The Ionic Basis of Oscillatory Responses of Skate Electroreceptors

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A B S T R A C T When physiological conditions are simulated, skate electroreceptors produce small maintained oscillatory currents. Larger damped oscillations of similar time-course are observed in voltage clamp. Subtraction of leakage in voltage clamp data shows that the oscillations involve no net outward current across the lumenal surface of the epithelium. The oscillations are much faster than the late outward current generated by the lumenal membranes of the receptor cells. Treatment of the basal surface of the epithelium with tetraethyl ammonium (TEA), high K, Co, or EGTA reversibly blocks the oscillations in voltage clamp, but has little or no effect on the epithelial action potential in current clamp or on the current-voltage relation. The TEA sensitivity of the oscillations indicates that they involve a potassium conductance in the basal membranes of the receptor cells. Treatment of the basal membranes with TEA and high calcium, with strontium, or with barium causes these membranes to produce large regenerative responses. Direct stimulation of the basal membranes then elicits a lumen-positive action potential whereas stimulation of the lumenal membranes elicits a diphasic action potential. Excitability of the basal membranes is abolished by extracellular Co, Mn, or La. Modulation of the lumenal membrane calcium conductance by the basal membrane conductances probably gives rise to the oscillatory receptor currents evoked by small voltage stimuli. The slower calcium-activated late conductance in the lumenal membranes may be involved in sensory accommodation.

I N T R O D U C T I O N

The preceding paper (Clusin and Bennett, 1979) shows that the shunted electroreceptor of the skate produces oscillatory epithelial currents, both spontaneously and in response to small voltage stimuli. The spontaneous oscillations are superimposed on a larger mean current, and the total current presumably represents summation of oscillatory regenerative responses occurring at random phase in a large number of excitable receptor cells. In voltage clamp the receptor epithelium produces an early inward Ca current through the lumenal faces and a late Ca-activated outward current (Clusin and Bennett, 1977 b). In addition, clamping in the negative slope region often produces oscillatory currents that are similar in time-course to the oscillations of the shunted
receptor. We initially thought that the oscillations involved escape from the clamp allowed by the series resistance of the basal membranes and intermittent activation of the late outward current across the lumenal membranes. Interaction between inward and outward currents gives oscillatory responses in barnacle muscle fibers under conditions of inadequate voltage clamping (Keynes et al., 1973). However, this explanation has two important deficiencies. First, the oscillations are much faster than the turn-off of the late conductance. Second, oscillatory responses are frequently absent in electroreceptors where the current-voltage relation and the time-course of the late current are indistinguishable from those seen in electroreceptors that give prominent oscillations.

The basal membranes of the receptor cells were treated as passive in previous studies for several reasons. No obvious regenerative response can be elicited by stimuli that directly depolarize the basal membranes enough to produce transmitter release (Clusin and Bennett, 1977a). Perfusion experiments show that there is little change in the form of the epithelial action potential despite drastic changes in the medium perfusing the basal faces. Finally, the early inward current is linear over a broad voltage range which includes and exceeds the reversal potential. The early current depolarizes the basal faces at voltages less than the reversal potential, but hyperpolarizes them at greater voltages.

However, these observations do not exclude the presence of conductances in the basal membranes that are very small, or are observed over only a small portion of the current-voltage relation, for example, in the negative slope region. Indeed the suppression of transmitter release by removal of calcium from the basal membranes (Steinbach, 1974) suggested that some excitable presynaptic calcium channels are present. In the squid giant synapse, the presynaptic calcium influx does not ordinarily produce a regenerative response, and the terminal becomes inexcitable when the sodium current is blocked by tetrodotoxin. However, if the presynaptic potassium channels are then blocked by 4-aminopyridine or intracellular tetraethyl ammonium (TEA), a large calcium-dependent regenerative response is observed (Katz and Miledi, 1969; Llinás et al., 1976). Similar calcium-dependent regenerative responses have been demonstrated in photoreceptors (Fain et al., 1977; Ross and Stuart, 1978) and crustacean muscle fibers (Fatt and Katz, 1953; Fatt and Ginsborg, 1958).

In this paper we present evidence that excitable potassium and calcium conductances are also present in the basal membranes of skate electroreceptors. Although the epithelial action potential and current-voltage relation are essentially unaffected by perfusion of the basal membranes with TEA, EGTA, or cobalt, all of these treatments reversibly block the oscillatory responses observed in the negative resistance region. Moreover, after treatment with high Ca and TEA, the basal membranes produce large calcium-dependent regenerative responses. These observations suggest that the basal membranes have an excitable calcium conductance and a TEA-sensitive potassium conductance that repolarizes the receptor cells during the declining phase of the oscillatory responses.

A brief preliminary communication has appeared (Clusin and Bennett, 1973).
MATERIALS AND METHODS

The experimental preparation and recording techniques have been described elsewhere (Clusin and Bennett, 1977a and b; 1979). In voltage clamp experiments, the ampullary epithelium was held at its resting potential, and voltage across the epithelium was directly controlled using a microelectrode thrust through the canal wall at the neck of the ampulla (Clusin and Bennett, 1977b). Series resistance compensation was unnecessary, because voltage at the ampulla was measured directly. Perfusion of the basal surface of the epithelium was accomplished by a continuous flow system and complete exchange of solutions was possible in under 2 min. An additional 2-3 min was allowed for ionic equilibration across the several hundred micrometers of gelatinous material that adheres to the basal surface of the ampulla. The perfusate consisted of modified Fühner's saline (Clusin and Bennett, 1979) at 10°C. KCl, TEA-Cl, CaCl₂, MnCl₂, or LaCl₃ were added to the perfusate by isosmotic substitution for NaCl. The pH of all solutions was 7.4; no precipitates were seen. The EGTA solution which contained the usual 1.8 mM Ca and a 2.5 mM Mg was prepared by isosmotic substitution of 5 mM EGTA and an additional 5 mM Hepes (Sigma Chemical Co., St. Louis, Mo.) for NaCl. The ionized calcium concentration of this solution was calculated as 0.2 μM according to the method of Portzehl et al., (1974). The total of 10 mM Hepes held the solution at pH 7.4 ± 0.1 during dilution with the control solution.

RESULTS

Oscillatory Responses of Voltage-Clamped Ampullae

When the ampullary epithelium is abruptly stepped to a voltage in its negative resistance region, damped oscillations in epithelial current are often seen, as shown in Fig. 1. The frequency of these oscillations is ~21 Hz for the −2-mV stimulus and increases to 25 Hz for the +7-mV stimulus. Each oscillatory inward peak is an inward current across the lumenal surface of the epithelium. This inward current flows outward across the presynaptic basal membrane of the receptor cells causing a corresponding but delayed peak in postsynaptic current (Clusin and Bennett, 1977b, 1979). As shown earlier, the inward-going phase of the oscillatory responses is generated by the voltage-sensitive calcium conductance in the lumenal membranes of the receptor cells. Calculation of the leakage in Fig. 1 (indicated by the horizontal line) shows that net active current is never outward during the first few oscillations. For weak stimuli (−2 to 6 mV) total current falls to the leakage level after the first inward current peak. For later peaks, and stronger stimuli, active current is consistently inward until onset of a sustained late outward current, which occurs with a long latency and coincides with cessation of the oscillations.

Oscillatory responses like those seen in Fig. 1 are frequently absent in electroreceptors that have normal leakage resistances and current-voltage relations and produce normal action potentials in current clamp (Clusin and Bennett, 1977b). Because the oscillations are not always present, their physiological significance was initially unrecognized. However, it now appears that oscillatory responses are essential for electroreceptor function. Oscillations are always obtained from receptors whose afferent discharge is sensitive to submil-
livolt stimuli. Oscillations are abolished by a variety of unphysiologic treatments that prevent afferent responses to small stimuli.

The oscillations shown in Fig. 1 can be explained in terms of inadequate clamping of the receptor cells, owing to the presence of the basal membranes. Cells responsible for the oscillations presumably undergo cyclic repolarization about 20 times per second. Because the lumenal membrane calcium conductance does not inactivate (Clusin and Bennett, 1977a and b), cyclic repolarization of

![Diagram](image)

**Figure 1.** Oscillatory responses do not involve outward current across the lumenal faces of the receptor cells. The ampullary epithelium is voltage clamped using a microelectrode thrust through the canal wall near the ampulla. Numbers to the left of each current trace represent absolute epithelial potential, the holding potential being -7 mV. Voltage steps into the negative slope region evoke damped oscillations whose frequency is ~21 Hz at -2 mV and 26 Hz at +7 mV. During each stimulus, the leakage current calculated from oppositely directed pulses is indicated by the solid lines. Outward current greater than the leakage current results from activation of outward current across the lumenal faces of the receptor cells and does not occur until the oscillations have ceased.

the receptor cells is presumably due to an outward current. As noted above, it is possible that repolarization during the oscillations is mediated by the calcium-activated outward current produced by the lumenal membranes. This possibility seems unlikely in view of the slow decay of this current when the epithelium is repolarized. However, in that membrane voltage is poorly controlled, epithelial voltage clamp does not permit measurement of true kinetics. It is therefore conceivable that faster decay of the late outward current under certain conditions could explain the oscillations. Other evidence indicates that activatable conductances in the basal membranes are necessary for the oscillations.
Dependence of the Oscillations on Basal Membrane Potassium Current

Treatment of the basal membranes with 10 mM TEA abolishes the oscillations in 1-3 min, as shown in Fig. 2. After TEA treatment, only a single inward current peak is observed, whose latency is somewhat prolonged. In some experiments, the peak inward current occurs as long as 200 ms after the onset of a weak stimulus, but latency is diminished with increasing stimulus strength. The peak inward current is followed by the sustained late outward current, which activates with little change in time-course. Oscillatory responses are abolished by as little as 2 mM TEA, and recover within 5-10 min when the
control solution is restored. The control records in Fig. 2 were obtained after recovery from TEA treatment.

Other than blocking the oscillations, application of TEA to the basal surface has little effect on the epithelium. A current-voltage relation obtained from the experiment in Fig. 2 is shown in Fig. 3 A. There is no significant change in the resting potential, the resting resistance, or the late outward current. Although there may be a small reduction in peak early current between 30 and 80 mV, the peak early current at 80 mV is unchanged. Suppression of the late outward current occurs at the equilibrium potential for the early current, which is unchanged. Treatment of the basal surface with TEA also has little effect on the

![Figure 3](image)

**Figure 3.** The epithelial current-voltage relation is little affected by perfusion of the basal surface with TEA or cobalt. In part A, (■) represent the current-voltage relation from an epithelium perfused with the control saline (Fig. 2, left column). The current-voltage relation after perfusion with 10 mM TEA (○) is little different even though the oscillatory responses in the negative resistance region are abolished (Fig. 2, right column). In part B, (□) represent early and late currents after ~10 min of perfusion of the basal faces with 10 mM Co (Fig. 6, middle column); (●) represent the early and late currents obtained when the basal surface is perfused with the control solution (Fig. 6, left column). The leakage resistance of 322 kΩ in the control solution and 242 kΩ in cobalt has been subtracted giving net active currents. A gradual irreversible fall in leakage resistance is commonly observed in prolonged perfusion experiments irrespective of the perfusate, and is not a specific effect of cobalt. Comparison of the active currents shows that they are little affected by perfusion of the basal faces with cobalt, even though the 20-Hz oscillations are abolished (Fig. 6).

current-clamped epithelium. The amplitude and duration of epithelial action potentials are essentially unchanged at all stimulus strengths. Application of TEA to the basal membranes therefore selectively abolishes the oscillatory currents observed in the negative resistance region while having little effect on luminal membrane excitability.

Involvement of the basal membranes in the oscillatory responses is further indicated by the fact that prominent oscillations can be elicited from nonoscillating receptors after bathing the basal faces in a saline containing zero potassium. Restoration to the control saline then abolishes the oscillations or makes them smaller. Furthermore, treatment of the basal surface of the ampulla with salines
containing >20 mM K abolishes oscillatory responses, even when they are prominent in the control solutions. All of these treatments affect oscillations within <2 min and produce little change in other electrical properties of the epithelium except for small changes in epithelial resting potential.

TEA presumably acts by blocking potassium channels in the basal membranes of the receptor cells. The voltage-sensitive potassium conductance in myelinated nerve fibers is reduced 75% by extracellular perfusion with 3 mM TEA (Hille, 1970) while both calcium-activated and voltage-sensitive potassium conductances in *Helix* neurones are reduced 75% by extracellular application of 50 mM TEA (Meech and Standen, 1975). The absence of a large change in epithelial current during perfusion of the basal membranes with TEA indicates that the basal membrane K conductance is relatively small, short lasting, or activated only over a small region of the current-voltage relation. In particular, TEA doesn't change the current-voltage relation during the late outward current when the resistance of the luminal membranes is lowest, and a change in the series resistance of the basal membranes would be most easily detected. Thus, blockage of the oscillations by a change in series resistance is unlikely. Also, a change in resting potential is probably not a contributing factor, because no significant change in epithelial potential is produced by TEA perfusion and application of a holding potential of either sign does not restore the oscillations.

**Evidence for an Excitable Calcium Conductance in the Basal Membranes**

Evidence for excitability of the basal membranes was obtained by other perfusion experiments. Whereas TEA alone has essentially no effect on the epithelial action potential, the combination of TEA and 20 mM Ca produces a marked change that apparently results from the development of a calcium action potential in the basal membranes of the receptor cells. In Fig. 4 A and B, the epithelium is perfused with the control saline. In Fig. 4 A, a near-threshold lumen-negative stimulus evokes a typical 70-mV action potential (upper trace) and a corresponding postsynaptic response (middle trace) in the afferent nerve. Presumably excitation of the luminal membranes depolarizes the basal membranes causing transmitter release. In Fig. 4 B, a very large lumen-positive stimulus is applied which directly depolarizes the basal membranes, producing transmitter release and an excitatory postsynaptic response. The presence of a small nonlinearity concealed in the artifact cannot be excluded, but there is no obvious regenerative response. The small relaxation in epithelial voltage that occurs during the stimulus is probably due to breakdown of the membrane, because prolonged lumen-positive stimuli >150 mV produce an irreversible decline in epithelial resistance. In Fig. 4 C, the basal surface of the same electroreceptor has been perfused for several minutes with 2 mM TEA and 20 mM Ca. Stimulation of the luminal membranes now evokes a diphasic action potential. The normal lumen-negative response is followed by a lumen-positive potential which continues beyond termination of the stimulus. The polarity of the lumen-positive potential is appropriate for a conventional depolarizing action potential in the basal membranes of the receptor cells.

Several observations suggest that the lumen-positive potential in Fig. 4 C is due to a calcium conductance increase in the basal membranes. First, large
prolonged lumen-negative stimuli, which hyperpolarize the basal membranes, can block the lumen-positive potential, leaving a normal lumen-negative response. Second, large lumen-positive stimuli, which depolarize the basal membranes, can directly evoke the lumen-positive response as shown in Fig. 4 D. Relatively large stimuli are required because most of the voltage drop occurs across the lumenal faces. Third, the postsynaptic response is greatly prolonged and lasts at least the duration of the lumen-positive potential. A prolonged postsynaptic response is consistent with a prolonged calcium influx across the basal membranes. Finally, the lumen-positive potential has the expected ionic requirements and pharmacological properties of a calcium-dependent response.

![Figures A to D showing recordings of action potentials](image-url)
Barium and strontium substitute effectively for calcium, in both the basal membrane action potential and postsynaptic response. In Fig. 5 A a typical diphasic action potential is obtained in an epithelium whose basal surface has been perfused with 20 mM strontium, 1 mM TEA, and zero calcium. Strontium and barium differ from calcium in that they can induce excitability of the basal membranes in the absence of TEA. Thus, ampullae whose basal surfaces are treated with Ca-free salines containing 30 mM Sr or Ba produce diphasic action potentials similar to those in Figs. 4 C and 5 A, whereas treatment with 30 mM Ca (and no TEA) has no effect. Similar results have been obtained in photoreceptors (Fain et al., 1977; Ross and Stuart, 1978). Werman and Grundfest (1961) suggested that Ba and Sr ions are capable of blocking potassium channels like TEA, and that this action is unrelated to their ability to substitute for calcium as charge carriers.

The basal membrane action potential is unaffected by 10 μM TTX, 1% procaine, or by complete replacement of external sodium by choline. The response is abolished by reduction of the calcium concentration bathing the basal membranes below 10 mM, substitution of magnesium for calcium, or
addition of 7 mM Co ++ or 7 mM Mn ++. 2 mM La +++ also blocks the basal membrane response although not reversibly. Abolition of the strontium-dependent response in Fig. 5 A by addition of 7 mM Co is illustrated in Fig. 5 B. Absence of a postsynaptic response in cobalt is consistent with its blocking presynaptic strontium influx. Extracellular Co has an identical effect on basal membrane excitability observed in high calcium and TEA.

**Dependence of the Oscillations on Basal Membrane Calcium Current**

Calcium current entering the basal membrane should depolarize the receptor cells to some degree. The 20-Hz oscillations observed in the negative slope region during voltage clamp are reversibly abolished by treatment of the basal membranes with 10 mM Co (Fig. 6). However, this treatment has little effect on the magnitude of the inward and outward currents generated by the luminal membranes (Fig. 3 B). The dependence of the oscillations on basal membrane calcium influx is also shown by their disappearance during perfusion of the basal surface of the epithelium with the Ca-EGTA solution given above (not illustrated). We previously reported that blockage of the presynaptic calcium influx by extracellular cobalt produces no change in the form of epithelial action potentials in current clamp (Clusin and Bennett, 1977 a). This observation is
confirmed in Fig. 7 which show that application of 10 mM Co has essentially no effect on the amplitude and duration of the epithelial action potential or on suppression of repolarization by very strong stimuli. Voltage clamp data from the same experiment show oscillations that are blocked by the cobalt treatment and recover when cobalt is removed.

The role of the basal membrane Ca current in the generation of the oscillations is uncertain. The contribution of this current to depolarization of the receptor cells may be necessary for activation of voltage-sensitive K channels in the basal membrane. Alternatively, Ca influx may activate a voltage-insensitive K conductance. However, Ca-activated currents generally persist for several hundred ms which is much longer than the period of the oscillations. Relatively greater TEA sensitivity may (Thompson, 1977) or may not (Meech and Standen, 1975) distinguish voltage-sensitive K currents from those activated by calcium.

**Figure 7.** Perfusion of the basal membranes with cobalt does not affect action potentials generated by the electrically isolated ampulla. Epithelial current is shown in the upper trace and epithelial voltage in the lower trace. In (A) the basal surface of the epithelium is bathed in the control saline. The epithelial resting potential is 19 mV. Lumen-negative stimuli evoke a single action potential, whose duration is progressively increased with increasing stimulus strength. In (B) the basal surface of the epithelium has been perfused for several minutes with a solution containing 7 mM Co++. The action potential is essentially unchanged at all stimulus strengths. Voltage clamp records obtained from the same receptor show oscillatory responses just before (A), which are suppressed by Co++ before (B).

**DISCUSSION**

*Ionic Basis of the Oscillatory Responses*

The foregoing experiments suggest that the oscillatory responses of skate electroreceptor cells result from interaction of active conductances in the lumenal and basal membranes shown in the equivalent circuit of Fig. 8. The lumenal membranes are represented by a fixed conductance, $r_{LUM}$, in parallel with the voltage-dependent calcium conductance, $r_{Cal(L)}$, and the late conductance, $r_{LATE}$. The outward current is known to be activated by intracellular calcium and its onset is slow (Clusin and Bennett, 1977 b). Its ionic battery has
not been identified but the late current is relatively insensitive to extracellular TEA, which is more characteristic of calcium-activated K currents. The basal membrane in Fig. 8 is represented by a fixed conductance, \( r_{\text{BAS}} \), in parallel with two variable conductances, a basal membrane calcium conductance, \( r_{\text{Ca}(B)} \), and a basal membrane potassium conductance, \( r_{\text{K}(B)} \). Pharmacological blockers of these two conductances have little effect on the epithelial action potential or current-voltage relation, but their existence can be inferred from two observations: (1) the basal membranes become excitable when they are perfused with TEA and high calcium, with strontium, or with barium; and (2) the oscillatory responses disappear when the basal membranes are perfused with TEA or cobalt.

![Figure 8](image-url)

**FIGURE 8.** Equivalent circuit of a skate electroreceptor with variable conductances of the basal membranes included. Capacitative elements are omitted. The luminal membranes of the receptor cells are represented by a fixed resistor, \( r_{\text{LUM}} \), in parallel with two variable resistors which give rise to the inward calcium current (\( r_{\text{Ca}(L)} \)) and the late outward current, (\( r_{\text{LATE}} \)). The basal membranes are represented by a fixed resistor, \( r_{\text{BAS}} \), in parallel with two variable resistors representing the presynaptic calcium conductance (\( r_{\text{Ca}(B)} \)) and the basal membrane potassium conductance (\( r_{\text{K}(B)} \)). \( r_{\text{LUM}} \) is much larger than \( r_{\text{BAS}} \) so that most of the voltage across inactive receptor cells acts on the luminal membranes. \( r_{\text{LATE}} \) has very slow kinetics, and is activated by intracellular calcium, but its ionic selectivity is uncertain. \( r_{\text{K}(B)} \) turns on and off rapidly and is sensitive to extracellular TEA, but its gating mechanism is unknown. Leakage current in the isolated receptor flows mainly through \( r_{\text{SH}} \), a passive shunt not involving the receptor cells. Under physiological conditions the receptor cells generate current that flows mainly through the low resistance of the canal.

The variable conductances in the basal membranes are believed to produce oscillatory responses in the following manner. Depolarization of the luminal membranes by an excitatory stimulus leads to an inward calcium current which flows outward across the basal membranes. Because they have a significant resistance, the basal membranes are depolarized, leading to activation of their calcium channels and synaptic transmitter release. The basal membrane potassium conductance is subsequently activated, either as a consequence of the presynaptic calcium influx or of the voltage change. Increased potassium conductance would then repolarize the receptor cells, leading to a turning off of the variable conductances in both membranes and to a restoration of excitability.
In the proposed model, the declining phase of the oscillations results not from an outward current through the luminal membranes but from outward current through the basal membranes. The initial effect of the basal membrane outward current would be to increase inward current through the luminal membranes. This inward current would decline only after the cell was sufficiently repolarized to close the calcium channels in the luminal faces. Another factor to be considered is calcium activation in the basal faces which should reduce inward calcium current across the luminal faces. However, the timing of the postsynaptic response, which should provide a somewhat delayed and distorted measure of calcium activation in the basal faces, indicates that this mechanism is not primarily responsible for the decline in inward current across the epithelium. In ampullae that produce oscillations, onset of the postsynaptic response precedes the first inward current peak rather than following it. Furthermore, at least partial repolarization of the receptor cells between the inward current peaks is indicated by the oscillations in postsynaptic response (Clusin and Bennett, 1979).

Effects of the Variable Conductances in the Basal Membranes on the Magnitude of Epithelial Currents

The stability of the epithelial current-voltage relation during treatment of the basal membranes with pharmacological blockers was initially interpreted as evidence that the basal membranes were passive. It now appears that the basal membranes have two variable conductances whose presence is not reflected in the magnitude of the peak inward and late outward currents. The basal membrane K conductance is large enough to turn off the luminal membrane calcium current, and the model indicates that it should increase the peak inward current across the epithelium. However, this increased inward current would not be conspicuous if activation of $r_{K(B)}$ occurred only in the negative slope region where the current-voltage relation is very steep.

The variable conductances in the basal membranes definitely do not contribute to the current-voltage relation during stimuli that exceed the reversal potential for the early current, because the basal membranes are not depolarized under these conditions. Smaller excitatory stimuli do lead to depolarization of the basal membranes, but the magnitude of this depolarization ought to vary substantially with stimulus strength. Our previous calculations indicate that the basal membrane resistance, $r_{BAS}$, is more than twofold higher than the resistance of the luminal membrane after activation of $r_{Ca(L)}$ (Clusin and Bennett, 1977b). The tendency of excitatory stimuli to hyperpolarize the basal membranes will therefore be much greater than at rest. For example, a 30-mV increase in stimulus strength after activation of $r_{Ca(L)}$ would produce a relative hyperpolarization of 20 mM in the basal membrane. Thus, activation of a voltage-sensitive K channel might well be suppressed during all but the weakest excitatory stimuli. Suppression of the oscillations by treatment of the basal membranes with cobalt could occur in the same manner. If cobalt treatment produced some reduction in the peak internal potential of excited receptor cells, then activation of voltage-dependent K channels in the basal membranes could be reduced and the oscillations thereby suppressed.
Although the basal membrane K conductance may be substantial, the basal membrane Ca conductance need not be. Unlike the oscillations, postsynaptic responses are observed during voltage stimuli that approach the reversal potential for the early current. The basal membrane calcium channels must therefore be activated over a wide range of transepithelial potentials. The linearity of the current-voltage relation and its stability during treatment of the basal faces with cobalt or EGTA indicate that the basal membrane calcium current is small under physiological conditions.

It should be noted that the earlier calculations of receptor cell resistances were based on an equivalent circuit in which the basal membranes were assumed to be passive. However, all of the measurements used in these calculations come from regions of the current-voltage relation where the basal membranes are not depolarized. The previously calculated values of $r_{LUM}$, $r_{CA(L)}$, $r_{LATE}$, and $r_{BAS}$ are therefore unaffected by the presence of excitable calcium and potassium conductances in the basal membrane.

Direct evidence for the presence of variable conductances in the basal membranes is the presence of small notches in some of the voltage clamp records. Although onset of the active currents is obscured by large capacity currents, careful examination of individual tracings sometimes reveals that a small notch is present on the rising phase of the inward current which may represent activation of basal membrane conductances. This notch is not obvious in most of the current traces in this paper, but is clearly discernible in Fig. 5 C. The outward peak at this notch is in the proper direction for an influx of cations across the basal membranes. In other records the beginning of the notch was found to precede onset of the excitatory postsynaptic potential by < 2 ms. The notch is absent during stimuli that exceed the reversal potential for the luminal membrane calcium current and therefore fail to depolarize the basal membranes. Furthermore, when a series of increasingly strong voltage stimuli is applied, the notch is found to disappear at the voltage where synaptic transmission fails (see Fig. 4 B of Clusin and Bennett, 1977 b). These observations indicate that the notch results from a variable conductance in the basal faces of the receptor cells that is associated with synaptic transmitter release, and hence involves a presynaptic calcium current.

Similar notches are sometimes noted in the tails of the calcium-activated outward currents after termination of an excitatory stimulus. These tail currents are always inward (relative to the luminal membrane) when the epithelium is repolarized to the resting potential, but are outward for lesser degrees of repolarization. Outward tail currents, which should hyperpolarize the basal membranes, produce no transmitter release (see Clusin and Bennett, 1977 b, Fig. 4 A), but inward tail currents produce large excitatory postsynaptic potentials indicating that they depolarize the basal membranes. A notch in inward tail currents is shown in Fig. 5 C and in Fig. 1 (28-mV stimulus). The outward component of this notch represents inward current through the basal faces and is roughly synchronous with onset of the postsynaptic response although it is somewhat obscured by the capacitative transient. Moreover,

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1 Clusin, W. T., and M. V. L. Bennett. Unpublished data.
during a series of records in which an increasingly strong repolarizing pulse is superimposed on the same excitatory stimulus, the appearance of the notch corresponds with the appearance of the excitatory postsynaptic response.

The presence of these notches indicates that the basal membrane conductances do affect the epithelial currents. The notches appear to be much smaller than the luminal membrane currents on which they are superimposed, but the exact magnitude and time-course of the basal membrane components is not easily assessed. Notches are barely discernible in many experimental preparations, and they are less stable than other features of the epithelial currents. We have not yet made a systematic attempt to determine whether the notches are altered by perfusion of the basal membranes with cobalt or TEA.

**Absence of the Oscillations in Current Clamp**

Another factor tending to obscure the origin of the oscillations is their absence in current clamp. Passage of an excitatory current across the isolated ampulla typically evokes an action potential whose duration is about 120 ms, whereas depolarization of the voltage-clamped ampulla by small stimuli evokes a train of oscillatory peaks with a period of ~50 msec (Waltman, 1968, Clusin and Bennett, 1977a). Both responses are repeatedly demonstrable in the same ampulla if one switches back and forth between current and voltage clamp modes. Comparable phenomena have not been described in cells with a uniform surface membrane. In barnacle muscle fiber, for example, the oscillatory peaks recorded under conditions of inadequate voltage clamping have approximately the same duration as the action potentials recorded in current-clamped fibers under appropriate conditions (Hagiwara and Naka, 1964; Keynes et al., 1973).

The absence of rapid oscillations in the current-clamped epithelium is readily explained by reference to the equivalent circuit in Fig. 8. In this model, depolarization of the receptor cells during electrochemical excitation results primarily from a calcium conductance increase in the luminal faces, whereas rapid repolarization during the oscillatory response results from an increased potassium conductance in the opposite faces. In voltage clamp, a permeability change in one membrane produces an identical voltage change in both membranes because the potential across the epithelium is held constant by the voltage clamp, whose effective internal resistance is zero. Close interaction of the two faces also occurs under physiological conditions when they are connected by the relatively low resistance of the canal, \( r_{\text{CANAL}} \). However, when the epithelium is electrically isolated (indicated schematically by the open switch in Fig. 8) the two faces become separated by the epithelial leakage resistance, \( R_{\text{SH}} \). Any change in basal membrane potential will then be divided between the luminal membranes and the shunt resistance, \( R_{\text{SH}} \), according to the voltage divider relation. While the epithelial leakage resistance is 200–400 kΩ, previous calculations indicate that the resistance of the luminal membranes in parallel is only 48 kΩ after activation of the early current (Clusin and Bennett, 1977b). Thus, most of the change in potential produced by activation of \( r_{K(B)} \) would be across the shunt resistor. The effective uncoupling of the luminal and basal membranes by the interposed epithelial shunt resistance would account for the absence of rapid oscillations in the current-clamped ampulla. Absence of rapid oscillations in
current clamp would not be expected if they were due to interaction of two conductances in the same membrane.

Another way to understand the absence of oscillations in current clamp is to view the epithelium as a whole. During the epithelial action potential, flow of current through the shunt resistor produces a 60-100-mV lumen-negative action potential. The large potential across the epithelium will prevent repolarization of individual receptor cells by \( r_{sh} \) just as occurs in voltage clamp in the range where oscillations are blocked. If only a few cells were active in a current-clamped epithelium they presumably could oscillate because the resistance of a single cell is much greater than the shunt resistance. Apparently, activation of relatively few cells causes a large enough regenerative response to block the oscillations.

**Role of Receptor Cell Excitability in Electroreception**

Calculations indicate that escape of receptor cells from the voltage clamp during the oscillations evoked by small stimuli involves large changes in intracellular potential. Regenerative depolarizations of large amplitude probably also occur in the shunted electroreceptor, but the pattern of the intracellular response is unknown. The function of regenerative responses in electroreceptor cells is presumably to increase sensitivity. As pointed out by Cole et al. (1970) the gain of excitable cells poised near threshold approaches infinity, and it is reasonable that oscillating cells which are repetitively passing through threshold would also be very sensitive to stimulation (Bennett and Clusin, 1979). The steepness of the stimulus-response curve of the ampulla indicates that small voltage changes produce large changes in receptor cell activity.

The spontaneous 20-Hz fluctuations in epithelial current suggest that individual receptor cells can oscillate at this frequency. Because there is little change in the frequency of evoked oscillations recorded from the whole epithelium over a stimulus range of several millivolts, the frequency of oscillations in individual receptor cells is likely to be relatively constant rather than modulated. To produce evoked changes in overall activity with little change in frequency, single cells could exhibit bursts of 20-Hz activity as shown in Fig. 9 A. Changes in the number of oscillations per unit time would then result from changes in burst duration or interburst period. During each burst, calcium would enter across the luminal membranes in a pulsatile manner. With sufficient calcium accumulation, the late outward current would activate, terminating the burst and producing a prolonged undershoot. Removal of calcium would lead to gradual depolarization and a subsequent burst. Excitatory stimuli would increase the number of oscillatory peaks per burst, or reduce the interval between bursts. Inhibitory stimuli would shorten the burst or increase the interburst interval. The degree of damping of the responses might be less in individual cells than in the epithelium as a whole, because damping of epithelial currents could result from desynchronization or from burst termination in individual cells. Summation of asynchronous bursts of oscillatory current could produce the mean active current and superimposed sinusoidal fluctuations that are recorded from the tonically active ampulla (Clusin and Bennett, 1979) as shown by the computer simulation in Fig. 9 B. Synchronization as well as recruitment of bursting
receptor cells would account for the oscillatory responses produced by small voltage steps.

A possible alternative to the burst model is that the spontaneous oscillations result from random summation of low frequency, more or less rhythmic, but asynchronous firing of many receptor cells. This alternative was tested by computer simulation and appears untenable. Summation from many periodic sources gives rise to a distribution which is difficult to distinguish from that produced by a Poisson process (Cox and Smith, 1953). In the simulation a large number of Poisson-distributed impulses are summated at several mean frequencies of impulses of different monophasic shapes. In all cases the computed current as a function of time is formless; no sinusoidal pattern like that generated by the epithelium can be discerned.

Given that receptor cells repetitively fire, the effective thresholds of these cells (defined here as a transepithelial voltage) must be quite uniform. Cells must be near the threshold for initiation or termination of regenerative responses or
must approach or pass through it frequently. As noted in the preceding paper, skate electroreceptors accommodate to prolonged stimuli without loss of sensitivity. The properties of the off-responses in the epithelial current indicate that this accommodation is at least partly mediated by changes in receptor cell activity. Granted that high sensitivity results from regenerative responses in the receptor cells, the effect of accommodation is to adjust all the cells to the same threshold without changing the character of the regenerative responses.

Similarity between a component of the off-responses observed in the short-circuited receptor after small excitatory stimuli and the outward tail currents produced by isolated epithelia under voltage clamp suggests that accommodation is mediated by the calcium-activated conductance in the lumenal membranes (Clusin and Bennett, 1979). During prolonged excitatory stimuli, increased activity of the receptor cells would cause accumulation of calcium near the lumenal membrane. Increased ionized calcium would cause increased activation of the lumenal membrane outward current tending to bring the membrane below threshold and terminate the oscillatory response. During prolonged inhibitory stimuli, decreased oscillatory activity of the receptor cells would cause a decline in ionized calcium near the lumenal membranes and the receptor cells would depolarize thus increasing activity. This interaction between the inward calcium current and the calcium-activated outward current may lead to both high sensitivity and accommodation by keeping the receptor cells near threshold at rest and during maintained stimuli. A similar scheme of regulation of spontaneous activity has been proposed for molluscan neurones (Eckert and Lux, 1976).

Receptor Cell Excitability in Plotosus Electroreceptors

The inference that regenerative responsiveness contributes to sensitivity is supported by comparison with tonic electroreceptors of teleosts (see Bennett and Clusin, 1979). The marine catfish Plotosus has receptors that are sensitive to stimuli in the microvolt range and these receptors also exhibit regenerative responses (Obara, 1974, 1976). In contrast the maximum sensitivity of the receptors of freshwater teleosts is in the range of tenths of a millivolt and these receptors do not have regenerative responses. Presumably the marine environment is quieter electrically which allowed greater sensitivity to evolve. But the greater sensitivity may well require regenerative responsiveness.

In Plotosus receptors the regenerative responses are also Ca-mediated, but they arise in the basal rather than the lumenal membranes (Akutsu and Obara, 1974). When the receptor epithelium is shunted, moderate excitatory stimuli produce damped oscillatory responses similar to those seen in skate ampullae (Obara, 1974, 1976). If the ampulla is then isolated by placing the canal in air, the excitable membrane is hyperpolarized and oscillatory responses no longer occur. Instead, the receptor produces long-lasting all-or-none responses, which do not spontaneously repolarize and which must be terminated by applied current. Afferents from Plotosus electroreceptors have a resting discharge, but spontaneous oscillatory epithelial currents have not been demonstrated. It remains to be determined whether the in situ receptor cells undergo repetitive
depolarizations by means of interaction between calcium and potassium conductances in opposed membranes.

**Fast and Slow Potassium Conductances in Other Cells**

The presence of more than one type of potassium conductance mechanism is well established in many excitable cells, most notably in neurones. *Helix* neurones (Meech and Standen, 1975; Heyer and Lux, 1976) have two potassium conductance mechanisms, a voltage-dependent conductance, and a slower calcium-activated conductance. Both potassium currents are fairly insensitive to small concentrations of extracellular TEA, although both are partially blocked by high concentrations (Meech and Standen, 1975). In contrast, intracellular injection of TEA preferentially blocks the voltage-sensitive current (Heyer and Lux, 1976).

Evidence for fast and slow potassium currents with different sensitivity to TEA has also been obtained for vertebrate neurones. Barrett and Barrett (1976) found that the afterhyperpolarization following the action potential in frog motoneurones has two phases, a fast phase that is selectively abolished by extracellular perfusion with 2-5 mM TEA, and a slow phase that is selectively abolished by low calcium or cobalt. The Barretts infer that these neurones have a fast TEA-sensitive K conductance that is activated by depolarization, and a slow TEA-insensitive K conductance activated by the presence of intracellular calcium. Both K systems are also found in cat motoneurons (Krnjevic et al., 1975).

Recent experiments indicate that TEA-sensitive potassium currents are also present in photoreceptors. Ross and Stuart (1978) recorded a calcium-dependent action potential in the presynaptic terminals of barnacle photoreceptors in the presence of TEA. Fain et al., (1977) demonstrated calcium-dependent oscillatory potentials and action potentials in rods perfused with 6-12 mM extracellular TEA. Although the appearance of these regenerative responses presumably results from blocking of a potassium conductance by TEA, the hyperpolarizing afterpotentials indicate that a TEA-insensitive outward current is also present. Repolarization of the photoreceptor action potentials could be mediated by a calcium-activated conductance.

The present study shows that skate electroreceptors also have fast and slow outward current mechanisms, but that they are located in different regions of the receptor cell membrane. The calcium-activated late outward current arises from the lumenal membrane. Its complete suppression by stimuli that exceed the equilibrium potential for the early calcium current indicates that few voltage-sensitive potassium channels are present in the lumenal membranes. Fast potassium channels in skate electroreceptors are located in the basal membranes. Their duration of opening and TEA sensitivity are characteristic of voltage-activated potassium channels in other vertebrate excitable membranes. However, the dependence of the fast oscillations on the small basal membrane calcium current could mean that the basal membrane potassium current is mediated by a separate calcium-activated channel. The faster operation of this channel could then result from more rapid fluctuation in intracellular calcium.
near the basal membranes. Well-circumscribed changes in cytoplasmic free calcium have been inferred from studies with aequorin (Rose and Loewenstein, 1975).

Most prior studies of potassium currents in excitable cells are based on intracellular recordings, which provide little information about the spatial distribution of the conductance changes. Furthermore, selective perfusion of different membrane areas is not easily accomplished in most cells. It is therefore possible that the fast and slow potassium conductances described in other excitable cells are segregated in specific regions of membrane, as is often true of Ca channels (Meech, 1976).

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