Effects of Divalent Ions and Drugs on Synaptic Transmission in Phasic Electroreceptors in a Mormyrid Fish

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ABSTRACT   We recorded impulses in afferent nerve fibers innervating two kinds of phasic electroreceptors in a mormyrid fish. We used an isolated preparation of skin, receptors, and sensory nerves to estimate synaptic delays, and to change solution in contact with the receptor-nerve synapse. The minimum delays between stimuli and sensory nerve responses, which must be slightly larger than synaptic delays, are about 0.7 msec in medium receptors and about 0.25 msec in large receptors. This result supports previous suggestions that transmission is chemically mediated in medium receptors and electrically mediated in large receptors. Furthermore, Mg^{+2} depresses synaptic transmission in medium receptors, and has little effect on transmission in large receptors. A complex dependence of response on both Mg^{+2} and Ca^{+2} masks divalent ion dependence of transmission, but a large excess of Mg^{+2} cannot completely block transmission in medium electroreceptors. L-glutamate, and not cholinergic drugs, produces a sequence of excitation and depression of medium receptor response which indicates that a similar chemical is the transmitter in the afferent synapse.

INTRODUCTION

Electroreceptors, which occur in a number of groups of fish, are receptors of the acousticolateralis system that are specialized for the detection of electric fields. Their properties have been recently reviewed (Bennett, 1970, 1971). In these receptors the initial active transformation of the stimulus is carried out by nonneural receptor cells that synapse on afferent fibers. The receptor cells are acted upon by the electrical potential across the epidermis in which they lie and are arranged so that their outer faces are in electrical contact with the external medium while their inner faces are in contact with the body interior. The afferent fibers constitute part of the lateral line nerves. Comparative physiological and morphological data suggest that synaptic transmission in electroreceptors is similar to that in mechanoreceptors of the 8th

cranial nerve, and that the evolutionary changes in the receptor cells that lead to electrical sensitivity are primarily in the mechanosensitive part of the cells. Study of transmission at these synapses is therefore likely to be of general relevance to receptor synapses of the acousticolateralis system.

Electroreceptors can be divided into two general types, tonic and phasic. The nerve fibers from tonic receptors carry a regular tonic impulse activity in the absence of stimulation; they respond to low frequency electric fields by increasing or decreasing the rate of this activity. Fibers from phasic receptors do not have a tonic discharge; spontaneous activity, if present, is irregular. They are sensitive only to the higher frequency components of a stimulus and respond with one or more impulses only to the onset or termination of a long-lasting rectangular stimulus. Apparently the outer faces of the receptor cells act as blocking capacitors that limit the low frequency response of the receptors.

We report here properties of synaptic transmission at two kinds of phasic receptors found in mormyrids, an African family of weakly electric fishes. Mormyrids have an additional type of electroreceptor that is tonic in function. The distribution and the innervation of the receptors have been carefully mapped by Harder (1968). Because of their appearance in the skin the three receptor types have been termed large, medium, and small (Bennett, 1967). Szabo refers to the same receptors as mormyrid type C, B, and A, respectively (Szabo, 1965). About 60% of the receptors are medium, 20% are large, and 20% are small (Harder, 1968).

Large receptors are phasic in function. One to seven receptor cells are innervated by a single myelinated fiber. All-or-none responses are generated by the receptor cells, presumably by their inner faces, and can be recorded as small initially positive spikes at the receptor opening on the skin surface. The externally recorded spike is evoked with very little latency at the onset of stimuli making the exterior of the skin positive with respect to the inside and at the termination of stimuli of opposite polarity ("anodal" and "cathodal" stimuli, respectively). At low frequencies of receptor cell activity, each spike response is followed by a single nerve impulse, but the nerve skips responses and may fail altogether at high frequencies. The evidence indicates that synapses in the large receptors are electrically transmitting. The delay of impulse transmission across the synapse is about 0.3 msec at 25°C, which is very short for chemically mediated transmission at this temperature. Also, antidromically stimulated impulses excite the externally recorded spike in a way that indicates that there is an electrotonic synapse between receptor cells and the afferent fiber.

Medium receptors are also phasic. They are innervated by three nerve fibers. There are two types of receptor cells, but it is not yet clear whether a single fiber can synapse with both. Potentials are elicited external to medium

receptors at both onset and termination of a rectangular stimulus. These potentials are graded with intensity of stimulation and cannot be evoked by antidromic stimulation. The response to onset of anodal stimuli probably is due to graded regenerative depolarizing activity of the inner faces of one type of receptor cell. The response to termination of anodal stimuli may represent anomalous rectification in the inner faces of the other type. Afferent fibers from medium receptors carry trains of impulses lasting for several milliseconds after an adequate stimulus. The relations between impulse activity and external response are such as to indicate that transmission from receptor cell to nerve fiber is chemically mediated.

Small receptors are tonic and are innervated by a single nerve fiber, the impulses of which can be recorded external to the receptor. Excitation caused by a brief stimulus can long outlast the stimulus and the after-discharge is virtually unaffected by a strong inhibitory stimulus. Furthermore, there is a minimum delay for nerve excitation of about 1 msec. These findings indicate that transmission is chemically mediated at the synapses between receptor cells and afferent fiber.

It is useful to outline our picture of the operation of the two kinds of phasic receptor under our experimental conditions. Because the external faces of the receptor cells act as blocking capacitors, the potential across the inner faces is only transiently changed at the onset of a rectangular stimulus, and it returns to its resting (prestimulus) level during a sufficiently long-lasting stimulus. At termination of such stimuli the potential across the inner face is oppositely changed. We have generally employed rectangular cathodal stimuli, that is, those making the outside of the skin negative with respect to the inside. These stimuli at onset hyperpolarize the inner faces of the receptor cells and at termination depolarize them (V_s and E_r in Fig. 1). The depolarization of the inner faces can be amplified by regenerative behavior in large receptors and perhaps also, but to a lesser extent, in medium receptors.

In large receptors the spike generated by the receptor cells is electrotonically transmitted to the nerve across a low resistance junction between them. The resulting depolarizing postsynaptic potential (PSP) initiates an impulse in the nerve. In medium receptors depolarization of the inner faces (at least of one type of receptor cell) causes them to release a transmitter chemical in an amount that depends on the degree of depolarization. The transmitter diffuses across the synaptic gap to the sensory nerve terminal where it reacts with specific sites to generate a depolarizing PSP (E_{nt} in Fig. 1). The PSP, if large enough, excites one or more impulses in the nerve. For example, in Fig. 1, $n1$ and $n2$ indicate impulses that might be evoked at threshold and at three times threshold.

In large receptors only a single receptor spike and nerve impulse are evoked by the onset of an anodal stimulus. Only small (but equal) decreases in latency

are produced in both responses as the stimulus strength is increased from threshold to many times this value. In medium receptors, on the other hand, marked decreases in latency are produced by increasing stimulus strength, and in many fibers the number of impulses increases.
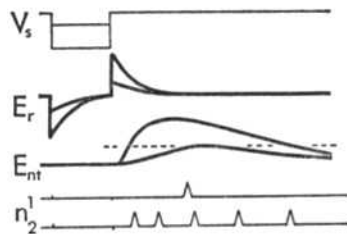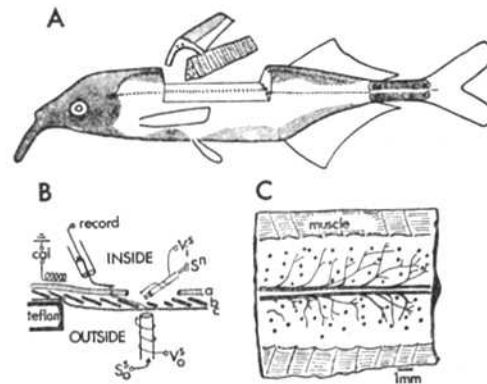

FIGURE 1   FIGURE 2

FIGURE 1. Operation of medium receptors, schematic representation of potentials at various sites as a function of time. V_s: stimuli applied across the skin making the outside positive ($V_s = V_o^* - V_i^*$ in Fig. 2 B), at threshold and at three times threshold. E_r : resulting hyperpolarization followed by depolarization of inner faces of receptor cells (alteration of potential form by responsiveness of the membrane is not shown). E_{nt} : the PSP's produced in the postsynaptic nerve terminal by transmitter released from the receptor cells; impulses are omitted and threshold is indicated by horizontal dashed line. $n1$ and $n2$: impulses evoked by the two stimuli as they might be recorded externally from a nerve fiber.

FIGURE 2. Preparation of excised skin and receptors from *G. petersii*. (A) Chunk cut from dorsal surface that includes an area of skin bearing electroreceptors (stippled areas) and both dorsal and medial pairs of lateral line nerves (dotted lines). The electric organ is in the caudal region as indicated. (B) Simplified side view of a portion of preparation pinned over a window cut in the Teflon bottom of experimental chamber. Three layers of skin shown are (a) fascia, (b) scales, and (c) epidermis. Fascia and scales are removed over a receptor. The nerve bundle containing the fiber(s) to the receptor studied is picked up to record impulses relative to the indifferent electrode in the bath. The transskin potential produced by stimuli applied between the indifferent electrode (not shown) and the capillary on the external surface of the skin (S_o^*) is measured by Ag-AgCl electrodes ($V_s = V_o^* - V_i^*$). A bipolar electrode (S^m) is used to stimulate nerves directly. (C) Drawing of the inside of excised skin flattened following removal of attached muscle; d.l.l. nerves and branches to receptors (black dots) are shown.

We used an excised preparation of skin, receptors, and sensory nerve to estimate transmission delay at synapses of both large and medium receptors more accurately than was possible in the intact preparation. We also tested the effects of Ca^{+2} and Mg^{+2} and of several drugs. Our results strengthen the earlier indications of chemically mediated transmission in medium receptors

and of electrically mediated transmission in large receptors. We found that synapses of medium receptors do not exhibit as clear an antagonism between Ca^{2+} and Mg^{2+} as do neuromuscular and probably many other synapses (Katz, 1969; Nicholls and Purves; 1970). We also obtained evidence that the transmitter in medium receptors is L-glutamate or a related substance.

METHODS

The Preparation

The animals used were the mormyrid *Gnathonemus petersii*, about 10 cm in length, and a few species of *Gnathonemus* lacking a chin proboscis, or tubular mouth. The fish originated in West Africa and were purchased locally and maintained in tanks at about 27°C before use. Experimental fish were killed quickly, and a section of the dorsal trunk was removed (Fig. 2 A). The section contained portions of the dorsal branch of the posterior lateral line nerve (d.l.l. nerve) and considerable muscle. Muscle was trimmed off and the skin with attached d.l.l. nerves was transferred rapidly to the experimental chamber and bathed with flowing physiological saline. The upper compartment of the experimental chamber had a Teflon floor with a window cut in the bottom that was somewhat smaller than the area of the skin to be studied. The window communicated with an underlying L-shaped compartment, the rising part of which reached the same level as the upper compartment. Solutions were maintained at about the same level in the two compartments so that there was little hydrostatic pressure difference across the skin. The skin was first pinned loosely over the window, inside surface uppermost (Fig. 2 B). Muscle remaining over the d.l.l. nerves was dissected away, and the skin was then stretched and pinned more tightly. Branches containing 2–20 fibers leave the d.l.l. nerve at intervals of about 2 mm and pass posteriorly and laterally along the skin (Fig. 2 C). Eventually small nerve branchlets pass through a thick layer of fascia and run between scales and connective tissues to reach electroreceptors in the epidermal layer (Fig. 2 B). The branches are easily freed of connective tissue and can be picked up for electrical recording. The fascia keeps solutions in the upper chamber from reaching the receptors. Therefore, a small area of fascia and several scales were removed just above (i.e., inside) the receptor being studied.

Electrical Recording and Stimulation

A polyethylene tube of 0.5 mm inside diameter and 1.5 cm long was filled with mineral oil and mounted on a manipulator. A hook on the end of a fine chloridated silver wire passed through the tube and could be moved in and out of the tip by a micro-drive. Nerve branches were hooked up and pulled into the oil for electrical isolation. In branches containing more than 20 fibers, single unit impulses were usually 0.5 mv or larger, and the system was mechanically and electrically stable for hours.

The stimulating voltage across the skin (V_s) was monitored by differential recording with two chloridated silver wires insulated except at the tip. One was placed close to the inside of the skin near the receptor being studied (V_i' in Fig. 2 B). The other (V_o') was attached to the external stimulating capillary and thus brought close to the

outside of the skin. Electroreceptors were stimulated by passing current from an rf isolation unit between a large chlorided silver electrode in contact with the inside of the skin (not shown in Fig. 2) and a saline-agar–filled capillary (tip diameter about 200 μ) close to the external surface of the skin (S_o^s in Fig. 2 B). The capillary was bent into a U shape and attached to a manipulator so as to pass down into the L-shaped compartment, and then up towards the underside of the preparation. Using transillumination, receptors, which are unpigmented, appeared as bright spots in the darkly pigmented skin; the stimulating capillary appeared as a dark anulus. By moving the capillary and determining minimum thresholds, single receptors could be localized. In some cases nerve branches were cut to block impulses in fibers inner-vating neighboring receptors. The usual stimulus applied across the skin was a 2 msec duration rectangular pulse that made the skin inside positive. This polarity was picked to stimulate on the break of the pulse and allow recording of the response without electrical interference by the stimulus. The duration was long enough to minimize changes in response with slight fluctuations in pulse duration. Some afferent fibers respond to the onset of stimuli making the inside of the skin positive as well as to stimuli of the usual polarity (Bennett, 1965). Such responses were not observed in the present experimental conditions. Responses consisting of one or more impulses evoked by transskin stimulation are called *synaptically evoked responses* henceforth. Most responses were obtained from medium receptors, corresponding to their greater number.

Bipolar chloridated silver electrodes were made of 125-μ in diameter Teflon-coated wires cemented together and connected to an RF isolation unit. These electrodes were used to apply brief stimuli to nerve fibers close to their receptors (S^n, Fig. 2 B). A response evoked by this procedure consisted of a single impulse and is called a *directly evoked response*.

Solutions

The physiological saline used contained 167 mM NaCl, 5 mM KCl, 3 mM CaCl$_2$, 1.5 mM MgCl$_2$, 4 mM tris(hydroxymethyl)aminomethane (Tris buffer) adjusted to pH 7.2, and 1 g of glucose/liter (based on several reported teleost saline solu-tions). Solutions with altered divalent ion concentration were made by mixing standard saline with three modified salines: (*a*) 30 mM Mg^{+2} substituted for Na, (*b*) 30 mM Ca^{+2} substituted for Na, and (*c*) no divalent ions at all. Osmolality was not mea-sured; addition of 1–3 g/liter sucrose did not significantly alter responses.

At the outset the upper chamber was perfused with oxygenated physiological saline using an air lift for recirculation at about 200 cc/min. Continuous perfusion of oxygenated, glucose-containing saline maintained medium and large receptor re-sponses for 2–3 hr. Without these precautions synaptically evoked responses began to fail within minutes of killing the fish, although directly evoked responses could be obtained for a much longer time. Failure of synaptically evoked responses typically was preceded by increased threshold, decreased number of impulses per response, and increased fatigue on repetitive stimulation. Tonic responses from small receptors were observed only rarely, and then failed within minutes after dissecting the preparation.

Drugs added to the bathing solution included acetylcholine bromide (Merck &

Co., Inc., Rahway, N.J.), d-tubocurarine chloride (Calbiochem, Los Angeles, Calif.), atropine sulfate (Burroughs Welcome & Co., Inc., Tuckahoe, N.Y.), sodium propionate (Mann Research Labs. Inc., New York) and D-, L-, or DL-glutamate or L-aspartate (obtained from Mann as the acid and made up with NaOH to pH 7.3). Each test solution was applied between applications of control solutions (usually physiological saline) and any deterioration of response with time was corrected by linear interpolation. All changes were made by opening a gravity feed outlet in the recirculation system, flushing the chamber with 100 cc of the new solutions, and then adding 200 cc of new solution before beginning recirculation; the chamber volume was about 10 cc.

RESULTS

Impulse Latency and Transmission Delay

Medium receptors in the excised skin preparation responded with thresholds between 2.5 and 100 mv minimum amplitude; threshold was usually about 25 mv measured across the skin. These values are somewhat higher than in the intact fish (Bennett, 1965, 1967; Szabo and Hagiwara, 1967). The estimated transskin resistance of the excised preparation was at least an order of magnitude smaller than the 50 kohm cm² measured in the intact fish (Bennett, 1967). A lowered skin resistance could decrease receptor sensitivity. Possibly the skin was injured in dissection, also saline on the outside of the skin probably lowers its resistance.

Synaptically evoked responses from medium receptors (Fig. 3) consisted of single impulses at threshold; in many fibers the number of impulses increased as a function of stimulus strength to some maximum number of impulses (three to seven usually). The latency of the first impulse decreased as stimulus strength increased. We measured the latency between the stimulus and the first impulse at threshold (T_t1) and at three to five times threshold when this latency became a minimum (T_m1). We also measured the maximum number of impulses in a train (n), and the latency of the last impulse at three to five times threshold $(T_m n)$. These parameters were used to evaluate effects of solution changes. Even with very large stimuli, there was considerable variability in the latency of the first impulse and in intervals between impulses in successive synaptically evoked responses. In a single fiber the mean latency of the first impulse and intervals between successive impulses (in milliseconds) in 24 successive responses to four times threshold stimuli delivered at intervals of 10 sec were (with the range, in milliseconds, given in parentheses) 0.8 (0.7–0.9), 1.3 (1.2–1.4), 1.7 (1.5–2.1), 2.0 (1.6–2.7), 2.5 (1.8–3.5). There was a greater variation when these parameters were calculated for eight single fibers from different preparations (values shown are the mean of the mean of three responses in each fiber and the range of the means for each impulse in parentheses): 0.9 (0.7–1.2), 1.4 (1.1–1.6), 1.7 (1.5–2.1), 2.1 (1.6–3.0), 2.7 (2.3–3.0).

(Only six fibers were tabulated for the last two values.) The results presented for medium receptors were obtained from nerve fibers that gave at least three impulses for suprathreshold stimulation and that showed little change in response over a 15 min initial observation period. Occasionally deterioration of
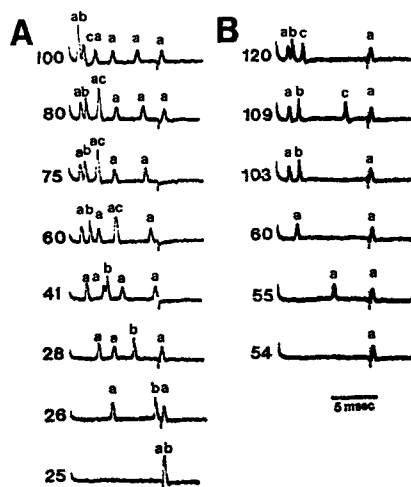

FIGURE 3. Responses of medium receptors. Each trace shows first the response to a 2 msec rectangular pulse applied across the skin (amplitude in millivolts is shown by the number to the left of the trace). The records begin with termination of the stimulus. Also shown on each trace is the response to direct stimulation of the afferent nerve (artifact indicated under each trace). The synaptically evoked response involved impulses in three fibers, designated *a*, *b*, and *c*, as indicated by examination of records in both (A) and (B). The directly evoked response was adjusted to involve only fiber *a*. (A) Response in physiological saline (3.0 mM Ca^{+2} and 1.5 mM Mg^{+2}) after treatment with excess Mg^{+2} and washing. Fibers *a* and *b* were first excited at about the same stimulus strength; fiber *c* required stronger stimulation. All fibers responded at shorter latency as stimulus strength was increased; the response of fiber *a* also increased in number of impulses. The directly evoked response was blocked by closely preceding impulses in fiber *a* (traces 41, 60, 75), but not in fiber *b* (trace 26). (B) Records in saline with 15 mM Mg^{+2} and 3.0 mM Ca^{+2} taken before (A). Compared to records in (A), threshold was increased (from 26 to 55 mv, approximately) and number of impulses in fiber *a* was reduced without change in minimum impulse delay.

response during a long experiment required up to 20% corrections after comparing early and late responses in normal saline.

Responses from large receptors were identified by low threshold and very small latency changes (less than 0.15 msec) near threshold. The latency of the single impulse ranged from 0.4 to 1.1 msec at threshold. These responses were studied wherever they were found, often without localizing the receptor. Thus the absolute values of the measured thresholds should not be considered significant.

In actual experimental records, the synaptically evoked response attributed to one medium receptor usually consisted of responses of two or three fibers that could be distinguished by shape or amplitude of the single impulse (Fig. 3). Quite exhaustive attempts to improve localization convinced us that we were observing responses of two or three nerve fibers from one medium receptor. One of the fibers usually had a considerably lower threshold, and gave more impulses for intense stimulation than did the other(s). All fibers appeared to be affected similarly by solution changes, but the data presented in tabular form concern only fibers having a low threshold.

Conduction time was estimated from the latency of the impulses evoked by direct stimulation of the nerve fiber involved in the synaptically evoked response. The small bipolar electrode was carefully positioned to find a minimum threshold point very near the receptor. By appropriate timing of synaptic and direct stimulation it was possible to show that the synaptically and directly evoked responses were carried by the same nerve fiber (Fig. 3 A).

Conduction times averaged about 0.6 msec for medium receptors, and 0.17–0.30 msec for large receptors. Measured conduction time was subtracted from the measured minimum latency of the first impulse ($T_m 1$) to give transmission delay. For the five large receptors studied this was 0.25 msec (range 0.23–0.30). The mean transmission delay at medium receptors was found to be 0.76 msec (range 0.6–1.2 msec, 35 units tabulated).

Effects of Divalent Ions on Medium Receptors

Measurements were made 15–30 min after solution changes unless otherwise stated; this may not have allowed for complete equilibration but did allow observed effects to reach an apparent steady state.

HIGH MAGNESIUM IONS Changing from normal saline (1.5 mM Mg^{+2}, 3.0 mM Ca^{+2}) to a solution containing higher Mg^{+2} always reduced the number of impulses and increased the threshold for synaptically evoked responses (Figs. 3, 4; Table I, A). The increase in threshold seemed not to be due simply to an increase in nerve threshold, since Mg^{+2} did not affect direct nerve excitation very much. Mg^{+2} did not alter the minimum transmission delay at high stimulus strength (Fig. 3). In three experiments medium receptors were exposed to solutions containing 30 mM Mg^{+2} and no Ca^{+2} for up to 2 hr. The threshold for direct stimulation of the nerve increased slightly, while the threshold for synaptic excitation increased greatly. However, all fibers continued to produce at least one impulse in response to synaptic activation. The latencies recorded at threshold were within the normal range, which indicates that the single impulse was not due to direct stimulation of the afferent nerve.

HIGH CALCIUM IONS Raising the Ca^{+2} above the usual level of 3 mM also increased threshold for synaptically evoked responses and reduced the

number of impulses (Table I, B). However, Ca^{+2} increased threshold for direct excitation of the nerve enough to account for much of its effect on synaptically evoked responses (Table I, B, right-hand column).

LOW DIVALENT IONS Removal of divalent ions from the perfusing solution rapidly produced a considerable decrease in threshold for synaptic acti-

TABLE I

CHANGES IN RESPONSE OF MEDIUM RECEPTORS AS
A FUNCTION OF EXCESS Mg^{+2} OR Ca^{+2}

For A initial Mg^{+2} concentration was 1.5 mM, final concentration is given in column 2; 3 mM Ca^{+2} was used at all times. For B initial Ca^{+2} concentration was 3 mM, final concentration is given in column 2; 1.5 mM Mg^{+2} was used at all times. Maximum correction for deterioration was 20%; 15–30 min equilibration was allowed after each solution change. Column 3 gives the number (n) of impulses per initial response, and column 4 (Δn) the decrease produced by high Mg^{+2}. $\Delta T_t l$ = latency of the first impulse evoked by a threshold stimulus in high Mg^{+2} minus latency in the initial (normal) saline. $\Delta T_m n$ = latency of last impulse evoked by a three to five times threshold stimulus in high Mg^{+2} minus latency of last impulse in normal saline. $\Delta S.E.R.$ = threshold of synaptically evoked response in high Mg^{+2} divided by threshold in normal saline. $\Delta D.E.R.$ = threshold of directly evoked response in high Mg^{+2} divided by threshold in normal saline.

A. Effects of excess Mg^{+2}

Prep.	Mg	n	Δn	$\Delta T_t l$	$\Delta T_m n$	$\Delta S.E.R.$	$\Delta D.E.R.$
	mM			msec	msec		
4-25, p1	3.0	5	−1	0	+1	1.25	1.05
4-23, p1	3.7	6	0	0	0	1.05	1.00
4-18, p1	7.5	4	−2	−0.5	0	1.10	1.00
4-16, p2	15.0	5	−2	−0.5	−4	2.00	1.00
4-16, p1	30.0	4	−2	−1.5	−2.5	2.66	1.60
5-10, p1	30.0	5	−3	−2.0	−4.0	3.10	1.40

B. Effects of excess Ca^{+2}

	Ca						
4-23, p2	7.5	6	0	+2.0	0	0.90	1.30
4-16, p2	15.0	5	−1.5	−0.5	−2.0	2.00	1.50
4-16, p1	30.0	4	−2.0	−2.0	−2.5	2.66	2.50

vation (Table II, Fig. 5). The number of impulses in a response was greatly increased, and with stronger stimuli a very cluttered response was often observed as a consequence of neighboring receptors beginning to respond. Thresholds for direct stimulation of the nerve were decreased somewhat less than those for synaptic activation (Table II, A). Exposure to solutions lacking divalent ions for 45 min or more, or briefer exposure to solutions containing 3 mM of the chelating agent ethylene glycol bis(β-aminoethyl ether)N,N,N',N'-

tetraacetic acid (EGTA), diminished synaptically evoked responses, usually irreversibly.

After exposure to saline lacking divalent ions for 15–30 min, a change to saline containing either 3 mM Ca^{+2} or 1.5 mM Mg^{+2} always tended to depress synaptically evoked responses (Table II, B). Ca^{+2} depressed direct excitation

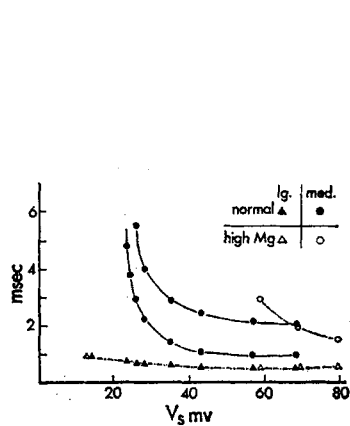FIGURE 4   FIGURE 5

FIGURE 4. Effects of high Mg^{+2} on synaptically evoked response. Abscissa, stimulus strength (V_s); ordinate, latency in milliseconds for impulses in a single fiber. Filled symbols: 3.0 mM Ca^{+2}, 1.5 mM Mg^{+2}; open symbols: 3.0 mM Ca^{+2}, 15 mM Mg^{+2}, 25 min after solution change; round symbols: impulses from a medium receptor (in normal saline two impulses were evoked by stimuli just above threshold); triangular symbols: impulses from a large receptor. High Mg^{+2} had no effect on the responses of the large receptor but raised the threshold and reduced the number of impulses in responses of the medium receptor. A stimulus larger than 80 mv in high Mg^{+2} presumably would have reduced the latency of the medium receptor response (see Fig. 3).

FIGURE 5. Effects of removal of divalent ions on synaptically evoked responses of a medium receptor. As in Fig. 4, but open symbols show impulses after 15 min in saline lacking divalent ions. Open bars indicate that responses in test solution consisted of too many impulses to count single events. Vertical lines on symbols indicate range of latency for each impulse.
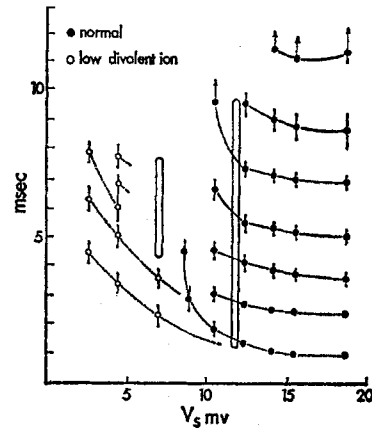
of the nerve, while Mg^{+2} appeared to have no significant effect on this parameter.

Effects of Divalent Ions on Large Receptors

Up to 30 mM Mg^{+2} had little effect on the threshold or latency of synaptically evoked responses observed in three large receptor fibers (e.g., Fig. 4); 15 mM Ca^{+2} produced a slight increase in threshold for two large receptors that could be attributed to decreased excitability of the receptor cells.

Effects of High Mg on Nerve-Electrocyte Synapses

High Mg^{+2} blocks the PSP at teleost nerve-muscle junctions (Hidaka and Toida, 1969). In a nerve-electrocyte preparation from *Gymnotus carapo*, a South American weakly electric gymnotid (Bennett and Grundfest, 1959), we found that 15 mM Mg^{+2} and 3.0 mM Ca^{+2} reduced PSP's by 90% within 1–2 min and eliminated detectable response to nerve stimulation within 5 min. In mormyrid muscle, the same high Mg^{+2} solution blocked visible twitches

TABLE II

CHANGES IN RESPONSES OF MEDIUM RECEPTORS IN GOING FROM NORMAL
SALINE TO SALINE LACKING DIVALENT IONS
AND IN READDING DIVALENT IONS

Normal saline contained 1.5 mM Mg^{+2} and 3.0 mM Ca^{+2}. Tabulation is as in Table I. For B preparations were soaked in divalent ion-free saline for 15–30 min and then placed in saline containing either 1.5 mM Mg^{+2} or 3.0 mM Ca^{+2} as indicated in Column 2.

A. Effects of saline lacking divalent ions

Prep.		n	Δn	$\Delta T_t l$	$\Delta T_m n$	$\Delta S.E.R.$	$\Delta D.E.R.$
				msec	*msec*		
4-23, p1		6	0	+1.0	4	0.44	0.90
4-23, p2		5	+3	+1.5	−2	0.31	0.90
4-25, p1		3	+3	−1.0	+7	0.30	0.93
4-25, p3		3	+2.5	+1.0	+4	0.76	0.80

B. Effects of readding divalent ions

Prep.	Ion	n	Δn	$\Delta T_t l$	$\Delta T_m n$	$\Delta S.E.R.$	$\Delta D.E.R.$
4-23, p2	Mg	5	−2	0	−1	3.00	1.00
4-25, p1	Mg	5	−3	−2.0	−8	6.00	1.00
4-23, p1	Ca	5	−0.5	−3.0	+2	3.00	1.00
4-23, p2	Ca	7	−2	−2.0	−1	1.80	1.40
4-25, p3	Ca	5	−1	−2.0	−1	1.70	1.30

evoked by neural stimulation within minutes. Thus these neuroeffector synapses in electric fishes appear to be blocked by high Mg^{+2} concentrations in the same manner as neuromuscular synapses in other vertebrates.

Pharmacological Data Concerning the Transmitter at Synapses of Medium Receptors

Perfusion of the excised skin preparation with 5 mM *d*-tubocurarine, (three experiments), 2 mM atropine (two experiments), or 2 mM acetylcholine (four experiments) produced only negligible transient changes in synaptically evoked responses. This argues against any conventional cholinergic step in synaptic transmission at electroreceptors. In contrast, synaptically evoked responses were depressed and then eliminated by application of 5 mM DL- or

L-glutamate. 10 mM D-glutatmate did not effect the responses, nor did 10 mM propionate, or 10 mM acetate. Because of the apparently specific effect of L-glutamate, this chemical was further investigated. Three concentrations of L-glutatmate were used: 0.5 mM, 2 mM, and 5 mM.

At a concentration of 0.5 mM, L-glutamate transiently enhanced synaptically
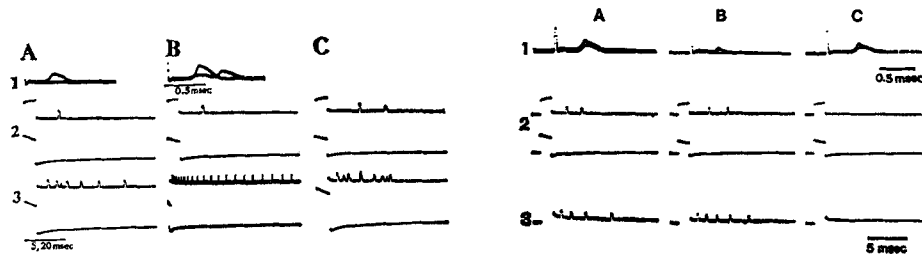

FIGURE 6   FIGURE 7

FIGURE 6. Effect of 0.5 mM L-glutamate on responses of a medium receptor. (A) Normal saline. (B) 30 sec after perfusing with 0.5 mM L-glutamate. (C) 7 min after perfusing with 0.5 mM L-glutamate. *1*, directly evoked response (in B1 there are signs of separate components, perhaps due to nodes of Ranvier). *2* and *3*, upper trace: synaptically evoked responses; lower trace: stimulating voltage. Stimulation was near threshold in *2* and suprathreshold in *3*. Time calibrations, 20 msec for B3, 5 msec for A2, A3, B2, C2, and C3, 0.5 msec for A1 and B1. This concentration of L-glutamate produced a transient hyperexcitability and reduced threshold. The directly evoked response after 7 min perfusion (C1) is not shown; it was normal but a suitable photograph was not taken during the experiment.

FIGURE 7. Effect of 2 mM L-glutamate on responses of a medium receptor. (A) Normal saline. (B) 30 sec after perfusing with L-glutamate, except for direct response (B1) taken after synaptically evoked responses had failed, about 3 min after beginning perfusion. (C) After 7 min perfusion. *1*, directly evoked responses, note second small fiber excited by slight increase in stimulus in (A) and (C); only the small fiber was excited in (B). *2*, upper trace: synaptically evoked responses; lower trace: stimulating voltage near threshold (7 mv for A2, lower for B2). *3*, synaptically evoked response at about five times threshold. The second fiber in the direct response did not respond synaptically nor was it affected by glutamate, perhaps because its receptor was covered by connective tissue or was not functionally connected to its synaptic region. This concentration of L-glutamate first reduced the threshold for synaptic excitation, then blocked both synaptic and direct excitation. During continued perfusion the directly evoked response returned, but synaptically evoked responses did not recover.

evoked responses. Threshold decreased significantly and suprathreshold stimulation produced up to 20 impulses with a $T_m n$ of at least 60 msec (contrasted to the usual 8 msec) (Fig. 6). This increased excitability was transient and after 3–10 min of exposure, the maximum number of impulses in a response returned to or below the usual, although threshold remained lowered for up to 30 min (the longest exposure).

A concentration of 2 mM L-glutamate initially reduced the threshold for

synaptic excitation (Fig. 7), and then rapidly eliminated synaptically evoked responses, beginning with the last impulses, as threshold increased again. In one case out of four, a transiently increased number of impulses occurred similar to that observed routinely with 0.5 mM L-glutamate. Synaptically evoked responses were generally impossible to elicit within minutes of the application of 2 mM L-glutamate. Directly evoked responses had increased thresholds and often failed completely. Over a period of 10 min, direct excitability of the afferent fiber returned to normal, but synaptically evoked responses were not observed so long as L-glutamate was present (Fig. 7 C). A similar although more rapid sequence of events was observed with 5 mM L-glutamate (three experiments), and a similar although slower sequence with 5 mM L-aspartate (one experiment). In two cases tonic impulses at a frequency of about 60/sec were observed during application of L-glutamate, one each at 2 and 5 mM concentrations. In both cases the frequency of this discharge increased to about 200/sec, then ceased abruptly at about the same time as that at which direct excitation failed. The tonic discharge was not affected by DC components of synaptic stimuli, indicating that small receptors were not involved. During washing with normal saline following glutamate application, synaptically evoked responses returned gradually, generally without any obvious transient hyperexcitability. However, in the two cases in which transient tonic firing was produced by glutamate, tonic firing began again during washing as synaptically evoked responses returned to normal.

DISCUSSION

Modes of Transmission at Receptor Synapses

The observations reported here strengthen the conclusion that transmission in medium receptors is chemically mediated while transmission in large receptors is electrically mediated. The transmission delay in medium receptors is about 0.7 msec, compared with about 0.25 msec in large receptors. These delays, which must be slightly larger than the actual synaptic delays, are consistent with chemically and electrically mediated transmission, respectively (Bennett, Pappas and Nakajima, 1967; Katz, 1969). The marked effects of excess divalent ions on medium receptors and the minimal effects on large receptors are further indications of the two modes of transmission.

At many sites where electrotonic transmission has been demonstrated physiologically, electron microscopy has revealed close membrane appositions (see Bennett, Pappas, Giménez, and Nakajima, 1967; Brightman and Reese, 1969; Pappas et al., 1971). To date such close appositions have not been observed at large receptors. The synaptic relations are similar at large and medium receptors (Derbin and Szabo, 1968; Szabo and Wersäll, 1970; unpublished studies in this department), and are consistent with chemically mediated transmission.

Pre- and postsynaptic membranes are separated by a distinct gap. At apparent sites of transmission there are presynaptic dense bodies and large numbers of vesicles as are found in many other receptor synapses. (cf. Bennett, 1970; Bunt, 1971). Close appositions have been observed at one class of 8th nerve receptor synapses, the chalice synapses of the vestibular sensory epithelium (Spoendlin, 1966). At these synapses the arrangement of receptor cell and enfolding nerve terminal further suggests electrically mediated transmission, but physiological data are lacking. The absence of morphological evidence for electrotonic transmission at large receptors is not conclusive. The morphological characteristics associated with chemically mediated transmission do not constitute proof that this process occurs, since in several other instances electrotonic synapses have presynaptic vesicles and related structures without there being a significant chemically mediated component of transmission (Bennett, Nakajima, and Pappas, 1967; Bennett, Pappas, Giménez and Nakajima, 1967).

Medium Receptors and Divalent Ions

Our data suggest that Mg^{+2} decreases the amount of transmitter released from medium receptor cells by a given stimulus. The threshold of synaptically evoked impulses is increased and the maximum number of impulses in a response to a strong stimulus is reduced to one (Figs. 3 B and 4). As observed in other preparations (e.g., Ottoson, 1965), Mg^{+2} also depressed excitability of the afferent nerve. This factor must contribute to the observed decrease in excitability but seems unlikely to account for the entire effect (Table I, A). It should be admitted that the relation between direct and synaptic threshold need not be linear. In order to prove a decrease in transmitter release under our experimental conditions, the excitability of the nerve must stay constant or increase and there must be no change in postsynaptic sensitivity. (We have no reason to anticipate a marked change in postsynaptic sensitivity; see Takeuchi, 1963.)

High Mg^{+2} does not significantly change the minimum latency of synaptically evoked responses of medium receptors. This observation suggests that the synaptic delay and the time for the PSP in the nerve terminal to reach spike threshold are little changed in high Mg^{+2}. Since Mg^{+2} decreases the number of impulses in a response train without producing detectable increases in the nerve refractory period, it is likely that Mg^{+2} reduces the duration of the PSP. Decrease in the electrical excitability of the receptor cells could contribute to the reduced release of transmitter in high Mg^{+2}. Since stimuli were applied that were much larger than externally recorded responses, this factor seems unlikely to be sufficient and there is probably a direct action of high Mg^{+2} on the release process itself.

Medium receptors soaked for a long time in solutions containing 30 mM Mg^{+2} and 0 Ca^{+2} continue to respond with one impulse, although threshold is greatly increased. Since the rapid effects of L-glutamate observed make it un-

likely that Mg^{+2} does not reach a high concentration near the receptor after 2 hr of exposure, it appears that these receptor synapses are not "shut off" completely by high concentrations of Mg^{+2}.

High Ca^{+2} has effects on medium receptors that are attributable to post-synaptic effects. Unlike Mg^{+2}, excess Ca^{+2} significantly increases the threshold for direct excitation, presumably because of a "stabilizing" action on the excitable membrane comparable with that observed in frog node of Ranvier (Hille, 1968). Nonetheless, it is possible to say that excess Ca^{+2} produces less depression of synaptically evoked responses than does excess Mg^{+2}.

Removal of Ca^{+2} and Mg^{+2} decreases thresholds for both synaptically and directly evoked responses. We interpret this to mean that at least the nerve is made more excitable and perhaps also the release of transmitter is facilitated by removal of divalent ions. An additional possibility is increased excitability of the receptor cells. These effects would tend to obscure a dependence of transmitter release on divalent ions.

Our results do not demonstrate a divalent ion requirement for transmitter release at receptor synapses. The lack of depression by low Ca^{+2} and low divalent ion solutions could be ascribed to retention of Ca^{+2} in the neighborhood of the receptors. The drug effects indicate that there is not an impermeable barrier around the cells, but a low concentration of Ca^{+2} might remain near the cells. The barrier argument is less applicable to the limited effects of high Mg^{+2} since the Mg^{+2} concentration near the cells should rise to near the full solution value. Thus, we are justified in concluding that transmitter secretion does not involve a Ca^{+2}-requiring step that can be blocked by Mg^{+2}. If there is a Ca^{+2}-requiring step, it is likely that Mg^{+2} can substitute for Ca^{+2} to some extent. Similar conclusions can be drawn for the receptor synapses in the carotid sinus of the cat (Eyzaguirre and Zapata, 1968 *a*, *b*). A depression of response following Ca^{+2} removal occurs only after a 1 hr period of up to 10-fold increase in responses, and excess Mg^{+2} does not eliminate responses in afferent nerves.

It is perhaps relevant that the electrical activity of receptor cells of electroreceptors is not blocked by tetrodotoxin, although impulses in the nerve fibers are (Zipser, 1971). The receptor cell activity may result from inward flow of divalent ions. Responses dependent on divalent ions and resistant to tetrodotoxin have been demonstrated in presynaptic nerve terminals, and are implicated in the release of transmitter (Katz and Miledi, 1969 *a*, *b*). The receptor cells can be considered equivalent to presynaptic terminals, but as yet no data have been obtained as to the divalent ion dependence of their electrical responses.

Medium Receptors and Glutamate

We conclude that acetylcholine is not the transmitter at the electroreceptor-sensory nerve synapse. Curarization of intact preparations has no effect on

synaptically evoked responses (Bennett, 1965, 1967). High concentrations of acetylcholine, curare, or atropine do not effect synaptically evoked responses in the excised preparation. In the mammalian carotid body (Liljestrand and Zotterman, 1954; Eyzaguirre and Koyano, 1965 *a*, *b*, *c*; Eyzaguirre and Zapata, 1968 *a*, *b*) and perhaps in vertebrate taste buds (Landgren et al., 1954; Duncan, 1964) there is evidence that acetylcholine is involved in synaptic transmission. Acetylcholine alters cochlear microphonics and auditory nerve responses in vertebrates, but this effect may be due to an influence on efferent rather than afferent synapses (Tanaka and Katsuki, 1966). Efferent inhibition of *Xenopus* lateral line receptors appears to be cholinergically mediated (Russel, 1968).

At synapses of medium receptors L-glutamate and L-aspartate rather than cholinergic drugs act like transmitters. L-glutamate at 0.5 mM lowers the threshold and enhances the duration of synaptically evoked responses. These effects can be attributed to depolarization of the postsynaptic nerve terminal. Maintained impulse discharges are sometimes observed in the absence of electrical stimuli. These might be due to activation of small, "tonic" receptors, but the lack of effect of DC stimuli argues against this hypothesis. We think that the maintained discharges occur in medium receptor fibers when the L-glutamate concentration becomes sufficient to depolarize the nerve terminal to threshold. Cessation of these responses is ascribable to excessive depolarization and cathodal depression of the nerve. The elimination of synaptically evoked responses by 2-5 mM L-glutamate is also ascribable to excessive depolarization and later postsynaptic desensitization. During the desensitization phase, synaptically evoked responses remain blocked, but directly evoked responses recover their normal threshold. The sequence of excitation and desensitization has been demonstrated for L-glutamate at invertebrate motor synapses (Beranek and Miller, 1968; Takeuchi and Takeuchi, 1964).

No attempt was made to evaluate the minimum effective concentration of L-glutamate, although it is probably much less than the lowest concentration we used, to judge from the very rapid onset of effects of the applied drug. Our minimum concentration (0.5 mM) is very high when placed on the scale of effectiveness of suspected transmitter chemicals (see Usherwood, 1967: Usherwood and Machili, 1968). It is the successive occurrence of excitation, depression and recovery of nerve excitability, rather than the concentration used to produce the effects, that we find most suggestive of physiological action.

Note Added in Proof   We have recently studied synapses in ampullae of Lorenzini, which are tonic electroreceptors of the lateralis system in elasmobranchs. Unlike that in mormyrid receptors, transmission is blocked in low Ca^{+2} or high Mg^{+2} salines. Similar to that in mormyrid receptors, transmission appears to be glutaminergic (Steinbach, A. B., and M. V. L. Bennett. 1971. Presynaptic actions of glutamate at a sensory synapse. *Biol. Bull.* In press.).

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