Localization and Function of the Electrical Oscillation in Electroreceptive Ampullary Epithelium from Skates

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ABSTRACT A steady, spontaneous current oscillation (1 nA p-p) occurs in voltage-clamped, isolated ampullary organs (canal, ampulla, and nerve) from skates (*Raja*). Spectral analysis showed that energy in the oscillation was confined to a narrow band of frequencies (3 Hz) about a fundamental frequency (32 Hz at 20°C) and in harmonics. The frequency of the oscillation was temperature dependent (increasing from 21 to 33 Hz for increases in temperature from 13°C to 21°C). The addition of 0.5 μM tetrodotoxin to the basal side of the ampullary epithelium eliminated afferent nerve activity but had no effect on the epithelial oscillation, indicating that the oscillation is not generated or induced by afferent nerve activity. Nitrendipine (2 μM) added to the solution bathing the basal side of the ampullary epithelium abolished the oscillation rapidly (within minutes), but a steady-state negative conductance (i.e., real part of the complex admittance <0) generated by the preparation remained for 36 min. Conversely, nitrendipine (50 μM) added to the perfusate (artificial sea water) of the apical side eliminated the negative conductance rapidly (18.5 min) but had no effect on the spontaneous oscillation for more than 1 h. The effect and the elapsed time for an effect of nitrendipine after separate applications to the basal and apical membrane surfaces of ampullary epithelium suggest that 1) the negative conductance and the oscillation are generated independently in apical and basal membranes, respectively, and 2) both processes involve L-type Ca channels. Furthermore, the addition of tetraethylammonium (2 mM) to the basal side eliminated both the oscillation and the postsynaptic response to voltage clamps ($\leq$100 μV) of the ampullary epithelium in the operational voltage range of the afferent nerve. This result suggests that the basal membrane oscillation functions in neurotransmitter release from presynaptic (basal) membranes.

INTRODUCTION

Ampullary organs excised from skates and isolated electrically respond to microvolt stimuli. Clusin and Bennett (1979a) obtained postsynaptic responses for constant current stimuli that produced transampullary voltages of ~5 μV. For voltage clamps of ampullary epithelia as low as 3 μV, Lu and Fishman (1994) measured changes in the firing rate of the afferent nerve that innervates the basal membranes of the ampullary epithelium. Measurable change in afferent nerve activity in response to such low transepithelial voltages (μVs) requires neurotransmitter release from depolarized (mVs) presynaptic membranes, which implies signal amplification by the ampullary epithelium. From steady-state complex admittance determinations and from step voltage clamp data, Lu and Fishman (1994) also found that an isolated organ produced a net negative conductance (see Fig. 1). They deduced that the net negative conductance resulted from the generation of a negative conductance by ion channels in apical membranes (i.e., inwardly directed current for membrane depolarization) and a positive conductance by ion channels in basal membranes (i.e., outwardly directed current for membrane depolarization) of the ampullary epithelium. On the basis of these findings, amplification of transampullary voltage, sufficient for neurotransmitter to be released from basal (presynaptic) membranes, seemed possible with a series circuit consisting of the apical membrane negative conductance and the basal membrane positive conductance.

Here we show by use of ion channel blocking agents that the negative conductance is generated in apical membranes of the ampullary epithelium. We also describe the properties and location of another important phenomenon involved in electroreception, i.e., a spontaneous, steady oscillation emanating from an excised ampullary organ (Clusin and Bennett, 1979a). Stimulus-response properties of an organ preparation were obtained from rapid complex admittance determinations (Fishman, 1992). The characteristics of the spontaneous oscillation were also discernible in admittance spectra from a Fourier analysis. From spectral analysis of transampullary admittance during voltage clamp of an organ, we determined quantitatively the value of the net negative conductance and obtained simultaneously from the spectrum the amplitude and frequency content of the oscillation generated under various experimental conditions. Our data suggest that the negative conductance is generated by Ca channels in apical membranes, whereas the spontaneous oscillation is generated by interaction of different channel types (e.g., Ca and K) located in basal membranes of the ampullary epithelium. The oscillation may function in neurotransmitter release, which controls primary afferent nerve activity.

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MATERIALS AND METHODS

The isolation of an ampullary organ (the canal, the ampulla, and the attached portion of afferent nerve), the various electrical measurements (harmonic analysis, admittance determinations, and voltage-clamp technique), and the ionic composition of the bathing solutions were described previously (Lu and Fishman, 1994). Tetrodotoxin (TTX) and tetraethylammonium (TEA) chloride (Sigma Chemical, St. Louis, MO) were prepared in stock solutions and added to bathing solutions. Nitrendipine (Research Biochemicals International, Natick, MA) was dissolved in methanol and diluted to final concentration with a methanol concentration of ≤1%. We also determined that concentrations of methanol as high as 2%, when added to the basal side, did not affect the steady-state negative conductance or spontaneous oscillations.

In perfusion experiments, a 100-μm diameter plastic and fused silica cannula (MicroFil, World Precision Instruments, Sarasota, FL) was connected to one end of a microelectrode holder containing an Ag-AgCl pellet (EH-2FR, E. W. Wright, Guilford, CT), which was mounted on a micromanipulator. The other end of the microelectrode holder was connected by polyethylene tubing to a reservoir (12-ml syringe) located 20 cm above the chamber and filled with perfusate. The "dead space" was eliminated by opening a valve in series with the reservoir, which allowed the perfusate to flow to the tip of the cannula. The valve was then shut, and the tip of the cannula was inserted into the canal through a cut made in the canal wall 3 cm away from the ampulla. The tip was advanced until it was ~1 mm from the apical surface of the ampullary epithelium. Flow was initiated from the reservoir by opening the valve. The pressure head caused perfusion of the lumen and backflow of perfusate along the cannula and out the cut end of the canal. Measurement of transampullary voltage was made with a pair of high conductance Ag-AgCl (pellet) electrodes, one in the basal bathing solution and the other in the cannula holder. Phenol red was used to visualize the perfusion pathway (Clusin and Bennett, 1977) and to monitor pH. Perfusates containing phenol red usually took 15–20 min to travel a distance of 1 mm. Consequently, equilibrium concentrations of blocking agents were not established for relatively long times. To observe short term (<30 min) effects, a higher concentration of these agents was used in luminal perfusates.

The pH of all solutions was adjusted to 7.4 at room temperature. The osmolality of all solutions was measured with an osmometer (Wescor, Model 5100, Logan, UT) and adjusted by the addition of sucrose, if required, to assure iso-osmolality (1025 mOsm) with skate blood. The temperature of solutions was monitored with a digital thermometer (Model BAT-12, Sensortek, Clifton, NJ). All experiments were carried out at room temperature (20°-24°C) except for those stated otherwise in the text.

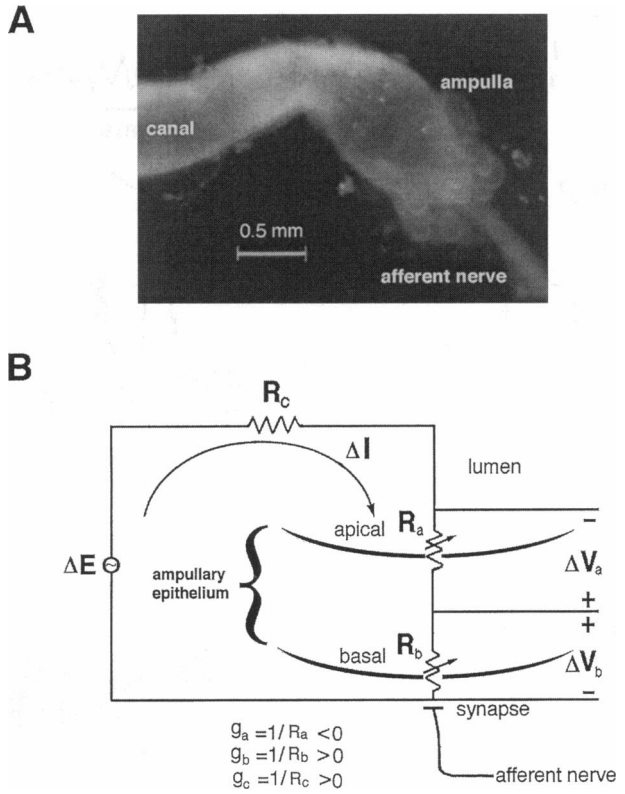

FIGURE 1 (*A*) The component parts of an ampullary organ excised from the skate *Raja erinacea* (Fishman, 1987). Ampulla (bulbous part) innervated by its afferent nerve and transition region into the canal. (*B*) Diagram of the direction of current flow and the incremental changes in voltages generated by a net negative conductance in apical membranes of the ampullary epithelium in the electrical pathway through an isolated ampullary organ under short-circuit steady-state conditions (i.e., transorgan voltage clamped at 0 mV and with a superposed small signal voltage, ΔE, (<100 μV) applied), as determined from frequency domain (admittance) analysis by Lu and Fishman (1994). An ohmic canal resistance (R_c) leads to the lumen of the ampullary epithelium (a single layer of cells) whose conduction properties are dominated by voltage-sensitive conduction in apical membranes (R_a) and basal membranes (R_b). The ampullary epithelium responds to the small signal voltage with a steady-state inwardly directed (apical to basal) current (ΔI) and by changes in both apical and basal membrane voltages, as indicated by the polarities of ΔV_a and ΔV_b (the common point between R_a and R_b corresponds to the interior of the epithelial cells). The current direction through and the polarity of the voltage change across apical membranes are indicative of a net negative conductance ($g_a < 0$) generated by ion channels in apical membranes, whereas the polarity of the voltage change across basal membranes is indicative of a net positive conductance ($g_b > 0$) generated by ion channels in basal membranes. From complex admittance [$Y(jf) = G(f) + jB(f), j = \sqrt{-1}$] determinations in the frequency (f) range 0.125 to 50 Hz, the net conductance in the transorgan pathway is negative (i.e., $G(f) < 0$), and thus the $g_a < 0$ generated by ion channels in apical membranes dominates ampullary organ conduction. Batteries corresponding to ion driving forces are not included in the diagram because the information was derived from incremental (admittance) analyses. Portions of basal membranes constitute the presynaptic membrane of synapses formed with the afferent nerve, which conveys encoded information about the transampullary epithelial voltage to the brain of the animal.

RESULTS

Characteristics of the ampullary organ oscillation

Under short-circuit conditions (voltage clamp of an organ to 0 mV), the current through an isolated viable ampullary organ with no stimulus applied showed a small, spontaneous oscillation (Fig. 2 *A*), which lasted for hours. The amplitude of the oscillation varied with time. The maximum peak-to-peak amplitude of the oscillation shown in Fig. 2 *A* was ~1 nA.

Spectral analysis of the oscillatory current, obtained under short-circuit conditions, showed that the oscillation consists of several frequency components (Fig. 2 *B*). Energy in the oscillation was distributed over a narrow frequency band (3 Hz, measured between half-peak power points) about a fundamental frequency and in second and third harmonics (*arrows* in Fig. 2 *B*). In the spectrum of Fig. 2 *B*, the fundamental frequency occurred at 35 Hz, determined in a preparation at 22°C. Under open-circuit conditions and for preparations exhibiting response state I or II (Lu and Fishman, 1994), an oscillation in transorgan voltage was also evident in spectral analysis (Fig. 2 *C*).

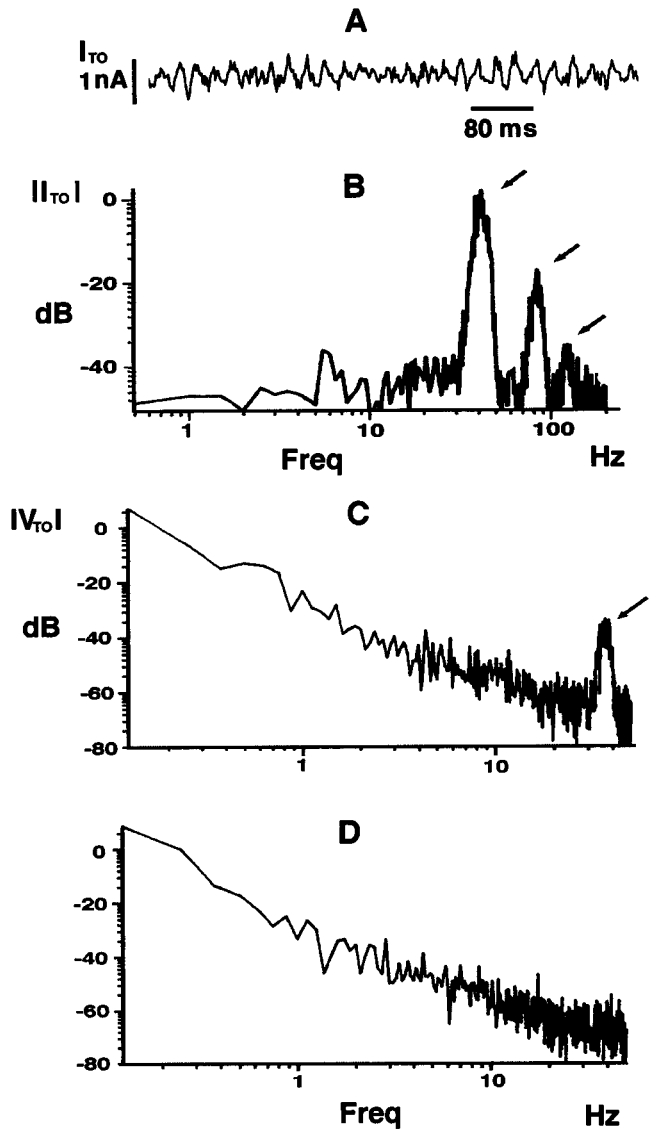

FIGURE 2 Spontaneous, steady oscillatory current from isolated ampullary organs voltage clamped at a holding potential of 0 mV (*A* and *B*) or current clamped at a holding current of 0 nA (*C* and *D*) and its spectral distribution. (*A*) A short time segment from recorded spontaneous oscillatory current through an organ (I_{TO}). (*B*) Spectral distribution from Fourier analysis of the oscillatory current in *A* showing energy confined to narrow frequency bands, indicated by *arrows*, at harmonically related frequencies. The spectrum in *B* is the average of the spectra of eight data samples; each sample length was 2 s. (*C*) Spectral distributions from Fourier analysis of the transorgan voltage (V_{TO}) from an open-circuit organ. The oscillation is evident (indicated by *arrows*) in this spectrum despite large extraneous components at other frequencies. (*D*) Transorgan voltage spectrum of the organ in *C* after cutting the ampulla.

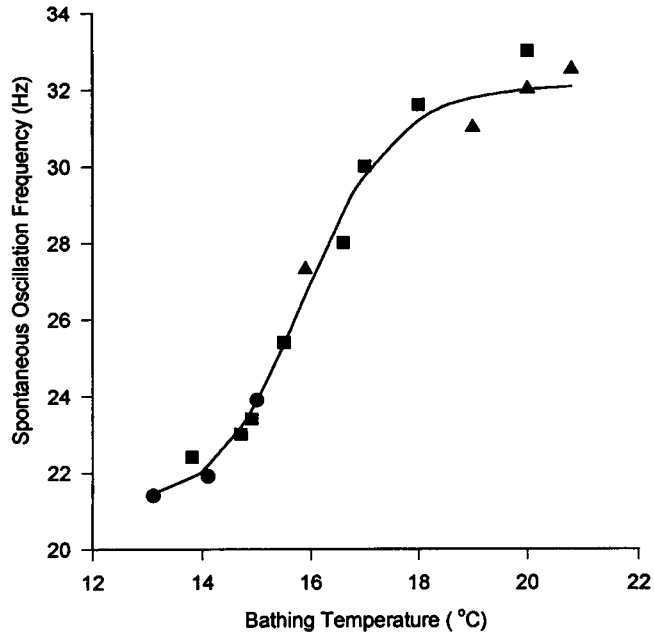

FIGURE 3 Temperature dependence of the fundamental frequency component of spontaneous current oscillations from three different (●, ■, ▲) voltage-clamped preparations (isolated ampullary organs). The temperature coefficient (slope of the curve) is 3 Hz/°C at 16°C. The solid curve is a best fit (mean square error minimization) of the logistic function $f = [(32.13 - 21.2)/(1 + (T/15.91)^{-19.04}) + 21.2]$ to the data.

To demonstrate that the oscillation relates to a viable ampullary organ, we examined the effects of ampullary damage and of temperature variation. Cutting the ampulla or its replacement by an agar bridge resulted in an immediate loss of the oscillation (Fig. 2 *D*). Changes in bath temperature also had a marked effect on the oscillation (Fig. 3). The fundamental frequency of the spontaneous oscillation was altered with a temperature coefficient of 3.0 Hz/°C at 16°C. At 13°C the fundamental frequency of the oscillation dropped to 21 Hz, which is close to the value obtained previously (Clusin and Bennett, 1979a,b). The frequency of the oscillation was determined quantitatively down to 13°C, and the oscillation was present as low as 10°C. Raising the bath temperature to 21°C increased the fundamental frequency of the oscillation to 33 Hz. The above results are consistent with the interpretation that the oscillation is generated by the ampullary organ.

Assessment of the current oscillation in spectra of the acquired complex admittance of ampullary organs was also possible. When an ampullary organ was clamped to 0 mV and a 16-μV (rms) synthesized voltage signal (the sum of 400 sinusoidal components; Fishman, 1992) was applied, admittance spectra (e.g., Fig. 4) showed that the oscillatory behavior was discernible in both amplitude and phase functions (32 Hz indicated by *arrow*). At frequencies below 20 Hz, the phase of the admittance function was between 90° and 270°, indicating that the ampullary organ generated a steady-state negative conductance (Lu and Fishman, 1994) simultaneously with the oscillations. However, the current oscillation was evident in spectra even without the applied admittance-determining stimulus (compare Fig. 2 *B* with no stimulus applied).

Site of generation of the oscillation

To find the location of the source of the current oscillation, we first determined whether the oscillation in an ampulla

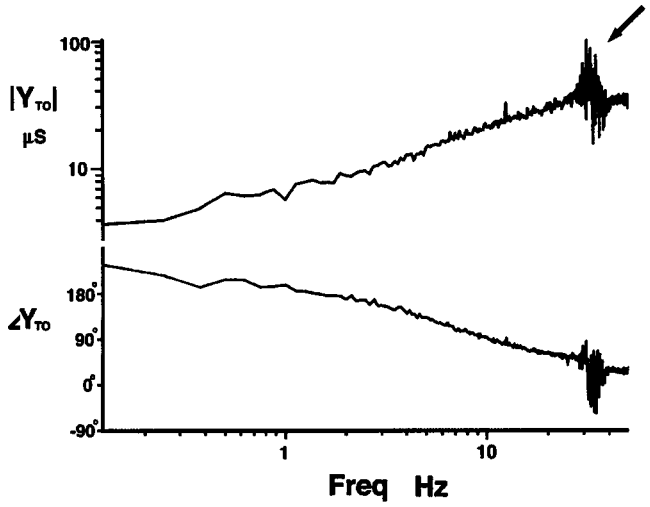

FIGURE 4 The transorgan complex admittance $[Y_{TO}(jf) = G(f) + jB(f)]$ determined under voltage-clamp conditions and plotted as magnitude $\{|Y_{TO}(jf)| = [G^2(f) + B^2(f)]^{1/2}\}$, and phase angle $\{\angle Y_{TO}(jf) = \tan^{-1}[B(f)]/[G(f)]\}$ functions of frequency (f). The steady-state generation of an ampullary current oscillation (narrow band of spectral components indicated by *arrow*) and negative conductance $\{G(f) = |Y_{TO}(jf)|\cos \angle Y_{TO}(jf) < 0$ for $f < 10$ Hz$\}$ are both discernible in admittance magnitude and phase functions. The current oscillation was evident in spectra even without the applied admittance-determining stimulus (compare Fig. 2 *B* with no stimulus applied). 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-μV rms synthesized signal containing 400 sinusoidal components (Fishman, 1992) was applied to the voltage-clamp system for the admittance determination.

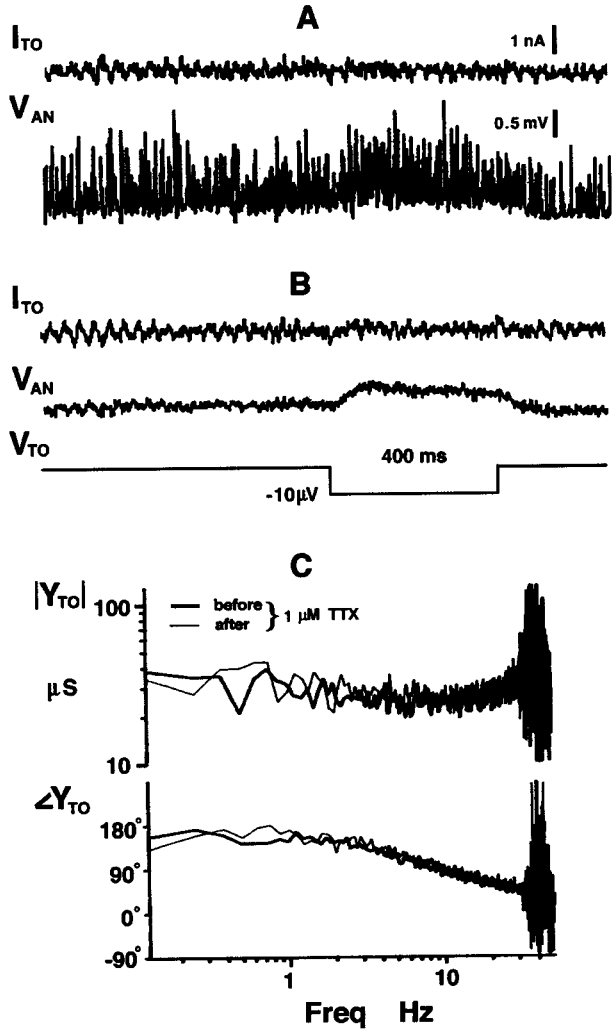

FIGURE 5 Tetrodotoxin (TTX, 0.5 μM) added to the basal-side elasmobranch saline to block afferent nerve conduction did not alter the organ oscillatory current, synaptic transmission, or ampullary generation of a negative conductance. (*A*) Transorgan current (I_{TO}) and afferent nerve voltage (V_{AN}) in response to a 10-μV step (lumen-negative) voltage clamp (V_{TO} shown in *B*) across an isolated organ. I_{TO} was oscillatory and V_{AN} showed an increased rate of spike firing in response to the step clamp. (*B*) Responses of I_{TO} and V_{AN} to the same step clamp as in *A* after addition of 0.5 μM TTX to the basal-side solution and elimination of afferent nerve firing. The oscillation in I_{TO} and postsynaptic voltage response in V_{AN} were still evident even after elimination of afferent (spike) activity (V_{AN}). (*C*) Both magnitude and phase functions of the transorgan complex admittance $[Y_{TO}(jf)]$ showed no significant change after addition of 1 μM TTX in another preparation. 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 an 8-μV rms synthesized signal was applied to the voltage clamp system for the admittance determination.

arose from currents originating in the afferent nerve. Fig. 5 *A* shows oscillatory current and afferent nerve responses to a 10-μV lumen negative stimulus (polarity is with respect to the basal bathing solution). After the addition of 0.5 μM TTX (a Na channel blocker) (Narahashi et al., 1964) to the basal-side solution of the ampullary epithelium, afferent nerve spike activity was eliminated, but the oscillatory current was unaffected. However, synaptic transmission was evident because the afferent nerve recordings showed clear postsynaptic voltage changes in response to the 10-μV lumen negative stimulus (Fig. 5 *B*). The complex admittance of another ampullary organ further confirmed that neither the negative conductance nor the oscillation was affected by the addition of TTX to the basal membrane solution (Fig. 5 *C*). Thus the oscillation is not caused by afferent nerve activity.

To determine which of the surfaces of the ampullary epithelium is the site of the oscillation, we used the L-type Ca channel blocker nitrendipine (Janis and Triggle, 1991) and the K channel blocker TEA (Tasaki and Hagiwara, 1957) to suppress the current oscillation or the negative conductance. The site of the oscillation was determined by comparison of the relative elapsed times for cessation of the oscillation after application of the blocking agents to basal and apical sides of isolated organs.

For these measurements, the ampullary epithelium was voltage clamped. Fig. 6 *A* shows an ampullary epithelial admittance locus of data points in the complex plane. The admittance was determined at 400 frequencies in the range from 0.125 to 50 Hz. For the low frequency portion of the locus, the real part of the admittance is negative (data points lie in the left half of the plane), indicative of a steady-state negative conductance. At higher frequencies, data points are scattered. A replot of the same data (Fig. 6 *A*) as an admit-

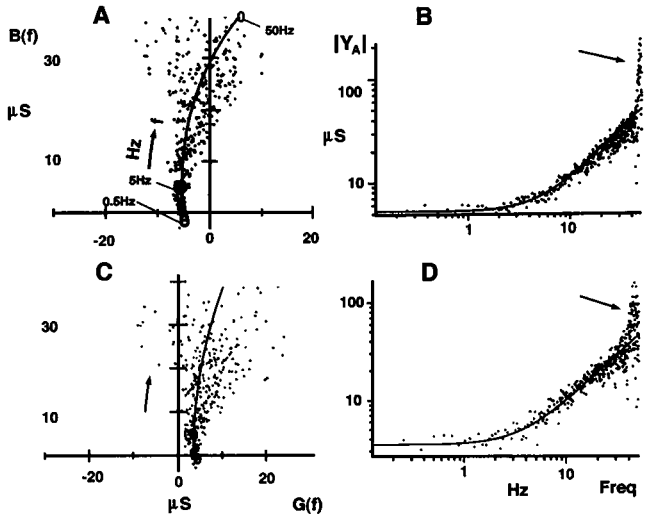

FIGURE 6 Nitrendipine (50 μM) added to the artificial seawater perfusing the apical side of an ampulla eliminated generation of the negative conductance, but the ampullary current oscillation persisted. (*A*) Control admittance before addition of nitrendipine. Complex plane plot {*B(f)* versus *G(f)*} of the admittance locus of frequency (*f*) points of the ampullary epithelium shows a steady-state negative conductance {$G(f) < 0$ for $f < 20$ Hz}. (*B*) Replot of the magnitude function {$|Y_A(jf)|$} of the complex admittance in *A* shows the presence of the oscillation (*arrow*). (*C*) Admittance determined after perfusion of apical membranes with artificial seawater + 50 μM nitrendipine. The ampullary epithelial steady-state conductance was now positive {$G(f) > 0$}. (*D*) Replot of the magnitude function of the complex admittance, after nitrendipine perfusion in *C*, shows that the oscillation (*arrow*) persisted. 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 *C* is due to the spectral components in the oscillation seen (*arrows*) in the magnitude functions in *B* and *D*. Admittance data obtained on an internally perfused organ with the transampullary epithelium clamped at 0 mV and acquired during a 1.5-min interval in which a 16 μV-rms synthesized signal was applied to the voltage clamp system for the admittance determination.

tance magnitude spectrum (Fig. 6 *B*) shows clear oscillatory behavior, made evident by the sharp peak (narrow frequency band indicated by the *arrow*). The scattered data points in the admittance locus plot (Fig. 6 *A*) at the uppermost frequencies are thus due to the components contributed by the oscillation. At 18.5 ± 0.5 min (± SD; number of preparations, $n = 2$) after addition of 50 μM nitrendipine to the solution perfusing the apical membrane, the locus of all admittance data points became positive at low frequencies (<20 Hz), indicating a net positive conductance after nitrendipine treatment (Fig. 6 *C*). Conversely, the current oscillation persisted after nitrendipine addition to the apical side, as seen by the presence of the scattering patterns in Fig. 6, *A* and *C*, and the sharp peaks (*arrows*) in the admittance magnitude plots of Fig. 6, *B* and *D*. The oscillation was evident for more than an hour after nitrendipine treatment. The relatively quick (18.5 min, including diffusion time for the perfusate to reach the apical surface) elimination of the negative conductance by nitrendipine when added to the apical perfusate suggests that the negative conductance is generated by L-type Ca channels and that these channels reside in apical membranes, as previously inferred by Lu and Fishman (1994).

The addition of nitrendipine to the basal membrane solution had the opposite effect on the negative conductance and the current oscillation compared with the effect produced by addition to the apical side. The control admittance before addition of nitrendipine again showed the presence of a negative conductance and oscillation (Fig. 7, *A* and *B*). Within minutes of the addition of 2 μM nitrendipine to the basal-side solution, the oscillation disappeared (Fig. 7, *C* and *D*), whereas the negative conductance was eliminated after 36.2 ± 13.7 min (± SD; $n = 4$). Consequently, the markedly different elapsed times for elimination of the oscillation by nitrendipine when added to apical versus basal sides (an hour or more against a few minutes) suggest that the current oscillation is generated in basal membranes. Also, the different elapsed times for elimination of the negative conductance by nitrendipine when added to apical versus basal sides (18.5 min, which includes the diffusion time for an effect of nitrendipine in the apical perfusate, versus 36.2 min) suggest that the negative conductance is

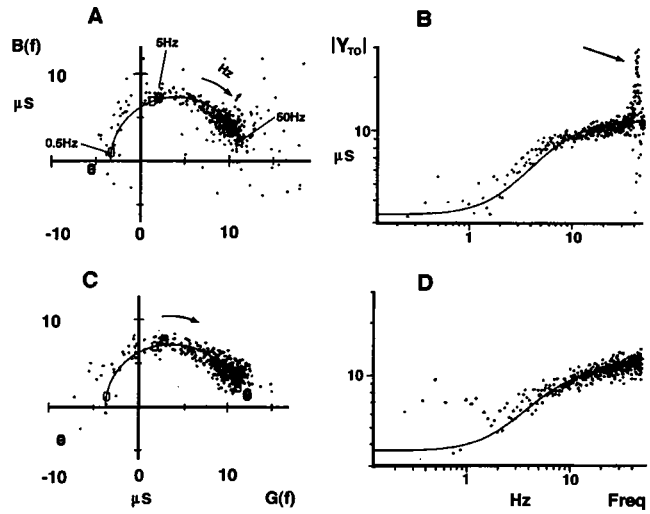

FIGURE 7 Nitrendipine (2 μM) added to the elasmobranch saline bathing the basal side of an ampulla rapidly eliminated the current oscillation, but the isolated organ negative conductance remained. (*A*) Control admittance before addition of nitrendipine. Complex plane plot {*B(f)* versus *G(f)*} of the admittance locus of frequency (*f*) points of an isolated organ shows a steady-state negative conductance {$G(f) < 0$ for $f < 5$ Hz}. (*B*) Replot of the magnitude function {$|Y_{TO}(jf)|$} of the complex admittance in *A* shows the presence of the oscillation (*arrow*). (*C*) The admittance locus determined 3 min after addition of 2 μM nitrendipine to the basal-side solution shows that the steady-state negative conductance remained. (*D*) Replot of the magnitude function of the complex admittance shows that the oscillation was eliminated after basal-side treatment with nitrendipine. 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* is 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-μV rms synthesized signal was applied to the voltage clamp system for the admittance determination.

generated in apical membranes. Furthermore, the negative conductance and the oscillation seem to be generated independently of each other.

Additional suppressors of basal membrane oscillations and apical membrane negative conductance

In addition to nitrendipine, we found several other agents that were effective in the suppression of the current oscillation, when added to the basal-side solution. After the addition of 2 mM TEA (K channel blocker) to the solution bathing the basal membrane, the current oscillation disappeared (Fig. 8) in 10 min, but the negative conductance persisted in this interval. This result suggests that K channels in basal membranes also play a role in the oscillation. In addition to nitrendipine and TEA, we found other suppressors of the oscillation: Ca channel blockers (Cd^{2+}), K channel blockers (4-AP, Ba^{2+}), Cl channel blockers (niflumic acid, DIDS), an antagonist of Na^+-Ca^{2+} exchange (clonazepam), and an antagonist of the Na^+/K^+ pump (ouabain). The effect of these agents will be discussed in more detail in a subsequent paper. The addition of these agents to the solution bathing the basal side of the ampullary epithelium either eliminated the oscillation exclusively or eliminated the oscillation before subsequent effects on the negative conductance. These temporal differences further support the interpretation that the oscillation is generated in basal membranes of the ampullary epithelium. Furthermore, the disappearance of the negative conductance after addition of 50 mM Cd^{2+} or 20 mM EGTA with no added Ca^{2+} to apical side perfusates occurred rapidly (<20 min), which further demonstrated that the negative conductance resides in apical membranes of the ampullary epithelium.

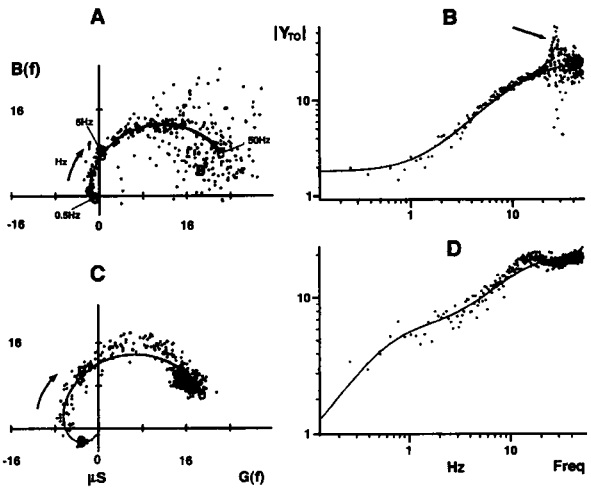

FIGURE 8 TEA (2 mM) added to the basal-side elasmobranch saline of an ampulla rapidly eliminated the current oscillation, but the negative conductance generated by the isolated organ persisted. (*A*) Control admittance before addition of TEA. Complex plane plot of the admittance locus of frequency (*f*) points of the ampullary organ shows a steady-state negative conductance $\{G(f) < 0, f < 5 \text{ Hz}\}$. (*B*) Replot of the magnitude function of the complex admittance in *A* shows the presence of the oscillation (*arrow*). (*C*) The admittance locus determined at 10 min after addition of TEA (2 mM) to the basal-side solution shows that the steady-state negative conductance remained. (*D*) Replot of the magnitude function of the complex admittance shows that the current oscillation was eliminated after basal-side treatment with TEA. 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* is 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-μV rms synthesized signal was applied to the voltage clamp system for the admittance determination.

The function of the oscillation in basal membranes

To elucidate the function of the spontaneous electrical oscillation in basal membranes, we determined the effect of abolishing the oscillatory current in basal membranes on postsynaptic responses. For a lumen-positive step voltage clamp (100 μV) of an ampullary organ held at 0 mV and bathed in saline containing 0.6 μM TTX, the ampullary epithelium exhibited a negative conductance in the admittance locus, but the afferent nerve discharge was eliminated. The 100 μV change in transorgan voltage was also reflected in the postsynaptic response recorded from an isolated portion of the attached afferent nerve (Fig. 9 *A*). The addition of a low concentration of TEA (2 mM) to the basal solution eliminated the oscillation (as in Fig. 8) in 10 min and markedly reduced the postsynaptic response (Fig. 9 *B*) to the same 100 μV step clamp, whereas a net negative conductance persisted. Thus synaptic transmission was impaired after suppression of the oscillation. In other experiments ($n = 3$), after raising the concentration of Mg^{2+} in the basal solution to 25 mM, both the current oscillation and the afferent nerve discharge disappeared in 20 min, whereas the apical membrane negative conductance was not affected. In summary, the oscillation seems to be associated with neurotransmitter release for the following reasons: 1) it is

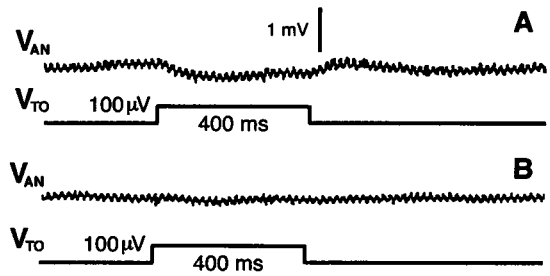

FIGURE 9 TEA added to the basal-side elasmobranch saline blocked synaptic transmission. (*A*) The postsynaptic potential (V_{AN}) response to a 100-μV (lumen-positive) step voltage clamp (V_{TO}) of an organ. (*B*) The absence of a postsynaptic response to the same step clamp as in *A* after addition of 2 mM TEA to the basal-side solution blocked the oscillation in basal membranes. The elasmobranch saline contained 0.6 μM TTX to prevent afferent nerve firing during the recording of the postsynaptic response. The extraneous 60-Hz signal on the traces of V_{AN} in *A* and *B* was due to power line induction.

generated in basal (presynaptic) membranes, 2) its abolition seems to also eliminate postsynaptic responses and afferent discharge, and 3) it is suppressed by extracellular increase of Mg^{2+} concentration, which is known to suppress neurotransmitter release from presynaptic membranes (Del Castillo and Engbaek, 1954).

DISCUSSION

The location and function of the ampullary epithelial oscillation

An important characteristic of the electroreceptor cell/afferent nerve synapse is the resting tonic spike discharge (30–50/s) that is present in afferent nerves in the absence of an applied stimulus to the ampullary organ (Murray, 1962). At most synapses, release of neurotransmitter from the presynaptic terminal requires sufficient depolarization of the presynaptic membrane to activate Ca channels through which Ca enters and initiates neurotransmitter release from vesicular stores (Del Castillo and Engbaek, 1954). For nerve, depolarization of the presynaptic terminal usually occurs only upon arrival of propagated action potentials. In the case of an ampullary organ, the resting tonic discharge in the innervating afferent nerve that occurs without an external organ stimulus implies that neurotransmitter is steadily released from presynaptic membranes. How could neurotransmitter be released steadily from presynaptic (basal) membranes of an ampullary epithelium without an exogenous depolarizing stimulus? One way would be to generate a steady endogenous stimulus that could regularly depolarize presynaptic membranes on the basal face of ampullary epithelial receptor cells.

Endogenous current oscillations at 20 Hz were recorded previously by Clusin and Bennett (1979a,b) in a salt-bridge shunted ampullary organ bathed in solutions at 10°C. Broun and Govadovskii (1983a,b) were unable to confirm the existence of these oscillations in the Black Sea skate *Raja clavata*. However, we measured current oscillations through organs held in a voltage clamp at 0 mV (Fig. 2) similar to those reported by Clusin and Bennett (1979a,b). Furthermore, from the rapid suppression of the oscillation by agents that block ion channels (TEA, nitrendipine), when added to the basal side solution in comparison with a lack of suppression of the oscillation when added to the apical side, we conclude that the site of generation of the oscillation is in basal membranes.

The presence and site of generation of the oscillation are consistent with the hypothetical endogenous stimulus suggested above because basal (presynaptic) membrane generation would be the most effective location for influencing neurotransmitter release. The oscillation also has two other characteristics that an endogenous stimulus might be expected to have. Spectral analysis showed that the fundamental frequency of the basal membrane oscillation at room temperature was 32 Hz, which seems to correspond to the frequency of the resting, mean afferent spike discharge. However, our measurements were inconclusive with respect to demonstration of a synchronous relationship between the basal membrane oscillation and the mean afferent firing rate. Additionally, elimination of the ampullary current oscillation after blockage of K channels with TEA or upon raising basal solution Mg^{2+} concentration produced substantial attenuation of postsynaptic potentials (reduced electroreceptive sensitivity) or abolition of afferent discharge, respectively, in response to externally applied transampullary electrical stimuli. These attributes suggest that the basal membrane oscillation is associated with neurotransmitter release and that the oscillation may affect the resting, mean firing rate. One explanation for the inhibition of neurotransmitter release by TEA is that basal membrane Ca channels were inactivated upon depolarization of the ampullary epithelium after K channel blockage. Alternatively, release of neurotransmitter from presynaptic membranes may not normally occur without the oscillation.

There are two ways in which a transampullary stimulus could interact with the basal membrane oscillation to effect a change in afferent nerve firing rate. First, the amplified stimulus could simply be added to the oscillation. The enhancement or diminution of the presynaptic membrane voltage would then open more or less Ca channels and consequently produce an increased or decreased neurotransmitter release. A second possibility is that the amplified stimulus interacts (nonlinearly) with the oscillation generated in basal membranes such that the product (modulation) of the two signals occurs across presynaptic membranes. In this case, the shape rather than the amplitude of the waveform (i.e., the amplitudes of the harmonics relative to the fundamental) of the driving voltage across presynaptic membranes would determine the extent of transmitter release. In both processes, signal transmission from transducing electroreceptor cells to the central nervous system would be critically dependent on the oscillation generated in basal membranes.

The location and function of the negative conductance

The other property that is essential for electroreception in an isolated ampullary organ, under short-circuit conditions, is a steady-state negative conductance (Lu and Fishman, 1994). The rapid suppression of the negative conductance by nitrendipine added to the apical side and the relatively longer time for an effect of nitrendipine on the negative conductance when added to the basal side suggest that the negative conductance is generated by long-lasting (nonactivating or slowly inactivating) L-type Ca channels in apical membranes. The effect and elapsed time for an effect of nitrendipine, TEA, and other blockers (Lu and Fishman, 1995) on the negative conductance and the oscillation also suggest that these two essential processes for electroreception are generated independently.

Clusin and Bennett (1979b) showed that the current oscillation could be suppressed by blockage of basal mem-

brane K channels with TEA or by blockage of Ca channels with Co^{2+} or EGTA, indicating a dependence of the oscillation on these basal membrane ion channels. However, they suggested that the oscillation could not be generated by ion channels residing solely in basal membranes, but instead required the interaction of Ca channels in apical membranes to activate basal membrane conductances. We have shown that a steady-state negative conductance (the property that underlies apical membrane spiking under open-circuit conditions) is generated in apical membranes in the absence of the current oscillation and that the current oscillation occurs in basal membranes after elimination of the apical membrane negative conductance. Thus we conclude that the oscillation is generated by the concerted action of the different ion conductances that reside solely in basal membranes. Furthermore, this interpretation is consistent with the occurrence of an attenuated oscillation in the spectrum of voltage measured (across the ampullary epithelium paracellular shunt resistance) in an open-circuit organ preparation (Fig. 2 *C*), which Clusin and Bennett (1979a,b) were not able to detect.

The generation of the oscillation in basal membranes

What are the essential elements for generation of an oscillation in a single membrane (such as the basal membranes of electroreceptor cells)? A simple analogy from linear circuit theory is useful. A "tank" circuit consisting of the parallel combination of a conductance, G, a capacitor, C, and an inductor, L, can produce a maximum value in the circuit impedance function of frequency at the resonant frequency, $f_0 = 1/(2\pi(LC)^{1/2})$. The voltage across the tank circuit in response to an applied current step is a damped (decaying) oscillation at f_0. If the shunt conductance in the tank circuit goes to zero, a sustained oscillation will occur spontaneously if the circuit is connected to a source of energy. However, in any real physical system, the other elements in the circuit will dissipate energy (e.g., the windings of an inductance have resistance), and the oscillation will be damped. If the shunt conductance is sufficiently small or a source of energy is available to offset the energy loss, the voltage across the circuit will oscillate. These principles can also explain oscillation in electroreceptor cells. A very small conductance can be approached by the neutralization of a negative conductance (generated by L-type Ca channels in basal membranes, Fig. 7) with a positive conductance (K channels in basal membranes, Fig. 8) in receptor cells. Chandler et al. (1962) and Mauro et al. (1970) showed that Na conductance kinetics contributed a capacitance-like susceptance and K conductance kinetics produced an inductance-like susceptance in the membrane complex admittance of a squid axon. Thus by analogy to a tank circuit, these two voltage- and time-variant conductances generated damped oscillatory behavior in squid giant axon (Mauro et al., 1970). Fishman et al. (1979) demonstrated from membrane complex admittance determinations in the squid giant axon that the generation of a steady-state negative conductance is associated with Na conduction and that a capacitance-like susceptance arises from Na channel activation kinetics. In addition, K conductance yields a steady-state positive conductance and an inductance-like susceptance arising from K channel activation kinetics. Ca channels can substitute for Na channels because they also generate a negative conductance (which can occur in a steady state for long-lasting channels), and their activation kinetics should also contribute a capacitance-like susceptance to membrane admittance.

An oscillation is not unusual in sensory cells. Baylor et al. (1979) recorded membrane current from the outer segment of a single toad rod with a suction electrode. At low-level light intensity, the recorded membrane oscillatory current was similar in waveform to the oscillation that we observed emanating from ampullary receptor cells. In a turtle cochlear hair cell, damped ringing was observed in response to a current step (Art and Fettiplace, 1987). Hudspeth and Lewis (1988) also showed in hair cells that damped oscillatory behavior could be generated by the interaction of Ca- and K(Ca)-channels. Thus the minimum requirement for damped and sustained oscillatory behavior is one type of channel (either Na or Ca) that produces a negative conductance and capacitance-like kinetics and one type of K channel that produces a positive conductance and inductance-like kinetics. The difference between damped and sustained oscillation then depends on the extent of neutralization of the positive and negative conductances, which determines the degree to which the oscillation is damped.

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