Calcium-Activated Conductance in Skate Electroreceptors

Current Clamp Experiments

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ABSTRACT When current clamped, skate electroreceptor epithelium produces large action potentials in response to stimuli that depolarize the lumenal faces of the receptor cells. With increasing stimulus strength these action potentials become prolonged. When the peak voltage exceeds about 140 mV the repolarizing phase is blocked until the end of the stimulus. Perfusion experiments show that the rising phase of the action potential results from an increase in calcium permeability in the lumenal membranes. Perfusion of the lumen with cobalt or with a zero calcium solution containing EGTA blocks the action potential. Perfusion of the lumen with a solution containing 10 mM Ca and 20 mM EGTA initially slows the repolarizing process at all voltages and lowers the potential at which it is blocked. With prolonged perfusion, repolarization is blocked at all voltages. When excitability is abolished by perfusion with cobalt, or with a zero calcium solution containing EGTA, no delayed rectification occurs. We suggest that repolarization during the action potential depends on an influx of calcium into the cytoplasm, and that the rate of repolarization depends on the magnitude of the inward calcium current. Increasingly large stimuli reduce the rate of repolarization by reducing the driving force for calcium, and then block repolarization by causing the lumenal membrane potential to exceed $E_{Ca}$. Changes in extracellular calcium affect repolarization in a manner consistent with the resulting change in $E_{Ca}$.

INTRODUCTION

Ampullae of Lorenzini are exquisitely sensitive electroreceptors found beneath the skin of elasmobranch fishes. These receptors are capable of detecting gill and muscle potentials of prey, and are most numerous around the mouth. Kalmijn (1971) showed that electrical signals emitted by prey can elicit feeding behavior. Dijkgraaf and Kalmijn (1963) demonstrated behavioral responses to potential gradients as small as 0.01 μV/cm². Murray (1967) found that individual ampullae respond to voltage stimuli of a few microvolts, but can accommodate without loss of sensitivity to maintained voltages that are much larger. Excised ampullary electroreceptors give transient responses to mechanical stimuli (Lowenstein, 1960), changes in salinity (Murray, 1960; Lowenstein and Ishiko, 1962),
and changes in temperature (Murray, 1967; Sand, 1958). However, Kalmijn (1975) showed that in free-swimming animals, the responses of the ampullary nerves to thermal and mechanical stimuli are insignificant compared to the responses evoked by electrical stimuli.

The resistivity of the skin and body tissues of the skate are low, and a field in the environment is not significantly distorted by the presence of the fish (Murray, 1967). On the other hand, the canals of the ampullae have a very long space constant (Waltman, 1966). Thus the receptor epithelium in the ampulla is acted upon by the voltage difference between the canal opening and body interior adjacent to the ampulla. The longest canals give the greatest sensitivity to uniform fields while a spectrum of canal lengths and orientations provides information about local variations.

An important property of these receptors is their electrical excitability, which was first suggested by Murray's (1965) report that low voltage oscillations can be recorded when a wire is thrust into the pore of the ampullary canal of a freshly killed skate. Waltman (1966) subsequently reported that excitatory (lumen negative) voltage stimuli applied between the pore of the canal and the ampulla evoke graded oscillatory responses across the ampullary epithelium. Obara and Bennett (1968, 1972) evoked similar responses by passing constant current into the lumen of the canal with a microelectrode. They showed that these responses are regenerative. Moreover, 30-mV action potentials with a well-defined threshold sometimes occur. Waltman (1968) used electronic feedback to prevent current from flowing around the edges of the epithelium, so that the epithelium was current clamped. Under these conditions, long trains of "all-or-none" action potentials are generated. The action potentials reach 60 mV in amplitude and have a duration of 100 ms. Under voltage clamp conditions, when the epithelium is effectively short-circuited by the feedback amplifier, an N-shaped current-voltage relation is obtained (Waltman, 1968). From Waltman's experiments it appears that, as the conductance shunting the epithelium is reduced from the voltage clamped condition, one obtains graded regenerative responses, and finally, action potentials with a well-defined threshold.

By penetrating the afferent fibers, Obara and Bennett (1972) recorded postsynaptic potentials that followed the regenerative responses of the ampullary epithelium. They concluded that the ampullary potentials are generated by the receptor cells. They suggested that the response of the electroreceptors to physiological stimuli results from a depolarizing excitability of the lumenal faces of the receptor cells which depolarizes the passive presynaptic basal faces. Experiments with large stimuli supported this proposed mechanism. Obara and Bennett also discussed the possibility that excitability of the lumenal membranes of the receptor cells might be due to an active inward calcium current. The evidence was primarily comparative: that is, the electrically excitable responses of teleost phasic receptors are insensitive to TTX (Zipser and Bennett, 1973), while the receptor potential in ampullary electroreceptors of Plotosus varies as the Nernst potential for calcium (Akutsu and Obara, 1974).

In this and the following paper (Clusin and Bennett, 1977) the permeability changes responsible for action potentials in skate electroreceptors are analyzed. The unusual anatomic features of the electroreceptors have permitted detailed
analysis of membrane properties without impalement of the receptor cells, which are very small and invested with connective tissue. This paper deals with current clamp and the following paper with voltage clamp experiments. We find that excitability of the ampulla involves a permeability process that has not been found in axons, but has been described in several nerve cell bodies (Meech and Standen, 1975). The rising phase of the ampullary action potential results from an increase in calcium permeability in the lumenal membranes of the receptor cells. The conductance increase responsible for repolarization is caused not by the change in voltage but by the calcium influx across the lumenal membranes. Large stimuli that prevent this calcium influx by exceeding the calcium equilibrium potential also prevent repolarization.

Preliminary communications have appeared (Clusin and Bennett, 1973; Clusin et al., 1974; Clusin et al., 1975).

Anatomical Considerations

Fig. 1 is a diagram of a typical ampullary electroreceptor. It consists of a lobulated bag, or ampulla, connected to an external orifice by a long canal. The canal wall is comprised of two layers of flattened cells. The cells of the basal layer are separated from each other by a uniform 100-Å extracellular gap. However, the cells abutting on the lumen are joined together by apical tight junctions which completely occlude the extracellular space (Waltman, 1966), and presumably form a barrier to ionic movement. The canal wall is electrically linear and has a very large resistivity (10⁷ Ω/cm²) in parallel with a capacity of about 0.5 μF/cm². To account for the large mural resistivity, Waltman proposed that the inner and outer membranes of cells abutting on the lumen have an extremely high

![Diagram of skate ampullary electroreceptor and canal](image.png)
resistance. He further suggested that the mural capacity is the capacity of the two membrane faces in series.

The epithelium of the ampulla is diagrammed in Fig. 2 A. It consists of a single sheet of cells that is continuous with the high resistance layer of the canal. As in the canal, adjacent cells are joined by occluding junctions that form a collar around each cell and partition its membrane into lumenal and basal faces. However, two types of cells are present in the ampulla. Spherical receptor cells are interspersed among columnar supporting cells. These receptor cells have cytoplasmic features that are quite distinct from those of the supporting cells and canal wall cells. Each receptor cell has a small lumenal face and a much larger basal face. The lumenal face bears an apical cilium (not shown) and the basal face forms several synaptic contacts with branches of afferent fibers from the eighth cranial nerve (Waltman, 1966). Afferent fibers ramify profusely and five to seven of them innervate the several thousand receptor cells in each ampulla. The electroreceptor synapses resemble other sensory synapses of the acoustical lateralis system in that they have presynaptic ribbons.

Both the ampulla and the canal are filled with a gelatinous substance whose conductivity is slightly greater than that of seawater (Murray and Potts, 1961). This jelly contains fine protein fibers that are anchored to the lumenal surface of the epithelium at hemidesmosomes. The neck of the ampulla is called the
marginal zone by Waltman (1966) and is a zone of transition between the epithelium of the ampulla and the epithelium of the canal. There are no receptor cells in the marginal zone. Its epithelial cells are modified supporting cells which interdigitate extensively below the tight junctions (Fig. 2 B). As a result, the basal faces of cells in the marginal zone have many times more area than the lumenal faces, and may contain much of the surface membrane in an ampulla (Szamier, personal communication).

MATERIALS AND METHODS

The Preparation

In order to fit the recording apparatus and simplify numerical treatment of data, electroreceptors of a uniform size were used in these experiments. Skates (Raja oscellata and R. erinacea) 45–55 cm in length were obtained by trawling near Woods Hole, Mass. The canals that run posteriorly from the mandibular capsule along the dorsal surface (Murray, 1966, Fig. 1 A) were exposed and transected within 4 cm of their terminal pores. Other canals and the afferent nerve were transected close to the capsule. The capsule, with attached posterior canals, was then removed from the fish and placed in a transparent, ice-cooled dissecting dish containing either cerebrospinal fluid or Ringer’s solution (composition below). Individual electroreceptors were detached under a dissecting microscope, along with several millimeters of the ampullary nerve branch. The average length of excised canal was 7 cm and the average diameter near the ampulla was 1 mm.

The recording apparatus in these experiments is diagrammed in Fig. 3. The canal was

![Diagram of experimental apparatus](image-url)

**Figure 3.** Experimental apparatus for passing constant current across the ampullary epithelium. Current was measured with a current-voltage transducer made from an operational amplifier with output voltage proportional to I. The ampullary potential was measured with a microelectrode thrust through the canal wall. Postsynaptic activity was recorded by drawing the ampullary nerve into oil on chlorided silver hooks attached to the input terminals of a differential amplifier.
suspended on thin pins across an air gap between two saline pools. The transected end of the canal was in one saline pool and the ampulla in another. The portion of canal in the air gap was washed with a solution of 526 mM sucrose and 350 mM urea to increase the resistance of the air gap.

**Electrical Stimulation and Recording**

The voltage across the ampulla was measured with a Bioelectric NF1 preamplifier and a microelectrode thrust through the canal wall at the neck of the ampulla. Conventional glass pipettes filled with 2 M KCl and having a resistance of 1 MΩ or higher were sufficient to impale the canal without measurably reducing its resistance. The reference electrode, which also served as a current return, was a chlorided silver plate with a surface area of 1 cm² and a resistance of less than 100 Ω. In some experiments, the silver plate was embedded in an agar-filled glass tube, the resistance of which was less than 500 Ω. The reference electrode was held at "virtual ground" by a current-voltage transducer made from an Analog Devices 40J operational amplifier (Analog Devices, Inc., Norwood, Mass.).

Zero voltage was established by short-circuiting the two saline pools through a low resistance (2 kΩ) salt bridge made with physiological saline and 2% agar. The potential across the ampulla is within 1 mV of zero when the distal end of the canal is short-circuited to the basal surface of the ampulla, as occurs under physiological conditions (Obara and Bennett, 1972).

Pulses of constant current were produced by using a voltage source in series with a 100 MΩ resistor. Current was applied through a chlorided silver wire to the pool containing the open end of the canal. Because the portion of the canal in the air gap had been washed with an ion-free solution, current passed between the saline pools flowed down the lumen of the canal and across the ampullary epithelium. The resistance along the external surface of the sucrose-treated canal was estimated as 7.5 MΩ/cm by determining the distance from the ampulla at which a cut produced about 10% reduction in input resistance. From Waltman’s figure for wall resistivity one can calculate that the mural resistance of each centimeter of the canals used in these experiments was 15 MΩ. Since the resistance along the lumen was only 2.3 kΩ/cm, it is unlikely that significant current would have crossed the canal wall and flowed along its external surface.

Postsynaptic activity was recorded extracellularly by draping the nerve over a chlorided silver hook that was then retracted into an oil-filled glass capillary. Differential recordings were sometimes obtained with a Metametrix (DC-coupled) amplifier and a second silver wire placed near the opening of the capillary. Postsynaptic potentials (PSPs) as large as 1 mV were often recorded with superimposed action potentials that were even larger. Sometimes 10⁻⁷ M TTX was added to the saline to abolish the postsynaptic action potentials, leaving a smooth PSP (Steinbach, 1974; Steinbach and Bennett, 1971).

**Solutions**

Stable recordings could be obtained for several hours if the ampulla was bathed in cerebrospinal fluid (CSF). For experiments involving ionic substitutions, a modified Fühner’s (1908) elasmobranch Ringer was nearly as satisfactory as CSF. This solution contained 415 mM urea, 340 mM NaCl, 6 mM KCl, 2.5 mM MgCl₂, 1.8 mM CaCl₂, 2.5 mM NaHCO₃, and 5 mM HEPES buffer (pKₐ = 7.55) adjusted to pH 7.4. The temperature of solutions bathing the ampulla was regulated by using a Peltier device activated by a small thermistor. Experiments were performed at 10°C unless otherwise indicated.

Solutions used in perfusing the lumen of the ampulla were made from a control solution containing 428 mM NaCl, 13 mM KCl, 50 mM MgCl₂, 10 mM CaCl₂, 75 mM urea, and 5 mM HEPES at pH 8.1. In a few preliminary experiments, a control solution was made by dissolving 75 mM urea in seawater.
The 100 mM CaCl₂ solution was made by replacement of 150 mM NaCl in the control solution. The 100 mM CaCl₂ solution was made by substitution of 90 mM CaCl₂ for 135 mM NaCl.

The EGTA solutions were prepared by substitution of 20 mM EGTA and 55 mM HEPES for NaCl in the control solution. The usual 10 mM CaCl₂ was present in the Ca-EGTA solution, but was replaced by MgCl₂ in the Ca-free EGTA solution. Both solutions were neutralized with NaOH so that the final pH was 8.1 and the final osmolarity was equal to that of the control solution. The total of 60 mM HEPES in both solutions stabilized them against changes in pH caused by chelation of additional calcium. Addition of 10 mM CaCl₂ to either solution caused a pH reduction of only 0.4.

**Internal Perfusion of the Ampulla**

Internal perfusion of the ampulla was difficult because the lumenal surface of the high resistance cells in the canal wall is not protected by connective tissue. Moreover, the canal is tortuous and must be straightened to prevent damage to these high resistance cells during cannulation.

Cannulation was accomplished by using the chamber shown in Fig. 4. This chamber had two saline pools separated by an air gap but, in addition, the portion of the canal nearest the ampulla was forced into a Vaseline-smeared groove in a Sylgard resin block. The Vaseline-filled groove served to straighten the canal and to immobilize it during cannulation. In the initial experiments, it was found that the saline solution bathing the ampulla tended to seep into the groove along the Vaseline-coated surface of the canal,

![Figure 4](https://i.imgur.com/3Q5Q5Q.png)

**Figure 4.** A diagram of the apparatus used for cannulation and internal perfusion of ampullary electroreceptors. The ampulla lay in one saline pool, while the canal was draped across an air gap which ran perpendicular to the Vaseline groove into a second saline pool (not shown). Perfusate was forced out of the cannula under pressure and flowed back along its outer surface to the nick in the canal wall where it was aspirated. The stimulating and recording apparatus was the same as in Fig. 3, except that the perfusion cannula was used in place of a microelectrode to measure the ampullary voltage.
reducing its sensitivity. This problem was solved by cutting a 1-mm wedge in the Sylgard block perpendicular to the groove.

After the canal was straightened, its wall was nicked at the edge of the Sylgard block furthest from the ampulla. The perfusion cannula was introduced through this nick in the canal wall. If the outer aspect of the canal was adequately deionized, this nick had no effect on the input resistance.

The perfusion cannula was a glass capillary several centimeters long and about 100 μm in diameter. This cannula was also used to record voltage. The perfusate was forced out of the cannula under modest pressure produced by compressed air. The maximum rate of flow was 100 μl/min. The cannula was mounted on an XYZ micromanipulator and was advanced down the straightened portion of the canal. Fluid forced into the lumen of the canal flowed back along the cannula to the nick in the canal wall where it was aspirated. Because the ampullary jelly was viscous and the cannula was somewhat flexible, the tip of the cannula could be steered as it advanced. Since the Sylgard block was transparent, the position of the tip could be viewed directly from above and also from the side by using a 45° front-silvered mirror. By viewing the tip in three dimensions, it was possible to prevent the cannula from touching the inner aspect of the canal wall as it advanced. Jelly in the canal was removed by perfusing with protease as the cannula was advanced (1 mg/ml of Sigma Type VI protease [Sigma Chemical Co., St. Louis, Mo.] in the control solution). The input resistance of the preparation was unaffected. Perfusion with a protease-free solution was begun when the tip of the cannula was within 2 mm of the neck of the ampulla.

With the tip of the cannula at the neck of the ampulla, ionic exchange was limited by diffusion into individual ampullary lobules, a distance of up to 1 mm. When the lumen was perfused with fluorescein or phenol red, about 20 min was required before the intensity of dye within the ampullary lobules was indistinguishable from that of the perfusate flowing out of the cannula. This is approximately the amount of time one would estimate for free diffusion of a substance across 1 mm of gelatin. Because of this prolonged diffusion time, it was not possible to adjust ionic concentrations to specified levels or to eliminate completely a particular ion from the ampullary lumen. However, it was quite feasible to introduce a new ion or chelating agent into the ampulla by perfusing with a fairly high concentration.

RESULTS

Passive Properties

When the ampulla is electrically isolated by the air gap and no current is passed across it, a lumen-positive transepithelial potential is recorded. This resting potential is 10-30 mV in freshly dissected preparations. Little resting potential is present under physiological conditions (Obara and Bennett, 1972) when the ampulla is shunted by the resistance of the canal (20-30 kΩ in the present experiments). When physiological conditions are mimicked by short-circuiting the two saline pools with a salt bridge, the transepithelial potential is close to zero. (The somewhat greater canal length in vivo should make no significant difference.) The resting potential in the isolated preparation presumably corresponds to a difference in the potentials across the two faces of the receptor cells. Under physiological conditions, the membrane potentials of the two faces must be nearly equal because the faces are connected through the low resistance of the canal.

The effects of constant current are shown in Fig. 5. Modest currents of either
polarity cause the epithelial voltage to approach a value that is proportional to applied current (Figs. 5 A, 7 B). The ampulla is typically linear between 0 and 150 mV lumen positive. In these experiments intact ampullae had input impedances ranging from 0.2 to 0.4 MΩ. Waltman (1968) reports a passive input impedance of 0.3 MΩ in ampullae of similar dimensions isolated by electronic feedback. In Fig. 5 B the logarithm of voltage minus final voltage is plotted against time for two traces. The plot shows that voltage rises nearly exponentially when a step of
current is applied. This result suggests that the resistance in series with the capacity of the ampullary epithelium is small, although voltage clamp experiments show that part of the capacity is in series with a measurable resistance (Clusin and Bennett, 1977).

Lumen-positive stimuli applied to the opening of the canal can directly depolarize the secretory membranes of the receptor cells and cause transmitter secretion (Obara and Bennett, 1972). However, lumen-positive stimulation of the isolated ampulla produces no postsynaptic potentials until the epithelial voltage exceeds 120 mV, lumen positive (Fig. 5 D). This finding implies that most of the voltage drop across inactive receptor cells occurs in the uninnervated luminal membranes, and that the resistance of these membranes is high.

**Action Potentials Evoked by Weak Stimuli**

Lumen-negative stimuli greater than 50–100 nA evoke action potentials across the epithelium as in Fig. 5 C. The action potential has a well-defined threshold at about 0 mV (20 mV lumen negative from the resting potential) as shown. The active character of the response is indicated by the fact that it occurs at the end of the stimulus and that the voltage continues to rise after the stimulus is terminated. The notch on the rising phase of the action potential corresponds to the termination of the stimulus. After the action potential there is a lumen-positive afterpotential lasting several hundred milliseconds. In deteriorating preparations, the resting transepithelial potential declines, and constant lumen-positive current has to be applied to keep the ampullary epithelium from becoming spontaneously active. In such preparations, lumen-negative rather than lumen-positive afterpotentials follow the action potential.

Recordings from the afferent nerve of an electrically isolated ampulla show postsynaptic potentials whose onset coincides with the rising phase of the ampullary action potential (Fig. 5 C). Under physiological conditions when the opening of the canal is short-circuited to the basal surface of the epithelium, graded responses of the epithelium and graded postsynaptic responses are usually obtained (Murray, 1967; Obara and Bennett, 1972). Lumen-positive stimuli produce graded hyperpolarizing PSPs presumably resulting from a reduction in spontaneous activity of the receptor cells and reduced transmitter release (Obara and Bennett, 1972), while lumen-negative stimuli produce graded depolarizing PSPs. Graded responses to voltage stimuli of a few microvolts can be obtained in vitro if the two saline pools shown in Fig. 3 are short-circuited by a salt bridge (Clusin and Bennett, 1974). In the electrically isolated preparation, however, no hyperpolarizing postsynaptic potentials are seen and no depolarizing PSPs are seen until the epithelium reaches threshold for the production of an all-or-none response. Presumably, the luminal faces of the individual receptor cells of an isolated ampulla are not spontaneously active and become synchronously excited when they are depolarized by applied current.

Maintained stimuli evoke trains of action potentials (Fig. 6 A). In an intact, freshly dissected preparation which has not been stimulated for at least 10 s the amplitude of the first action potential is between 60 and 100 mV and the rise time is about 60 ms. Subsequent action potentials of a train are uniform, but are 50% smaller in amplitude and duration. When brief stimuli are repeated at 2-s
intervals the amplitude and duration of the action potential is progressively reduced (Fig. 6 B), reaching a steady state after several stimuli. The basis for this refractoriness is discussed in the following paper (Clusin and Bennett, 1977).

**Effects of Strong Stimuli**

As in other excitable cells (Fitzhugh, 1961), large long-lasting stimuli evoke only a single action potential, which occurs at the beginning of the stimulus. Action potentials for a family of stimuli are shown in Fig. 7 A. In Fig. 7 B, voltage is plotted as a function of current. The peak voltage and the minimum voltage after the action potential but before termination of the stimulus are both linearly related to current for moderate stimulus strengths. The resistance of the inactive epithelium is 331 kΩ and the resistance immediately after an action potential is 84 kΩ. When brief excitatory stimuli are superimposed on a large long-lasting stimulus just after the action potential no additional active response occurs and the current voltage relation is identical to that obtained by using a single stimulus and plotting the minimum voltage after the action potential. Thus, for large stimuli the slope conductance of the epithelium is increased fourfold after the action potential. Obara and Bennett (1972) reported a less than twofold conductance increase associated with the ampullary action potential. However, since their results were obtained in ampullae that were shunted by the resistance of the canal, there is no disagreement.

If the increase in slope conductance associated with the action potential results entirely from increased conductance in the lumenal membranes of the receptor cells (see below), then the resting resistance of the lumenal membranes must be at least three times greater than the resting resistance of the basal faces. Calculations based on voltage clamp experiments show that the ratio is actually much larger (Clusin and Bennett, 1977). This asymmetry of membrane resistance would be consistent with the observations on evoked transmitter release described above.

A striking feature of the ampullary action potentials is that they become greatly prolonged when large stimuli are applied as shown in Fig. 7 A. With
increasing stimulus strength, the onset of repolarization occurs later and the rate of repolarization is slowed. Above 158 mV, no repolarization occurs during the stimulus (Fig. 7A, Fig. 10A), even when the stimulus lasts more than 1 s. However, after termination of the stimulus, there is an inflection in the voltage trace (arrow) beyond which the trace is nearly superimposable on the falling phase of an action potential evoked by a weak stimulus.

![Figure 7](image.png)

**Figure 7.** Effects of constant current stimuli and the resulting current voltage relation. In A, the voltage responses (upper traces) to a family of current stimuli (lower traces) are shown. The resting transepithelial potential is 30 mV, lumen positive. A current voltage relation is plotted in B. The peak voltage of the action potential (filled circles) and the minimum voltage between the peak of the action potential and the termination of the stimulus (open circles) are linearly related to current over a broad range. With large stimuli repolarization is incomplete and the voltage at the end of the stimulus is plotted.

Thus, with large stimuli repolarization is progressively slowed and delayed until a "suppression potential" is reached, above which repolarization is indefinitely blocked. The time course of the voltage trace after termination of a large stimulus suggests that the process which normally repolarizes the action potential is delayed until the stimulus ends.

**Ionic Substitution outside the Basal Faces**

In order to investigate the ionic basis for the ampullary action potential, it is necessary to vary the composition of the saline perfusing the two surfaces of the
epithelium. It is assumed that the ampullary action potential results from changes in the permeability of membranes to ions, and that the total transepithelial potential represents the sum of ionic potentials generated in the lumenal and basal membranes. If the action potential results from significant permeability changes in both the lumenal and basal membranes, then it should be possible to change the form of the action potential by varying one or more ionic gradients across either membrane. If the permeability changes are confined to one face, only substitutions at that face will affect the action potential.

The basal membranes are most readily studied because they are more accessible to perfusion. Perfusion experiments show that a variety of changes in the basal membrane's ionic milieu do not significantly affect the form of the ampullary action potential. Replacement of 90% of extracellular Na with K or with choline does not substantially alter the rising phase or the repolarizing phase of the action potential within 5 min. Steinbach (1974) previously reported that application of TTX to the basal membrane blocks action potentials in the postsynaptic nerve but does not affect the ampullary potential. In the present study total replacement of Na has no immediate effect on the ampullary action potential, although there is a gradual increase in epithelial conductance over several minutes. The significance of this observation is discussed below.

When calcium is completely removed from the Ringer and 1 mM EGTA is added, there is no effect on the ampullary action potential but synaptic transmission is abolished in less than 1 min. The rapid action of the calcium-free solution suggests that ionic exchange across the gelatinous material adhering to the basal surface of the epithelium is rapid. The dependence of synaptic transmission on extracellular calcium suggests that there is a voltage-dependent calcium conductance in the basal membranes of the receptor cells (Steinbach, 1974). This observation further suggests that any calcium crossing the lumenal membranes during the action potential does not diffuse across the receptor cells in sufficient quantity to mediate transmitter release. Since the transepithelial action potential is unaffected by removal of calcium from the basal face, we can conclude that, as in squid giant synapse (Katz and Miledi, 1969), the active calcium permeability in the secretory membrane has little effect on its potential under physiological conditions. Moreover, we conclude that calcium entering across the basal membranes does not affect the permeability of the lumenal membranes during the action potential (Clusin and Bennett, 1973). Rose and Lowenstein (1975) inferred from aequorin studies that at low concentrations calcium diffuses only a few micrometers in epithelial cells of an insect salivary gland before it is sequestered.

Even more drastic ionic substitutions in the saline bathing the basal membranes do not affect the form of the action potential. Fig. 8 B shows an action potential recorded in a calcium-free saline containing 1 mM EGTA in which 80% of the NaCl has been replaced by potassium acetate. Exposure to potassium acetate saline causes the epithelium to become spontaneously active, so that a steady hyperpolarizing current has to be applied to maintain the resting potential. As in other experiments where a DC holding current is used to prolong viability (Steinbach, 1974), a lumen negative afterpotential is recorded. However, the shape of the action potential is normal, and the regenerative character of the response (not shown) is preserved. Moreover, there is little change when
the epithelium is returned to the control saline (Fig. 8 A). Since neither the rising
nor the falling phase of the action potential is significantly affected by drastic
perturbation of ionic gradients across the basal membranes, the action potential
and the associated increase in conductance must result from permeability
changes in the lumenal membranes.

**Ionic Substitution outside the Lumenal Faces**

Because of the long diffusion times involved, no attempt was made to remove
sodium from the lumen of the canal. However, when the lumen is perfused with
1 μM tetrodotoxin (TTX), the action potential is unchanged as shown in Fig. 9 D.
TTX selectively abolishes the voltage-dependent sodium permeability of axons
and of many other excitable tissues, though TTX-resistant sodium conductances
have been described (Kao, 1966).

When the lumen is perfused with cobalt, excitability is abolished. Cobalt is
known to be a selective blocker of voltage-dependent calcium permeabilities. 30
mM cobalt nearly abolishes the inward calcium current in barnacle muscle fibers
(Hagiwara et al., 1969), but has little effect on the outward potassium current. Fig. 9 B shows the effects of constant-current pulses applied across an ampullary
epithelium after the lumen has been perfused with a 100 mM cobalt solution for
25 min. Not only is the action potential abolished, but there is very little delayed
rectification. The concentration of cobalt required to produce these effects is
undoubtedly lower than 100 mM, but because of diffusion delays, the concentra-
tion within the alveoli is not known. Fig. 9 C shows the same epithelium after 35
min of perfusion with the control solution. The normal form of the action
potential at different peak voltages is largely restored. Complete reversibility is
not achieved, presumably because the preparation deteriorates before complete washout of cobalt occurs.

In most excitable membranes, delayed rectification causes repolarization during large excitatory stimuli, even if the active inward current has been blocked. Reversible loss of excitability during perfusion of the lumen with cobalt suggests that the action potential in skate electroreceptors is ascribable to a calcium permeability increase in the luminal membranes. However, the absence of delayed rectification during perfusion with cobalt suggests that cobalt somehow interferes with the repolarizing process as well.

**Figure 9.** Effects of perfusing the lumen with cobalt and TTX. In A the lumen is perfused with the control solution. In B the ampulla has been perfused for 25 min with a 100-mM cobalt solution. The cobalt concentration at the alveolar epithelium is not known because of the long diffusion time. Perfusion with cobalt renders the ampulla inexcitable. Considerable recovery occurs when the ampulla is perfused with the control solution for 35 min, as shown in C. There is, to be sure, a progressive irreversible decline in leakage resistance, which is invariably seen in preparations more than 3 h old and need not be attributed to cobalt. D shows a normal action potential recorded after perfusing the lumen for 25 min with a solution containing 1 μM tetrodotoxin. The vertical bar represents 1 μA and 88 mV in A–C and 0.2 μA and 20 mV in D. The horizontal bar represents 0.35 s in A–C and 0.2 s in D. The resting potential was 20 mV in all four records.

The role of calcium can be clarified by perfusion of the lumen of the electroreceptor with a solution containing 20 mM EGTA, 10 mM calcium, and 50 mM magnesium. The ionized calcium concentration in this perfusate is 0.2 μM at pH 8, according to the method of Portzehl et al. (1964). However, because the movement of EGTA into the lumen is limited by diffusion, it is unlikely that the ionized calcium concentration at the receptor cells reaches this level. After 15 min of perfusion, the repolarizing phase of the action potential in Fig. 10 C is slowed at all voltages, and the suppression potential is reduced from 145 to 91 mV. The time course of delayed repolarization is also slowed in low calcium.

If perfusion is continued for another 10 min (Fig. 10 D) repolarization no longer occurs at any voltage. The action potential evoked by a just-threshold stimulus is a sustained plateau, which can last at least 10 s and has to be terminated by applied current. In several experiments, perfusion of the lumen
with a calcium-free solution containing EGTA was found completely to abolish electrical excitability and to render the epithelium electrically linear within 30 min.

The relationship between the extracellular calcium concentration and the size of the action potential is complex. A comparison of current and voltage clamp data (Clusin and Bennett, 1977) indicates that the repolarizing process begins before the peak of the action potential, so that the latency of the repolarizing process affects spike amplitude. However, the rate of repolarization varies inversely with extracellular calcium (Fig. 10). Thus a decrease in extracellular calcium would have antagonistic effects: it would tend to decrease spike amplitude by decreasing inward calcium current, but it would tend to increase spike amplitude by slowing the onset of repolarization.

Fig. 10 B is from the same preparation as above after 30 min of perfusion with a solution containing 100 mM Ca and no EGTA. The effect of calcium chelation on the action potential has been clearly reversed. The duration of the action potential is shorter at all voltages than it is in normal calcium and repolarization is no longer suppressed at 145 mV. The suppression potential in high calcium was not determined for fear of damaging the epithelium.

The effects of changing the calcium concentration in the lumen of the ampulla
indicate that electrical excitability results from a voltage-dependent calcium conductance in the luminal membranes of the receptor cells. Moreover, it appears that calcium influx is necessary for the repolarizing process to occur. The rate of repolarization for a given stimulus and the suppression potential for repolarization both depend on the gradient of ionized calcium across the luminal membranes.

**Effects of Dinitrophenol**

Another way of altering the calcium concentration gradient across cell membranes is treatment with 2,4-dinitrophenol (DNP), which causes efflux of mitochondrial calcium into the cytoplasm (Drahota et al., 1965). When the electroreceptor epithelium is treated with 10^{-4} M DNP, a fourfold transepithelial conductance increase develops over a period of 10 min. During this period there is progressive loss of electrical excitability. Excitability and a normal transepithelial resistance cannot be restored by passing large inhibitory currents which hyperpolarize the luminal membranes. This suggests that the transepithelial conductance produced by DNP is insensitive to voltage. Interpretation of these results is complicated because dinitrophenol could act directly, or by some metabolic effect other than liberation of calcium. However, the result is consistent with the suggestion that ionized intracellular calcium produces a conductance increase in the normally high-resistance luminal membranes of the receptor cells.

The effects of DNP are similar to those observed when the basal surface of the epithelium is bathed in a solution in which all of the sodium has been replaced by choline. Over a period of 10 min the transepithelial conductance increases and excitability is lost. A normal resting resistance cannot be restored by passing large lumen-positive currents. In squid axons, Blaustein and Hodgkin (1969) showed that extrusion of calcium is reduced in sodium-free saline, while Baker et al. (1971) used aequorin to show that the cytoplasmic concentration of ionized calcium in squid axons rises when the extracellular sodium is replaced. In skate electroreceptors, calculations based on Fig. 32 of Waltman (1966) show that the basal faces comprise more than 98% of the receptor cell surface membrane. Removal of extracellular sodium from the basal faces could therefore lead to a rise in the cytoplasmic calcium concentration. Katz and Miledi (1969) suggested that a similar mechanism may account for the disappearance of the calcium-dependent action potential when presynaptic fibers of squid stellate synapse are bathed in a sodium-free saline.

**Discussion**

**Origin of the Epithelial Potentials**

Voltage clamp experiments (Waltman, 1968; Clusin and Bennett, 1977) show that excitability of the ampulla results from an active current which flows inward across the luminal membranes and outward across the basal membranes of cells in the epithelium. Since the action potential is associated with transmitter release, at least some of this current must flow outward across the basal membranes of the receptor cells. The present data do not exclude the possibility that both the receptor cells and the supporting cells are electrically excitable. How-
ever, on morphological and functional grounds we suspect that the supporting cells are inexcitable high-resistance cells like those of the canal wall, and that only the receptor cells produce active current. Voltage clamp data (Clusin and Bennett, 1977) support this inference.

The perfusion experiments clearly suggest that it is the lumenal membranes of the receptor cells that are excitable. Various alternatives, such as a hyperpolarizing potassium-dependent response in the basal membranes, are excluded by the fact that the action potentials persist when every conceivable ionic gradient across the basal membrane is altered. The effects of perfusing the lumen with high Ca, EGTA, and cobalt suggest that both the depolarizing and repolarizing phases of the action potential are attributable to permeability changes in the lumenal membranes of the receptor cells.

The Synchronization of Receptor Cells

Under current clamp conditions, the ampullary epithelium behaves like a single sheet of excitable membrane, generating large action potentials with a well-defined threshold. Activity of individual patches of excitable membrane in the lumenal faces of the receptor cells in an electrically isolated ampulla must therefore be closely synchronized. The basis for this synchronization is illustrated in Fig. 11. The lumenal faces are represented by a fixed resistance, $r_{\text{LUM}}$, in parallel with an active calcium conductance, $g_{\text{Ca}}$, while the basal membrane is represented by a fixed resistance, $r_{\text{BAS}}$. There are two reasons to believe that the resistance of the basal membranes is low compared with the resting resistance of the lumenal membranes:

![Figure 11](https://example.com/supplementary_material.png)

**Figure 11.** Equivalent circuit illustrating spread of electrical excitation between two receptor cells in the ampullary epithelium. The lumenal faces are represented as an active calcium conductance, $g_{\text{Ca}}$, in parallel with a fixed resistance $r_{\text{LUM}}$. (The corresponding batteries $E_{\text{Ca}}$ and $E_{\text{LUM}}$ are drawn but not labeled.) The basal faces are represented as fixed resistors, $r_{\text{BAS}}$. A single resistance $R_{\text{SH}}$ represents shunt pathways across inexcitable cells in the epithelium and through the intercellular clefts. The numbered arrows indicate possible return pathways for inward current generated by an active response in the lumenal membrane of the receptor cell on the right.
There is a large increase in the slope conductance across the epithelium during the action potential. In the following paper, (Clusin and Bennett, 1977) it will be shown that this increase in slope conductance is due to an increase in the instantaneous conductance of the epithelium, and that the conductance increase across the receptor cells is considerably larger than the fourfold transepithelial conductance increase. Since the transepithelial conductance increase during the action potential is due to an increased conductance of the lumenal membranes, it follows that the resistance of the basal membranes is low.

The transepithelial potential must exceed 100 mV lumen positive in order to directly excite the presynaptic calcium conductance in the basal faces and cause release of transmitter, while lumen-negative potentials of less than 20 mV directly excite the voltage-dependent calcium permeability of the lumenal membranes.

The basal membrane resistance need not be lower than the active resistance of the lumenal membranes. In fact, under voltage clamp conditions the basal membranes appear to constitute a significant series resistance as discussed in the following paper.

The low resting resistance of the basal membranes has two consequences: (a) most of the voltage drop across an inactive receptor cell will occur in the lumenal membrane; (b) before onset of the late outward current most of the early inward current through the lumenal membrane of an excited receptor cell will flow outward across the basal membrane.

In Fig. 11, some of the current generated by a response in the receptor cell on the right flows outward across the lumenal membrane of the adjacent receptor cell, thereby depolarizing it (current path 1). Because of the low resistance of the basal membrane, active current is not greatly shunted by the leakage resistance of the membrane in which it arose (current path 3).

Leakage of current through the intercellular clefts (included in current path 2) is minimized by the zonulae occludentes. The supporting cells must also be of high resistance as evidenced by the large input impedance of the isolated ampulla. However, introduction of an external low resistance pathway across the ampullary epithelium loads down active receptor cells and shunts current away from adjacent inactive receptor cells so that interaction among them is reduced (current path 4). This shunting can be done reversibly by cutting open the canal wall in the air gap and short-circuiting the incision to the saline pool containing the ampulla with a salt bridge. When an isolated ampulla with an input resistance of 300 kΩ is shunted by the remaining canal resistance of 15 kΩ, the response becomes graded and no threshold can be demonstrated.

The disappearance of the well-defined threshold when the epithelium is shunted excludes the possibility that the receptor cells are synchronized mainly by way of electrotonic junctions between cells in the epithelium. Voltage clamp experiments in the following paper indicate that the lumenal faces probably remain excitable when the canal is short-circuited because of the series resistance of the basal faces. If synchronization of the receptor cells were mediated primarily by electrotonic junctions between them, the threshold characteristic would be little affected by reducing the input resistance of the epithelium. The
above experiments therefore demonstrate that the synchronization is mediated mainly by extracellular pathways.

Since there have been no freeze-fracture studies of skate electroreceptor epithelium, the possibility of small gap junctions within the zonulae occludentes cannot be excluded. However the existence of such gap junctions, with a small degree of coupling between receptor cells, would not affect the conclusions in this or the following paper.

Calcium Dependence of Repolarization

Sufficient reduction in the extracellular free calcium concentration in the lumen of the ampulla causes the action potential to become plateau shaped with no signs of repolarization more than 20 s after excitation. The inhibition of the repolarizing process by low calcium and by cobalt could be interpreted in several ways. For example, the reduction in extracellular calcium could lead to inactivation of the repolarizing process by a nonspecific effect on the membrane not involving intracellular calcium. The effect of cobalt could be explained by a direct action on the repolarizing process. However, when the similar effects of cobalt and low calcium are considered together, the simplest explanation is that the repolarizing process is initiated by an influx of calcium into the cytoplasm. Since the calcium influx associated with the plateau-shaped action potential evoked in the low calcium solution was not sufficient to initiate repolarization, it appears that a certain minimum level of intracellular calcium is required.

There is a marked similarity between the effect of lowering the extracellular calcium concentration in the lumen and that of passing large excitatory current pulses across the epithelium. In both cases, the rate of repolarization becomes progressively slowed until repolarization is completely blocked. The effect of reduced calcium and the effect of large excitatory currents are additive, as shown by the fact that the suppression potential for repolarization is reduced when the lumen is bathed in low calcium saline and increased in high calcium saline.

In squid giant synapse, passage of large outward currents was found to block another calcium-dependent process, release of synaptic transmitter. Katz and Miledi (1967) and Kusano et al. (1967) injected TEA into the presynaptic terminal of squid giant synapse so that large depolarizations could be achieved. They found that depolarization of the terminal beyond +130 mV blocks transmitter release until the end of the stimulus. The lowest potential at which transmission is blocked was termed the suppression potential, and the postsynaptic potential occurring at the end of the stimulus was attributed to "delayed release." The suppression potential was presumed to be the calcium equilibrium potential. Stimuli causing the presynaptic membrane to exceed this voltage block the influx of calcium into the cytoplasm. When the stimulus is terminated, a significant calcium influx occurs before the membrane returns to its resting potential and the calcium channels close. This calcium influx produces the delayed release. Using aequorin, Llinás and Nicholson (1975) demonstrated that the suppression potential corresponds to the voltage at which the calcium influx is blocked. An influx of calcium after termination of the stimulus accompanies delayed release. Blockage of aequorin luminescence during large excitatory stimuli, with delayed
luminescence on repolarization, had previously been demonstrated in *Aplysia* neurons by Stinnakre and Tauc (1973).

In skate electroreceptors with a normal concentration of calcium in the lumen, excitatory stimuli which cause the epithelial voltage to exceed 150 mV block repolarization. Since most of the transepithelial voltage is developed across the excitable lumenal membrane, the effect of large stimuli can be explained by supposing that they cause the luminal membrane to exceed the calcium equilibrium potential. Repolarization is delayed until the end of the stimulus because the repolarizing process is initiated by a calcium influx which does not occur until the luminal membrane falls below the calcium equilibrium potential. The rate at which the repolarizing conductance develops should depend on the rate of calcium influx. Thus the falling phase of an action potential evoked by a weak stimulus and the time course of delayed repolarization after a strong stimulus are faster in higher calcium concentrations (Fig. 10). Moreover, in both high and low calcium solutions repolarization is slowed as the suppression potential is approached. Voltage clamp data to be presented in the following paper (Clusin and Bennett, 1977) support this interpretation.

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