Mode of Operation of Ampullae of Lorenzini of the Skate, *Raja*

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ABSTRACT Ampullae of Lorenzini are sensitive electroreceptors. Applied potentials affect receptor cells which transmit synaptically to afferent fibers. Cathodal stimuli in the ampullary lumen sometimes evoke all-or-none "receptor spikes," which are negative-going recorded in the lumen, but more frequently they evoke graded damped oscillations. Cathodal stimuli evoke nerve discharge, usually at stimulus strengths subthreshold for obvious receptor oscillations or spikes. Anodal stimuli decrease any ongoing spontaneous nerve activity. Cathodal stimuli evoke long-lasting depolarizations (generator or postsynaptic potentials) in afferent fibers. Superimposed antidromic spikes are reduced in amplitude, suggesting that the postsynaptic potentials are generated similarly to other excitatory postsynaptic potentials. Anodal stimuli evoke hyperpolarizations of nerves in preparations with tonic activity and in occasional silent preparations; presumably tonic release of excitatory transmitter is decreased. These data are explicable as follows: lumenal faces of receptor cells are tonically (but asynchronously) active generating depolarizing responses. Cathodal stimuli increase this activity, thereby leading to increased depolarization of and increased release of transmitter from serosal faces, which are inexcitable. Anodal stimuli act oppositely. Receptor spikes result from synchronized receptor cell activity. Since cathodal stimuli act directly to hyperpolarize serosal faces, strong cathodal stimuli overcome depolarizing effects of lumenal face activity and are inhibitory. Conversely, strong anodal stimuli depolarize serosal faces, thereby causing release of transmitter, and are excitatory. These properties explain several anomalous features of responses of ampullae of Lorenzini.

INTRODUCTION

In a number of groups of fishes certain lateral line receptors have become specialized for the detection of electric fields. The existence of these electroreceptors was proposed by Lissmann (1958) on the basis of behavioral experiments. He correctly surmised that certain specialized lateral line receptors that had been described morphologically were in fact the postulated receptors

(Lissmann, 1958). Electroreceptors are found in nonelectric as well as electric fish and their properties have been recently reviewed (Bennett, 1970, 1971 *a,b*). They, like other acoustico-lateralis receptors, have receptor cells that synapse with the afferent fibers, and the initial active transformation of the stimulus is carried out by the receptor cells.

Ampullae of Lorenzini are electroreceptors which are found in most if not all elasmobranchs. A substantial body of electrophysiological and behavioral evidence establishes their electroreceptive function (Murray, 1962, 1967; Dijkgraaf and Kalmijn, 1966; Kalmijn, 1971; Waltman, 1966) although they have also been considered to be mechano- or thermal receptors. The most sensitive ampullae respond to extraordinarily small voltages, only a few microvolts (Murray, 1967). Individual ampullae are grouped together in several capsules located in the cranial region. Canals, one from each ampulla, radiate out to terminate in the skin and open to the exterior. The canal walls are of very high resistance and the canals have a space constant so long that there is negligible decrement of DC potentials along them (Waltman, 1966). The receptor cells, which are in the walls of the ampullae, are acted on by the difference in potential between the canal opening and the fish's interior adjacent to the ampullae. Information as to field orientation is obtained by there being many canals of different lengths oriented in different directions. Longer canals are sensitive to smaller gradients than are shorter canals, but shorter canals provide more information about local variations in the field. In *Raja*, which we have studied, each ampulla contains hundreds of receptor cells in a single epithelial layer. The receptor cells are separated by supporting cells. Apical *zonulae occludentes* connect all cells lining the ampullary lumen and canal, and can be expected to prevent leakage of current through intercellular clefts, thus maximizing flow through the receptor cells (Waltman, 1966; cf. Bennett, 1971 *a,b*). The *zonulae occludentes* divide the membrane of the receptor cells into lumenal and serosal faces (cf. Fig. 11). Electrical stimuli applied across the receptor epithelium cause current to enter through one face of the receptor cells and leave through the other. Five or more afferent nerve fibers innervate each ampulla. Efferent innervation is absent. The receptor cells and afferent fibers form typical receptor synapses at which there are dense presynaptic ribbons and associated vesicles (Waltman, 1966).

The ampulla of Lorenzini provides a preparation that in several respects is quite suitable for electrophysiological study of transmission at a receptor synapse. The ampulla and its innervating fibers can be isolated and maintained in vitro (Sand, 1938; Loewenstein, 1960), and, as shown here, the nerve fibers are accessible for microelectrode penetration. The present investigation indicates that the receptor cells of the ampulla are electrically excitable and capable of generating spikes, and that they transmit chemically to the innervating fibers. It is concluded that an adequate stimulus modulates

electrical activity of the receptor cells which in turn modulates the release of transmitter controlling the afferent discharge frequency. The electrical excitability of the receptor cells can account for several anomalous features of the responses of these receptors. A preliminary communication of this work has appeared (Obara and Bennett, 1968). An abstract by Waltman (1968) of an independent study is in agreement with the finding of excitability of the receptor cells. His work will be referred to in relevant sections of this paper.

METHODS

Common skates, *Raja oscellata* and *R. erinacea*, 50–75 cm in length, were obtained by trawling near Woods Hole, Mass., and were kept in running seawater until use. The mandibular group of ampullae was employed for most experiments (Murray, 1962). After pithing the animal, the ampullae and their innervating nerve were removed from the fish together with the overlying skin. The preparation was placed with its external surface down in a small Petri dish illuminated from the bottom, and further dissection was carried out in skate saline under a dissecting microscope. Subcutaneous muscle layers were removed in order to visualize the ampullae which are contained in a capsule of connective tissue. The entire course of their canals was also exposed. The preparation was then pinned down in a wax-bottomed chamber containing saline, the capsule being positioned over a transparent window to permit illumination from below. Activity of the preparation was monitored by external recording from the nerve trunk, while gross wire electrodes in the bath were used for field stimulation of the receptors.

Most of the experiments were carried out with two microelectrodes penetrating the canal, one for recording placed as close to the ampulla as possible and the other for current application a few millimeters to a centimeter down its canal. With this arrangement of electrodes, much of the applied current flows through the external opening of canal so that current magnitude could be used only as a rough measure of the effective stimulus.

Since the currents applied generated negligible potentials outside the ampulla (that is at its serosal surface), single-ended recording was adequate to measure the potential difference across the ampullary wall. In a few experiments differential recordings across the wall were made using a small external wire electrode close to the ampulla. Usually a single large silver-silver chloride (Ag-AgCl) electrode grounded the preparation, but in a few cases a separate current return electrode was employed. The currents applied were small and no difference between the two methods was noted.

Gross nerve activity was recorded from the whole ampullary nerve through a pair of Ag-AgCl wire electrodes. Single unit activity was obtained either by a third microelectrode penetrating the ampullary nerve close to the ampulla (N in Fig. 1), or by isolating a strand of one to a few units from the trunk (N' in Fig. 1). In the latter case, the corresponding ampulla was identified by probing the canal openings with a small wire electrode insulated except at the tip (Stim. in Fig. 1) and then tracing the canal back to the ampulla of origin. While this circuit tracing was tedious and sometimes in error, microelectrode recording required partial removal of the capsule

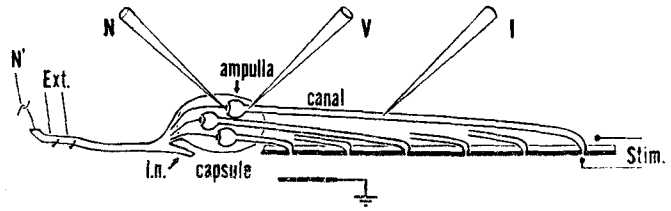

FIGURE 1. Diagram of ampullae of Lorenzini in the mandibular capsule and of recording and stimulating electrodes. The mandibular group consists of 20–30 ampullae enclosed in a fibrous capsule from which a bundle of individual canals runs beneath the skin to open in a regular pattern to the outside (downward through the heavy horizontal line in the figure). Only three ampullae are shown. *l.n.*, nerve branch to lateral line organs; *I*, current electrode; *V*, voltage electrode for recording the potential change in the ampulla; *N*, microelectrode for recording from a nerve terminal, *N'*, single unit recording from a fine filament of the ampullary nerve; *Ext.*, external electrodes for recording afferent discharge or for antidromically stimulating the entire afferent nerve; *Stim.*, external stimulating electrodes one of which is placed on the opening of a canal.

around the ampulla, and this manipulation often led to loss of spontaneous activity as described in the results section. Removal of the capsule was limited to exposing the nerve fibers of superficial receptors close to the ampulla.

Microelectrodes were conventional glass pipettes filled with 2 M KCl and with resistances between 10 and 50 MΩ. The higher resistance electrodes were required to penetrate the axons.

In a small number of experiments single ampullae with a short length of nerve and canal were isolated from the largest (hyoid) capsule and immersed in mineral oil. For stimulation and recording of the ampullary response, two large pipettes, about 100 μ in diameter, were passed down the canal. A large indifferent electrode contacted the serosal surface of the ampulla. Small stainless steel hooks were used to record the nerve response between the cut end of the nerve and its junction with the ampulla. This technique allowed recording of the response of the entire nerve bundle from a single ampulla.

The saline contained 287 mM NaCl, 4.1 mM KCl, 10 mM $CaCl_2$, 1.0 mM $MgCl_2$, 444 mM urea, and 2 mM tris(hydroxymethyl)aminomethane (Tris)-HCl buffer. The pH was adjusted to 7.4. More recent studies indicate that saline containing 245 mM NaCl, 3 mM KCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$, 350 mM urea, and 5 mM glucose plus Tris buffer better maintains the spontaneous activity that is characteristic of in vivo preparations (Steinbach and Bennett, unpublished observations). Isolated ampullae were studied either without artificial saline or with cerebrospinal fluid obtained before pithing the animal (Murray, 1965). Several milliliters could be obtained from each animal. All experiments were carried out at room temperature of 20°–25°C.

RESULTS

In the isolated preparation the ampullary nerve trunk usually showed a high level of spontaneous activity that was maintained over several hours. On field stimulation with gross electrodes immersed in the bath, the spontaneous dis-

charge was either increased or decreased depending upon polarity of the field (Murray, 1962). However, removal of the capsular sheath, even if partial, tended to cause gradual decline of the spontaneous activity in the nerve. Most of the data reported here were obtained from preparations with little or no spontaneous discharge. In spite of the change in the resting discharge, opening the capsule seemed to have little effect on responses recorded in the ampulla and on evoked afferent discharges.

Receptor Responses Recorded in the Ampulla

There was little if any resting potential between ampullary lumen and exterior. Often on penetration, and more reliably on withdrawal of a microelectrode from the ampulla, a potential difference of no more than 1 mv was recorded, which can be ascribed to a change in tip potential. The absence of resting potential is consistent with the long space constant and open and hence short circuited distal end of the canal (Waltman, 1966).

Anodal current applied in the ampulla (or canal) caused a positive potential change in the ampulla with a gradual rise and fall. The effective time constant was dependent on relative distance between the voltage and current electrodes as well as the length of the canal. The steady-state level changed linearly with current intensity over a range of at least 20–30 mv (Waltman, 1966).

Nonlinearities in the ampullary potential did appear on cathodal stimulation, i.e., with the current electrode negative with respect to the outside. In many of the preparations studied, cathodal current pulses could evoke a negative-going spike response in the ampulla. This type of response appeared in a discontinuous or all-or-none manner, although peak amplitude could increase with increasing stimulus strength (Fig. 2 A_1, A_2). The voltage threshold for the ampullary spike varied widely from one preparation to another, but had a more or less stable value in a given ampulla over considerable periods of time. In most of the ampullae that showed this type of response, the voltage threshold ranged from a few tenths of a millivolt to 5 mv. Spontaneous spikes were observed occasionally (Fig. 2 E). The ampullary spike could also appear as a "break response" after the termination of anodal stimuli (Fig. 3 B).

The active nature of the ampullary response is indicated by the fact that it can occur spontaneously as well as after an evoking stimulus is terminated (Fig. 2 A_1, A_2, Fig. 3 B). The ampullary response is thus comparable to the spikes of nerve and muscle and cannot be analogized to responses due to a regenerative increase in resistance which occur in frog skin (Finkelstein, 1964) and certain muscle fibers and muscle-derived electrocytes (Bennett and Grundfest, 1966). The input resistance of the ampulla was reduced by as much as one-half during the spike. The change in resistance of the generating membrane was presumably considerably greater, because the input resistance is re-

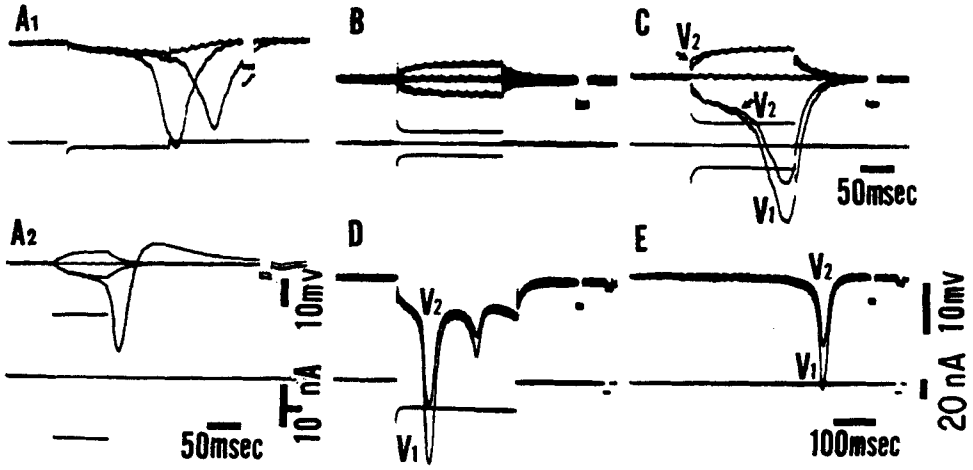

FIGURE 2. Receptor spikes evoked by cathodal stimulation in the ampulla. Ampullary voltages on upper traces; lumenal positivity up in this and subsequent figures. Currents on lower traces; those making the lumen positive shown as upward deflections in this and subsequent figures. (A_1) and (A_2), threshold stimulation of two preparations. Several superimposed sweeps in which spikes did and did not occur. Record (A_2) was obtained from a different ampulla than the responses in the remainder of the figure. (B) to (E), recordings were made simultaneously near to (V_1) and far from (V_2) the ampulla, with a current electrode placed farther down in the canal. (B), subthreshold cathodal stimulation and equal anodal stimulation superimposed. (C), just supra-threshold stimulation. The receptor spike is larger nearer the ampulla. The electrotonic potentials both to anodal and small cathodal pulses are of equal steady-state amplitude at the two recording sites (B), but the rise and fall are faster at the site which is closer to the current electrode (C). (D), a larger cathodal current evoked two successive receptor responses with progressively declining size, indicating a long refractory phase after the initial spike. (E), spontaneous appearance of a receptor spike, which also was larger in amplitude near the ampulla. Voltage and current calibrations in (E) apply to (A_1) and (B) to (D). Voltage calibration pulses appear near the end of the traces in this and several of the later figures.

duced by shunting through the open canal and because there appears to be an inactive membrane in series with the generating membrane as will be discussed below.

In general the ampullary response amplitude was small, from 5 to 10 mv, but it could reach 20–30 mv. The duration halfway between peak and resting level averaged about 50 msec. On stimulation with long current pulses, repetitive spike responses sometimes occurred, but always with decreasing amplitude, suggesting a long refractory period (Fig. 2 D). When tested by a pair of short pulses, complete recovery of amplitude required 0.5–1 sec in most cases. The negative spike was often followed by a positive afterpotential.

In several experiments the potential was observed at two points in a single canal simultaneously, one electrode close to the ampulla (V_1) and another one

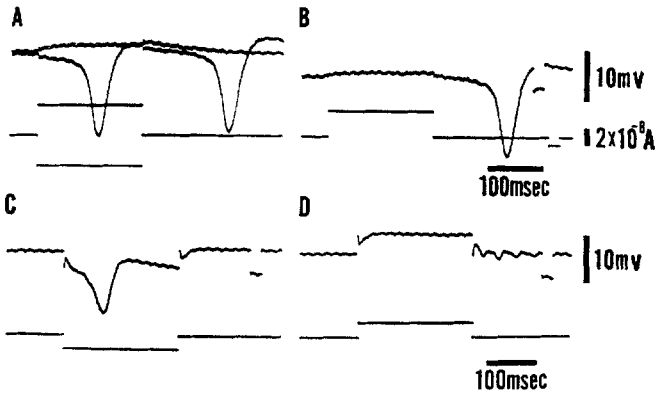

FIGURE 3. Responses in the ampulla to anodal stimuli. In (A) and (B), the receptor spike is evoked either during a cathodal pulse or after an anodal pulse of the same intensity is terminated. Stimulating current on lower traces. There were no spontaneous spikes. (C) and (D), a different preparation. A cathodal pulse triggered a receptor spike which was somewhat graded and showed an inflection on its rising phase (C). An anodal pulse induced a train of small oscillations after the end of the pulse (D). Calibrations are the same in (A) and (B) and in (C) and (D).

some distance away (V_2). In these experiments the ampullary spike was always larger closer to the ampulla suggesting that this is the site at which it is generated (Fig. 2 C–E). The steady-state potentials to both anodal and small cathodal current pulses were very nearly the same at the two recording sites (Fig. 2 B,C), although their rise and fall were more rapid closer to the polarizing electrode than farther out in the canal (Fig. 2 C). The difference in attenuation of spike and steady-state potentials in these experiments is ascribable to two factors. First, the time constant of the canal wall is very long and attenuates higher frequencies more rapidly. Second, the ampulla "looks" into a cable that is terminated by a short circuit at the open end of the canal, while for spread towards the ampulla, the cable is terminated by a high impedance, as the canal has an essentially closed end (Waltman, 1966). Thus, steady-state potentials decrement less spreading towards the ampulla than away from it. The ampullary origin of the spike is also indicated by the fact that passing a microelectrode through the ampullary wall tended to block the response, although there was little effect on the input resistance of the canal. In contrast careful penetration of the canal wall was not injurious, and, as Waltman (1966) has shown, isolated canals are electrically linear. Presumably the elements generating the active responses are the receptor cells as will be considered more fully in the discussion. For simplicity these responses will be termed receptor spikes throughout the remainder of this paper.

Receptor spikes, while commonly observed, were evoked less frequently than were graded oscillations. No clear threshold could be defined for the

oscillations as the amplitude gradedly increased with increasing stimulus strength (Fig. 4). These responses could occur at the termination of anodal stimuli (Fig. 3 D), although anodal stimulation could also have little effect on the responses (Fig. 4 B). In contrast to the amplitude changes, the frequency of oscillation appeared more or less independent of stimulus intensity (Fig. 4).

It is likely that the oscillations are generated by the same response mechanism as the receptor spikes, and the oscillations were larger recorded in the ampulla than distally in the canal. The same ampulla was observed to generate either oscillations or spikes at different times or under different conditions of stimulation (Fig. 3 C,D). Change of a response from an oscillatory to a spikelike form was sometimes produced by increasing the stimulus strength (Fig. 4 A).

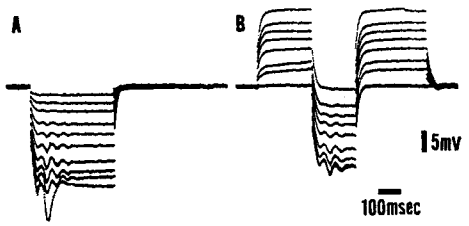

FIGURE 4. Receptor oscillations. (A), rectangular cathodal current pulses of increasing amplitude induced graded oscillatory responses in the ampulla. The frequency of the oscillation changed little with increasing strength of stimulation. In this particular ampulla, the second negative peak was increased markedly and the next two negative peaks were eliminated in the response to the largest stimulus. (B), anodal polarization before the test cathodal pulse had only a small effect on the response shape.

Responses of the Afferent Nerve Fibers

When a single fiber was isolated from the ampullary nerve (*N'* in Fig. 1), or was recorded from externally with a microelectrode (*N*), the data were generally in agreement with those obtained by previous workers (Murray, 1962, 1965; Sand, 1938). Initially at least, the fibers were tonically and fairly rhythmically active, and cathodal stimulation in the ampulla increased the resting discharge (Fig. 5) while anodal stimulation decreased it. The excitatory or inhibitory effect of brief stimuli could long outlast the stimluus (Fig. 7; Murray, 1965). In silent preparations the threshold ampullary potential to evoke a nerve spike could be as low as 0.1–1 mv. This level is far higher than that required to cause a 10% change in the resting discharge of the most sensitive spontaneously active preparations (a few microvolts, Murray, 1967). Usually the threshold for nerve discharge in silent preparations was well below the level required to evoke receptor spikes or detectable oscillations (Fig. 5, Fig. 6 D–F). A receptor spike was in general accompanied by only a small increase or acceleration of the nerve discharge in the nerve (Fig.

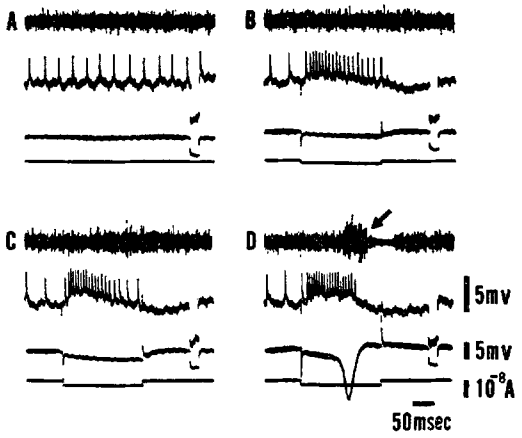

FIGURE 5. Relation between nerve and receptor responses. Interaction among the ampullae mediated by a receptor spike. First trace, recording from the afferent nerve from the capsule. Second trace, microelectrode recording from a single fiber, AC coupled amplification so that the slow negative potential shifts are not significant. Third trace, ampullary potential. Fourth trace, stimulating current in the canal. (A), the spontaneous rate of afferent discharge in the axon. (B) to (D), cathodal stimuli increased the rate of nerve discharge during the stimulus; the response was followed by a silent period. (D), a receptor spike was evoked by the same current strength as in (C). The single fiber response was accelerated, but terminated at the end of the spike. The spike was also accompanied by a burst discharge and silent period in the nerve trunk (arrow). Evidently the receptor spike stimulated most or all of the other ampullae in the capsule.

5D, Fig. 6 F). In a few experiments the receptor spike appeared to be required for nerve discharge (Fig. 6 A,B). In occasional preparations of the mandibular capsule a receptor spike was accompanied by a burst discharge and silent period in the afferent nerve from the entire capsule (Fig. 5 D). Evidently the potential field outside the ampulla due to the receptor spike was adequate to excite all the ampullae in the capsule, perhaps causing some of them to generate receptor spikes also.

Evoked impulses recorded from the nerve bundle leading from an isolated ampulla had similar properties. The first few discharges in response to strong stimuli were relatively synchronous and apparently involved most if not all fibers (Fig. 7 A,B). Successive discharges decreased in amplitude and broadened indicating decreased synchrony. Later the discharge became quite asynchronous but still involved many fibers.

The nerve impulses could sometimes be recorded by an electrode inside the ampulla or in the nearby canal. The impulses appeared as brief positive-negative potentials, commonly less than 100 μv in amplitude (Fig. 6 E,F, Fig. 8). Simultaneous recording from single afferent fibers or from the afferent nerve bundles demonstrated the association with the afferent impulses. An

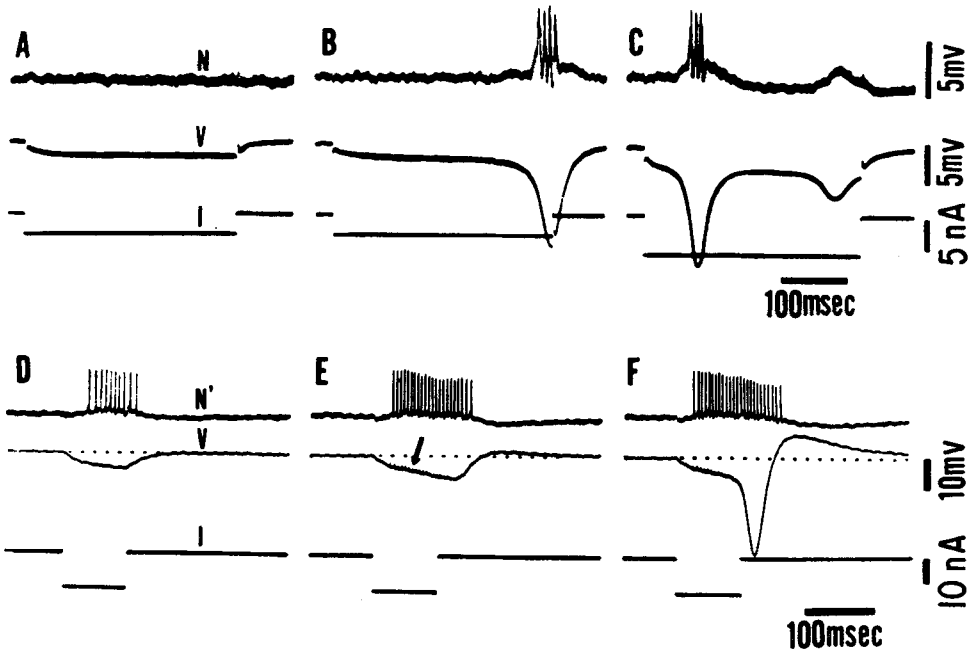

FIGURE 6. Direct relation of the receptor spike to impulse discharge and the generator potential. Recorded as in Fig. 5 except that the recording from the nerve trunk is omitted. (A) to (C), a preparation with a high threshold for nerve discharge. (A), a cathodal current pulse evoked no response. (B), a pulse of the same intensity elicited a receptor spike and a short burst of nerve impulses. (C), a larger stimulus triggered an initial, larger receptor spike and a second, much smaller receptor response. The first was accompanied by a burst of nerve impulses, but for the second there was only a small generator potential. A larger generator potential appears to underly the impulses. (D) to (E), a different preparation. The fixed current pulse was given at 1/sec. As a subthreshold response (E) developed, the afferent discharge was prolonged to outlast the pulse duration. A receptor spike (F) was associated with further increase in the nerve discharge. Small brief potentials in the ampulla are associated with the nerve spikes (arrow in E). Same calibrations for (A) to (C) and for (D) to (F).

antidromic stimulus to the nerve trunk could evoke a similar positive-negative spike in the ampulla.

The nerve spikes in the ampulla are recorded through the impedance of the thin ampullary wall. That the responses are initially positive on both orthodromic and antidromic stimulation suggests that orthodromic spikes arise somewhat centrally in the axon and then propagate back to the terminal as well as centripetally. The negative phase may represent activity propagating back into the terminals or capacitative current through inactive membranes in series with the input resistance of the ampulla (cf. Freygang and Frank, 1959; Bennett, 1966).

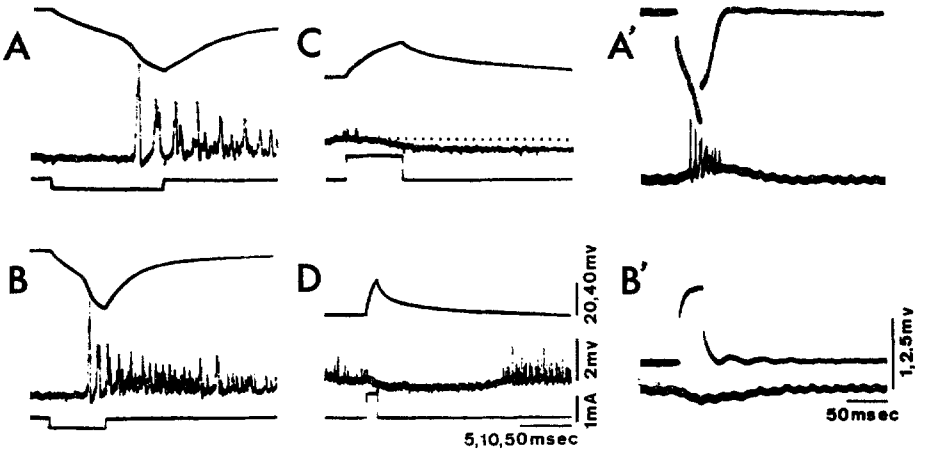

FIGURE 7. Recordings from an isolated ampulla and its afferent nerve bundle. First trace, potential in the ampulla. Second trace, recording from the nerve, one electrode at the edge of the ampulla and one on the nerve bundle several mm centrally. Positivity of the central lead recorded upwards. Third trace, stimulating current (omitted in A′ and B′). (A) and (B), responses to a cathodal stimulus at two sweep speeds. A long lasting burst of impulses was evoked in the afferent nerve bundle. These discharges started quite synchronously but became progressively less synchronous. There was some receptor response indicated by the inflection on the ampullary potential. (C) and (D), responses to an anodal stimulus at two different sweep speeds (lower gain for potential in the ampulla). A low rate of spontaneous nerve discharge was blocked, and there was long lasting negativity of the central lead indicating hyperpolarization of the nerve terminals. Termination of the hyperpolarization was accompanied by a burst of impulses. (A′) and (B′), from a different preparation. Cathodal stimulation evoked a burst discharge (A′) and anodal stimulation evoked a hyperpolarization of the terminals (B′). Same calibrations for (A) to (D) and for (A′) to (B′).

Generally the nerve spikes recorded in the ampulla decreased or disappeared after the first few impulses of a long lasting afferent discharge (Fig. 6 E,F, Fig. 8). This reduction of the nerve spikes in the ampulla is ascribable to desynchronization of the firing of the innervating fibers (cf. Fig. 7 B). Desynchronization would cause an especially large reduction, because the diphasic potentials adding out of phase would tend to cancel out. Another factor may be decreased spread into the terminals due to refractoriness and short circuiting by the generator potential (see below).

Generator or Postsynaptic Potentials in the Nerve

Microelectrode penetrations of the afferent fibers were signalled by a sudden negative shift of the base line and recording of a large antidromic spike. Usually the spike deteriorated within a minute, but in a few cases, a stable recording was maintained for many minutes.

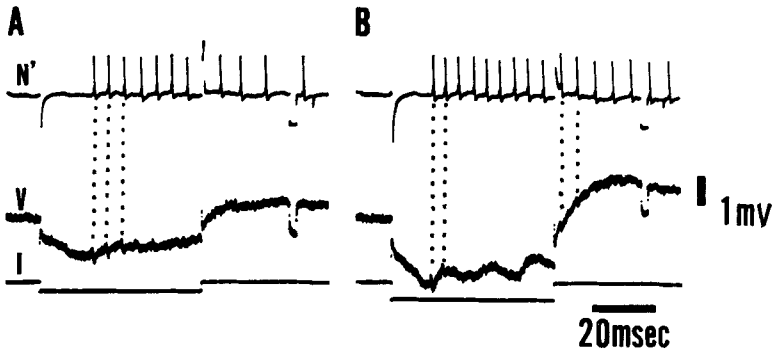

FIGURE 8. Correlation of small brief potentials recorded in the ampulla with nerve impulses. Recorded as in Fig. 5 except that the recording from the nerve trunk is omitted. Small biphasic potentials in association with the first few afferent impulses are superimposed on a receptor oscillation. These potentials broaden and disappear later during the afferent discharge. The ampullary potential is recorded by AC coupled amplification.

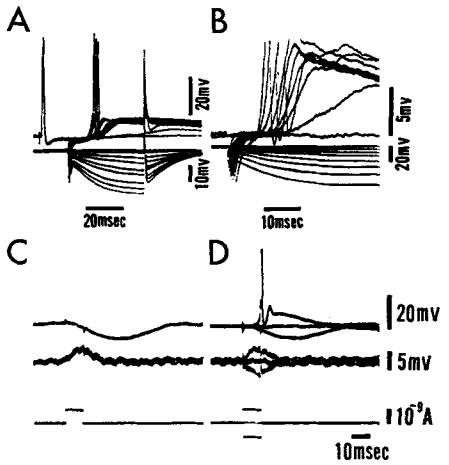

FIGURE 9. Generator potential in the afferent fibers. First trace, DC coupled intra-axonal recording. Second trace, ampullary potential. Third trace, stimulating current (omitted in A and B). (A), an antidromic spike precedes the responses to graded cathodal stimuli (superimposed sweeps). Increasing stimulus strength evokes slow potentials that shorten in latency and increase in amplitude until a spike is initiated. (B), as in (A) but at higher gain and faster sweep speed. (C) and (D), a different ampulla. (C), an anodal stimulus evokes a hyperpolarization of the nerve. (D), superposition of equal amplitude anodal and cathodal stimuli that evoke hyperpolarization and depolarization, respectively.

When a recording was made in an axon close to the ampulla, a cathodal current pulse in the canal evoked a small slow depolarization in the axon, which could outlast the potential change in the ampulla (Fig. 9 A,B,D). On increasing stimulus intensity the slow potential increased gradedly until it initiated an orthodromic spike. Further increase of current intensity often

evoked a repetitive afferent discharge. The intra-axonally recorded slow potential was generally less than 10 mv in amplitude, but in the terminals of unpenetrated fibers the amplitude may have been considerably larger. The slow potential evidently is a generator potential or postsynaptic potential transmitted from the receptor cells and it appears to be responsible for initiating the nerve impulses. The lack of correspondence between the generator potential and the potential in the ampulla indicates that transmission is chemically mediated, which is supported by the fine structure of the receptor synapses as well as other data described below.

When the polarity of stimulation was reversed and the ampullary lumen was made positive, almost no potential change was observed in the axon in most experiments. In a few cases there was a hyperpolarization (Fig. 9 C,D) corresponding to the suppressive effect that this polarity of stimulation has on the discharge of spontaneously active preparations. In many other experiments the hyperpolarization was recorded externally from the nerve bundle of an isolated ampulla (Fig. 7 C,D,B′), perhaps because spontaneous activity was better maintained if, as in these experiments, artificial saline was not used. The depolarizing generator potentials also could be recorded externally, although in Fig. 7 they are obscured by impulse activity (Loewenstein, 1960; Steinbach and Bennett, 1971 *b*).

The latency of the depolarizing generator potentials in response to weak stimuli could exceed 20 msec (Fig. 9 A). Some part of the delay was due to the slow rise of potential in the ampullary lumen. Moderately strong stimuli reduced the latency to about 10 msec (Figs. 9, 10). The minimum latency of externally recorded postsynaptic potentials and nerve impulses in response to

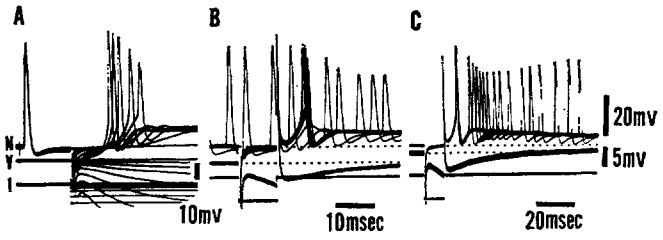

FIGURE 10. Evidence for a conductance increase during the generator potential. (A), an antidromic spike was followed by graded cathodal stimulation to the ampulla. As the stimulus was increased progressively, the generator potential reached threshold for an orthodromic spike which became larger on stronger stimulation. (B) and (C), a shorter stimulus pulse was adjusted to evoke a long-lasting generator potential with a single orthodromic spike. Antidromic spikes were superimposed on the generator potential at various times (one in each of the superimposed sweeps). The generator potential returned to its normal time-course after the undershoot of the antidromic spike which indicates maintained transmitter action. The spike amplitude was reduced throughout the generator potential which suggests that there was an associated conductance increase to a reversal potential below the spike peak.

maximally effective cathodal stimuli was about 4 msec (Fig. 13 E). These values are long compared to those for transmission at electroreceptors of freshwater fish (ca. 1 msec; Bennett, 1971 *a,b*) and for postsynaptic potentials in general including at the nerve-electrocyte synapse of the skate (Bennett, 1961; Eccles, 1964; Katz and Miledi, 1965). The long latency is ascribable, at least in part, to the indirect action of the stimulus on the secretory (presynaptic) membrane of the receptor cells as will be discussed in the next section.

The orthodromic spike in response to threshold stimulation was smaller than the antidromic spike. With increasing stimulus intensity the amplitude of the orthodromic spike increased until it reached or slightly exceeded that of the antidromic spike (Fig. 10 A).

When antidromic spikes were superimposed on the generator potential, their amplitude was reduced. In Fig. 10 B and C, a short current pulse to the ampulla was adjusted so that a single orthodromic spike was followed by a sustained generator potential with little fluctuation in successive responses (at a rate of 1/sec). Each antidromic spike superimposed on the generator potential was followed by an undershoot, but the generator potential promptly recovered to resume the control time-course, a result which indicates prolonged transmitter action or prolonged release. The peak amplitude of each superimposed spike was reduced. This reduction suggests that the depolarizing generator potential involves a conductance increase to a reversal or "equilibrium" potential below the spike peak, in the same way as do excitatory postsynaptic potentials in nerve and muscle (Eccles, 1964; Katz, 1969). The effect in the ampullary nerve fibers is probably contributed to by refractoriness or inactivation of the spike-generating mechanism, because the time-course of spike reduction and generator potential are not strictly parallel.

DISCUSSION

Mode of Operation of the Receptors

From the foregoing data a consistent theory of the operation of the receptors can be derived. A single receptor cell is diagrammed in Fig. 11 and the potentials that would be recorded from it are shown in Fig. 12. The response recorded in the ampullary lumen can be assigned to the receptor cells. This response sometimes leads to the discharge of action potentials, but is not directly related to any potential recorded from the nerve fibers; thus it is involved in reception, but is not generated by the nerves. The polarity of the ampullary response is consistent with its being a conventional depolarizing response of the lumenal or outer faces of the receptor cells. The serosal or inner faces of the receptor cells are apparently inexcitable, because anodal stimuli that from the morphological relations (Fig. 11) would depolarize these faces produce no response in the ampulla during the stimulus; only anode break

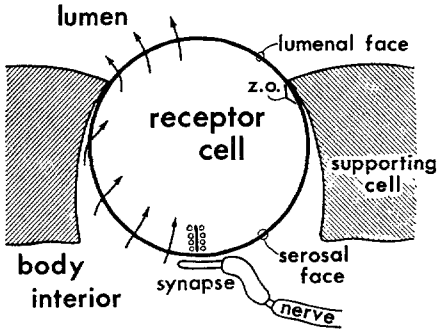

FIGURE 11. Diagram of a receptor cell. The afferent synapse is shown as having a presynaptic ribbon with associated vesicles. The *zonula occludens* (z. o.) which seals off extracellular space between receptor and supporting cells is indicated on the right. The arrows show direction of current flow through one side of the cell when a cathodal stimulus is applied in the ampullary lumen. Current is inward through the serosal face and outward through the lumenal face.

responses are observed which are ascribable to responsiveness of the lumenal faces.

Several lines of evidence indicate that transmission from receptor cells to innervating fibers is chemically rather than electrically mediated: (*a*) transmission is blocked by high Mg, low Ca solutions (Steinbach and Bennett, 1971 *b*, cf. 1971 *a*); (*b*) the relation between potential in the ampulla and generator potential is at best indirect; and (*c*) the ultrastructure of the synapse is typical of chemically transmitting synapses and unlike that of electrically transmitting synapses (Waltman, 1966).

The morphological relations indicate that cathodal stimuli applied in the ampulla tend to depolarize the lumenal faces of the receptor cells and to hyperpolarize the serosal faces (Fig. 12 A). If depolarization causes the serosal faces to release transmitter as at other synapses, cathodal stimuli should tend to prevent secretion of transmitter by the serosal faces, whereas actually they are excitatory. Evidently depolarizing responses are evoked in the lumenal faces. The associated inward current through the lumenal faces flows outward through the (inexcitable) serosal faces overcoming the hyperpolarizing effect of the stimulating current itself and leading to increased depolarization. Thereby the release of an excitatory transmitter is increased. This effect is diagrammed in Fig. 12 C. For reasons to be described below, the lumenal faces are indicated as tonically active, the stimulus increasing this frequency of firing. For convenience a sharp and fixed threshold is given for onset of transmitter secretion, although we expect that the actual relation between pre- and postsynaptic potentials is sigmoidal comparable to that at the squid giant synapse (Katz and Miledi, 1967 *b*; Kusano, 1968).

Anodal stimuli act oppositely to cathodal stimuli, and they would tend to

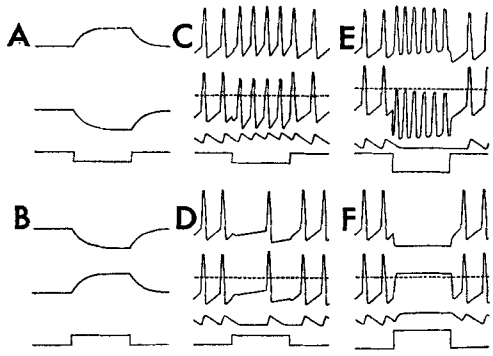

FIGURE 12. Diagram of hypothesized potential changes across the faces of a receptor cell and contribution to generator potentials in an afferent fiber. Top line, potential differentially recorded across the lumenal face. Second line, potential differentially recorded across the serosal face. The level at which transmitter release begins is indicated by the dotted line (C to F only). Third line (C to F only), contribution from this particular receptor cell to the generator potential in an afferent fiber. Bottom line, stimulating current pulse. (A), potential changes produced by a cathodal current pulse applied in the ampulla, excluding responsiveness of the receptor cell. The lumenal face is depolarized; the serosal face is hyperpolarized. (B), potential changes produced by an anodal pulse, excluding responsiveness as in (A). The lumenal face is hyperpolarized; the serosal face is depolarized. (C), effects of a weak cathodal stimulus on a responding receptor cell. Tonic activity of the lumenal face is increased in frequency which leads to an increased frequency of depolarizations of the serosal face, which is inexcitable. These depolarizations cause an increased release of transmitter and a larger generator potential. (D), effects of a weak anodal stimulus. Tonic activity of the lumenal face is decreased in frequency and transmitter release by the serosal face is decreased. (E), effects of a strong cathodal stimulus. Activity of the lumenal face is markedly increased, but the stimulating current itself hyperpolarizes the serosal face enough to block the release of transmitter. (F), effects of a strong anodal stimulus. Activity of the lumenal face is completely blocked, but the stimulating current depolarizes the serosal face enough to cause release of transmitter.

hyperpolarize the lumenal faces and depolarize the serosal faces (Fig. 12 B). Although the direct action on the serosal faces should increase the release of transmitter, this polarity of stimuli can decrease ongoing nerve activity and as shown in Figs. 6 and 7 hyperpolarize the nerve. To explain these inhibitory effects we postulate that there is spontaneous, tonic activity of the lumenal faces that is decreased by anodal stimuli, which leads to a relative hyperpolarization of the serosal faces and reduced transmitter release (Fig. 12 D). This spontaneous receptor cell activity generally must be asynchronous in different receptor cells, because the spontaneous nerve discharge usually is not accompanied by any detectable potentials in the ampulla. Similarly, we expect that each nerve fiber receives PSP contributions from many asynchronously active receptor cells and that the impulses in a single fiber are not

synchronized with activity of any single receptor cell. The generator potentials we have recorded do not show large discrete components (although they may not be absolutely smooth, Fig. 9 B). Furthermore, the nerve impulses can occur at much higher frequency than the receptor spikes which are presumably generated by synchronous activity of many receptor cells.

From this proposed mechanism there follow several predictions as to the effects of strong stimulation. Strong cathodal stimuli should hyperpolarize the serosal faces sufficiently that even the depolarizing effect of the activity of the lumenal faces fails to cause the release of transmitter (Fig. 12 E). Strong anodal stimuli should depolarize the serosal faces sufficiently to override any decrease in depolarization due to reduced activity of the lumenal faces (Fig. 12 F). Responses to strong anodal stimuli should occur at a shorter latency than responses to cathodal stimuli since the stimulus acts directly on the secretory membrane without the intervention of activity of the lumenal faces. These predictions were verified experimentally as is shown in Fig. 13. As the strength of a cathodal stimulus was increased well above threshold, the latency of the nerve response first decreased to a minimum of about 3 msec (Fig. 13 E). As the stimulus was further increased the latency gradually increased to a maximum of ca. 4 msec, and then transmission failed altogether (Fig. 13 F–H). The blocking cathodal stimulus in Fig. 13 H was ca. 50 mv in amplitude, which was not so large as to damage the cells. (A response still followed the termination of this stimulus as well as that of weaker ones. A possible cause of this response is rapid decay of the hyperpolarization of the serosal faces which were then depolarized by remaining depolarization of the lumenal faces. The effects of different time constants of the two faces are not considered in Fig. 12.) As the strength of an anodal stimulus was increased, nerve responses were evoked at relatively short latency (Fig. 13 A–D). The maximum latency to anodal stimulation in this experiment was about 3 msec and occurred when the stimulus had reached a threshold amplitude of ca. 30 mv (Fig. 13 B). The latency then gradually decreased to about 2 msec as the stimulus was increased and then a still earlier response appeared that had a latency of 0.5 msec or less. Presumably the longer latency responses evoked by anodal stimulation were synaptically mediated, the anodal stimulus acting directly on the serosal, secretory faces of the receptor cells to cause release of transmitter. The earliest response is ascribable to direct stimulation of the afferent fibers; similar short latency responses are evoked by very strong anodal stimuli applied to other electroreceptors (Bennett, 1967).

The opposite effects of strong compared to weak stimuli were noted earlier by Murray (1965). He used long current pulses and measured neither stimulating voltage nor initial response latency. He ascribed his results to direct action on the innervating nerve rather than to action on the secretory membrane of the receptor cells, but the absolute magnitude of the applied voltages as well as the latencies makes his explanation unlikely.

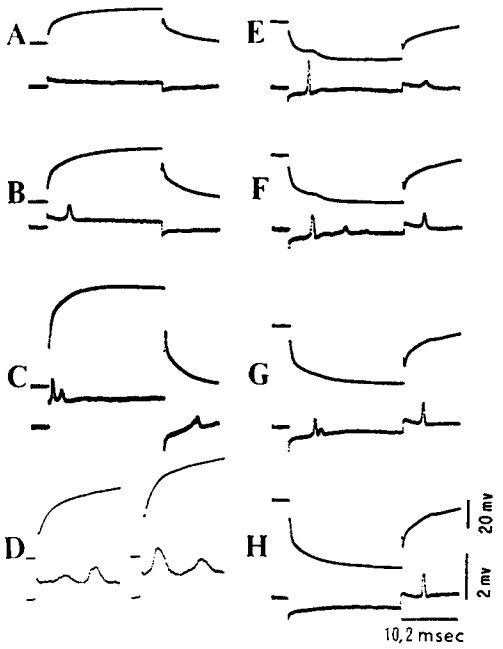

FIGURE 13. Effects of very strong stimuli on transmission to the afferent fibers. Recorded as in Fig. 7 except that the rectangular current pulses are omitted. (A), a moderate strength anodal stimulus that would be capable of blocking any spontaneously active preparation. (B), a stronger anodal stimulus evokes a response at its onset with a latency of ca. 3 msec. (C) to (D), with stronger stimuli this response moves to a latency of just under 2 msec and an earlier response with a latency as low as 0.5 msec appears. The earlier response partially occludes the later one indicating the same nerve fibers are involved. (E), a moderately strong cathodal stimulus that evokes a response at a minimum latency of about 3 msec. (F) to (H), increasingly strong cathodal stimuli delay and block the response at the onset of the stimulus. Expanded sweep for the two records in (D).

If anodal stimuli inhibit by decreasing tonic activity of the receptor cells, the hyperpolarizing generator potentials in the nerve should be accompanied by an increase in resistance. This prediction has not yet been tested in the skate ampulla. In electroreceptors of the marine catfish *Plotosus* the peak height of the antidromic spikes is increased during hyperpolarizing generator potentials, suggesting increased resistance (Obara, unpublished observations). For small stimuli of both polarities the relation between discharge frequency and stimulus strength is quite linear in the ampulla (Murray, 1965) as well as in a number of other electroreceptors (Bennett, 1971 *a,b*). This result also suggests that the same continuous process is affected by the two polarities of stimuli, rather than there being separate excitatory and inhibitory transmitters.

The electrical excitability of the lumenal faces of the receptor cells will have to be established by intracellular recording. Nevertheless, the combined results

of weak and very strong stimulation, the location of the receptor synapses, the fine structural relations between cells, and comparative considerations all support the hypothesis we have presented.

In our proposal the receptor epithelium of the ampulla is equivalent to an inside out cell. Because of the high input resistance of the ampulla, inward current through the lumenal face of one receptor cell due to its spontaneous or evoked activity makes the lumen more negative which tends to further excite this cell and also to excite other receptor cells. The lumenal negativity produced by the tonic resting activity of the receptor cells must generally be too small for the cells to excite each other. Normally it would be only when a large number of receptor cells became active simultaneously that there would be sufficient feedback for the response to become regenerative. Whether the receptor epithelium generated a spike or a graded oscillation would depend on a number of factors, the most important of which is likely to be loading by the impedance looking from the ampullary lumen peripherally down the canal (Bennett et al., 1970). Waltman (1968) has apparently obtained spikes in most or all of his preparations by using feedback circuitry to compensate for the canal impedance. In respect to magnitude of transmembrane potentials the receptor spikes are probably comparable to ordinary action potentials. Waltman reports a maximum amplitude of about 60 mv for the receptor spike recorded across the ampullary wall. The amplitude of responses in single receptor cells during asynchronous resting activity is unclear. There may be spikes that are only moderately reduced by loading or there may be graded subthreshold oscillations (Mauro et al., 1970). Which kind of response occurs will depend upon the resistance of the serosal face because it limits the degree of loading provided by the current path through the serosal membrane and back around through the canal into the ampullary lumen. Whatever the response amplitude, the duration would probably be comparable to that of the spikes of the entire receptor epithelium. There are many hundreds of receptor cells, which is probably enough that a full sized spike response in a single cell need not produce a significant potential in the ampulla. The amplitude of a cathodal stimulus adequate to block transmitter release—about 50 mv—suggests that a sizable fraction of this potential is developed across the serosal membrane and that therefore its resistance must be comparable to or greater than that of the lumenal membrane.

Comparison to Other Electroreceptors

The scheme of receptor function derived for the ampulla presents interesting similarities to and differences from electroreceptors of freshwater teleosts (Bennett, 1970, 1971 *a*,*b*). The teleost receptors can be divided into two general categories, the tonic and phasic receptors. The afferent fibers from tonic receptors carry a spontaneous rhythmic discharge similar to that of elasmo-

branch ampullae of Lorenzini. However anodal stimuli excite and cathodal stimuli inhibit this discharge, a polarity of sensitivity opposite to that of ampullae of Lorenzini.

Intracellular recording from receptor cells of a teleost tonic receptor shows that most of a stimulating voltage is developed across the inner or serosal face of the cells (Bennett, 1971 *b*). No active electrical responses of the receptor cells are seen in this case, nor does external recording reveal any electrical response of receptor cells in tonic receptors of other freshwater teleosts. Thus, it appears that stimuli act directly on the serosal face, which is the presynaptic membrane of the receptor cells, to increase or decrease the release of transmitter and thereby affect the nerve discharge. The direct action of the stimulus on the secretory membrane is confirmed by the short synaptic delay, about 1 msec; at the same time the length of the delay excludes direct action of the stimulus on the afferent fiber. The receptor cells of teleost tonic receptors are electrically linear or nearly so in contrast to receptor cells of ampullae of Lorenzini.

As in respect to ampullae of Lorenzini, it can be concluded that there is a tonic release of transmitter in teleost tonic receptors that is increased by excitatory stimuli, which are those that depolarize the secretory membrane, and decreased by inhibitory stimuli, which are those that hyperpolarize this membrane. Conductance changes during the generator potentials in the afferent fibers have not been measured (except as noted above with respect to the marine catfish), but the linearity of the input-output relation for small stimuli of either sign makes the hypothesis of separate excitatory and inhibitory transmitters very unlikely.

The direct action of stimuli on the secretory membrane in teleost tonic receptors contrasts to the indirect action in the ampullae of Lorenzini. This difference explains the opposite polarity of sensitivity in the two classes of receptor. It also accounts for much of the longer latency of the responses of the ampullae of Lorenzini.

The receptor cells of phasic receptors of freshwater teleosts generally are excited by electrical stimuli to give electrical responses which can be graded regenerative oscillations or all-or-none spikes. In this respect they resemble ampullae of Lorenzini. However, in teleost phasic receptors it is the inner receptor cell membranes, that is the serosal faces, that are excitable. Presumably the electrically excitable membrane lies interspersed with the secretory membrane.

Entry of Ca ions has been implicated in the release of transmitter at a number of interneuronal and neuromuscular synapses (see Katz, 1969). Presynaptic depolarization appears to act by increasing Ca influx which then mediates transmitter release. By increasing the Ca gradient and pharmacologically blocking both Na and K activation, it has been possible to demonstrate Ca spikes in presynaptic terminals of the squid giant synapse and (less

directly) in those of the frog neuromuscular junction (Katz and Miledi, 1969 *a,b*). It is to be expected that Ca has a similar role at electroreceptor synapses, for high Mg reduces or blocks transmitter release at a teleost phasic receptor and at the ampulla of Lorenzini, and in the latter preparation low Ca solutions reduce transmitter release (Steinbach and Bennett, 1971 *a,b*). It might be expected therefore, that there are some electrically excitable Ca channels in receptor cells of teleost tonic receptors, only that there are too few to cause a readily detectable electrical nonlinearity. In this respect it is interesting that the electrically excitable responses of teleost phasic receptors are insensitive to tetrodotoxin (TTX) which is known to block Na activation in many excitable tissues and blocks nerve and muscle excitability in the same fish (Zipser, 1971). TTX blocks nerve impulses of the ampullary afferents, but does not block the ampullary responses or generator potentials recorded externally from the nerve (Steinbach and Bennett, 1971 *b*). These findings indicate that any direct electrical action on the inner faces of the receptor cells is TTX resistant, but do not yet establish the TTX insensitivity of the ampullary response. TTX applied to the serosal surface of the ampulla would not be expected to cross the receptor epithelium which is well sealed off from the lumen as indicated by the high electrical resistance of the ampulla. Since TTX acts on the external surface of the squid axon (Narahashi et al., 1967), it should be injected into the ampullary lumen to determine the sensitivity of the lumenal faces of the receptor cells.

The TTX insensitivity of the electrically excitable responses of teleost electroreceptors does allow the suggestion that their evolution involved an increase in the number of Ca channels, the capability to form them in the secretory membrane already being present. This mode of evolution may for unknown cellular reasons have been more likely than development of electrically excitable Na channels. There is no evidence for regenerative responses in receptor cells other than electroreceptors. Yet the parallel evolution of this trait in at least three different lines (the elasmobranchs, the gymnotids, and the mormyrids) suggests that it is a feature which may be found in other acousticolateralis receptors.

Electroreceptors of marine elasmobranchs are significantly more sensitive than those of freshwater teleosts, about 50–100 times. Given a particular kind of Ca channel that opens in response to a small depolarization, the sensitivity becomes greater when the parallel conductances are so small that the responses are regenerative. Indeed the gain of a system responding in an all-or-none manner is infinite when the system is poised at threshold (Cole et al., 1970). It is a reasonable conjecture that greater sensitivity can be achieved by an oscillating regenerative system than by the same channels electrically loaded down to a point where the system is fairly linear. Thus the greater sensitivity of the elasmobranch receptors is possibly a result of there being tonic activity of the receptor cells.

As noted above we anticipate that the relation between pre- and postsynaptic potentials in the ampulla is sigmoidal comparable to that at the squid giant synapse (Katz and Miledi, 1967 *b*; Kusano, 1968). In the ampullae the slope of the curve would not be required to be particularly steep, if the regenerative responses of the receptor cells were responsible for this high receptor sensitivity. In tonic receptors of teleosts, however, the slope is likely to be many times greater than in the squid synapse, for stimuli of the order of a millivolt can modulate nerve discharge frequency from several hundred impulses per second down to zero frequency. The teleost tonic receptors also differ in the apparent large release of transmitter in the absence of stimulation. Thus, compared to the squid synapse the input-output relation of the receptor synapses is shifted along the presynaptic voltage axis in the negative direction. Another probably important factor in receptor function is shift of the synaptic input-output relation as a result of prior stimulation (see Katz and Miledi, 1971). All the receptors show some degree of accommodation, but the contribution of altered transmitter release and postsynaptic changes have not been disentangled.

One can still question why tonic receptors of freshwater teleosts have not evolved a mechanism to increase sensitivity similar to that of marine elasmobranchs. One possible explanation of the lower sensitivity of freshwater teleost receptors is the presence of electrical noise. The dilute solutions in which freshwater fish live would be more liable to surface and streaming potentials and perhaps also to atmospheric interference of various kinds. Whatever the difference in the two environments some preliminary comparative data suggest that there is a valid difference in the useful sensitivity in the two media. The freshwater sting ray *Potamotrygon* has ampullae of Lorenzini that have the same polarity of sensitivity as in marine forms but a much lower sensitivity that is comparable to that of freshwater teleosts (Szamier and Bennett, 1971). The marine catfish *Plotosus* has tonic receptors with the same polarity of sensitivity as in other teleosts, but the sensitivity is considerably greater, approaching that of marine elasmobranchs (Obara, unpublished observations). There are indications of a regenerative response of the *Plotosus* receptor cells, but by membrane located in the serosal rather than lumenal faces.

There are differences in the accessory structures of the receptors of marine and freshwater fish; the ampullary canals in *Potamotrygon* are very short and the receptors resemble tonic receptors of freshwater teleosts. In *Plotosus* the canals are much longer than in other teleosts and the receptors have been called ampullae of Lorenzini because of their resemblance to the receptors of marine elasmobranchs. These differences in accessory structures can be explained in terms of maximizing sensitivity in the environments of greatly different conductivity (Bennett, 1971 *a,b*).

The ampulla of Lorenzini appears to be an excellent preparation for further study of several aspects of transmission at receptor synapses. The preparation

is readily maintained in vivo and a variety of test solutions can be applied to it. A suggestion as to the nature of the transmitter is provided by the observation that the nerve is excited by low concentrations of glutamate (Steinbach and Bennett, 1971 *b*). Further experiments should help to elucidate the ionic basis of the receptor cell response, the mechanisms of transmitter release, and the chemical identity of the transmitter.

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