J. Physiol. (1985), **358**, *pp.* 153–167
With 9 text-figures
Printed in Great Britain

PERMEABILITY CHANGES INDUCED BY L-GLUTAMATE IN SOLITARY RETINAL HORIZONTAL CELLS ISOLATED FROM *CARASSIUS AURATUS*

BY MASAO TACHIBANA

From the Department of Information Physiology, National Institute for Physiological Sciences, Okazaki, 444 Japan

(*Received 27 April 1984*)

SUMMARY

1. Solitary horizontal cells isolated from goldfish retinae are depolarized by L-glutamate (Glu) (Ishida, Kaneko & Tachibana, 1984), a possible candidate for the transmitter of photoreceptors. The underlying mechanisms were analysed under voltage-clamp conditions using 'giga-seal' suction pipettes in the whole-cell recording configuration.

2. Glu induced an inward current at the resting membrane potential (*ca.* −57 mV). Membrane depolarization decreased the amplitude of Glu-induced current and reversed its polarity to outward beyond approximately −3 mV.

3. Membrane hyperpolarization below the resting potential *decreased* the amplitude of the Glu-induced inward current. When a K current through the anomalous rectifier, which is activated by membrane hyperpolarization (Tachibana, 1983), was blocked by Cs ions, this phenomenon disappeared and the Glu-induced current increased in amplitude with hyperpolarization. Mg ions had no effect on the reduction of the Glu-induced current at hyperpolarized potentials.

4. It was strongly suggested that Glu produced two types of conductance change; a conductance increase due to an activation of Glu channels and a conductance decrease due to a blockage of the K current through the anomalous rectifier. The latter effect is analysed in detail in the following paper (Kaneko & Tachibana, 1985*b*).

5. The Glu-activated channel was permeable to cations (Na, K, Ca, Mg, Tris and choline ions) with low selectivity, but not to anions. The least effective dose of Glu was less than 10 μM. The relation between the Glu-induced current and the membrane potential curved upwards near the reversal potential, and this relation was not affected by Mg ions.

INTRODUCTION

There are several lines of evidence which support the idea that acidic amino acids, L-glutamate and/or L-aspartate, may serve as the transmitter released by vertebrate photoreceptors. These amino acids are accumulated in photoreceptors by high-affinity uptake mechanisms (Marc & Lam, 1981; Brandon & Lam, 1983), and are released from photoreceptors when the extracellular K concentration is raised (Miller &

Schwartz, 1983). The post-synaptic effects of these agents resemble those of the endogenous transmitter of photoreceptors; horizontal and off-bipolar cells are depolarized by application of these agents, while on-bipolar cells are hyperpolarized (Murakami, Ohtsu & Ohtsuka, 1972; Kaneko & Shimazaki, 1976).

Recently, solitary horizontal cells were dissociated from enzyme-treated retinae (Johnston & Lam, 1981; Tachibana, 1981, 1983; Shingai & Christensen, 1983) and their sensitivity to these amino acids was examined (Lasater & Dowling, 1982; Ishida, Kaneko & Tachibana, 1984) without interference from other retinal cells. Solitary horizontal cells respond to L-glutamate with membrane depolarization, though L-aspartate produces no detectable response. The glutamate-induced depolarization has been suggested to be accompanied by a conductance increase to Na ions (Ishida *et al.* 1984). In the present study, the glutamate-induced responses were analysed in further detail under voltage-clamp conditions. It will be shown that these responses are evoked mainly by a conductance increase to cations and in part by a conductance decrease due to a blockage of a K current through the anomalous rectifier. The latter component will be described in the following paper (Kaneko & Tachibana, 1985*b*).

METHODS

Preparation

Experiments were performed on solitary horizontal cells, which were dissociated from the retinae of pithed adult common goldfish, *Carassius auratus*, and cultured at 10 °C for 1–10 days before recording. Dissociation and culture techniques have been described in detail elsewhere (Tachibana, 1983). Solitary horizontal cells were identified from their characteristic morphology under the inverted microscope with phase-contrast optics (Nikon TMD, Tokyo, Japan) (Tachibana, 1981, 1983).

Recording procedures

Solitary cells were continuously superfused with solutions and the temperature was kept at 15 °C (Tachibana, 1983). The composition of superfusates is listed in Table 1.

Since the somata of horizontal cells are characteristically small in diameter (10–25 μm), an improved patch-clamp technique was applied to solitary horizontal cells to record membrane currents in the whole-cell recording configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The recording procedures have been described in detail elsewhere (Kaneko & Tachibana, 1985*a*). The pipette solution contained (in mM): NaCl, 10; KCl, 120; EGTA, 5; HEPES, 10, and the pH was adjusted to 7·3 with KOH (final concentration 18 mM). Resistance of these pipettes was approximately 10 MΩ. An agar salt bridge served as the ground electrode. When superfusates were exchanged in experiments, a change of liquid junction potential was measured by a pipette filled with 3 M-KCl at the end of each experiment, and the data were appropriately corrected. Both current and voltage outputs of the amplifier (List electronic, EPC-5, Darmstadt, F.R.G.) were displayed on an oscilloscope, recorded on a pen recorder, and stored on a magnetic tape.

Application of L-glutamate (Glu)

In most experiments, Glu (Katayama Chemical Industries Co. Ltd., Osaka, Japan or Peptide Inst. Inc., Osaka, Japan) was applied to the recorded solitary horizontal cells by a pressure-ejection system. Glu was dissolved in solutions, each of which had the same composition as the superfusate, and the pH was readjusted to 7·5. When two types of superfusate were used in an experiment, each barrel of a double-barrelled glass pipette was filled with the corresponding solution with Glu. The tip of the glass pipette was 10–20 μm in diameter and positioned 40–60 μm away from the recorded cells. Pipette solutions were ejected by applying positive pressure (0·3 kg/cm^2) for a controlled amount of time. A small amount of negative pressure (*ca.* 10 mmH_2O) was continuously applied to minimize the leakage of solutions from the pipettes (Ishida *et al.* 1984).

The concentration of Glu applied by the pressure-ejection system was calibrated by the following method using K ions as a test substance, since solitary horizontal cells behave as a K electrode (Tachibana, 1981). Solitary horizontal cells bathed in control solution I (10 mM-K ions) were clamped at the resting membrane potential and the membrane current was monitored when 20 mM-K ions were pressure applied or bath applied to the recorded cells (Fig. 1). It was found that the K ion concentration near the cell surface increased to approximately the same level as that of the pipette solution in 1 s after the onset of the pressure pulse, and that the concentration remained at the same level for a few seconds after the termination of the pulse and then decreased with a slower time course. The time course of the concentration change was reproducible when the pressure was applied to the same pipette at a constant interval, although there were some variations in time course from pipette to pipette. The pressure ejection caused a mechanical movement of the recorded cells but the drift of the membrane current was negligible. Thus, it was estimated that the concentration of Glu near the cell surface reached that of the pipette solution not later than 2 s after the application of pressure.

TABLE 1. Composition of superfusates (in mM)

	NaCl	KCl	$CaCl_2$	$MgCl_2$	Choline Cl	HEPES	Tris	Sucrose	CsCl
Control I	118	10	2·5	1	—	2	—	—	—
Low Na (sucrose)	2	10	2·5	1	—	2	—	232	—
Cs, low Na	2	10	2·5	1	—	2	—	212	10
Low Na (choline)	2	10	2·5	1	116	2	—	—	—
Low Na (Tris)	2	10	2·5	1	—	—	117	—	—
0 Ca, low Na	2	10	0	3·5	—	—	117	—	—
10 Ca, low Na	2	10	10	1	—	—	105	—	—
2 K, low Na	2	2	2·5	1	—	—	125	—	—
40 K, low Na	2	40	2·5	1	—	—	87	—	—

	NaCl	KCl	$CaCl_2$	$MgCl_2$	Choline Cl	HEPES	CsCl	4-AP	TEA Cl
Control II	81	10	2·5	1	37	2	—	—	—
0 Mg	81	10	2·5	0	38·5	2	—	—	—
0 Mg Cs	81	10	2·5	0	28·5	2	10	—	—
Cs	81	10	2·5	1	27	2	10	—	—
Cs, 4-AP, TEA, 0 Ca	81	10	0	3·5	2	2	10	10	20
2 K	81	2	2·5	1	45	2	—	—	—
20 K	81	20	2·5	1	27	2	—	—	—
40 K	81	40	2·5	1	7	2	—	—	—
10 Ca	81	10	10	1	26	2	—	—	—

	NaOH	KOH	$CaCl_2$	$MgCl_2$	HEPES	NaCl	Methanesulphonic acid
Low Cl	116	10	2·5	1	2	2	126

All solutions contained glucose (16 mM), bovine serum albumin (0·1 mg/ml), and phenol red 0·001 % (w/v). The pH was adjusted to 7·5 with NaOH or HCl.

In some experiments, Glu was ionophoretically applied to the recorded cells. Glass micropipettes filled with 1 M-Glu (Na salt) and measuring approximately 50–100 MΩ in resistance were positioned with their tip a few micrometres away from the cell surface. Glu was ejected by passing a negative current (−30 to −50 nA). A constant braking current (+1 to +5 nA) was continuously passed to minimize leakage of Glu.

Abbreviations

4-AP, 4-aminopyridine; EGTA, ethyleneglycol-bis (β-amino-ethyl ether) *N*, *N*, *N'*, *N'*-tetraacetic acid; Glu, L-glutamic acid; HEPES, *N*-2-hydroxyethyl-piperazine-*N'*-ethanesulphonic acid; TEA, tetraethylammonium; Tris, Tris (hydroxymethyl) aminomethane.

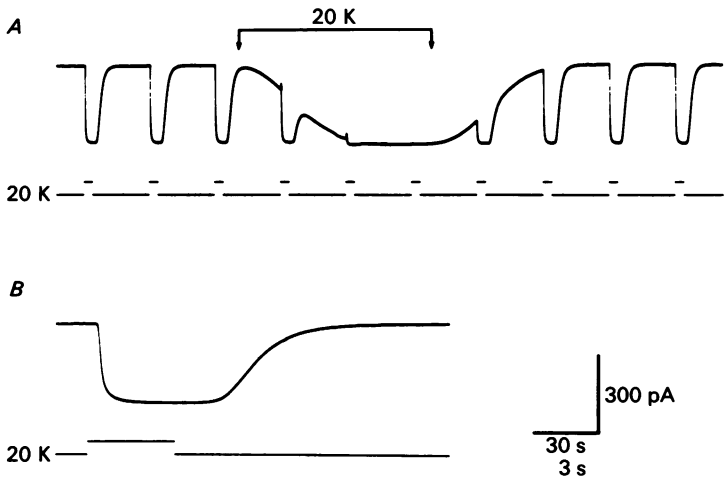

Fig. 1. Calibration of the pressure-ejection system. *A*, a solitary horizontal cell was voltage clamped at −55 mV and the membrane current (upper trace) was monitored when the solution containing 20 mM-K ions (20 K) was pressure applied for 4 s every 30 s (lower trace). The cell was superfused with the control solution II containing 10 mM-K ions, or with the solution of the same ionic composition as the drug-pipette solution (20 K). The amplitude of the K-induced current was almost the same whether the 20 K solution was pressure applied or bath applied. No change in amplitude was observed when the ionic composition of the superfusate was identical to that of the drug-pipette solution (see the current trace at the 6th pressure pulse). The starting level of the current trace corresponds to 0 pA and inward currents are shown as downward deflexions. The data are shown on a slow time base. *B*, the K-induced current on a faster time base, recorded from the same cell shown in *A*.

RESULTS

Glu-induced current in solitary horizontal cells

A total of 237 cells were examined under voltage-clamp conditions.

Application of 100 μM-Glu by a pressure pulse produced a net inward current when the membrane potential was held at the resting level ($-56{\cdot}9 \pm 2{\cdot}6$ mV, $n = 81$; mean ± S.D., number of cells examined) in control solution I (118 mM-Na ions, 10 mM-K ions; see Table 1). As illustrated in Fig. 2*A* (a trace at −57 mV), the evoked current reached a steady level within 1 s after the onset of a pressure pulse (2 s in duration). After the termination of the pulse, the current remained at the steady level for a few seconds and then decreased to zero. The steady level was prolonged when a pressure pulse of longer duration was applied (not illustrated), and there was no indication of desensitization (cf. Ishida *et al.* 1984).

The amplitude of the Glu-induced current (I_{Glu}) at the resting membrane potential was $-45{\cdot}2 \pm 24{\cdot}0$ pA ($n = 81$). This somewhat large variation in amplitude seemed to be related to the surface area of the cells, i.e. larger responses were observed in larger solitary horizontal cells irrespective of the period in culture. Current fluctuations increased in the presence of Glu (at the plateau of the responses), presumably due to random opening and closing of ionic channels activated by Glu. The Glu-induced fluctuations were not analysed further in this paper, however.

To examine the conductance change underlying I_{Glu}, the relation between I_{Glu} and the membrane potential was analysed. In the control solution, voltage-dependent ionic currents were activated (Tachibana, 1983) when the membrane potential was shifted to various levels by command pulses. After the currents reached a steady state, 100 μM-Glu was pressure applied (Fig. 2*A*). As the membrane potential was depolarized beyond approximately -50 mV, I_{Glu} decreased in amplitude, became undetectable at $-2{\cdot}8 \pm 2{\cdot}4$ mV ($n = 81$), and then reversed its polarity into outward. This observation strongly suggests that Glu increases a conductance of ion(s) whose reversal potential lies near -3 mV.

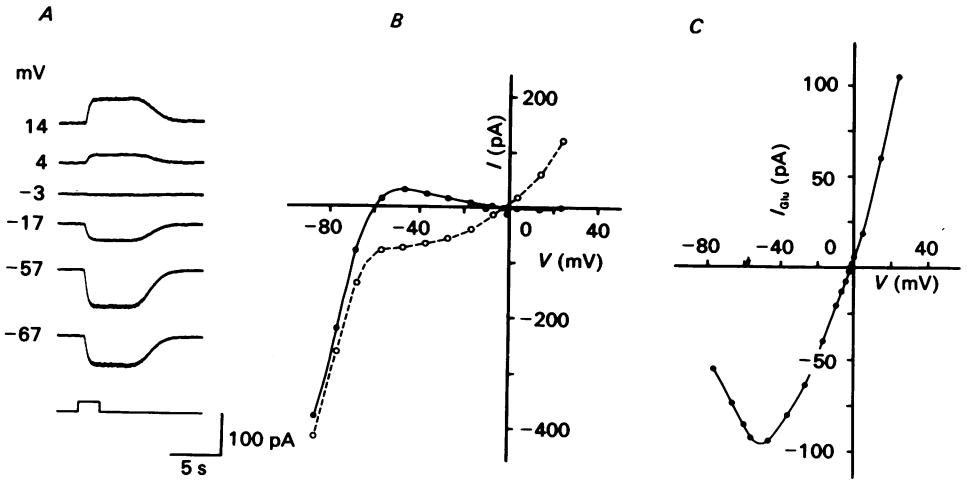

Fig. 2. The relation between the L-glutamate-induced current (I_{Glu}) and the membrane potential (V). A solitary horizontal cell was superfused with control solution I (118 mM-Na ions, 10 mM-K ions, see Table 1). The membrane potential was held at the resting level (-57 mV) and then command voltage pulses (20 s in duration) of various intensities were applied every 40 s. At 5 s after the onset of command pulses, 100 μM-Glu was pressure ejected. *A*, Glu-induced current. Current traces are displaced arbitrarily. Inward currents are shown as downward deflexions. The command voltages are shown at the left side of each current trace. The bottom trace shows the timing of pressure application. *B*, current–voltage (I–V) relations in the presence or absence of Glu. Amplitudes of the current were measured just before (0 Glu; filled circles) and during (100 μM-Glu; open circles) the application of Glu and plotted as a function of membrane potential. *C*, the I_{Glu}–V relation replotted from the data shown in *A*. An arrow indicates the resting membrane potential.

At membrane potentials more hyperpolarized than -50 mV, the voltage dependence of I_{Glu} was very different from that expected from the above hypothesis. Membrane hyperpolarization from -50 mV *decreased* the amplitude of the inward I_{Glu} (Fig. 2*A*). Thirty-two out of 206 cells examined reversed the polarity of I_{Glu} to outward at a hyperpolarized potential somewhere between -70 and -95 mV (Fig. 3).

The relation between the membrane current (I) and the membrane potential (V) is illustrated in Fig. 2*B*. The steady-state I–V relation without Glu was non-linear; a negative-conductance region was observed at potentials beyond -50 mV and a

strong inward-going rectification below the resting membrane potential (< -60 mV) (Fig. 2*B*, filled circles). In the presence of 100 μM-Glu, the *I–V* relation was changed; the negative-conductance region was not observed (Fig. 2*B*, open circles), or was much less prominent than that seen in the control solution (not illustrated).

The Glu-induced current (I_{Glu}) is defined as the difference in current amplitude measured at the same potential between two curves (Fig. 2*B*) and is plotted as a function of membrane potential (Fig. 2*C*). The I_{Glu}–*V* relation thus obtained in the control solution was always non-linear and V-shaped.

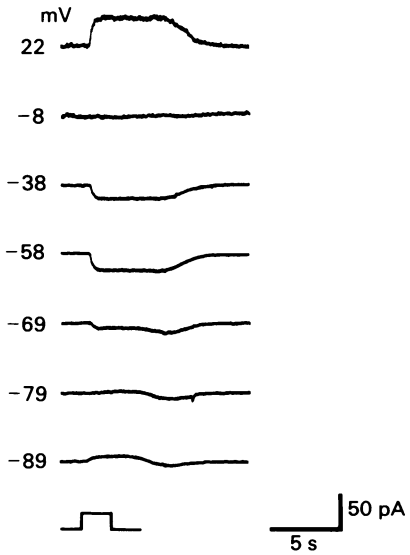

Fig. 3. An example of the reversal in polarity of I_{Glu} negative to the resting membrane potential. A solitary horizontal cell was superfused with control solution II (81 mM-Na ions, 10 mM-K ions) and 100 μM-Glu was pressure applied at various membrane potentials. Membrane polarization from the resting potential (-58 mV) in either direction reduced the amplitude of I_{Glu} and reversed its polarity to outward at potentials beyond -8 mV and below -79 mV. However, the response never nullified at potentials negative to the resting potential (see the trace at -79 mV). This would be explained if two components affected by Glu are different in their dose or time dependence. Current traces are displaced arbitrarily. The bottom trace shows the timing of the pressure application.

The reduction of I_{Glu} by membrane hyperpolarization was not observed when solitary horizontal cells were bathed in the solution containing a mixture of pharmacological agents (Cs, 4-AP, TEA and 0 Ca), which suppressed most of the voltage-dependent currents (Fig. 4, open circles). These voltage-dependent currents are a K current through the anomalous rectifier, which is activated by membrane hyperpolarization, and an A current, an outward K current and a Ca current, which are activated by membrane depolarization (Tachibana, 1983). The I_{Glu}–*V* relation was monotonic in the presence of these agents (open circles), while that in the control solution was V-shaped (filled circles). The difference between two curves was prominent at potentials below the resting membrane potential. This result suggests that Glu affects the voltage-dependent current activated by membrane hyper-

polarization, i.e. the K current through the anomalous rectifier, but not the currents activated by membrane depolarization. In fact, I_{Glu} increased in amplitude with hyperpolarization in cells bathed in the solution containing Cs ions, which block the anomalous rectifier selectively (Fig. 5*B*; see also Fig. 2 in the following paper, Kaneko & Tachibana, 1985*b*).

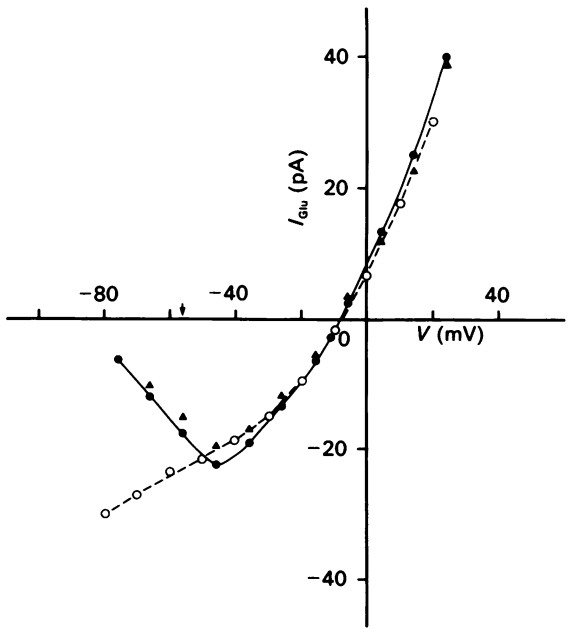

Fig. 4. The effect of Glu (100 μM) on voltage-dependent currents. A solitary horizontal cell was superfused with control solution II (81 mM-Na ions) and the I_{Glu}–V relation (filled circles) was examined by a similar procedure shown in Fig. 2. Then, the superfusate was switched to the solution containing 10 mM-Cs, 10 mM-4-AP, 20 mM-TEA and low Ca (nominally 0 mM), and the I_{Glu}–V relation was examined again (open circles). The concentrations of Na and K ions were kept constant in each solution (81 and 10 mM, respectively). The effect of blockers was reversible (filled triangles). An arrow indicates the resting membrane potential of the cell (−56 mV).

In solitary horizontal cells, withdrawal of Mg ions from the superfusate evoked no significant change in the V-shaped I_{Glu}–V relation (Fig. 5*A*), while it has been demonstrated in mouse central neurones that a non-linear I_{Glu}–V relation is caused by the voltage-dependent blockage of Glu-activated channels by Mg ions (Nowak, Bregestovski, Asher, Herbet & Prochiantz, 1984).

These results indicate that the effect of Glu on solitary horizontal cells can be separated into two components; an increase in conductance to ions which have a reversal potential at about −3 mV, and a decrease in the K conductance of the anomalous rectifier. The latter effect of Glu will be further analysed in the following paper (Kaneko & Tachibana, 1985*b*).

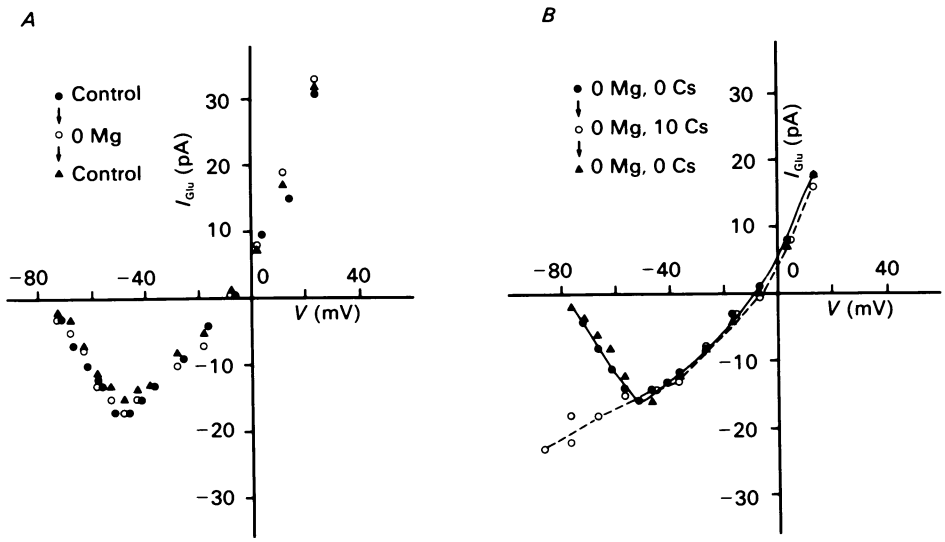

Fig. 5. Effects of Mg and Cs ions on the Glu-induced current. *A*, a solitary horizontal cell was bathed either in control solution II containing 1 mM-Mg ions (filled circles and triangles) or in Mg-free solution (open circles), and 100 μM-Glu was pressure applied. No significant change was observed in the I_{Glu}–V relation. *B*, the reduction of I_{Glu} at hyperpolarized potentials was prevented by an application of 10 mM-Cs ions (open circles). The absence of Mg ions in the superfusate could not linearize the I_{Glu}–V relation even after the anomalous rectifier was blocked by Cs ions. The effect of Cs ions was reversible (filled triangles). Glu (100 μM) was pressure ejected to a different cell from that shown in *A*.

Dose–response relation

The dose–response relation was examined to estimate the least effective concentration of Glu, which induced an increase in conductance. To isolate the conductance-increase component, Cs ions were added to the superfusate. Two different concentrations of Glu were applied to the same cell and two I_{Glu}–V curves at each Glu concentration were obtained (Fig. 6*A*). The voltage dependence of I_{Glu} was almost identical at different concentrations of Glu; the reversal potential was the same and two curves could be fitted to each other by normalization. The ratio of I_{Glu} amplitude at these two Glu concentrations showed little variation among cells (e.g. I_{Glu} at 30 μM/I_{Glu} at 100 μM = 0·56±0·03, n = 6). Thus, the ratio of the response evoked by a particular concentration of Glu to that evoked by 100 μM-Glu was calculated for each cell and plotted as a function of Glu concentration (Fig. 6*B*).

The least effective dose of Glu was a few micromolar. The relative amplitude of I_{Glu} became larger as the Glu concentration was raised, up to approximately 100 μM. Further increase in concentration decreased the amplitude. Glu (100 μM) does not seem to be the saturating dose, since fluctuations in I_{Glu} were clearly observed (Fig. 6*A*, inset) (almost no current fluctuations would be expected in saturated responses because the open probability of ionic channels becomes near 1·0). The reduction in amplitude of I_{Glu} at higher concentrations of Glu might be due to an antagonistic effect of Glu itself, similar to the antagonistic effect of high concentrations

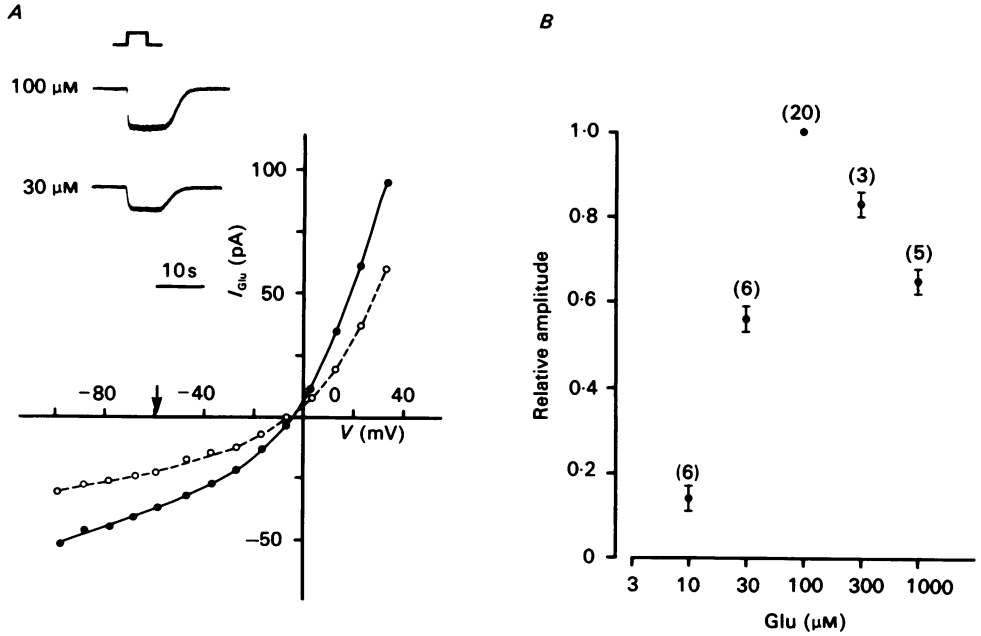

Fig. 6. Dose–response relation. *A*, I_{Glu}–V relations obtained by pressure applying 30 μM-Glu (open circles) and 100 μM-Glu (filled circles) to a cell bathed in the Cs solution (10 mM-Cs ions, 81 mM-Na ions, 10 mM-K ions; see Table 1). The continuous line was drawn to fit the data points (100 μM-Glu) by eye, and the dashed line was obtained by multiplying the current amplitudes of the continuous line by 0·6. The data points measured with 30 μM-Glu fit very well to the dashed line. An arrow indicates the resting membrane potential (−59 mV). Inset shows examples of current records at −59 mV. The top trace indicates the timing of pressure application. *B*, dose–response relation. Two different concentrations of Glu (100 μM as a reference) were applied to each cell and the ratio of current amplitudes measured at −60 mV was calculated. Means and standard deviations of these ratios were plotted as a function of Glu concentration. The numbers in parentheses indicate the number of cells examined.

of D-aspartate on I_{Glu} (Ishida *et al.* 1984), but this hypothesis was not examined further in this study.

The I_{Glu}–V relation of solitary horizontal cells was still non-linear even after the voltage-dependent currents were blocked (Fig. 4, open circles); the outward current was larger in amplitude than the inward when the two currents were compared at any symmetrical potential shifts from the reversal potential. This cannot be ascribed to the accumulation of ions near the cell membrane during a prolonged application of either Glu or command pulses, because a similar relation was also obtained either when Glu was briefly applied to the cells ionophoretically (not illustrated, but see Kaneko & Tachibana, 1985*b*), or when the membrane potentials were changed rapidly either by 100 ms voltage pulses or by ramps (200 mV/s) in the presence of pressure-applied Glu (not illustrated). Furthermore, in Mg-free solutions, application of Cs ions suppressed the reduction of I_{Glu} by membrane hyperpolarization but the I_{Glu}–V relation remained non-linear (Fig. 5*B*, open circles).

Ions which carry the Glu-induced current (I_{Glu})

Na ions. The I_{Glu} of solitary horizontal cells flowed inwards at the resting potential and reversed its polarity between 0 and −10 mV (Figs. 2–5 and 6*A*). These results indicate that I_{Glu} is carried by at least one ion species, the equilibrium potential of which is more positive than the K equilibrium potential (E_K; *ca.* −60 mV in 10 mM-extracellular K ions). Thus, the effect of extracellular Na ion concentration was examined. As shown in Fig. 7*A*, the reversal potential of I_{Glu} shifted to the hyperpolarizing direction when Na ions were replaced with sucrose (118 mM-extracellular Na ions, $-1{\cdot}5 \pm 1{\cdot}4$ mV, $n = 20$; and 72 mM-extracellular Na ions, $-10{\cdot}2 \pm 1{\cdot}5$ mV, $n = 5$), showing that Na ions carry I_{Glu}, at least in part (the channel was not permeable to Cl ions; see below).

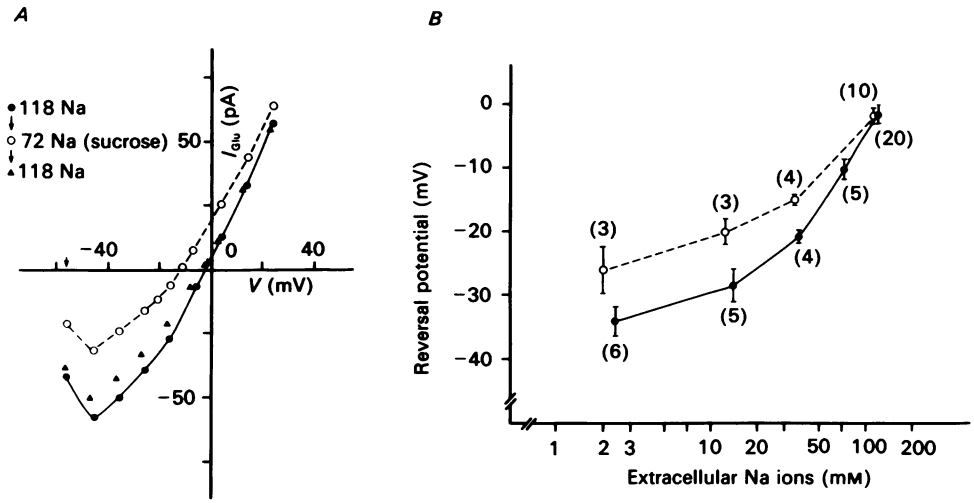

Fig. 7. The effect of extracellular Na ions on the Glu-induced current. *A*, I_{Glu}–V relations obtained in 118 (filled circles) and 72 mM-extracellular Na ions (open circles). Na ions were replaced with sucrose. Glu (100 μM) was pressure applied to a cell. When the extracellular Na ion concentration was decreased, the reversal potential shifted from −2 to −11 mV. The effect of extracellular Na ion concentration was reversible (filled triangles). An arrow indicates the resting membrane potential (−56 mV). *B*, the relation between the reversal potential and the extracellular Na ion concentration. The reversal potentials were measured at two different extracellular Na ion concentrations (118 mM as a reference) in each cell, and the means and standard deviations are plotted. Na ions were replaced with sucrose (filled circles) or $Tris^+$ (open circles). The number of cells examined is shown in parentheses.

The relation between the reversal potential and the extracellular Na ion concentration is illustrated in Fig. 7*B*. At >30 mM-extracellular Na ions, the reversal potential was almost linearly related to log extracellular Na ion concentration; the slope was 38 mV/10-fold change of extracellular Na ion concentration when sucrose substituted for Na ions (filled circles). This value is smaller than that expected from the Nernst equation (57 mV/decade) on the assumption that only Na ions are permeable. The result indicates that some other ions also pass through the Glu-

activated channel. This hypothesis is supported by the observation that the reversal potential deviated from the linear relation at lower extracellular Na ion concentrations (<30 mM), where the contribution of other ions to I_{Glu} would be more prominent. (The reversal potential at 2·4 mM-extracellular Na ions did not change significantly even when the Glu-induced conductance decrease was eliminated; $-31{\cdot}3 \pm 3{\cdot}1$ mV, $n = 6$, in the Cs, low-Na solution, and $-34{\cdot}0 \pm 2{\cdot}3$ mV, $n = 6$, in the low-Na, Cs-free solution.)

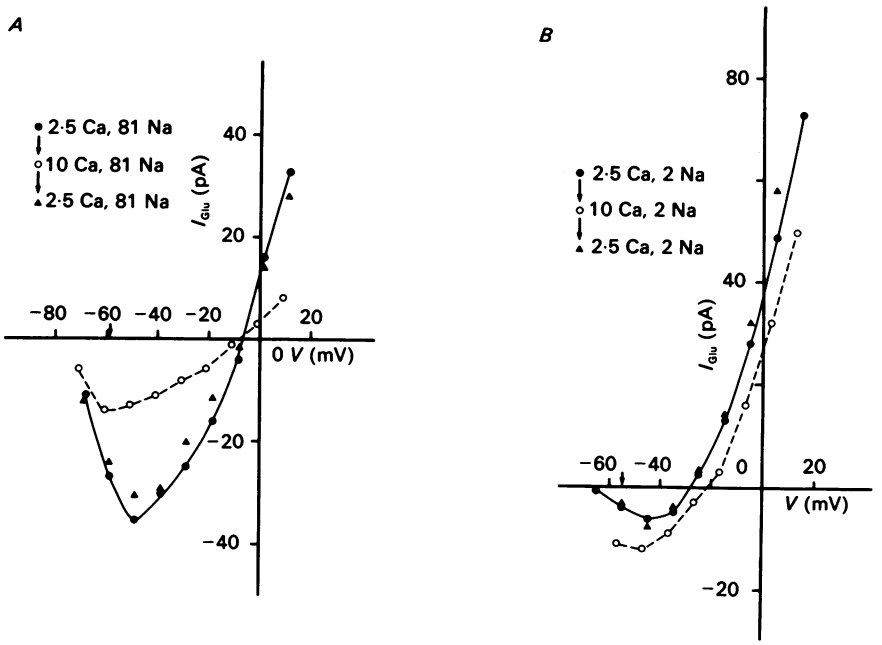

Fig. 8. The effect of extracellular Ca ions on the Glu-induced current. *A*, the concentration of Ca ions was changed from 2·5 (2·5 Ca, 81 Na; filled circles) to 10 mM (10 Ca, 81 Na; open circles) in the presence of 81 mM-Na ions, and the I_{Glu}–V relations were obtained by pressure applying 100 μM-Glu to a cell. Ca ions were replaced with choline$^+$. The effect of Ca ions was reversible (filled triangles). The holding potential was −59 mV, the resting potential of this cell in the control solution (arrow). *B*, most of the Na ions were replaced with Tris$^+$ and the extracellular Ca ion concentration was changed from 2·5 (2·5 Ca, 2 Na; filled circles) to 10 mM (10 Ca, 2 Na; open circles). Ca ions were replaced with Tris$^+$. Glu (100 μM) was pressure ejected to a cell and the I_{Glu}–V relations were measured in each solution. The effect of Ca ions was reversible (filled triangles). The holding potential was −55 mV, the resting potential in the control solution (arrow).

When the substitute for Na ions was Tris$^+$ or choline$^+$, the shift of the reversal potential was smaller than that obtained in Na–sucrose solution (26 mV/decade for Tris$^+$; Fig. 7*B*, open circles and 24 mV/decade for choline$^+$; not illustrated). These results suggest that even Tris$^+$ and choline$^+$ may pass through the Glu-activated channel to some extent.

Ca ions. When the extracellular Ca ion concentration was increased from 2·5 to 10 mM in the presence of 81 mM-extracellular Na ions, the reversal potential of I_{Glu} did not change significantly but the amplitude decreased to 50% (53 ± 12%, $n = 5$)

(Fig. 8*A*). However, in the low-Na solution (2 mM-extracellular Na ions), the same increase in extracellular Ca ion concentration shifted the reversal potential in the depolarizing direction from $-26{\cdot}2 \pm 1{\cdot}8$ mV (2·5 mM-Ca ions, $n = 5$) to $-19{\cdot}6 \pm 2{\cdot}1$ mV (10 mM-Ca ions, $n = 5$), and augmented the amplitude measured at the resting potential (Fig. 8*B*). These results suggest that Ca ions carry I_{Glu} at low extracellular Na ion concentration and that Ca ions may suppress the passage of Na ions through the Glu-activated channel. Similar observations have been reported in crayfish muscles (Onodera & Takeuchi, 1976).

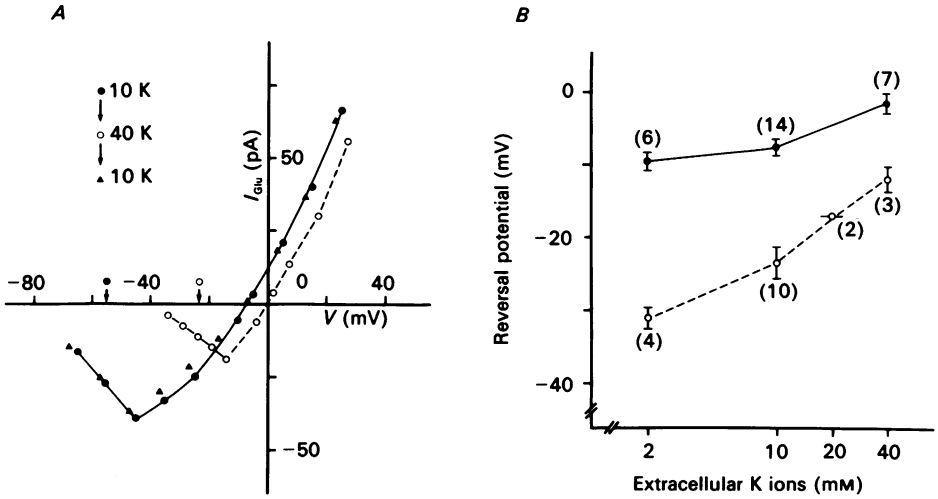

Fig. 9. The effect of extracellular K ions on the Glu-induced current. *A*, K ions were increased from 10 (10 K; filled circles) to 40 mM (40 K; open circles), and responses of a cell to 100 μM-Glu were examined. K ions were replaced with choline$^+$, while Na ions were kept constant (81 mM). The resting membrane potential shifted from -55 (10 K) to -23 mV (40 K), and the holding potential was set at each resting potential in each solution (arrows). Note the shifts of the reversal potential and of the potential which showed a maximum inward current. The effect of K ions was reversible (filled triangles). *B*, the relation between the reversal potential and the extracellular K ion concentration. The concentration of Na ions was kept constant at 81 (81 Na; filled circles) or 2 mM (2 Na; open circles). In the former case, substitutes for Na and K ions were choline$^+$, and in the latter case, they were Tris$^+$. The number of cells examined is shown in parentheses.

When Ca ions were reduced to nominally zero by replacing with an equimolar concentration of Mg ions in the low-Na solution, the reversal potential shifted only slightly (from $-25{\cdot}2 \pm 0{\cdot}8$ to $-27{\cdot}6 \pm 1{\cdot}1$ mV, $n = 5$). This result suggests that Mg ions may also permeate through the Glu-activated channels.

K ions. In the preceding sections, it was shown that Na, Ca and possibly Mg ions permeated through the Glu-activated channel. Since the equilibrium potentials of these ions should be far positive to the observed reversal potential of I_{Glu} (*ca.* -3 mV) in the control solution, the contribution of K ions to I_{Glu} was expected.

When the extracellular K ion concentration was increased from 10 to 40 mM in the presence of 81 mM-Na ions and 2·5 mM-Ca ions, the reversal potential shifted from

$-7{\cdot}8 \pm 1{\cdot}2$ to $-1{\cdot}6 \pm 1{\cdot}4$ mV ($n = 7$) (Fig. 9*A*). (The potential which showed a maximum inward current also shifted in the depolarizing direction and this will be considered in further detail in the following paper, Kaneko & Tachibana, 1985*b*.) On the other hand, when the extracellular K ion concentration was decreased to 2 mM, the reversal potential shifted in the hyperpolarizing direction (Fig. 9*B*). These results indicate that K ions also carry I_{Glu}.

The reversal potential shifted about 9 mV per 10-fold change in extracellular K ion concentration in the presence of 81 mM-extracellular Na ions and 2·5 mM-extracellular Ca ions (Fig. 9*B*, filled circles). Changes in K ion concentration in the solution containing 2 mM-Na ions (replaced with $Tris^+$) and 2·5 mM-Ca ions produced a larger shift of the reversal potential (*ca.* 20 mV/decade, Fig. 9*B*, open circles). The remaining current seems to be carried by Ca, Mg, Na and Tris ions.

Cl ions. The predominant anions in the superfusates were Cl ions. To examine whether this anion can carry I_{Glu}, Glu-induced responses were measured when most of the Cl ions were replaced with methanesulphonate ions. No significant changes were observed either in amplitude or in I_{Glu}–V relation (not illustrated). This result suggests that anions are impermeable through the Glu-activated channel, although we cannot exclude a possibility that methanesulphonate and Cl ions are equally permeable.

DISCUSSION

The present study strongly suggests that Glu induced two types of conductance change in solitary horizontal cells; an increase in conductance to cations (the Glu-activated channel) and a decrease in the K conductance of the anomalous rectifier.

The Glu-activated channel of solitary horizontal cells was permeable to cations. The selectivity of this cation channel seems to be very low, since not only inorganic cations (Na, K, Ca and Mg ions) but also cations as large as $Tris^+$ and $choline^+$ were able to permeate. These results suggest that the pore size of this channel would be very large. Similar cation channels with large pore size have been observed in the Glu-activated channel of the locust neuromuscular junction (Anwyl, 1977) and in the acetylcholine-activated channel of the frog neuromuscular junction (Dwyer, Adams & Hille, 1980) (see review by Edwards, 1982).

The present study showed that Ca ions suppressed the passage of Na ions through the Glu-activated channel, although Ca ions carried I_{Glu} when the extracellular Na ion concentration was low. Similar results have been reported in the Glu-activated channel of crayfish (Onodera & Takeuchi, 1976). If we assume that the interaction between Ca and Na ions is negligible at 2·5 mM-extracellular Ca ions and >30 mM-extracellular Na ions, and that the intracellular concentrations of ions are very close to those of the pipette solution (10 mM-Na ions and 138 mM-K ions), the relative permeability ratio of monovalent cations estimated from the Goldman–Hodgkin–Katz equation would be roughly $P_{Na}:P_K:P_{choline}:P_{Tris} = 1{\cdot}0:0{\cdot}96:0{\cdot}40:0{\cdot}35$. These values may be an over-estimation because the contribution of divalent cations to I_{Glu} was neglected.

The I_{Glu}–V relation of solitary horizontal cells curved upwards near the reversal

potential even after the voltage-dependent currents were blocked. This relation was not affected by withdrawal of Mg ions from the superfusate, suggesting that the Glu-activated channel of solitary horizontal cells is different from that reported in the mouse central neurones (Nowak *et al.* 1984). Single-channel current recordings and noise analysis of Glu-activated channels in other preparations have revealed that the duration of the channel lifetime decreased with membrane hyperpolarization (experiments were performed in Mg-free saline; Anderson, Cull-Candy & Miledi, 1978), although the single-channel conductance shows no potential dependence (Patlak, Gration & Usherwood, 1979; Cull-Candy, Miledi & Parker, 1980). These results may explain why the current through the Glu-activated channel of solitary horizontal cells did not increase in proportion to the membrane hyperpolarization from the reversal potential.

The steady-state I–V relation obtained in the absence of Glu did not show an obvious outward-going rectification, which was observed in the previous studies (Tachibana, 1983; Ishida *et al.* 1984). This discrepancy may be attributable to the difference in the recording technique; 'giga-seal' suction pipettes were used in the present experiments and fine-tipped conventional micropipettes in the previous studies. In the former case, EGTA contained in the pipette solution easily diffused into the recorded cells and partially prevented the Ca-mediated inactivation of the Ca current (Tachibana, 1983), thus it was difficult to detect net outward currents at potentials beyond -20 mV.

In the presence of 100 μM-Glu, the total I–V relation showed almost no negative-conductance region. The suppression of the negative-conductance region may be caused not only by a shunt of the membrane resistance due to the activation of Glu channels, but also by a blocking effect of Glu on the anomalous rectifier, which has a negative-conductance region positive to E_K (Tachibana, 1983).

The reversal potential of the Glu-induced current in solitary horizontal cells was approximately -3 mV, which is similar to the reversal potential of the photoresponses in horizontal cells *in situ* (Trifonov, Byzov & Chailahian, 1974; Byzov, Trifonov, Chailahian & Golubtzov, 1977). Furthermore, both solitary horizontal cells in the presence of Glu and horizontal cells *in situ* under the influence of the transmitter released from photoreceptors are permeable to Na ions (Kaneko & Shimazaki, 1975; Waloga & Pak, 1978). Thus, the present data are consistent with the hypothesis that the transmitter mediating the photoreceptor-to-horizontal cell synapse is Glu.

I thank Professor Akimichi Kaneko for his valuable advice, discussion and encouragement, Drs Shozo Yasui and Teruya Ohtsuka for their comments on the manuscript. Thanks are also to Miss Michi Hosono for her excellent technical assistance. This work is supported by Professor Kato Memorial Research Fund for Physiology and Medicine and by Grants in Aid from the Ministry of Education, Science and Culture (Nos. 58480177, 58870015 and 58106008 to A. Kaneko).

REFERENCES

ANDERSON, C. R., CULL-CANDY, S. G. & MILEDI, R. (1978). Glutamate current noise: post-synaptic channel kinetics investigated under voltage clamp. *Journal of Physiology* **282**, 219–242.

ANWYL, R. (1977). The effect of foreign cations, pH and pharmacological agents on the ionic permeability of an excitatory glutamate synapse. *Journal of Physiology* **273**, 389–404.

BRANDON, C. & LAM, D. M. K. (1983). L-glutamic acid: A neurotransmitter candidate for cone photoreceptors in human and rat retinas. *Proceedings of the National Academy of Sciences of the U.S.A., Biological Sciences* **80**, 5117–5121.

BYZOV, A. L., TRIFONOV, YU. A., CHAILAHIAN, L. M. & GOLUBTZOV, K. W. (1977). Amplification of graded potentials in horizontal cells of the retina. *Vision Research* **17**, 265–273.

CULL-CANDY, S. G., MILEDI, R. & PARKER, I. (1980). Single glutamate-activated channels recorded from locust muscle fibres with perfused patch-clamp electrodes. *Journal of Physiology* **321**, 195–210.

DWYER, T. M., ADAMS, D. J. & HILLE, B. (1980). The permeability of the endplate channel to organic cations in frog muscle. *Journal of General Physiology* **75**, 469–492.

EDWARDS, C. (1982). The selectivity of ion channels in nerve and muscle. *Neuroscience* **7**, 1335–1366.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

ISHIDA, A. T., KANEKO, A. & TACHIBANA, M. (1984). Responses of solitary retinal horizontal cells from *Carassius auratus* to L-glutamate and related amino acids. *Journal of Physiology* **348**, 255–270.

JOHNSTON, D. & LAM, D. M. K. (1981). Regenerative and passive membrane properties of isolated horizontal cells from a teleost retina. *Nature* **292**, 451–454.

KANEKO, A. & SHIMAZAKI, H. (1975). Effects of external ions on the synaptic transmission from photoreceptors to horizontal cells in the carp retina. *Journal of Physiology* **252**, 509–522.

KANEKO, A. & SHIMAZAKI, H. (1976). Synaptic transmission from photoreceptors to the second-order neurons in the carp retina. In *Neural Principles in Vision*, ed. ZETTLER, F. & WEILER, R., pp. 143–157. Berlin Heidelberg: Springer-Verlag.

KANEKO, A. & TACHIBANA, M. (1985*a*). A voltage-clamp analysis of membrane currents in solitary bipolar cells dissociated from *Carassius auratus*. *Journal of Physiology* **358**, 131–152.

KANEKO, A. & TACHIBANA, M. (1985*b*). Effects of L-glutamate on the anomalous rectifier potassium current in horizontal cells of *Carassius auratus* retina. *Journal of Physiology* **358**, 169–182.

LASATER, E. M. & DOWLING, J. E. (1982). Carp horizontal cells in culture respond selectively to L-glutamate and its agonists. *Proceedings of the National Academy of Sciences of the U.S.A., Biological Sciences* **79**, 936–940.

MARC, R. E. & LAM, D. M. K. (1981). Uptake of aspartic and glutamic acid by photoreceptors in goldfish retina. *Proceedings of the National Academy of Sciences of the U.S.A., Biological Sciences* **78**, 7185–7189.

MILLER, A. M. & SCHWARTZ, E. A. (1983). Evidence for the identification of synaptic transmitters released by photoreceptors of the toad retina. *Journal of Physiology* **334**, 325–349.

MURAKAMI, M., OHTSU, K. & OHTSUKA, T. (1972). Effects of chemicals on receptors and horizontal cells in the retina. *Journal of Physiology* **227**, 899–913.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBET, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**, 462–465.

ONODERA, K. & TAKEUCHI, A. (1976). Permeability changes produced by L-glutamate at the excitatory post-synaptic membrane of the crayfish muscle. *Journal of Physiology* **255**, 669–685.

PATLAK, J. B., GRATION, K. A. F. & USHERWOOD, P. N. R. (1979). Single glutamate-activated channels in locust muscle. *Nature* **278**, 643–645.

SHINGAI, R. & CHRISTENSEN, B. N. (1983). Sodium and calcium currents measured in isolated catfish horizontal cells under voltage clamp. *Neuroscience* **10**, 893–897.

TACHIBANA, M. (1981). Membrane properties of solitary horizontal cells isolated from goldfish retina. *Journal of Physiology* **321**, 141–161.

TACHIBANA, M. (1983). Ionic currents of solitary horizontal cells isolated from goldfish retina. *Journal of Physiology* **345**, 329–351.

TRIFONOV, YU. A., BYZOV, A. L. & CHAILAHIAN, L. M. (1974). Electrical properties of subsynaptic and nonsynaptic membranes of horizontal cells in fish retina. *Vision Research* **14**, 229–241.

WALOGA, G. & PAK, L. (1978). Ionic mechanism for the generation of horizontal cell potentials in isolated axolotl retina. *Journal of General Physiology* **71**, 69–92.