Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5315–5319, July 1988
Neurobiology

L-Glutamate-induced depolarization in solitary photoreceptors: A process that may contribute to the interaction between photoreceptors *in situ*

(glutamate channel/glutamate transport/synapse/retina/vision)

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Communicated by Tsuneo Tomita, March 23, 1988 (received for review January 26, 1988)

ABSTRACT **L-Glutamate is a leading candidate for the vertebrate photoreceptor transmitter. In addition to the signal transmission to second-order neurons, photoreceptors communicate with each other not only electrically but also chemically. In the present study, by using solitary turtle photoreceptors, we examined the possibility that L-glutamate mediates interreceptor communication. L-Glutamate evoked an inward current in all subtypes of photoreceptors voltage-clamped to the resting potential. The highest glutamate sensitivity was located at the axon terminal. Both stereoisomers of aspartate were effective, whereas kainate, quisqualate, *N*-methyl-D-aspartate, and D-glutamate were ineffective. The presence of Na^+ was essential to response generation; even Li^+ could not substitute for Na^+. The relation between L-glutamate-induced current and the membrane voltage was strongly inward-rectifying. These results favor the hypothesis that the L-glutamate-induced response is generated by an electrogenic uptake carrier. However, L-glutamate-induced current was always accompanied by an increase in current fluctuations, a phenomenon commonly observed in ion channels but not expected for an uptake carrier. Although the underlying mechanism needs further elucidation, it seems likely that L-glutamate is a transmitter for communication between photoreceptors.**

L-Glutamate and L-aspartate are the leading candidates for the chemical transmitter of vertebrate retinal photoreceptors. Photoreceptors accumulate exogenously applied L-glutamate or L-aspartate by a selective high-affinity uptake system (1, 2). These compounds are released when the cells are depolarized (3, 4), e.g., by exposure to a high concentration of extracellular K^+. L-Glutamate or L-aspartate evokes actions in the second-order neurons analogous to those of the endogenous transmitter: horizontal cells and off-type bipolar cells are depolarized and on-type bipolar cells are hyperpolarized (5–9).

It had been hypothesized that individual photoreceptors function independently, but accumulating evidence indicates that they interact with each other. Electrical (10, 11) and chemical (12) interactions have been reported. In the present study we examined the possibility that L-glutamate is a chemical transmitter in the interphotoreceptor interaction.

MATERIALS AND METHODS

Preparations. Solitary photoreceptors were obtained from the freshwater turtle *Geoclemys reevesii* by dissociating the retina with papain (Worthington), as described in detail elsewhere (13, 14). Isolated cells were plated in a culture dish, the bottom of which was replaced with a Con A-coated cover glass. Experiments were conducted on freshly isolated cells (within 10 hr after dissociation).

The turtle retina has photoreceptors of seven morphological subtypes (four types of single cone, one type of double cone, and one type of rod), each of which has been correlated with specific spectral sensitivity (15, 16). The subtype of solitary photoreceptors was identified by their morphology, which was retained after dissociation.

Recording Procedures. Solitary photoreceptors were voltage-clamped by the whole-cell variant of the patch–clamp technique (17). The recording pipette (tip diameter ≈ 1 μm, pulled from Pyrex tubing) was filled with a solution containing 124 mM KCl, 14 mM NaCl, 5 mM EGTA, and 10 mM Hepes (pH 7.2) (pipette resistance measured in the superfusate was ≈ 10 MΩ). Cells were continuously superfused with a solution of 116 mM NaCl, 10 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 16 mM glucose, and 2 mM Hepes (pH 7.4). The temperature of the superfusate was maintained at 15°C.

The electrode was connected to a patch-clamp amplifier [List Electronics (Darmstadt, F.R.G.) L/M EPC 7 or Nihon Kohden (Tokyo) CEZ-2100]. The data were displayed on an oscilloscope equipped with a pen recorder [Graphtec (Tokyo) model no. WR3101] and sampled by an A/D converter connected to a computer [Digital Equipment (Maynard, MA) VAX 11/750] after appropriate filtering [8-pole low-pass Bessel filter, NF Circuit Design Block (Yokohama, Japan), model FV-624A].

Test substances were applied either by iontophoresis from a fine-tip glass pipette, by pressure ejection from a pipette with a 20-μm tip or by addition to the bathing medium (for details, see refs. 18 and 19). L- and D-Isomers of glutamate and aspartate were purchased from the Peptide Institute (Osaka, Japan); kynurenic acid was from Tokyo Kasei Kogyo (Tokyo); kainic acid, quisqualic acid, and *p*-chloromercuriphenylsulfonic acid were from Sigma; and *N*-methyl-D-aspartic acid was from Tocris Chemicals (Essex, England).

Experiments were conducted on a total of 197 photoreceptors. In each experiment, similar results were obtained consistently from at least five cells.

RESULTS

L-Glutamate-Induced Responses. Solitary photoreceptors responded to L-glutamate with membrane depolarization. When the cells were voltage-clamped to the resting membrane potential (*ca.* -40 mV), an inward current was evoked by L-glutamate. Fig. 1*A* illustrates the responses obtained from a single cone with a red oil droplet (a red-sensitive cone) voltage-clamped to -63 mV, and Fig. 1*B* indicates the response amplitude (in pA) recorded in the other subtypes of cells.

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Abbreviations: I_{Glu}, L-glutamate-induced current; $[Na^+]_o$, external Na^+ concentration.

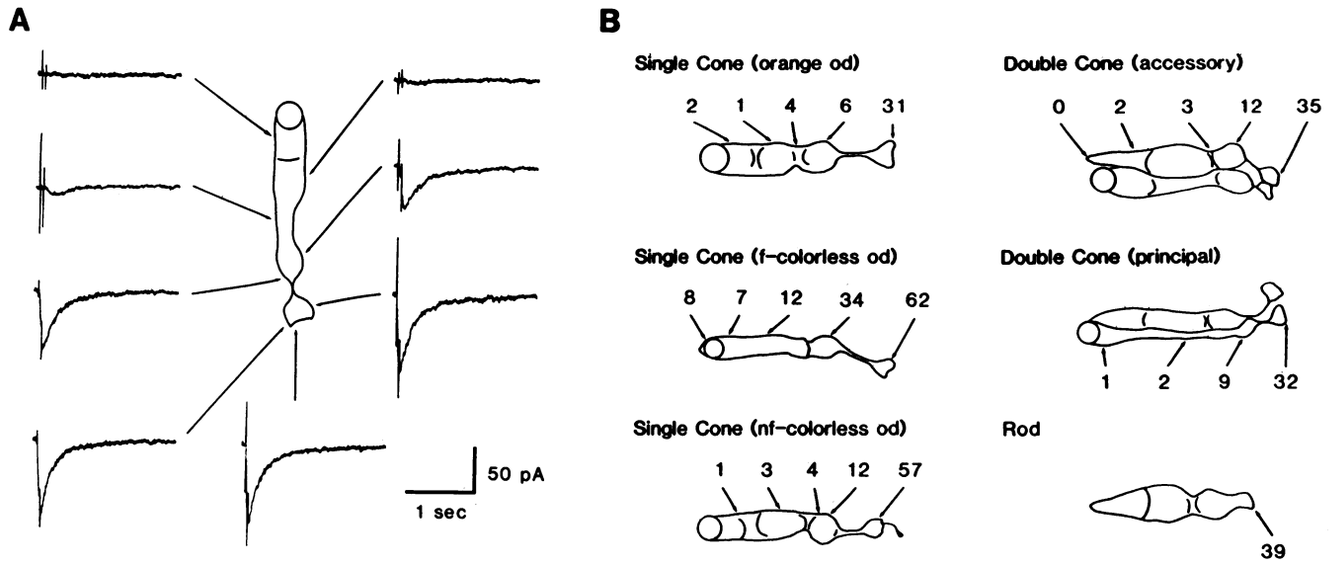

FIG. 1. Glutamate-evoked responses and distribution of glutamate sensitivity. (*A*) L-Glutamate-induced current (I_{Glu}) in a solitary photoreceptor containing a red oil droplet (a red-sensitive cone). The cell was voltage-clamped to −63 mV by a patch pipette in the whole-cell clamp configuration. The tip of a micropipette filled with 0.5 M sodium L-glutamate was positioned at various parts of the cell shown by arrows, and L-glutamate was applied locally by iontophoresis (duration, 50 msec; intensity, 40 nA). The transient biphasic deflection of the current trace is an artifact due to the iontophoretic current pulse. The resting membrane potential was −42 mV. (*B*) I_{Glu} in the other subtypes of photoreceptors. Description in parentheses denotes the color and presence of autofluorescence (f, with autofluorescence; nf, without autofluorescence) of the oil droplet (od) contained in single cones, or the member of double cones. Peak amplitudes of the responses evoked by iontophoretically applied L-glutamate are given in pA. The holding potential was at −63 mV.

Localization of L-Glutamate Sensitivity. To examine the distribution of L-glutamate sensitivity, the tip of the L-glutamate-containing pipette was positioned at various parts of the cell and an identical amount of L-glutamate was locally applied by iontophoresis. In all subtypes of cells, the response was maximal when L-glutamate was applied to the axon terminal; the response amplitude became smaller when L-glutamate was ejected to distal parts of the cell. Thus, the highest L-glutamate sensitivity is located at the axon terminal. This observation was supported by the observation that the L-glutamate sensitivity was very low in photoreceptors that had lost their axon terminal during dissociation.

Comparison of L-Glutamate Sensitivity Among Subtypes of Photoreceptors. All subtypes of photoreceptors responded to L-glutamate approximately equally. The maximal amplitude varied from cell to cell, and there was no statistical difference in response amplitude between subtypes. For example, the mean amplitude of response evoked by a saturating dose of L-glutamate was 36 ± 27 pA (mean ± SD, n = 20) in single cones with a red oil droplet (holding potential, −63 mV) and 31 ± 12 pA (n = 9) in the principal member of double cones (the same holding potential). Since all subtypes of photoreceptors responded to L-glutamate similarly, the subtype will not be specified in the following sections.

Dose–Response Relationship. The amplitude of I_{Glu} was dose-dependent. The data shown in Fig. 2 were obtained in an experiment in which various concentrations of L-glutamate were added sequentially to the bathing medium. (The lack of desensitization to L-glutamate was confirmed in several experiments in which a saturating dose of L-glutamate was ejected iontophoretically for >5 sec.) The minimal detectable response was evoked by 5 μM L-glutamate. The response amplitude became larger with increasingly larger doses and finally leveled off at ≈1 mM. The concentration at which a half-maximal response was evoked was 40 μM. The data points approximated a Michaelis–Menten relation—i.e., the Hill coefficient was ≈1. These observations indicate that at least one glutamate molecule occupies each binding site.

Responses to Glutamate Analogues. In the central nervous system, glutamate receptors have been classified into pharmacologically distinct subtypes: kainate type, quisqualate type, and *N*-methyl-D-aspartate type (20). To identify the types of glutamate receptor, 100 μM kainate, 100 μM *N*-methyl-D-aspartate, or 100 μM quisqualate was applied to photoreceptors. However, none evoked a detectable response (Fig. 3 *A* and *B*; data for quisqualate not illustrated).

Effect of stereoisomers of glutamate and aspartate was also investigated. As shown in Fig. 3*C*, L-isomers of both amino acids (at 100 μM) had roughly the same potency; the amplitude evoked by L-aspartate was ≈50% of I_{Glu}. However, effects of D-isomers were different. D-Aspartate was nearly as effective as L-aspartate (Fig. 3*D*), but D-glutamate (100 μM) evoked no detectable response (data not illustrated).

Kynurenic acid (1 mM), a blocker of glutamate receptors (21), did not suppress the I_{Glu} of photoreceptors (data not illustrated). On the other hand, I_{Glu} was blocked by 200 μM *p*-chloromercuriphenylsulfonic acid, a glutamate-uptake in-

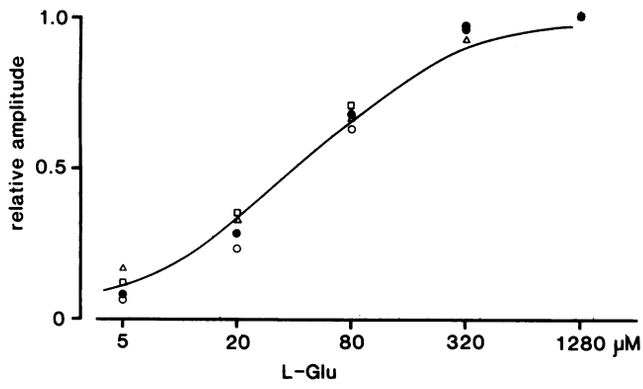

FIG. 2. Relation of normalized response amplitude of L-glutamate-evoked current (I) responses (I/I_{max}) to the dose of L-glutamate applied to the bathing medium. The curve represents the Michaelis–Menten relation $\{I/I_{max}$ = [glutamate]/([glutamate] + $K_m)\}$. K_m, the dose that evoked the half-maximal response, was 40 μM. Data were obtained from four cells.

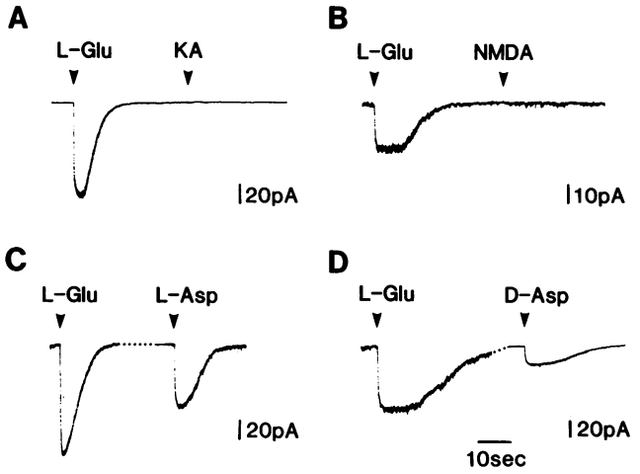

FIG. 3. Effects of L-glutamate agonists. L-glutamate and one of the agonists were applied to each cell voltage-clamped to −63 mV (*A–C*) or to −43 mV (*D*). L-Glutamate at 100 μM (*A–C*) or at 1 mM (*D*) or each agonist at 100 μM (*A–C*) or at 1 mM (*D*) was dissolved in a solution identical to the superfusate and was ejected from a large-tip pipette by pressure (0.3 kg/cm^2). KA, kainate; NMDA, *N*-methyl-D-aspartate.

hibitor (22) (data not illustrated). The blockade was partial (≈30%), but this amount of suppression was reported to be maximal in axolotl Müller cells (23) and in rat brain slices (22).

Ionic Selectivity. The presence of Na^+ was essential for the generation of I_{Glu} in photoreceptors. When the external concentration of Na^+ ($[Na^+]_o$) was reduced by replacing Na^+ with an equimolar concentration of choline, the amplitude of I_{Glu} decreased (Fig. 4) in a concentration-dependent manner. The curve relating the response amplitude to $[Na^+]_o$ had an S shape, similar to that expected from the generalized Michaelis–Menten relationship (the dose that produced a half-maximal response was ≈50 mM and the Hill coefficient was close to 3). It was surprising that Li^+ was unable to be substituted for Na^+. When Na^+ in the superfusate was totally replaced with Li^+, the L-glutamate-evoked response disappeared. A similar relationship between $[Na^+]_o$ and I_{Glu} was obtained when Na^+ was replaced with Li^+ or with choline. I_{Glu} was not affected by either external K^+ or external Cl^-; I_{Glu} did not change when the external concentration of K^+ was increased from 10 mM to 40 mM (without changing $[Na^+]_o$) or when Cl^- in the superfusate was totally replaced with methanesulfonate.

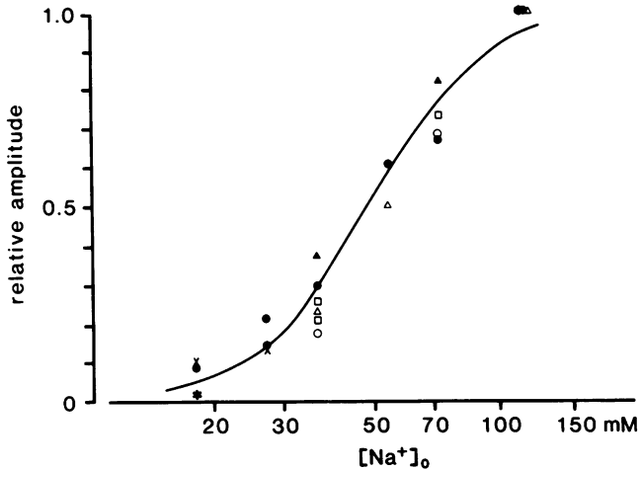

FIG. 4. Dependence of L-glutamate-evoked response on Na^+. Relative amplitude of the currents evoked by iontophoretically applied L-glutamate was plotted against the external concentration of Na^+. Na^+ was replaced with choline. The curve was drawn according to the relation $I/I_{max} = [Na^+]^3/([Na^+]^3 + K_m)$, with the K_m of 50 mM. Data were obtained from seven cells and presented with different symbols for each cell.

Voltage Dependence. The relationship between the I_{Glu} and the membrane potential was strongly inward-rectifying. When the membrane potential of a solitary photoreceptor was clamped to more negative values (e.g., −63 mV) than the resting potential, the amplitude of the inward I_{Glu} increased (Fig. 5*A*). As the cell was clamped at more depolarized potential levels, the amplitude of I_{Glu} became smaller, and at ≈0 mV the response was not detected. At positive potential values a small outward I_{Glu} (2–3 pA) was detected in about half of the preparations, but it was difficult to determine the reversal potential of I_{Glu} accurately due to the low signal-to-noise ratio, even after voltage-dependent K currents (24) were blocked (e.g., by CsCl in the patch pipette).

The I_{Glu}–membrane potential relationship was strongly affected by $[Na^+]_o$ (Fig. 5*B*). When $[Na^+]_o$ was reduced, the response amplitude decreased, and reversal potentials, estimated from I_{Glu}–membrane potential curves by eye, shifted to the hyperpolarizing direction by ≈20 mV when $[Na^+]_o$ was changed from 117 mM to 53 mM and by ≈30 mV when $[Na^+]_o$ was reduced to 27 mM.

Analysis of Current Fluctuations Induced by L-Glutamate. All responses induced either by L-glutamate (Fig. 6*A*) or by its related compounds (see Fig. 3) were accompanied by an increase in current fluctuations. It is generally believed that current fluctuation is a phenomenon associated with the opening and closing of channels. Although the mechanism underlying the generation of I_{Glu} in photoreceptors is subject to discussion (see *Discussion*), here we assumed tentatively that the current was generated by channel opening and presumed single-channel events were estimated by a noise-analysis technique.

Under such an assumption the single-channel current (i) can be calculated as a ratio of the variance (s^2) of I_{Glu} to the mean amplitude (A) of I_{Glu}. Suppose that photoreceptors have N independent and identical channels, each of which opens with a probability (p) and passes a current (i). According to the definition of variance, s^2 has the following relation with N, i, and p.

$$s^2 = Ni^2p - Ni^2p^2 \quad \text{and} \quad A = Nip.$$

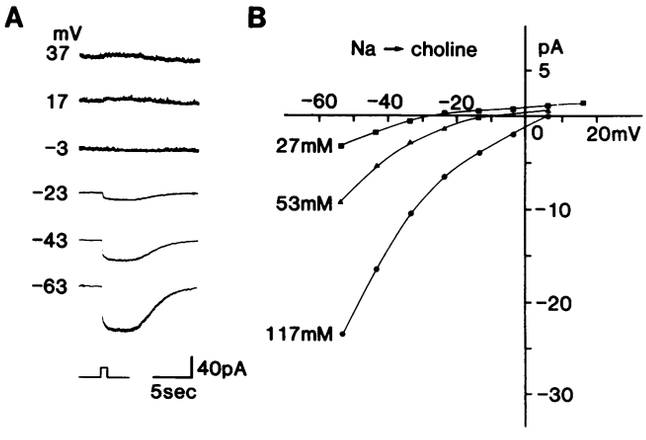

FIG. 5. Voltage dependence of glutamate-evoked responses. (*A*) Effect of membrane potential on I_{Glu}. The cone was clamped to various membrane potentials as indicated, and 100 μM L-glutamate was pressure-applied. Depolarization of the cell more positive than −3 mV increased the background noise, probably due to the activation of Ca-induced K current. (*B*) Relation between I_{Glu} and the membrane potential at three values of $[Na^+]_o$. All data were obtained from a single cell (different from that in *A*). L-glutamate was applied by iontophoresis (35 nA and 1 sec). Na^+ was replaced with choline.

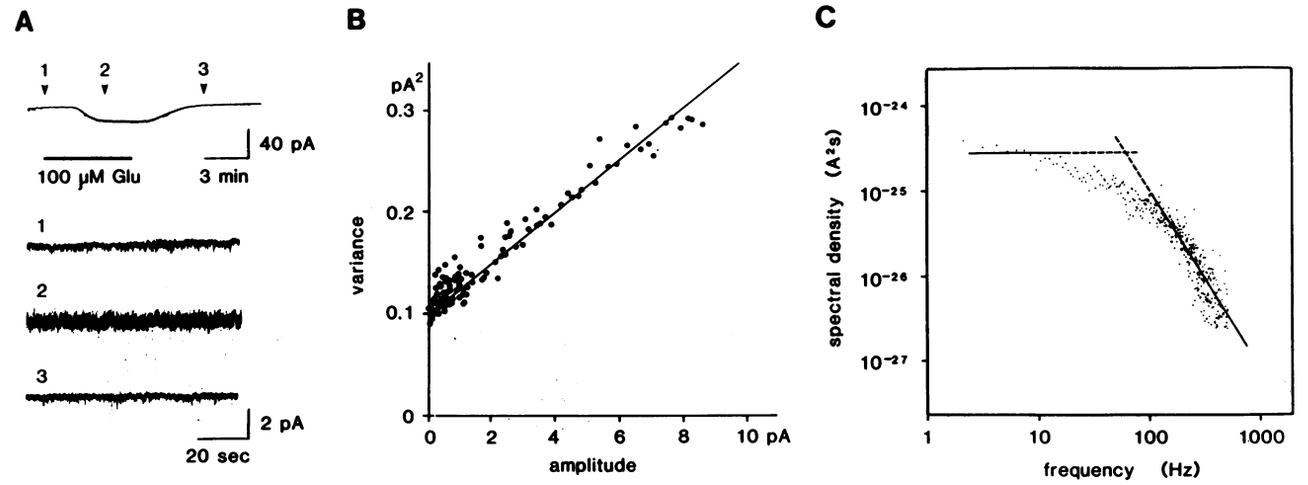

FIG. 6. Current fluctuations evoked by L-glutamate. (*A*) Current traces before (arrowhead 1), during (arrowhead 2), and after (arrowhead 3) the application of 100 μM L-glutamate to the bath recorded from a cell voltage-clamped to −53 mV. Current traces with a higher gain and faster time base are shown below. Traces were reproduced on a chart from a data recorder. (*B*) Variance of fluctuations plotted against mean amplitude of L-glutamate-evoked current to estimate the single-channel current. Current records were low-pass-filtered (cutoff frequency, 1 kHz) and sampled at 3 kHz. The slope of the straight line, fitted by the least-squares method, represents a single-channel current of 0.03 pA. (*C*) Power spectrum of the L-glutamate-induced current fluctuations. Two straight lines, one flat and another with a slope of 40 dB per decade, were fitted "by eye." The corner frequency of ≈80 Hz was obtained from the intersection of two lines.

Therefore,

$$i = s^2/A(1 - p).$$

When p is negligibly small, s^2/A gives the single-channel current i. The single-channel current thus obtained was ≈0.03 pA (Fig. 6*B*). By assuming that the driving force was 60 mV, the single-channel conductance of 0.5 pS was obtained.

The power spectrum of the glutamate-induced current fluctuations could be fitted by a single Lorentzian component with the corner frequency of 80 Hz within the resolution of our recording system (Fig. 6*C*).

DISCUSSION

The present study demonstrated that L-glutamate induced an inward current in all subtypes of turtle photoreceptors voltage-clamped near the resting membrane potential. The L-glutamate sensitivity was located at the axon terminal, suggesting that the L-glutamate-evoked responses are somehow related to the synaptic transmission in the outer plexiform layer. Interaction between cones and rods (25, 26) and between cones of different types (12) has been suggested in various animals; some of those interactions are mediated electrically (10, 11) and some are mediated chemically (12). It is highly likely that the L-glutamate-evoked depolarization observed in the present study contributes to the heterologous interaction of photoreceptors.

It might be argued that depolarization of photoreceptors by L-glutamate accelerates glutamate release, which in turn further depolarizes photoreceptors. However, such self-regenerative depolarization does not seem to occur, since depolarization would be counteracted by activation of outward K currents (24) and the membrane potential of the photoreceptor would settle at a new level by the combination of these currents.

The L-glutamate-evoked response in photoreceptors showed dual characteristics; one is common to uptake carriers and the other is common to ion channels. Therefore, we will discuss I_{Glu} in photoreceptors from these two aspects: (*i*) on the assumption that L-glutamate was generated by glutamate-uptake carriers and (*ii*) on the assumption that the current passed through the channels.

In the vertebrate retina, axolotl Müller cells have been shown to have a typical carrier system of glutamate transport (23). The properties of I_{Glu} in photoreceptors that resemble those of axolotl Müller cells are (*i*) agonist selectivity (effective agonists were L-glutamate, L-aspartate, and D-aspartate; ineffective agonists were D-glutamate, kainate, quisqualate, and *N*-methyl-D-aspartate), (*ii*) suppression by glutamate-uptake blockers but not by glutamate antagonists, and (*iii*) the requirement for Na^+. These observations tempt us to think that I_{Glu} in photoreceptors was generated by a glutamate-uptake carrier. From the cooperativity values obtained from the dose–response curves of L-glutamate and of $[Na^+]_o$ (*i* and *iii*, respectively), one glutamate molecule and three Na^+ are thought to couple with a "carrier" molecule and to be cotransported. Thus, there is a good reason to believe that the carrier is electrogenic. The reversal potential of the carrier can be calculated from the energetics (e.g., ref. 27) as follows.

$$E_R = (3E_{Na} - E_{Glu})/2,$$

where E_R is the reversal potential of the carrier-mediated current and E_{Na} and E_{Glu} are equilibrium potentials for Na^+ and glutamate, respectively. Under the present experimental conditions ($[Na^+]_o$ = 117 mM and intracellular $[Na^+]$ = 14 mM), E_R was estimated to be +22 mV (by assuming that the intracellular concentraton of glutamate was 10 mM) or +50 mV (1 mM intracellular glutamate). The observed reversal potentials are not identical to, but also are not far from, the above estimations. If the mechanism underlying the generation of I_{Glu} is the carrier, it would work quite efficiently to terminate the L-glutamate-induced responses in the second-order neurons; photoreceptors respond to light with membrane hyperpolarization, which reduces release of L-glutamate from photoreceptors and facilitates uptake of L-glutamate from the synaptic cleft.

The second aspect of the glutamate-evoked response in photoreceptors is that it has a property common to ion channels. In the vertebrate retina, the glutamate-induced current in goldfish horizontal cells is generated by ionic movement through channels (18, 28, 29). Glutamate-operated channels of horizontal cells are mainly of the kainate-type (sensitive to L-glutamate, kainate, and quisqualate, but are not sensitive to L-aspartate and D-aspartate) (18, 30), are blocked by glutamate antagonists (18, 31, 32), and are

permeable to cations with low selectivity (19). Furthermore, I_{Glu} in horizontal cells was accompanied by current fluctuations (28, 29). It is our general understanding that the current fluctuation is not seen in carrier-mediated responses (e.g., ref. 23) because each carrier molecule functions independently and the charges translocated by a single execution of a carrier molecule are too small (3×10^{-7} pC in the case of Na–glutamate cotransport system with 3:1 stoichiometry). Our observation of current fluctuations favors the channel mechanism, but as pointed out in the preceding section, I_{Glu} in photoreceptors has several properties that are unusual for an ion-channel current (e.g., a Na^+ requirement, a strong nonlinearity, etc.).

The final possibility, which is our tentative interpretation, is that photoreceptors have both carriers and channels. If a large fraction of I_{Glu} is evoked by the glutamate-uptake carrier, the major characteristics of the response in photoreceptors should resemble the properties of the response in Müller cells. Current fluctuations could be ascribed to the remaining minor fraction of current flowing through channels. Further studies—for example, demonstration of translocation of L-glutamate into photoreceptors—are required to elucidate the mechanism underlying the glutamate-evoked response in photoreceptors.

Note Added in Proof. A similar observation was made independently (33) on tiger salamander cones, which appeared after this paper was accepted for publication.

We thank Michi Hosono for technical assistance in preparing solitary photoreceptors and thank Masahiro Mori for electronics. This research was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture (61440026, 61304032, and 62121008).

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