore, Clusin and Bennett (1974) recorded the current produced by ampullae when the physiological condition was approximated by short-circuiting the two saline pools. They found that ampullae are tonically active and operate in the negative resistance region of the current voltage relation.

Voltage stimuli of a few microvolts produce oscillatory responses in the epithelial current. These findings suggest that, under physiological conditions, the luminal membrane potentials are near threshold, with many of the receptor cells firing spontaneously. Small changes in the voltage across the epithelium presumably produce a significant change in the proportion of active receptor cells or in their firing rates.

A most intriguing property of skate electroreceptors is that while they are sensitive to stimuli of a few microvolts they can accommodate without loss of sensitivity to maintained stimuli of several millivolts (Murray, 1967). In order to account for accommodation, it is necessary to propose that the threshold of the receptor cells can shift several millivolts during a sustained voltage displacement with little change in the character of the active response.

The calcium-activated outward current may explain accommodation since activation of the longlasting outward current would raise the threshold for a subsequent active response. Facilitation of the onset of the late current by residual calcium would also be involved. Mediation of sensory accommodation by intracellular calcium has been well documented in *Limulus* photoreceptors (Lisman and Brown, 1972, 1975 *a, b*).

Ampullary electroreceptors of the marine catfish *Plotosus* are both morphologically and physiologically similar to skate electroreceptors. In *Plotosus*, however, excitability appears to result from a voltage-dependent calcium conductance in the basal faces of the receptor cells (Akutsu and Obara, 1974). Whether there is also a calcium-activated repolarizing current remains to be established.

MOLECULAR BASIS FOR THE CALCIUM ACTIVATED OUTWARD CURRENT The simplest explanation for the effects of intracellular calcium on membrane permeability is to suppose that calcium ions interact directly with the inner surface of the membrane. Previous electrophysiological demonstrations of calcium-activated permeabilities have involved membranes that also had voltage-sensitive outward current channels. It was therefore suggested (Meech, 1974) that intracellular calcium might affect potassium permeability by interacting with certain voltage-dependent permeability channels. A mere screening of surface charges on the inner surface of the membrane was excluded by Meech and Standen's (1975) observation that many divalent cations do not affect potassium permeability when injected into the cytoplasm. However, a more specific interaction of calcium ions with voltage-sensitive potassium channels could cause them to open in the absence of any transmembrane potential change.

The alternative possibility is that there is a separate macromolecule that is insensitive to voltage and activated only by the presence of calcium at the inner surface of the membrane. Meech and Standen (1975) found that although the dose response curves for blockage of voltage- and calcium-dependent potassium conductances by TEA are identical, the two conductances have slightly different reversal potentials. They concluded that separate channels could be involved. The present study indicates that intracellular calcium activates a voltage insensitive macromolecule in the luminal membrane of skate electroreceptor cells. The absence of active outward current during large stimuli suggests that voltage-sensitive potassium channels are not involved.

We believe that the molecular basis for the calcium-activated conductance in skate electroreceptors is likely to be similar to that in other cells. Because of their electrophysiological simplicity, skate electroreceptors are in many respects a useful material for the study of calcium-activated conductance mechanisms.

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