# Ion Channels and Transporters in the Electroreceptive Ampullary Epithelium from Skates

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ABSTRACT Two ampullary epithelial properties necessary for electroreception were used to identify the types of ion channels and transporters found in apical and basal membranes of ampullary receptor cells of skates and to assess their individual role under voltage-clamp conditions. The two essential properties are (1) a steady-state negative conductance generated in apical membranes and (2) a small, spontaneous current oscillation originating in basal membranes (Lu and Fishman, 1995). The effects of pharmacological agents and ion substitutions on these properties were evaluated from transorgan or transepithelial complex admittance determinations in the frequency range 0.125 to 50 Hz measured in individual, isolated ampullary organs. In apical membranes, L-type Ca channels were found to be responsible for generation of the steady-state negative conductance. In basal membranes, K and Ca-dependent Cl (Cl(Ca)) channels were demonstrated to contribute to a net positive membrane conductance. L-type Ca channels were also evident in basal membranes and are thought to function in synaptic transmission from the electroreceptive epithelium to the primary afferent nerve. In addition to ion channels in basal membranes, two transporters ($Na^+/K^+$ pump and $Na^+$-$Ca^{2+}$ exchanger) were apparent. Rapid (minutes) cessation of the current oscillation after blockage of any of the basal ion channels (Ca, Cl(Ca), K) suggests critical involvement of each of these channel types in the generation of the oscillation. Suppression of either $Na^+/K^+$ transport or $Na^+$-$Ca^{2+}$ exchange also eliminated the oscillation but at a slower rate, indicating an indirect effect.

## INTRODUCTION

The transduction of an electric field stimulus into an encoded afferent nerve discharge that is conveyed to the brain of elasmobranch fishes occurs in specialized ampullary organs (Murray, 1962, 1965; Obara and Bennett, 1972; Clusin and Bennett, 1979a,b; Broun and Govardovskii, 1983; Bodznick et al., 1993; Lu and Fishman, 1994). Measurements on both intact and isolated organs also show that microvolt changes across the ampullary epithelium produce a measurable postsynaptic response and primary afferent nerve output (Murray, 1962; Clusin and Bennett, 1979a,b; Lu and Fishman, 1994). Consequently, the extraordinary sensitivity of elasmobranch fishes to electric fields (Kalmijn, 1966) seems to be due primarily to the properties of individual organs.

Viewed as a problem in signal processing and with the assumption of normal synaptic processes (Del Castillo and Engbaek, 1954), amplification of transampullary voltages (from $\mu$V stimuli to mV depolarizations of presynaptic membranes for transmitter release) could occur by the generation of separate steady-state negative and positive conductances located in apical and basal membranes, respectively, of ampullary epithelial cells (Lu and Fishman, 1994). In addition, an oscillation generated by the interplay of ion conductances in basal membranes (Lu and Fishman, 1995) seemed to be necessary for synaptic transmission. Here we used the effects of pharmacological agonists and antagonists on transorgan complex admittance spectra to identify the ion channels in apical and basal membranes, which produce the conductance characteristics and the oscillatory behavior at each surface of the ampullary epithelium that are necessary for electroreception.

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## MATERIALS AND METHODS

### Preparation and solutions

The dissection of ampullary organs from skates with attached afferent nerve, the ionic composition of elasmobranch salines, and the electrical measurements (voltage-clamp technique) were described previously (Lu and Fishman, 1994). The perfusion of the lumen of an isolated ampulla has also been described (Lu and Fishman, 1995). Nitrendipine, S(−)-BAY K 8644, clonazepam, and niflumic acid were prepared in methanol stocks and diluted to final concentrations at the time of usage. $Cd^{2+}$, $\omega$-conotoxin GVIA, tetrodotoxin (TTX), amiloride, ouabain, 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS), dendrotoxin ($\gamma$-DTX), charybdotoxin (ChTX), apamin, tetraethylammonium (TEA), 4-aminopyridine (4-AP), and $Ba^{2+}$-containing solutions were prepared in stocks with distilled water and diluted to final concentrations. $Na^+$-free solution was obtained by replacing $Na^+$ with equimolar tetramethylammonium. $Cl^-$-free solution was made by replacing $Cl^-$ with equimolar gluconate. Nitrendipine, S(−)-BAY K 8644, $\omega$-conotoxin GVIA, $\gamma$-DTX, and ChTX were obtained from Research Biochemicals Incorporated (Natick, MA). Other chemicals were purchased from Sigma (St. Louis, MO). The osmolality of all solutions was adjusted to 1025 mOsm by the addition of sucrose, when required, and by the use of an osmometer (Wescor, Logan, UT). The pH of all solutions was adjusted to 7.4 at room temperature (20–24°C).

### Driving-point function determinations

To characterize the transorgan conduction properties, we used complex admittance spectroscopy (Fishman, 1992). The time course of the current, $I_{TO}(t)$, through an organ was obtained in response to a small amplitude, synthesized waveform (<100 $\mu$V rms), consisting of the sum of 400

sinusoids from 0.125 to 50Hz, applied as a repetitive (waveform period of 8 s) transorgan voltage, $V_{TO}(t)$. Data were acquired in a steady state (after one or more cycles of the continuously applied synthesized signal had occurred). The total experimental time to acquire and process a single response to obtain a 400-point admittance function in the 50-Hz frequency band took ~1.5 min. The complex admittance, $Y_{TO}(jf)$, of the organ was computed as

$$Y_{TO}(jf) = \frac{\mathcal{F}[I_{TO}(t)]}{\mathcal{F}[V_{TO}(t)]} = \frac{I_{TO}(jf)}{V_{TO}(jf)} = G(f) + jB(f) \quad (1)$$

where $\mathcal{F}$ denotes a fast Fourier transform of the sampled functions of time $I_{TO}(t)$ and $V_{TO}(t)$, and $I_{TO}(jf)$ and $V_{TO}(jf)$ are the transformed functions of complex ($j = (-1)^{1/2}$) frequency ($f$). In Eq. 1, $G(f)$ is the real part and $B(f)$ is the imaginary part of $Y_{TO}(jf)$. The magnitude of $Y_{TO}(jf)$ is defined as

$$|Y_{TO}(jf)| = \sqrt{G^2(f) + B^2(f)} \quad (2)$$

Admittance data are presented as complex plane plots (imaginary part versus real part) and plots of log magnitude versus log frequency.

## RESULTS

### Cl channels in basal membranes

One type of channel that is often found in sensory epithelia and that generates a positive conductance is a Cl channel. At the resting potential of cells, $Cl^-$ is usually distributed approximately at equilibrium, thus intracellular $Cl^-$ concentration is lower than that of the extracellular concentration. If the cell membrane is depolarized, Cl channels open and the membrane potential tends toward the Nernst potential for $Cl^-$ ($E_{Cl}$) to oppose the depolarization in the cell (similar to the effect of K channels).

To probe for $Cl^-$ processes in basal membranes of the electroreceptive epithelium, the basal side of the ampullary epithelium was bathed in a $Cl^-$-free solution (isosmotic with elasmobranch saline solution but with gluconate replacement of $Cl^-$). The complex admittance of an ampullary organ plotted as the locus of frequency points in the complex plane ($B(f)$ versus $G(f)$) and its magnitude function ($|Y_{TO}(jf)|$) in regular saline are shown in Fig. 1, *A* and *B*. Within 3 min after replacement with the $Cl^-$-free solution (in $n = 4$ preparations), the amplitude of the oscillatory current decreased and the current baseline began to drift. After 8 min had elapsed, the oscillation and the negative conductance disappeared (Fig. 1, *C* and *D*). These $Cl^-$-free effects were reversible. Within 10 min of return to normal saline, the oscillatory current had recovered totally, and the negative conductance was evident; after 17 min, the negative conductance recovered fully (Fig. 1, *E* and *F*). The relatively rapid loss of the current oscillations in voltage-clamped ampullae after $Cl^-$-free replacement of the basal side solution suggested the presence of $Cl^-$ dependent processes located on basal membranes of the ampullary epithelium.

The existence of Cl channels or transporters in basal membranes was established by application of DIDS, which is a Cl channel blocker (Gögelein, 1988) and an inhibitor of $Cl^-$ transport (Russell and Brodwick, 1981). Addition of 100 $\mu$M DIDS to the basal solution abolished the ampullary oscillation within minutes, whereas DIDS did not eliminate the negative conductance for more than 20 min (Fig. 2, *A–D*). Higher concentrations of DIDS (200–400 $\mu$M) resulted in more rapid disappearance of both processes. Thus maintenance of basal-side $Cl^-$ processes is necessary for basal membrane generation of oscillations and has long-term effects on apical negative conductance.

Several studies have shown that DIDS blocks $Ca^{2+}$-dependent Cl (Cl(Ca)) channels in epithelia (Anderson and Welsh, 1991; Anderson et al. 1992). Cl(Ca) channels are widely distributed in olfactory receptor cells and neurons (Lowe and Gold, 1993; Kleene, 1993; Mayer, 1985). Because DIDS eliminated the ampullary oscillation when applied to the basal side, we further explored the possibility of Cl(Ca) channels in basal membranes. We used niflumic acid, which is a specific blocker of Cl(Ca) channels in other sensory cells and neurons (Kleene, 1993; Lowe and Gold, 1993), to determine whether Cl(Ca) channels are present in basal membranes of the ampullary epithelium. Addition of 0.5 mM niflumic acid to the basal solution had immediate effects. The baseline of the ampullary current began to shift. Because of the shift, the admittance function was not stationary. Nevertheless, 5 min after the addition of niflumic acid (number of preparations ($n$) = 4), the acquired data (compare Fig. 3, *A* and *B* with *C* and *D*) clearly show the absence of the oscillation and of the negative conductance (Fig. 3, *C* and *D*). The irreversibility of these effects after removal of niflumic acid from the basal-side solution indicated decay of the preparation. Rapid suppression of the oscillation by niflumic acid and DIDS is consistent with the presence of a Cl(Ca) channel in the basal membrane of the ampullary epithelium.

### K channels in basal membranes

The presence of K channels in basal membranes of the ampullary epithelium was reported previously (Clusin and Bennett, 1979a,b; Broun et al., 1984). In addition to Cl(Ca) channels, K channels could also generate a positive conductance in basal membranes of the electroreceptive epithelium. Again, our assay of drug effects was confined to the functionally relevant basal membrane oscillations and apical membrane negative conductance. Our results not only confirmed the existence of K channels but also showed K channel involvement in oscillations in basal membranes of ampullae (Table 1).

TEA is a general type of K channel blocker (Hagiwara and Saito, 1959). Low concentrations of TEA (0.5–2.0 mM) in the basal-side solution blocked the oscillation. Exposure of basal membranes to the low concentrations of TEA also completely eliminated the net negative conductance of the preparation in 15–20 min. The TEA effects were reversible. Higher concentrations of TEA (10 mM or more) abolished the oscillation and the negative conductance more rapidly. $Ba^{2+}$ was less effective than TEA, requiring 10 mM to

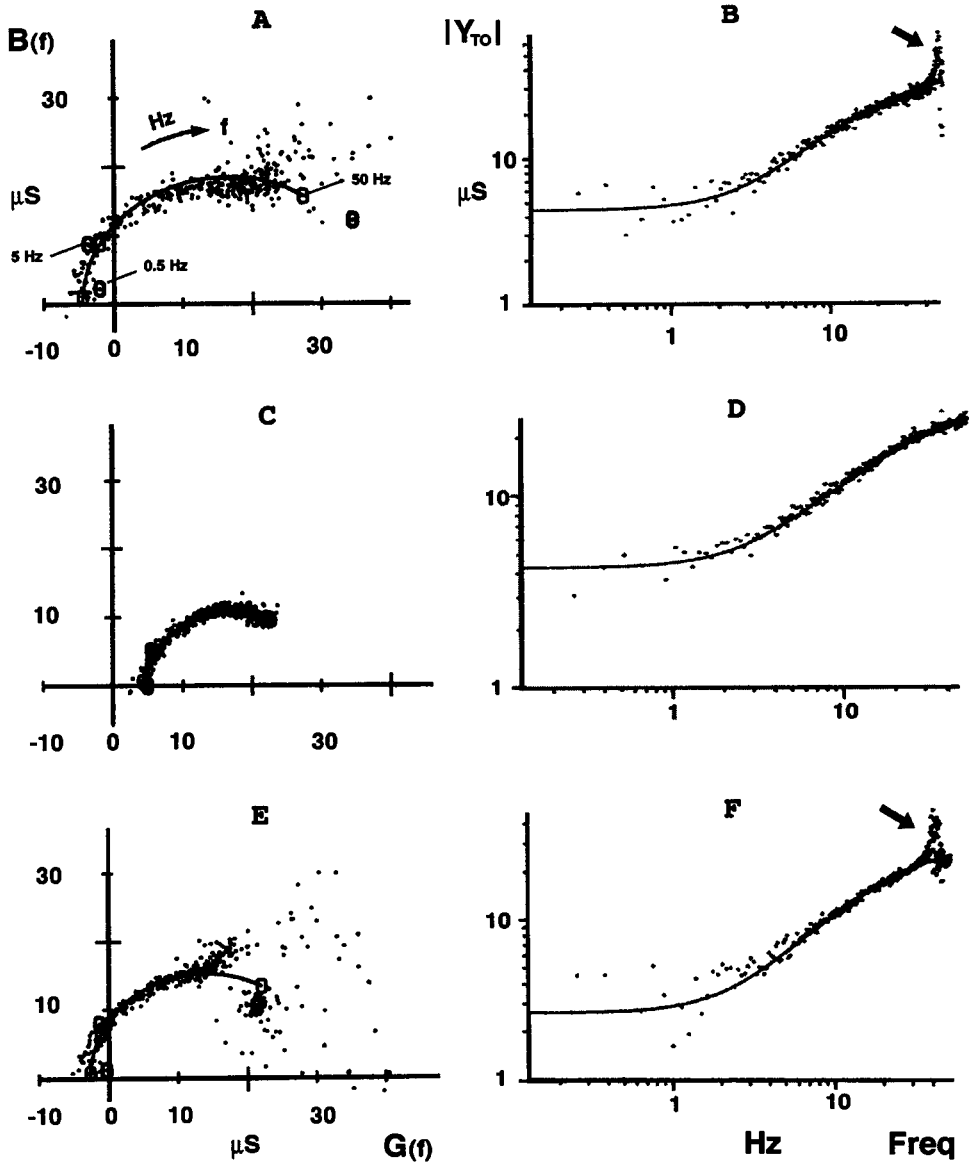


FIGURE 1 Reversal of the abolition of both the negative conductance and the current oscillation in an isolated, voltage- clamped ampullary organ after replacement of the basal-side elasmobranch saline by a $Cl^-$-free saline. (*A*) Control transorgan admittance $\{Y_{TO}(jf) = G(f) + jB(f)\}$ plotted as a locus of frequency ($f$) points in the complex plane $\{B(f)$ versus $G(f)\}$. Note that $G(f) < 0, f <$ 10 Hz, before basal-side solution replacement of elasmobranch saline. (*B*) Replot of the magnitude function $\{|Y_{TO}(jf)| = [G^2(f) + B^2(f)]^{1/2}\}$ of the complex admittance in *A* shows the presence of an oscillation (*arrow*). (*C*) The admittance locus determined 8 min after replacement of elasmobranch saline with isosmotic $Cl^-$-free solution. The transorgan steady-state conductance was now positive $\{G(f) > 0\}$. (*D*) Replot of the magnitude function of the complex admittance shows that the oscillation was also abolished. (*E* and *F*) Negative conductance and the oscillation returned 17 min after wash out. Solid-line curves are best fits (mean square error criterion) of an admittance model (Fig. 9 in Lu and Fishman, 1994). The scattering of locus points at the upper frequencies in *A* and *E* is due to the spectral components generated by the oscillation (*arrows*) in the magnitude functions in *B* and *F*. Admittance data obtained on an organ clamped at 0 mV and acquired during a 1.5-min interval in which a 16-$\mu$V rms synthesized signal was applied to the voltage-clamp system for the admittance determination.

eliminate the oscillation. Another established blocker of voltage-dependent K channels in axonal membranes is 4-AP (Meves and Pichon, 1977). Low concentrations of 4-AP (2 mM) suppressed delayed-rectifier K conductance markedly in molluscan neurons (Hermann and Gorman, 1981). In the ampullary organ preparation, low concentrations of 4-AP (2 mM) abolished the oscillation quickly but had no effect on the negative conductance for 30 min. A specific blocker for one type of delayed-rectifier K current, γ-DTX, which has a much smaller activating potential range than the other type of delayed-rectifier K current, in *Xenopus* (Brau et al., 1990) did not affect the oscillation and the negative conductance, even at high concentrations (10 nM). All of the above observations suggest the presence of delayed-rectifier K channels, which are not γ-DTX-sensitive, in basal membranes.

To identify other K channel types in basal membranes, charybdotoxin (ChTX), which blocks large, Ca-dependent K channels (K(Ca)) (Strong, 1990; Castle et al. 1989), and apamin, which blocks small K(Ca) channels (Blatz and Magleby, 1986), were added in separate experiments to the basal solution. None of these treatments affected the oscillation or the negative conductance, even at high concentrations (33 nM and 1.23 $\mu$M, respectively). Nevertheless, because of the existence of other Ca-activated K channels that are not ChTX or apamin sensitive (Sauvé et al., 1995), the possible existence of K(Ca) channels in basal membranes cannot be rejected.

## Ca channels in basal membranes

Cl(Ca) channels are important for the generation of the current oscillation in basal membranes of the ampullary epithelium. Because activation of this type of Cl channel depends on $Ca^{2+}$ entry from extracellular sources, we stud-

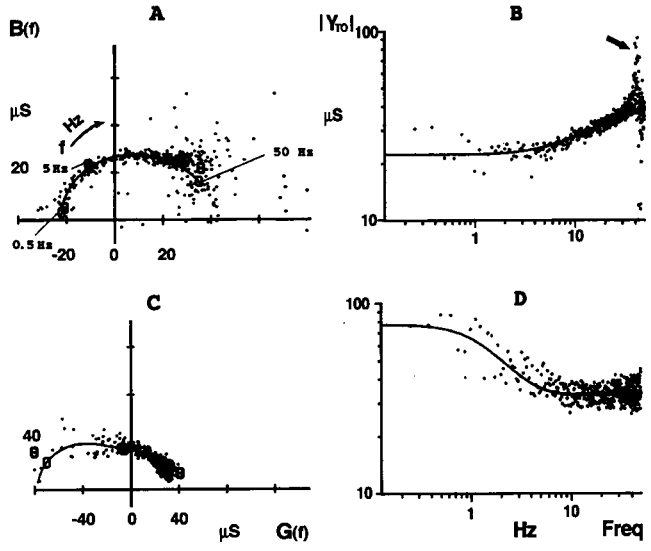


FIGURE 2 DIDS (100 $\mu$M) added to the basal-side elasmobranch saline solution rapidly eliminated ampullary organ generation of an oscillation, but generation of a steady-state net negative conductance persisted. (*A* and *B*) Controls before addition of DIDS are as described in the caption of Fig. 1. (*C*) Admittance determined 3 min after addition of 100 $\mu$M DIDS to the basal solution. The negative conductance remained, and its magnitude was substantially increased. (*D*) Replot of the magnitude function of the complex admittance after DIDS addition shows that the oscillation was abolished. Solid-line curves are best fits of an admittance model (Fig. 9 in Lu and Fishman, 1994). The scattering of locus points at the upper frequencies in *A* are due to the spectral components generated by the oscillation seen (*arrow*) in the magnitude functions in *B*. Admittance data obtained on an isolated organ held in a voltage clamp at 0 mV and acquired during a 1.5-min interval in which a 16-$\mu$V rms synthesized signal was applied to the voltage clamp system for the admittance determination.

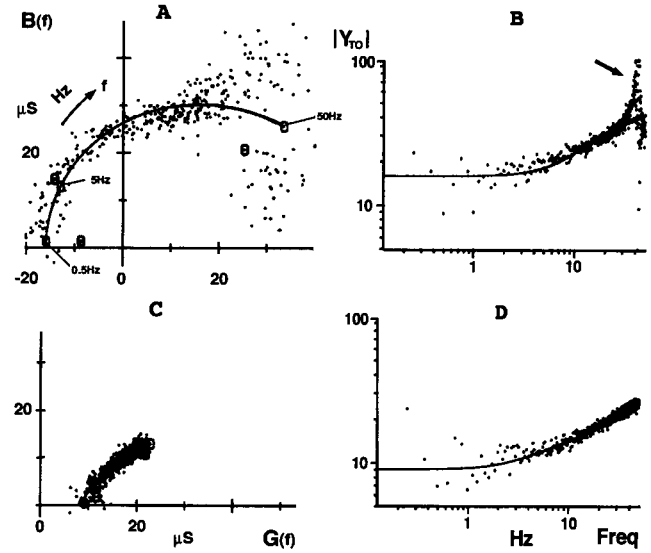


FIGURE 3 Niflumic acid (0.5 mM) added to the basal-side elasmobranch saline solution rapidly abolished isolated ampullary organ generation of both a negative conductance and an oscillation. (*A* and *B*) Controls before the addition of niflumic acid are described in the caption of Fig. 1. (*C*) The admittance locus determined 5 min after addition of 0.5 mM niflumic acid to the basal-side solution. The transorgan steady-state conductance was now positive $\{G(f) > 0\}$. (*D*) Replot of the magnitude function of the complex admittance after niflumic acid addition shows the oscillation was also abolished. Experimental conditions and curve fitting procedure are the same as those in the caption of Fig. 1.

ied the effect of an agonist and antagonists of $Ca^{2+}$ channels in ampullary epithelia. The results of these experiments are listed in Table 2.

$Cd^{2+}$ is a general blocker of Ca channels (Lakshminarayanaiah, 1991; Byerly and Hagiwara, 1982), and nitrendipine is a specific antagonist of L-type $Ca^{2+}$ channels whereas S(−)-BAY K 8644 is an agonist of L-type $Ca^{2+}$ channels (Janis and Triggle, 1991). Both $Cd^{2+}$ and nitrendipine when added separately to the basal-side solution eliminated oscillations immediately but did not eliminate the net negative conductance of the preparation for more than 30 min. The suppressive effect of $Co^{2+}$ (Clusin and Bennett, 1979b) on the steady-state oscillation was less than that of either $Cd^{2+}$ or nitrendipine. Within minutes of replacement of the control basal-side solution with one containing (S(−)-BAY K 8644), the current through the ampullary epithelium began to drift, and the amplitude of the oscillation increased. This result complements the nitrendipine finding and supports the interpretation that L-type Ca channels exist in basal membranes.

The N-type Ca channel blocker, $\omega$-conotoxin GVIA (Kasai et al., 1987), was also added to the basal-side solution of ampullary epithelia ($n$ = 3). Even at high concentration (20 nM), no significant effect was observed on either the preparation current oscillation or negative conductance, suggesting that basal membranes of ampullary epithelia do not have $\omega$-conotoxin GVIA-sensitive N-type Ca channels.

Finally, because $Na^+$-$Ca^{2+}$ exchange is essential for regulation of intracellular $Ca^{2+}$ concentration in many cells (Reuter et al., 1973) and particularly in sensory receptors (Yau and Nakatani, 1984, 1985; Yau and Baylor, 1989), we examined the effect of a known inhibitor of $Na^+$-$Ca^{2+}$ exchange, clonazepam (Cox and Matlib, 1993), when applied to the basal side. A control complex admittance (Fig. 4 *A*) and magnitude function (Fig. 4 *B*) were obtained from an ampullary organ in elasmobranch saline. The addition of

**TABLE 1 Effects of basal-side K channel blockers on ampullary epithelium properties (n = 3)**

| Agents | TEA | 4-AP | $Ba^{2+}$ | Apamin | γ-DTX | ChTX |
|---|---|---|---|---|---|---|
| Concentration | 0.5~2* | 1~2 | 10 | $10^{-3}$ | $10^{-5}$ | $3*10^{-5}$ |
| Oscillation | + | + | + | ○ | ○ | ○ |
| Negative conductance | + | ○ | + | ○ | ○ | ○ |

Note: + Indicates an agent has an effect. ○ Indicates an agent has no effect. * Concentrations: mM unless written in the text. A given symbol indicates the same effect in all experiments using the particular agent.

**TABLE 2 Effects of basal-side Ca channel blockers on ampullary epithelium properties (n = 3)**

| Agents | $Cd^{2+}$ | Nitrendipine | S (−)-BAY K 8644 | ω-Conotoxin GVIA |
|---|---|---|---|---|
| Concentration | 10 | $2*10^{-3}$ | $10^{-4}$ | $2*10^{-5}$ |
| Oscillation | + | + | + | ○ |
| Negative conductance | ○ | ○ | + | ○ |

Note: + and ○ are defined in Table 1.

150 μM clonazepam to the basal solution abolished the oscillation after an elapsed time ranging from 5 to 20 min (Fig. 4, *C* and *D*), but the net negative conductance of the preparation remained (compare Fig. 4, *A* and *B* with *C* and *D*). The action of clonazepam was reversible (Fig. 4, *E* and *F*). The relatively rapid suppression of oscillations by clonazepam without significantly affecting the negative conductance of the preparation again supports the conclusion that the oscillations are generated in basal membranes independently of apical membrane conduction (Lu and Fishman, 1995). The action of clonazepam also suggests $Na^+$-$Ca^{2+}$ exchange in basal membranes of ampullary epithelia.

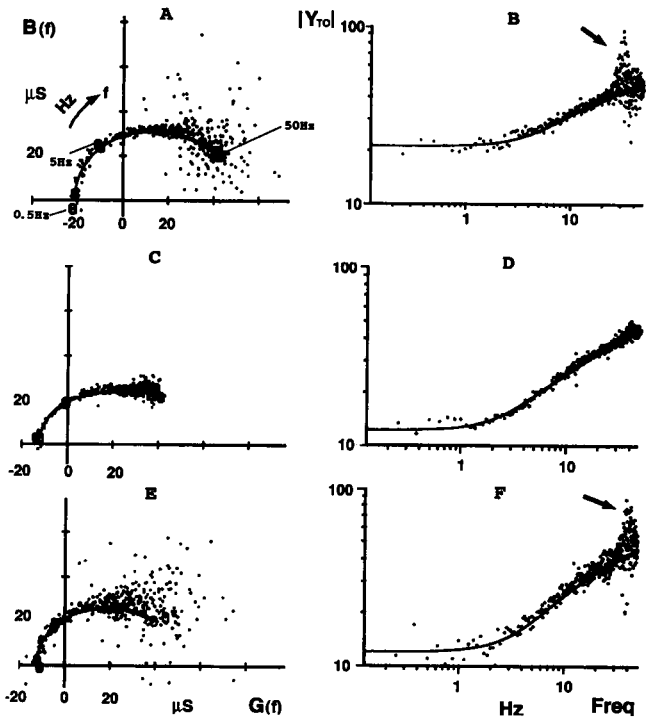


FIGURE 4 Reversal of the abolition of the oscillation after addition of clonazepam (150 μM) to the basal-side elasmobranch saline solution. (*A* and *B*) Controls before the addition of clonazepam are as described in the caption of Fig. 1. (*C*) The admittance locus determined 5 min after addition of 150 μM clonazepam to the basal-side solution. A steady-state negative conductance $\{G(f)<0, f< 5$ Hz$\}$ persisted. (*D*) Replot of the magnitude function of the complex admittance after clonazepam addition shows that the oscillation was eliminated. (*E* and *F*) Oscillation is reestablished after return to control solution on the basal side. Experimental conditions and curve fitting procedure are the same as those in the caption of Fig. 1.

## $Na^+/K^+$ pumps but no Na channels in basal membranes

$Na^+$-$Ca^{2+}$ exchange across cell membranes usually coexists with $Na^+/K^+$ transport to regulate intracellular $Ca^{2+}$ and $Na^+$ concentrations (Moore et al., 1993). We therefore used ouabain (Keynes, 1963; Russell, 1976) to suppress $Na^+/K^+$ pumps in basal membranes and to determine whether these pumps have a role in electroreception. The ampullary organ complex admittance and magnitude function in control elasmobranch saline showed a negative conductance and the presence of an oscillation under voltage-clamp conditions (Fig. 5, *A* and *B*). The oscillation ceased 8 min after the addition of 0.1 mM ouabain to the basal-side saline; after 10 min had elapsed, the negative conductance was affected. Fig. 5, *C* and *D*, show admittance data acquired 20 min after the addition of ouabain, at which time both the oscillation and the negative conductance were lost. These ouabain experiments demonstrated the existence of $Na^+/K^+$ pumps in basal membranes and a role for pumps, in addition to the one for channels, in sustaining oscillations.

To test for Na channel involvement in the oscillation, TTX (Narahashi et al., 1964) and amiloride (Benos, 1982; Wills, 1990), the blocking agents of different types of Na channels, were applied to the basal side of isolated ampullary organs (Table 3). No change in either the oscillation or the negative conductance was observed. However, when $Na^+$ was completely replaced by tetramethylammonium

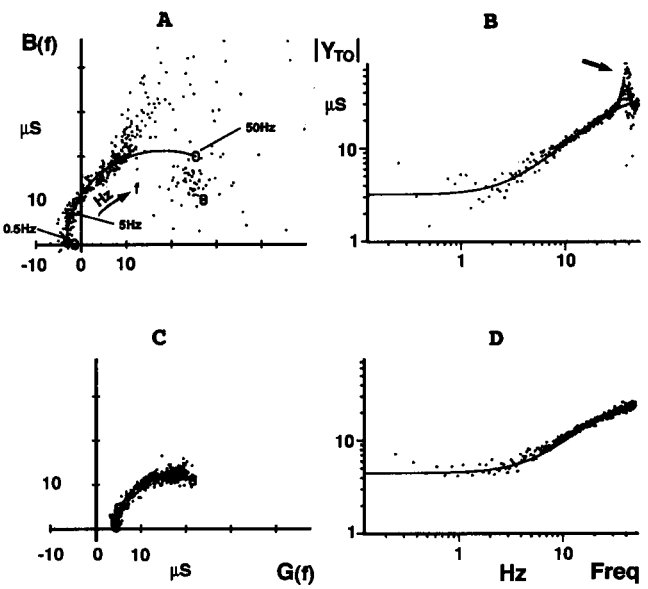


FIGURE 5 Ouabain (0.1 mM) added to the basal-side elasmobranch saline solution abolished the generation of both the negative conductance and the oscillation in an isolated ampullary organ. (*A* and *B*) Controls before the addition of ouabain are as described in the caption of Fig. 1. (*C* and *D*) Negative conductance and oscillation are eliminated within 20 min by addition of 0.1 mM of ouabain to the basal solution. Experimental conditions and curve-fitting procedure are the same as those in the caption of Fig. 1.

**TABLE 3 Basal-side $Na^+$-dependent effects on ampullary epithelium properties (n = 3)**

| Agents | Ouabain | TTX | Amiloride | $Na^+$-free |
|---|---|---|---|---|
| Concentration | 0.1 | $10^{-3}$ | 0.1~0.5 | |
| Oscillation | + | O | O | + |
| Negative conductance | + | O | O | + |

Note: + and O are defined in Table 1.

ions in the basal solution, the oscillation ceased and shortly thereafter the negative conductance disappeared, again implying effects mediated via $Na^+/K^+$ pumps and/or $Na^+$-$Ca^{2+}$ exchangers.

### Ca channels in apical membranes

Ca and Na channels are prospective channels for the generation of a negative conductance in apical membranes because they can produce inwardly directed current for membrane depolarization. To identify Ca channels in the apical membranes of receptor cells, we perfused the lumen of an organ with solutions containing either $Cd^{2+}$ or nitrendipine (Table 4). Both treatments eliminated the net negative conductance of the preparation within 20 min. $Cd^{2+}$ (50 mM) also affected the oscillation, most likely because we used a high concentration. Furthermore, luminal perfusion with a $Ca^{2+}$-free solution plus 20 mM EGTA eliminated the preparation negative conductance. These results suggest that Ca channels occur in apical membranes, and the effect of nitrendipine indicates that they are of the L-type. These channels seem to underlie generation of the net negative conductance in an isolated organ preparation.

To assess the possible existence of Na channels in apical membranes, high concentrations of conventional Na channel blockers (amiloride (0.5 mM) and TTX (40 $\mu$M)) were added to solutions used to perfuse apical membranes. No effect on the negative conductance or the oscillation was observed (Table 4). Using spikes of transampullary voltage (state I or II; Lu and Fishman, 1994) as an assay, we also confirmed the lack of an effect of these Na channel blockers when added to perfusates under open-circuit conditions.

In addition to Ca channels in apical membranes, we also used TEA and other blockers to determine whether K channels and transporters occur in apical membranes as well. We observed no changes in spikes or the negative conductance in admittance determinations after perfusion of apical membranes with solutions containing either 20 or 100 mM TEA under open-circuit conditions and with solutions containing 50 mM TEA under short-circuit conditions ($n$ = 2).

**TABLE 4 Effect of apical-side channel blockers on ampullary epithelium properties (n = 2)**

| Agents | $Cd^{2+}$ | Nitrendipine | Amiloride | TTX |
|---|---|---|---|---|
| Concentration | 50 | 0.05 | 0.5 | 0.04 |
| Oscillation | + | O | O | O |
| Negative conductance | + | + | O | O |

Note: + and O are defined in Table 1.

## DISCUSSION

### Functional aspects of the identified ion channels and transporters in basal membranes

We found several ion channel types (Cl(Ca), K, Ca channels) and two transporters ($Na^+$-$Ca^{2+}$ exchange and $Na^+/K^+$ pump) in basal membranes of ampullary epithelia (Fig. 6). Each of these processes was identified by application of specific agents that either eliminated or enhanced basal membrane oscillations, which are necessary for synaptic transmission (Lu and Fishman, 1995). Thus each of these membrane processes has a crucial role in electroreception. In a lumped admittance model of the ampullary organ (Eq. 2 in Lu and Fishman, 1994), the transepithelial conductance is the sum of a negative and a positive conductance. The blockage of Cl channels in basal membranes by DIDS produced an increase in the magnitude of the lumped ampullary epithelial negative conductance (compare low frequency portion of Fig. 2, *A* and *C*). This result indicates that conducting Cl channels contributed to the net positive steady-state conductance in basal membranes. On the basis of $E_{Cl}$ relative to the resting potential, Cl(Ca) channels can generate activity-dependent after-hyperpolarizations or after-depolarizations with significant effects on encoding of impulse firing (Mayer, 1985), and Cl(Ca) channels can also serve to amplify input signals (Lowe and Gold,

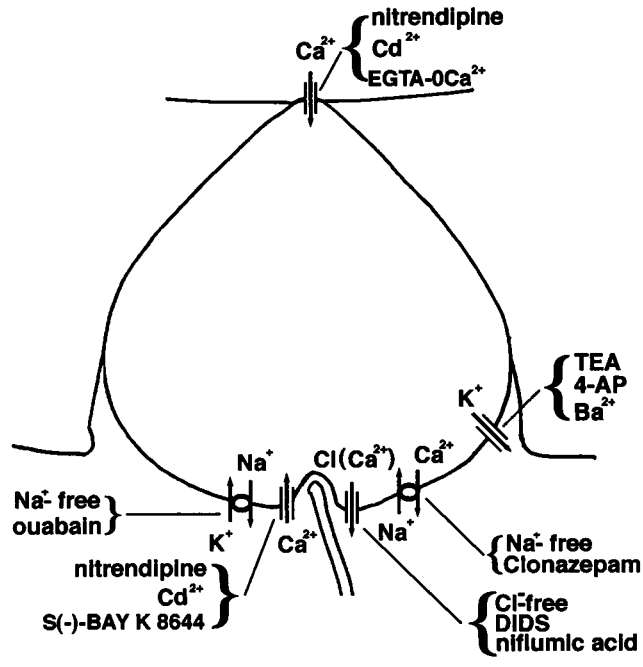


FIGURE 6 Diagram summarizing the ion channels and transporters in the ampullary epithelium identified by the effect of specific antagonists, agonists, or ion substitutions (in *brackets*) on the negative conductance and the oscillation, as interpreted from admittance spectra of isolated organs.

1993). We think that the function of activated basal membrane Cl(Ca) channels of the ampullary epithelium is to repolarize the basal membrane potential after membrane depolarization and the opening of basal membrane L-type Ca channels. Thus Cl(Ca) channels could participate in the generation of an endogenous (no stimulus applied) current oscillation in basal membranes by their delayed activation after $Ca^{2+}$ entry and their restorative effect on membrane potential. The subsequent hyperpolarization of membrane potential after activation of Cl(Ca) channels would deactivate Ca channels, which would result in less entry of $Ca^{2+}$ and deactivation of Cl(Ca) channels to enable a new cycle to begin again. The dependence of Cl(Ca) channels on $Ca^{2+}$ entry through Ca channels in presynaptic membrane suggests that they (like Ca channels) may be present only in the presynaptic portion of basal membranes.

Cl(Ca) channels occur in other sensory cells and in neurons. Barnes and Hille (1989) reported Cl(Ca) in a salamander cone photoreceptor. Lowe et al. (1993) and Kleene (1993) also found Cl(Ca) channels in rat olfactory receptor cells and amphibian olfactory receptor neurons, respectively.

In addition to Cl(Ca) channels, K channels were also identified in basal membranes by the TEA, 4-AP, and $Ba^{2+}$ experiments. In contrast to Cl(Ca) channels, K channels in basal membranes could also function to restore the resting potential but would be directly responsive to basal membrane voltage. The voltage sensitivity of these channels and their concerted conductances that generate a positive conductance in basal membranes would be necessary for signal amplification (Lu and Fishman, 1994) and for modification of the basal membrane endogenous oscillation produced by Ca and Cl(Ca) channels. The baseline of the current response of the electroreceptive epithelium declined rapidly after basal-side replacement of elasmobranch saline with high $K^+$ solution. This baseline shift indicates that $K^+$ conductance is important in the regulation of resting potential. Furthermore, the elimination of the oscillation within minutes of adding 2 mM 4-AP is consistent with delayed-rectifier K channel involvement in the generation and modification of oscillations (Hermann and Gorman, 1981). L-type Ca channels are found in many sensory neurons and are identified by their sensitivity to dihydropyridine (Tsien et al., 1988). Ca channels are also required for neurotransmitter release from chemically transmitting presynaptic membranes (Janis and Triggle, 1991). L-type Ca channels in basal membranes probably function, as they do in other presynaptic membranes, to allow an inflow of $Ca^{2+}$ that initiates neurotransmitter release upon depolarization of basal membranes.

The existence of Na-dependent processes in basal membranes was clearly demonstrated by the elimination of oscillations by $Na^+$-free solution. The failure of conventional epithelial and neural blockers of Na channels (amiloride and TTX) to produce any effect suggests that basal membranes lack Na channels. A $Na^+$ effect without Na channels suggests the presence of one or more $Na^+$ transport systems. Clonazepam reversibly eliminated the oscillations and left the negative conductance intact, which is indicative of a role for $Na^+$-$Ca^{2+}$ exchange. However, we cannot rule out the possibility that the cessation of the oscillation after inhibiting $Na^+$-$Ca^{2+}$ exchange is an indirect effect resulting from deregulation of receptor cell $Ca^{2+}$ homeostasis, even though this effect occurred rapidly (<20 min). Plasma membranes of other sensory cells (retinal rod photoreceptors) also have $Na^+$-$Ca^{2+}$ exchangers (Yau and Nakatani, 1984). The effect of ouabain on the oscillations suggests the presence in basal membranes of $Na^+/K^+$ pumps, which function in $Na^+$ homeostasis and also create the $Na^+$ gradient from which the $Na^+$-$Ca^{2+}$ exchange derives its energy. These transport processes may also be involved in the high degree of regulation necessary for stabilization of resting membrane voltage so that $\mu$V signals can be detected.

## Functional aspects of ion channels in apical membranes

Elimination of the negative conductance generated in apical membranes after application of either nitrendipine or $Cd^{2+}$ showed the presence of L-type Ca channels in apical membranes (Fig. 6). The generation of an apical membrane negative conductance is an essential property that underlies electroreception in an ampullary epithelium (Lu and Fishman, 1994). Furthermore, apical membrane Ca channels may play a key role in signal amplification by the ampullary epithelium. Our finding of Ca channels in apical membranes confirms and extends the findings of Clusin and Bennett (1979a) using $Co^{2+}$ and EGTA. We found no other channel types in apical membranes.

## Signal processing by the ampullary organ

From our results, detection of electric fields in receptor cells seems to be initiated by the activation of Ca channels above existing resting levels in apical membranes. How then is the response of the isolated ampulla to transepithelial voltage changes of a few microvolts reconciled with the fact that membrane depolarization of a few millivolts is usually necessary for the activation of a sufficient number of voltage-sensitive ion channels ($V$ relative to $kT/q$ (25 mV, where $k$ is Boltzman's constant, $T$ is temperature, and $q$ is charge)) to effect measurable change in membrane conductance? This apparent inconsistency has been explained by the possibility of a signal-to-noise (S/N) enhancement by an ampulla (Fishman, 1987; Pickard, 1988).

Each ampulla is composed of ~10,000 receptor cells arranged morphologically to respond to the same transampullary voltage. The outputs of these 10,000 cells converge on ~10 afferent nerve fibers that innervate the receptor cells. Such paralleled stages (receptor cells) whose outputs are summed (by afferents) can produce S/N enhancement over that in a single gain stage by the factor $1/\sqrt{n}$, where $n$ is

the number of paralleled stages. Assuming that 1000 receptor cells are innervated by each afferent nerve fiber, the S/N performance, compared with that of a single receptor cell responding to the transampullary voltage, would improve by a factor of 31.6. This enhancement of the signal (apical membrane conductance change) over the noise together with amplification by the epithelium (Lu and Fishman, 1994) could enable detection of a very small increase in the number of conducting Ca channels in apical membranes of receptor cells for microvolt changes across the ampullary epithelium.

Consequently, we propose that an electric field-induced increase in Ca channel conduction in apical membranes generates an increase in the existing negative conductance (a larger inwardly directed current for increased depolarization). The interplay of the negative conductance (generated by Ca channels in apical membranes) and the positive conductance (generated by Cl(Ca) and/or K channels that dominate conduction in basal membranes) results in signal amplification across basal (presynaptic) membranes (Lu and Fishman, 1994). This amplified signal modifies the steady endogenous oscillation generated in basal membranes. On further depolarization of basal membranes by the amplified signal, additional $Ca^{2+}$ flows into receptor cells locally through the increased number of conducting L-type Ca channels in the presynaptic region of basal membranes. This local inflow of $Ca^{2+}$ further activates Cl(Ca) channels, and the increased basal membrane depolarization activates K channels. Conduction in these two channel types opposes the depolarization and thereby constitutes the "opposing force" that results in the oscillatory character of the basal membrane voltage. Thus the apparent function of L-type Ca, Cl(Ca), and K channels in basal membranes is to modulate transmitter release and thereby encode afferent spike discharge, which conveys the electrical signal to the central nervous system of the skate.

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