Postsynaptic Response Kinetics Are Controlled by a Glutamate Transporter at Cone Photoreceptors

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[1]*Department of Molecular and Cell Biology, Division of Neurobiology, University of California at Berkeley, Berkeley, California 94720;* [2]*Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030; and* [3]*Moran Eye Center, University of Utah, Salt Lake City, Utah 84132*

Gaal, Lubor, Botond Roska, Serge A. Picaud, Samuel M. Wu, Robert Marc, and Frank S. Werblin. Postsynaptic response kinetics are controlled by a glutamate transporter at cone photoreceptors. *J. Neurophysiol.* 79: 190–196, 1998. We evaluated the role of the sodium/glutamate transporter at the synaptic terminals of cone photoreceptors in controlling postsynaptic response kinetics. The strategy was to measure the changes in horizontal cell response rate induced by blocking transporter uptake in cones with dihydrokainate (DHK). DHK was chosen as the uptake blocker because, as we show through autoradiographic uptake measurements, DHK specifically blocked uptake in cones without affecting uptake in Mueller cells. Horizontal cells depolarized from about −70 to −20 mV as the exogenous glutamate concentration was increased from ~1 to 40 μM, so horizontal cells can serve as ''glutamate electrodes'' during the light response. DHK slowed the rate of hyperpolarization of the horizontal cells in a dose-dependent way, but didn't affect the kinetics of the cone responses. At 300 μM DHK, the rate of the horizontal cell hyperpolarization was slowed to only $17 \pm 8.5\%$ (mean $\pm$ SD) of control. Translating this to changes in glutamate concentration using the slice dose response curve as calibration in Fig. 2, DHK reduced the rate of removal of glutamate from ~0.12 to 0.031 μM/s. The voltage dependence of uptake rate in the transporter alone was capable of modulating glutamate concentration: we blocked vesicular released glutamate with bathed 20 mM Mg^{2+} and then added 30 μM glutamate to the bath to reestablish a physiological glutamate concentration level at the synapse and thereby depolarize the horizontal cells. Under these conditions, a light flash elicited a 17-mV hyperpolarization in the horizontal cells. When we substituted kainate, which is not transported, for glutamate, horizontal cells were depolarized but light did not elicit any response, indicating that the transporter alone was responsible for the removal of glutamate under these conditions. This suggests that the transporter was both voltage dependent and robust enough to modulate glutamate concentration. The transporter must be at least as effective as diffusion in removing glutamate from the synapse because there is only a very small light response once the transporter is blocked. The transporter, via its voltage dependence on cone membrane potential, appears to contribute significantly to the control of postsynaptic response kinetics.

INTRODUCTION

The glutamate concentration at the cone terminal is determined by a dynamic balance among the rates of vesicular release, transporter uptake, and diffusion away from the synapse followed by uptake by retinal Mueller cells (Brew and Attwell 1987; Sarantis and Attwell 1990). With cone hyperpolarization, glutamate concentration is thought to be reduced by a voltage-dependent decrease in vesicular release (Ayoub et al. 1989; Copenhagen and Jahr 1989), in the presence of diffusion. Paradoxically, most of the calcium activation curve in cones lies at potentials more positive than the light response range (Bader et al. 1982). But the horizontal cells respond over a much larger part of the cone response range where there is little calcium activation. Glutamate also may be released into the synaptic region through a form of voltage-independent release as has been described by Rieke and Schwartz (1994). The misalignment between calcium activation and cone response voltage opens the possibility that glutamate concentration may be controlled through the voltage-dependent glutamate transporter (Eliasof and Werblin 1993; Grant and Dowling 1995; Grant and Werblin 1996; Marc and Lam 1981; Picaud et al. 1995a; Tachibana and Kaneko 1988).

We measured the contribution of the cone transporter in removing glutamate by blocking uptake with dihydrokainate (DHK), which we show here to be a very effective blocker of uptake in cones but not Mueller cells (Yang and Wu 1997). We monitored changes in the kinetics of glutamate concentration by measuring horizontal cell activity, which we show to be a monotonic function of glutamate concentration. DHK significantly slowed the rate of glutamate removal during cone hyperpolarization, consistent with the notion that uptake contributes significantly to the removal of glutamate.

Even in the absence of vesicular release, but with bathed glutamate substituted, glutamate concentration fell during cone hyperpolarization, suggesting that the transporter is voltage dependent and robust. Our results suggest that the rate of voltage-dependent transporter uptake is at least as great as diffusion and may serve as an essential link between cone membrane voltage and glutamate concentration (Gaal et al. 1995).

METHODS

Autoradiography

Isolated tiger salamander retinas were sliced at 40 μm and incubated for 10 min at room temperature in 25-μl droplets of physiological saline containing 2.5 μCi [^{3}H] D-aspartate (~5 μM total D-aspartate) and DHK (0, 10, 100, and 1,000 μM), rinsed in cold saline, fixed in mixed aldehydes, epoxy resin embedded, precision sectioned at 500 nm, and processed for light microscope autoradiography (Marc et al. 1978) with 5-day exposures. The radioactive label spread throughout the cone, so it was possible to measure

levels of activity corresponding to uptake at sites in the cone far from the synaptic terminal. Images of individual Mueller cells and cones for each DHK dose were captured as 512×480 pixel frames (Marc et al. 1990), and the integrated silver grain signal in a standard window measured in all identified cells. D-aspartate was used as a probe for the time-integrated transport because it is metabolically inert and therefore remained within the cell membranes and its activity spread throughout the cells (Fonnum 1984; Marc and Lam 1981).

Electrical recording

Horizontal cells were recorded in the tiger salamander retinal slice (Werblin 1978) with both the current- and voltage-clamp mode of the whole cell patch-clamp technique. Cells were stained with Lucifer yellow and viewed under UV epi-illumination at the end of the experiment to verify cell identity. Horizontal cell isolation was facilitated with papain. Isolated horizontal cells were identified because only their inward rectifying potassium current is blocked with 0.5 mM barium (Dong and Werblin 1996). Electrode resistance fell between 5 and 10 MΩ.

Solutions and drugs

Standard amphibian Ringer consisted of (in mM) 112 NaCl, 2.5 KCl, 2 or 0.1 CaCl$_2$ (we used either concentration with similar results), 1 MgCl$_2$, 10 glucose, and 5 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES); pH adjusted to 7.8 with NaOH. Vesicular release was blocked with Mg^{2+} substituted for Na$^+$, DHK was added without substitution, and γ-aminobutyric acid inputs to photoreceptors and neighboring HCs were blocked with 100 μM picrotoxin. All drugs were bath applied. Intracellular solution consisted of (in mM) 46 KCl, 59 potassium gluconate, 5 CsF, 0.5 MgCl$_2$, 0.5 CaCl$_2$, 5 HEPES, 1.3 bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid, 5 Na$_2$ATP, and 0.1 Na$_3$ guanosine 5'-triphosphate (Na$_3$GTP) and 0.01% Lucifer yellow and pH adjusted to 7.4 with KOH. E_{Cl} was set to -20 mV (Miller and Dacheux 1983).

Selecting cone-driven horizontal cells

We recorded horizontal cell potential in response to full field flashes eliciting maximum responses in light-adapted retinas as a measure of glutamate concentration at the cone synapse. Horizontal cells that were driven primarily by cones, not rods, were selected by eliminating most cells with ''tail currents,'' the slowly decaying hyperpolarizations after the termination of the light stimulus, traditionally associated with rod activity (Attwell and Wilson 1980).

Nystatin measurements

Nystatin-perforated patch (Horn and Marty 1988) was used to eliminate the run-down of the light response and Ca currents in cones. A stock solution of 50 mg/ml (in dimethyl sulfoxide) was diluted to a final concentration of 300 μM/ml in the intracellular solution. A capacitative transient appeared within 5 min after obtaining a gigaohm seal on the cone, indicating access to the cytoplasm, and increased to its final stable magnitude within 20 min.

R E S U L T S

Autoradiography shows that DHK blocks uptake in cones but not Mueller cells

If one compares the blocking effects of DHK in cones (Eliasof and Werblin 1993; Picaud et al. 1995a) and Muller's cells (Barbour et al. 1991) on the glutamate-elicited current, DHK does not appear to be selective for cones.
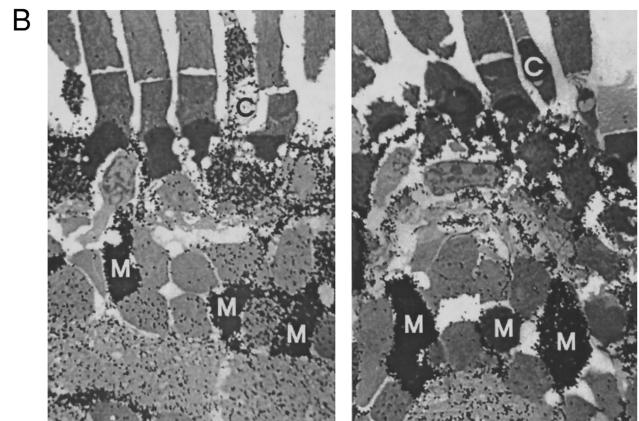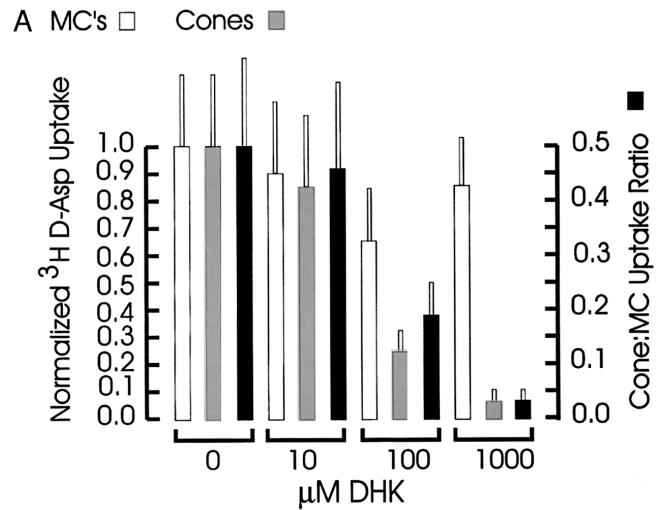

FIG. 1. Autoradiography of cone and Mueller cell uptake. *A*: bar graph showing the normalized D-aspartate uptake and the root mean square coefficient of variation for 12–20 cones and Mueller cells at dihydrokainate (DHK) doses of 0, 10, 100, and 1,000 μM. At each concentration, the graph shows the ratio of the mean grain densities for each type. Values of cone uptake in 100 and 1,000 μM DHK differ from Mueller cell uptake with $P < 0.01$. The mean Mueller cell uptake at 1,000 μM DHK was not significantly different from that in the absence of DHK. *B*: photomicrographs of grain density for normal uptake (*left*) and for uptake in the presence of 1,000 μM DHK (*right*). Grains appear throughout the cells, allowing measurement of uptake in regions far from the synaptic terminals where Mueller cells and cone terminals would be difficult to distinguish. C, cones; M, Mueller cells.

These results were based on measurements of a combination of transporter and transporter-gated chloride currents. But it has been suggested that the stoichiometric relationship between glutamate transported and the chloride current is not fixed (Billups et al. 1996; Eliasof and Jahr 1996; Fairman et al. 1995), so the earlier estimates of relative uptake may be misleading. To show that DHK is a selective blocker for uptake in cones but not Mueller cells, we measured uptake directly with autoradiography. D-aspartate was used rather than glutamate because L-aspartate is metabolized in Mueller cells, allowing us to measure the radiographic signature for aspartate in both cones and Muller's cells.
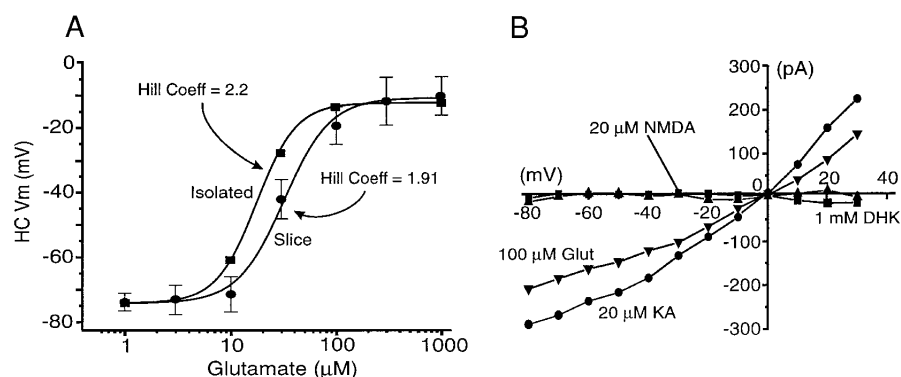
FIG. 2. Horizontal cell calibration curve. *A*: isolated horizontal cells: Glutamate dose-response curve for isolated horizontal cells showed an EC_{50} of 18 μM. This curve represents the dose response curve of glutamate receptors in horizontal cells assuming that there is no difference in sensitivity between intra- and extrasynaptic receptors (if they exist) on horizontal cells. Hill coefficient for the isolated cell was 2.2. Slice: dose-response curve for horizontal cells in the slice with 20 mM Mg^{2+} and 500 μM DHK to block vesicular release and transporter uptake had $EC_{50} = 32$ μM. This curve identifies the voltage response range but the true dose response curve may be shifted to the left because there exists an ambient glutamate concentration that was not blocked by Mg^{2+} and there exists an ambient uptake that was not blocked by DHK. Addition of Mg^{2+} also might affect the position of the curve. Hill coefficient was 1.91. Data for both curves was fit with a logistic function. *B*: direct effect of agents on an isolated horizontal cell. DHK (1 mM) and *N*-methyl-D-aspartate (NMDA; 20 μM, with 5 μM glycine and 1 μM strychnine) had no effect on isolated cells. Kainate (20 μM) evoked a current similar to 100 μM glutamate, suggesting that horizontal cells have kainate receptors. Traces represent evoked currents measured at each voltage step after the subtraction of the control recordings.

Figure 1*A* shows the DHK is a selective blocker of transporter uptake at cones but not Mueller cells. Autoradiographic measurement shows that the ratio of cone to Mueller cell uptake was reduced from 0.5 under control conditions to <0.05 in the presence of 1,000 μM DHK. Photomicrographs of these two conditions are shown in Fig. 1*B*. The absolute level of uptake in Mueller cells was not affected by the addition of DHK. At 300 μM DHK, the ratio of uptake was reduced to ~0.08 of the normal level. These measurements suggest that DHK can be used to specifically block uptake in cones without affecting uptake in Mueller cells (Wu et al. 1995).

Horizontal cells respond to glutamate concentrations between 1 and 50 μM

We calibrated horizontal cells with different concentrations of bath-applied glutamate as a basis for estimating the relationship between the endogenous glutamate concentration and horizontal cell response. In the slice, potential changes were recorded from horizontal cells in the presence of 500 μM DHK to block uptake and 20 mM Mg^{2+} to block endogenous glutamate release from rods and cones. Mg^{2+} reduces the rate of release to levels where quantal events can be measured in both the pre- (Larsson et al. 1996; Picaud et al. 1995) and postsynaptic (Maple et al. 1994) cells. Horizontal cells depolarized from about −70 to −20 mV over a concentration range from ~1 to 80 μM as shown in Fig. 2*A*. The data were fit with a logistic function with Hill coefficient of 1.91. This curve may be shifted to the right compared with the true dose-response curve for the glutamate receptors due to the presence of either residual uptake or release in the cone. Either of these conditions would increase the concentration at which the horizontal cells would begin to respond to applied glutamate.

To gain a measure of the *absolute sensitivity* of the gluta-

mate receptors, unaffected by the cone membrane properties, we recorded from isolated horizontal cells under whole cell patch clamp as shown in Fig. 2*A*. These data were fit with a logistic function with Hill coefficient of 2.2. The position of the dose-response curve under voltage clamp was not affected by the relative conductance of the glutamate-gated and resting channels. Glutamate elicited graded inward currents over the concentration range from 1 to 40 μM, but this curve is shifted to the left with respect to the "slice curve" of Fig. 2*A*. The effective glutamate concentrations are orders of magnitude lower than values (near 1 mM) found at spiking synapses in the central nervous system (CNS) (Clements et al. 1992).

In the following, we used DHK to reduce the rate of uptake and used Mg^{2+} to reduce the rate of vesicular release in cones. The curves in Fig. 2*B* control for the use of these blockers by showing that DHK alone had no direct effect on isolated horizontal cells. Figure 2*B* also shows that there were no *N*-methyl-D-aspartate receptors on isolated horizontal cells, consistent with the study of Yang and Wu (1991), so it is unlikely that Mg^{2+} would affect horizontal cells.

Voltage range of the calcium activation curve does not overlie the cone response range

Figure 3 shows that the voltage range for the calcium current in cones lies between −40 and 0 mV; this is similar to the results of Bader et al. (1982) and Maricq and Korenbrot (1989). However, the majority of the light response lies at more negative potentials, between −40 and −55 mV, although there is a slight slope to this curve even at more negative potentials within the cone response range. Because of this misalignment, it is unlikely that light-elicited, voltage-induced changes in rates of transmitter release, mediated by voltage-dependent changes in calcium entry, could modu-
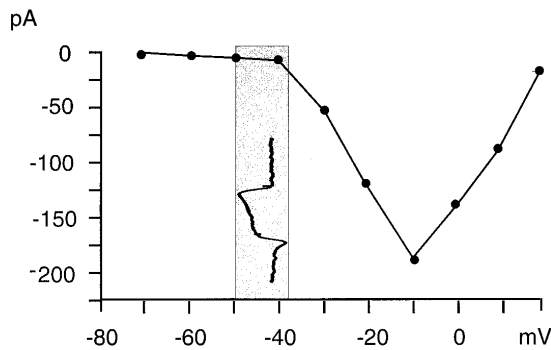
FIG. 3. Comparison of the voltages spanned by the activation curve for Ca^{2+} and cone light response range. Current-voltage relationship for the calcium current measured in an isolated cone. Calcium begins to activate at about -40 mV and is fully activated near -10 mV. Light response in the same cone lies between -40 and -55 mV. These curves show that there is very little calcium activation over the response range of the cone.

late glutamate concentration over most of the voltage range of the cone response. Schwartz (1986) has made a similar suggestion, and Reike and Schwartz (1994) have described a voltage-independent form of vesicular release that could supply glutamate to the synapse over the portion of the response range where voltage-dependent calcium-mediated release is either small or absent. There also might be some increase in calcium current with hyperpolarization due to the increase in driving force on calcium. In the absence of a significant increase in calcium current with depolarization, which would lead to an increase in voltage-dependent release, an additional voltage-dependent mechanism operating over the full cone response range from -40 to -55 mV would be required to link glutamate concentration to cone membrane potential. A likely candidate for this link is the glutamate transporter. The following experiments support the notion that the cone glutamate transporter is involved in controlling glutamate concentration in a voltage-dependent manner.

DHK slows horizontal cell ON response kinetics

We used the horizontal cell response as a measure of glutamate concentration, taking advantage of the monotonic relationship between concentration and potential as shown in Fig. 2. DHK, added to the bathing solution, depolarized both cones and horizontal cells as shown in Fig. 4. Depolarization in cones was probably due to the block of a transporter-gated chloride current, which has been shown to be outward at -40 mV (Eliasof and Werblin 1993; Picaud et al. 1995b). Cone depolarization then could lead to increase in glutamate concentration, either because voltage-dependent vesicular release was increased or because voltage-dependent uptake was slowed.

The glutamate concentration also would increase due to a partial DHK-mediated block of transporter uptake in cones causing the glutamate concentration to rise to a new, higher level. In similar measurements Yang and Wu (1997) found that DHK depolarized of horizontal cells without any depolarization in cones, suggesting that horizontal cell depolarization was due to partial block of the transporter alone. We cannot distinguish between concentration increases due to a

voltage-dependent shift in uptake and release due to cone depolarization or concentration increases due to partial block of the transporter. Because of this ambiguity, we do not use horizontal cell depolarization alone as a measure of DHK block of the glutamate transporter.

However, the *kinetics* of the horizontal cell response were significantly slowed in 300 μM DHK, whereas there was only a small change in kinetics of the cone response (to $98 \pm 8\%$, $n = 4$). The slowdown of the hyperpolarizing response was graded with DHK concentration, falling from $\sim$0.122 mV/ms in control to $<$0.017 mV/ms in the presence of 300 μM DHK over the range from 10 to 90% of the response. The rate was determined by linearizing the region from 10 to 90% of the response. Using the slice calibration curve in Fig. 2, the rate of glutamate removal would be reduced from 0.12 to 0.031 μM/ms. The light response was not fully blocked in 300 μM DHK, either because the transporter was not fully blocked or because there remained some voltage-dependent reduction in release in the presence of diffusion. In the presence of 300 μM DHK, the horizontal cell was never fully hyperpolarized in light probably because there remained some voltage-independent release (Rieke and Schwartz 1994) in the presence of an incomplete block of the transporter. Decreases in response kinetics in the presence of transporter blockers have been observed by Yang and Wu (1997) and by Vandenbranden et al. (1996).

Mg^{2+} slows horizontal cell OFF kinetics

The results above show that a DHK-induced reduction in the rate of uptake reduced the rate of hyperpolarization at
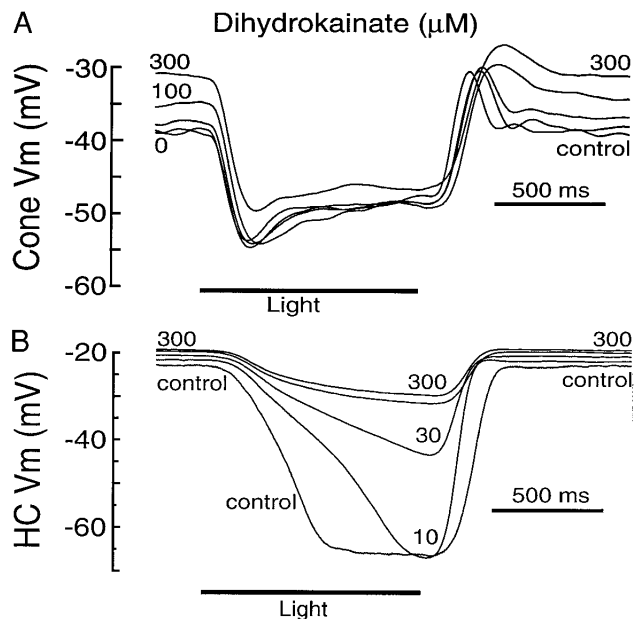

FIG. 4. Responses in different DHK concentrations at light ON. *A*: light responses of a cone. Cones depolarized to DHK, but the kinetics of the responses at light ON were only slightly reduced with 300 μM DHK to $98 \pm 8\%$ ($n = 4$) of control. *B*: responses of a horizontal cell. At 300 μM DHK, the rate of the response onset decreased to $17 \pm 8\%$ of control. Results similar to this figure were measured in 10 cells. Kinetics measured as the slope (linear regression) between 10 and 90% of the maximum size of each light response. Each trace is the average of 15 records.
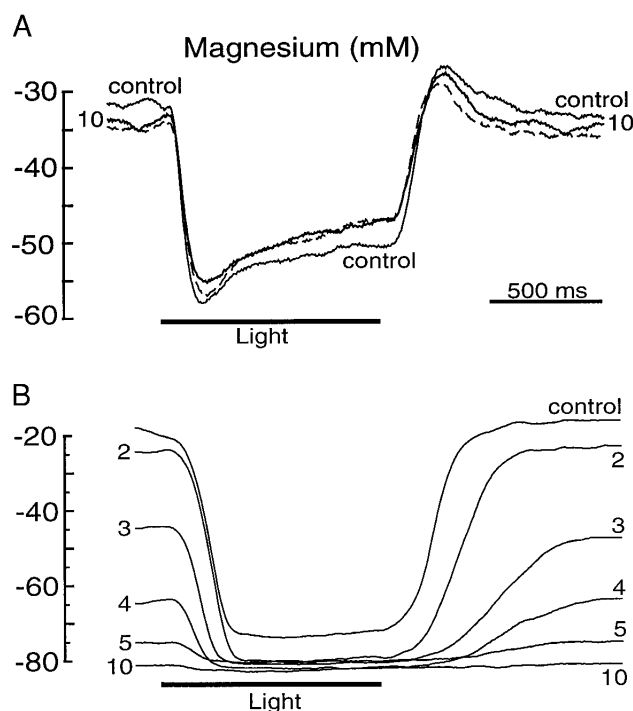
A

Magnesium (mM)

B

FIG. 5.   Responses in different Mg^{2+} concentrations at light OFF. *A*: light responses of a cone. Magnesium hyperpolarized cones but hardly affected the rate of the light response onset ($81 \pm 11\%$) and offset ($81 \pm 6\%$, $n = 6$). *B*: responses of a horizontal cell. At 5 mM Mg^{2+}, the depolarizing response at light OFF, an indication of glutamate increase, was slowed to $8 \pm 4\%$ control. These records are representative of the measurements in 6 cells. Similar but smaller effects were seen in an additional 7 cells. Kinetics measured as the slope (linear regression) between 10 and 90% of the maximum size of each light response. Voltage scale shown on *left,* time represented by bar, light where indicated. Cells were recorded separately from different slices.

light ON. Here we show that decreasing the rate of release reduced the rate of depolarization at light OFF. Figure 5 shows that addition of Mg^{2+} to the bath to partially block release caused the horizontal cells to hyperpolarize but had little effect on the cone potential level. The hyperpolarization in the horizontal cells is probably due to the reduction of release from the synapse (Dowling and Ripps 1973; Trifonov 1968). Mg^{2+} also slowed the horizontal cell OFF kinetics but had no effect on the kinetics of the cone OFF responses. As the Mg^{2+} concentration was increased, the OFF response rate decreased from $\sim77 \pm 4\%$ of control in 2 mM Mg^{2+} to $8 \pm 4\%$ in 5 mM Mg^{2+} ($n = 6$). This decrease in rate of depolarization is consistent with the notion that vesicular release serves to recharge the synapse with glutamate and that the recharging is slower because absolute release rate was decreased in the presence of Mg^{2+}.

Transporter alone can modulate glutamate concentration at the cone synapse

The DHK effects presented in the earlier section could have been generated by a voltage-independent transporter, slowed by DHK, operating in the presence of voltage-dependent release. To confirm that the transporter itself was volt-

age dependent like that in Mueller cells (Brew and Attwell 1987), we blocked release with 20 mM Mg^{2+}, causing the horizontal cells to hyperpolarize to -70 mV as shown in Fig. 6*A*. Then 30 μM glutamate was added to the bath, an action that depolarized the horizontal cells to -35 mV. The glutamate concentration at the cone-horizontal cell synapse was probably somewhat <30 μM because of the active uptake of glutamate at the cones and Muller's cells. The calibration curves of Fig. 2*A* suggest a concentration between 10 and 20 μM for a 35-mV depolarization in the horizontal cells. Under these conditions, a light flash elicited a hyperpolarization in the horizontal cells of 13 ± 7 mV ($n = 9$) as shown in Fig. 6*B*. In a separate set of measurements using 20 μM cadmium to block release (not shown here), light elicited a response of 9.7 ± 5 mV ($n = 5$). The response was slower than normal probably because the transporter uptake was opposed by glutamate diffusion into the synapse, whereas under normal conditions of low external glutamate, diffusion and transport act in the same direction, both moving glutamate out of the synapse. Figure 6*B* shows that the addition of DHK caused a further depolarization and diminution of the light response, consistent with the results of Fig. 4. This suggests that the light response was generated by the hyperpolarization-induced increase in the uptake rate of the DHK-sensitive transporter alone.

Figure 6*C* shows that in the presence of Mg^{2+}, when kainate (a glutamate agonist that is not transported) was substituted for glutamate, the horizontal cell depolarized but light elicited no response. This result confirms that the light-elicited horizontal cell hyperpolarization was mediated by voltage-dependent glutamate uptake in cones alone in the absence of release. This uptake is robust enough to significantly reduce glutamate concentration, even when uptake was opposed by diffusion of exogenous, bathed glutamate into the synapse. The light response is probably not due to light activation of the transporters in Mueller cells because
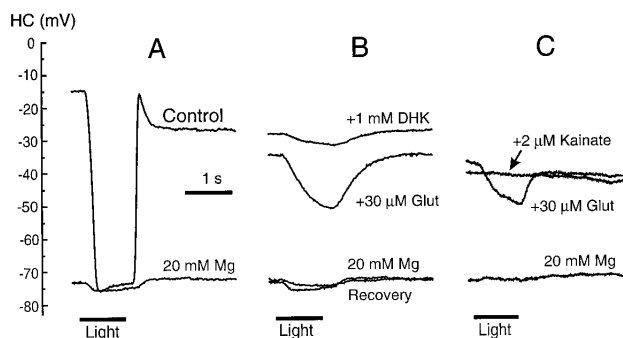
HC (mV)

FIG. 6.   Transporter removes glutamate even in the absence of release. *A*: normal light response in control conditions and in the presence of 20 mM Mg^{2+}. *B*: in the presence of 20 mM Mg^{2+}, the addition of 30 μM glutamate depolarized the horizontal cell to -35 mV, and a light flash elicited a 17-mV hyperpolarization to -52 mV. Similar responses were seen in 8 other cells. Further addition of 1 mM DHK depolarized the horizontal cell and blocked most of the light response. *C*: kainate, which is not transported, elicited no light response. Application of 20 mM Mg^{2+} hyperpolarized the cell and blocked the light response (*bottom*). In the presence of 20 mM Mg^{2+}, further addition of 2 μM kainate depolarized the cell to -39 mV but light did not elicit a response (*middle*, →). In the presence of 20 mM Mg^{2+}, a light response was recorded in the same cell when 30 μM glutamate was substituted for kainate.

these cells depolarize to light, thereby increasing extracellular glutamate concentration (Miller and Dowling 1970), which would have a depolarizing effect on the horizontal cells.

DISCUSSION

Comparison between transporter uptake at spiking and nonspiking neurons

There is a general understanding that postsynaptic kinetics at most CNS synapses are controlled either by postsynaptic receptor desensitization (Clements et al. 1992; Hestrin et al. 1990; Takahashi et al. 1995), channel inactivation (Hestrin et al. 1990; Jonas and Spruston 1994; Lester et al. 1990), or diffusion (Clements et al. 1992; Isaacson and Nicholl 1993; Sarantis et al. 1993). These mechanisms can be effective in rapidly terminating the synaptic signal after the arrival of an action potential. Transport appears to play little or no role in controlling postsynaptic kinetics (Hestrin et al. 1990; Isaacson and Nicholl 1993; Sarantis et al. 1993; Tong and Jahr 1994).

The situation at the nonspiking cone synapse is quite different because the concentration is maintained at elevated levels, which are modulated continuously by presynaptic voltage. Furthermore, the rates of change in postsynaptic activity are orders of magnitude slower, and the concentrations of glutamate are orders of magnitude lower (Clements et al. 1992). A mechanism is required to modulate glutamate concentration as a function of presynaptic (cone) membrane Potential. Desensitization, inactivation, or diffusion cannot link glutamate concentration to membrane potential. A likely candidate for this role is the glutamate transporter.

Possible mechanism linking cone membrane potential to glutamate concentration

Our results are consistent with the notion that both the ambient level and the kinetics of glutamate concentration at the cone terminal are controlled by a balance among vesicular release, uptake by transporters, and diffusion. Glutamate concentration is increased via voltage-dependent and voltage-independent vesicular release (Reike and Schwartz 1994) and decreased via diffusion and uptake. Release is probably not voltage dependent over most of the cone response range as shown in Fig. 2, so most of the voltage-dependent control of glutamate concentration is mediated by the transporter working against a voltage-independent release rate (Rieke and Schwartz 1994). The transporter is both voltage and concentration dependent (Eliasof and Werblin 1993; Picaud et al. 1995). At each steady-state level, the rates of uptake and diffusion are equal and opposite to release. When the cone hyperpolarizes, two changes take place to assure that uptake and diffusion rates remain equal to release: the driving force for uptake increases, causing uptake to increase and glutamate concentration to fall, and as glutamate concentration falls, the net uptake rate is reduced until it is equal once again to release. This mechanism could link membrane potential to glutamate concentration via the transporter.

Uptake, release, and diffusion are constrained by a diffusion barrier at the cone synapse

These processes of uptake and release appear to operate in a confined extracellular space from which diffusion probably is limited physically by the membranes of cells and glia surrounding the cone terminal (Lasansky 1973). The extracellular space also may contain an additional diffusion-limiting matrix as well (Marszalek et al. 1995). The concentration outside the diffusion limited space is maintained at low levels by the transporter at retinal Mueller cells (Barbour et al. 1991; Brew and Attwell 1987). Brew and Attwell (1987) suggest that diffusion from the cone terminal is fast enough to remove glutamate. They may have overestimated the rates of glial uptake because it recently has been shown that part of the uptake current is carried by chloride (Billups et al. 1996; Eliasof and Jahr 1996).

If diffusion were the primary pathway for removal, there would be no mechanism available to link glutamate concentration to cone membrane potential in the absence of voltage-dependent release, DHK would not dramatically reduce the rate of glutamate removal (Fig. 2), and it would be impossible for the transporter to modulate glutamate concentration in the presence of high extracellular glutamate concentration (Fig. 6). Further evidence for a diffusional barrier was found in salamander by Yang and Wu (1997). However, Vandenbranden et al. (1996) suggest that the cone pedicle does not limit the clearance of transmitter form the synapse.

Essential role for the glutamate transporter

The observation that most of the cone response range falls outside the activation range for calcium (Fig. 3) suggests that some other voltage-dependent process may link cone membrane potential to glutamate concentration. Our finding that DHK significantly slows the glutamate removal process (Fig. 4A) suggests that voltage-dependent uptake is at least as rapid as diffusion. If diffusion were predominant, blocking uptake would have had little effect on the rate of removal of glutamate from the synapse. Blocking vesicular release with Mg^{2+} precludes any possibility of a voltage-dependent process other than the transporter. Our finding that under these conditions light still can cause horizontal cells to hyperpolarize (Fig. 6) suggests that the transporter is robust enough to modulate glutamate concentration. The predominance of the transporter in removing glutamate from the synapse supports the notion that the voltage-dependent glutamate transporter in cones serves as the essential link between cone membrane potential and glutamate concentration.

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