Gadolinium Reduces AMPA Receptor Desensitization and Deactivation in Hippocampal Neurons

SAOBO LEI AND JOHN F. MACDONALD

Departments of Physiology and Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Received 4 December 2000; accepted in final form 27 March 2001

Lei, Saobo and John F. MacDonald. Gadolinium reduces AMPA receptor desensitization and deactivation in hippocampal neurons. J Neurophysiol 86: 173–182, 2001. The actions of the trivalent cation Gd\(^{3+}\) on whole cell AMPA receptor-mediated currents were studied in isolated hippocampal neurons, in nucleated or outside-out patches taken from cultured hippocampal neurons, and on miniature excitatory postsynaptic currents (mEPSCs) recorded in cultured hippocampal neurons. Glutamate, AMPA, or kainate was employed to activate AMPA receptors. Applications of relatively low concentrations of Gd\(^{3+}\) (0.1–10 \(\mu\)M) substantially enhanced steady-state whole cell glutamate and kainate-evoked currents without altering peak currents, suggesting that desensitization was reduced. However, higher concentrations (>30 \(\mu\)M) depressed steady-state currents, indicating an underlying inhibition of channel activity. Lower concentrations of Gd\(^{3+}\) also increased the potency of peak glutamate-evoked currents without altering that of steady-state currents. An ultrafast perfusion system and nucleated patches were then used to better resolve peak glutamate-evoked currents. Low concentrations of Gd\(^{3+}\) reduced peak currents, enhanced steady-state currents, and slowed the onset of desensitization, providing further evidence that this cation reduces desensitization. In the presence of cyclothiazide, a compound that blocks desensitization, a low concentration Gd\(^{3+}\) inhibited both peak and steady-state currents, indicating that Gd\(^{3+}\) both reduces desensitization and inhibits these currents. Gd\(^{3+}\) reduced the probability of channel opening at the peak of the currents but did not alter the single channel conductance calculated using nonstationary variance analysis. Recovery from desensitization was enhanced, and glutamate-evoked current activation and deactivation were slowed by Gd\(^{3+}\). The Gd\(^{3+}\)-induced reduction in desensitization did not require the presence of the GluR2 subunit as this effect was seen in hippocampal neurons from GluR2 null-mutant mice. Gd\(^{3+}\) reduced the time course of decay of mEPSCs perhaps as a consequence of its slowing of AMPA receptor deactivation although an increase in the frequency of mEPSCs also suggested enhanced presynaptic release of transmitter. These results demonstrate that Gd\(^{3+}\) potently reduces AMPA receptor desensitization and mimics a number of the properties of the positive modulators of AMPA receptor desensitization such as cyclothiazide.

INTRODUCTION

Glutamate activates three different families of ion channels including N-methyl-d-aspartate (NMDA), \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate receptors. One particular property of glutamate receptors is that they are modulated by divalent cations such as Mg\(^{2+}\) (Ault et al. 1980; Mayer et al. 1984; Nowak et al. 1984). The Mg\(^{2+}\) block of NMDA receptors is voltage dependent and plays an important physiological role. Currents mediated by these receptors are similarly blocked by other members of group IIb metal cations including cadmium, cobalt, nickel, and manganese (Mayer and Westbrook 1987; Mayer et al. 1989). Nevertheless, millimolar concentrations of Mg\(^{2+}\) also increase NMDA-evoked currents by interacting with the glycine-binding site of the receptor (Paoletti et al. 1995; Wang and MacDonald 1995). The effects of polyvalent cations on AMPA and kainate receptor-mediated currents have also been investigated, although to a lesser extent than for NMDA receptors. At micromolar concentrations the trivalent cations, La\(^{3+}\) and Gd\(^{3+}\) inhibit kainate receptor function in both neurons and cell lines expressing the GluR6 subunit (Huetten et al. 1998). In contrast, Zn\(^{2+}\) and Cd\(^{2+}\) enhance AMPA receptor function at low concentrations and inhibit at higher concentrations (Mayer et al. 1989; Rassendren et al. 1990). Zn\(^{2+}\) also reduces AMPA receptor desensitization in cultured superior colliculus neurons (Bresink et al. 1996).

We recently demonstrated that some positive modulators of AMPA receptors, such as cyclothiazide (CTZ), act to reduce a proton-induced enhancement of AMPA receptor desensitization (Lei et al. 2001). In the present paper, we examine the effects of Gd\(^{3+}\) on AMPA receptor function. Gd\(^{3+}\) is a highly effective blocker of a variety of ion channels likely because of its potent ability to reduce negative surface charges on the membrane (Xiong et al. 1997). Our results demonstrate that Gd\(^{3+}\) reduces AMPA receptor desensitization and deactivation while it also blocks AMPA receptors.

METHODS

Preparation of acutely isolated hippocampal CA1 neurons

The animals used in this work were handled under the regulations of the Canadian Institutes of Health Research. CA1 hippocampal pyramidal neurons were acutely isolated using modified procedures of Wang and MacDonald (1995) except that protease (type XIV, Sigma) instead of papain was used. Briefly, Wistar rats of 2- to 3-wk old were decapitated under halothane anesthesia using a guillotine. Hippocampi were quickly removed and placed in a dish containing cold oxygenated external solution consisting of (mM) 140 NaCl, 1.3 CaCl\(_2\), 5.4 KCl, 25 HEPES, 33 glucose, 1 MgCl\(_2\), and 0.0003 tetrodotoxin (TTX; pH, 7.4, osmolarity, 320–335 mosmol/l). The hippocampi were cut into 300- to 500-\(\mu\)m-thick slices by hand with a razor blade. The hippocampal slices were digested at room temperature (20–22°C) in...
the preceding solution containing 1.5 mg/ml protease. The incubation medium was stirred with pure oxygen blown in at the bottom of the vessel. After 40 min of enzymatic digestion, slices were rinsed three times with enzyme-free solution. The slices were maintained in the external solution, bubbled with oxygen, and used for up to 8–10 h. The CA1 region was dissected out with a scalpel under a phase-contrast microscope and then triturated with a fire-polished glass pipette. Data were obtained from the pyramidal cells that had a bright color and clear outline.

Whole cell recording

Whole cell recordings were performed with an Axopatch-1B amplifier (Axon Instruments) in voltage-clamp mode. Recording electrodes with resistances of 3–5 MΩ were constructed from thin-walled borosilicate glass (1.5-mm diam, WPI) using a two-stage puller (PP83, Narishige). Data were digitized, filtered (5kHz) and acquired on-line using the program pClamp6 (Axon Instruments). The standard external solution for recording electrodes consisted of the following (in mM) 140 CsF, 35 CsOH, 10 HEPES, 2 MgCl2, 2 tetrathyammonium, 11 EGTA, and 4 Na2ATP (pH 7.3, osmolarity, 300 mosmol/l). The time course of whole cell AMPA currents was similar when CsF was replaced with CsCl2 (Lei et al. 2000; Wang and MacDonald 1995). The standard external solution was the same as that previously described except that Mg2+ (3 mM) and AP5 (50 μM) were included to block N-methyl-d-aspartate (NMDA) receptors. After formation of the whole cell configuration, the recorded cells were voltage-clamped at −60 mV and lifted into the stream of solution. Saturating concentrations of glutamate (3–10 mM) were applied to the neurons for 2 s to evoke responses. Under these recording conditions, the currents evoked by glutamate were completely blocked by GYKI 53655, confirming their identity as AMPA receptor responses.

Nucleated or outside-out patch recording and ultra-fast perfusion

Outside-out patch recordings were carried out as previously described (Bai et al. 1999), and glutamate was applied using theta tubing connected to a piezoelectric translator (PZS-100, driven by PZ-150, Burleigh, Fishers). For solution exchange, τ is less than 200 μs as determined by measuring the open-tip junction potentials. Currents were filtered at 5 kHz and digitized at 50 kHz.

Nonstationary variance analysis

Nonstationary variance analysis was used to estimate conductance and the open probability of the channels at the peak of the response (Sigworth 1980). Generally, 60 responses evoked by applications of 10 mM glutamate for 100 ms at 5-s intervals were recorded for each outside-out patch. During an epoch of 60 responses, an average of 10% run-down was observed. Data from patches showing >20% run-down were not included for analysis. Responses from each patch were divided into 10–12 groups (5 for each group). After aligning each of the five responses to the peak, local means of each group were calculated to minimize the distortion originating from run-down. Each individual response was subtracted from the local mean of the group to compute the variance. Current responses 90 ms from the peak were selected for analysis. The mean current was divided into 100 equally sized bins, and the corresponding variances were pooled. The binned variance versus the mean current was plotted and fit with the equation: $\sigma^2 = il - I^2N + \sigma_{base}$, where $\sigma^2$ is the variance, $I$ is the mean current, $N$ is the number of channels activated at the peak, $l$ is the single channel current, and $\sigma_{base}$ is the background variance. Open probability at the peak, $P_{o, peak}$ was calculated by $P_{o, peak} = I_{peak} / (Nl)$, where $I_{peak}$ is the peak current; then, the single channel conductance was measured from $\gamma = I/Erev$, where $E$ is the holding potential, $E_{rev}$ is the reversal potential (0 mV under our recording conditions).

Miniature excitatory postsynaptic currents

Miniature excitatory postsynaptic currents (mEPSCs) mediated by AMPA receptors were recorded from mouse hippocampal neurons taken from wild-type or GluR2 null-mutant mice (Jia et al. 1996) cultured for 14–17 days as previously described (Lei et al. 2000). The extracellular solution was supplemented with TTX (0.5 μM), AP5 (50 μM), strychnine (1 μM), and bicuculline methiodide (20 μM). mEPSCs were filtered at 2 kHz. The synaptic events were acquired and analyzed using the SCAN program (Strathclyde Software). For detection, the trigger level was set approximately three times higher than the baseline noise. False events were eliminated by subsequent

\[ \text{FIG. 1. Effects of Gd}^{3+} \text{ on AMPA receptor currents evoked by glutamate or kainate. A: Gd}^{3+} \text{ enhanced the I_{peak} of glutamate-evoked current. B: summarized data (n = 10). C: AMPA receptor currents (I_{peak}) evoked by glutamate (1 mM) from a neuron were enhanced by different concentrations of Gd}^{3+}. D: concentration-response curve constructed from 7 neurons. Note the maximal effect occurred at 10 \mu M. At 30 \mu M, there was a slight inhibition. E: AMPA receptor currents evoked by kainate (100 \mu M) were enhanced by lower concentrations of Gd}^{3+} \text{ but depressed by higher concentrations of Gd}^{3+}. F: concentration-response curve constructed from 8 neurons. Note the maximal effect occurred at 3 \mu M.} \]
inspection of the raw data. Gd$^{3+}$ was dissolved in the extracellular solution and applied to the cells.

**Data analysis**

The time course of the onset of desensitization in outside-out patches was determined by fitting the decay of the response (beginning 10 points after the peak) with a single-exponential function. The decay of deactivation was fit with single-exponential function from 10 to 40% of the peak to exclude the influence of desensitization. Data were expressed as means ± SE. The values in parentheses refer to the number of cells used for the statistical analysis. Concentration-response curves were fit by Hill equation: $I = I_{\text{max}} \times \left[1/\left(1 + (EC_{50} / [\text{ligand}])^n\right)\right]$, where $I_{\text{max}}$ is the maximum response, $EC_{50}$ is the concentration of ligand producing a half-maximal response, and $n$ is the Hill coefficient. Statistical analyses were performed using Student’s t-test or by one-way ANOVA when appropriate. $P$ values less than 0.05 were taken as an indication of a significant difference.

**RESULTS**

**Modulation of whole cell AMPA receptor currents by Gd$^{3+}$**

We first studied the effect of Gd$^{3+}$ on glutamate-evoked AMPA receptor-mediated whole cell currents in isolated rat hippocampal CA1 neurons. Applications of Gd$^{3+}$ (5 μM) both in the control and glutamate barrels had little effect of the peak ($I_p$) of glutamate-evoked currents (Fig. 1, A and B). However, the steady-state currents ($I_{ss}$) were enhanced to 200 ± 25% of the control ($n = 10$, $P < 0.01$, Fig. 1B). The ratio of the $I_{ss}/I_p$ was, therefore, increased to 201 ± 25% of the control ($n = 10$, $P < 0.01$, Fig. 1B) suggesting that the extent of desensitization was decreased by Gd$^{3+}$.

We then constructed concentration-response relationships for Gd$^{3+}$ using glutamate as an agonist. At concentrations up to 10 μM, Gd$^{3+}$ potentiated $I_{ss}$ of glutamate-evoked currents in a concentration-dependent manner with a threshold of about 0.03 μM and an $EC_{50}$ value of 1.0 ± 0.2 μM ($n = 7$; Fig. 1, C and D). In contrast, higher concentrations (up to 30 μM or higher) of Gd$^{3+}$ depressed $I_p$ (Fig. 1D). This resembled the effects of Zn$^{2+}$ on AMPA receptor function (Rassendren et al. 1990).

AMPA receptor-mediated currents can also be evoked with applications of kainate that demonstrate much less complete desensitization than with glutamate. Similar to steady-state glutamate-evoked currents, the steady state of kainate responses was enhanced by low concentrations of Gd$^{3+}$ (0.1–10 μM; Fig. 1E) and inhibited by higher concentrations (Fig. 1, E and F). The $EC_{50}$ value for Gd$^{3+}$ was 0.63 ± 0.06 μM ($n = 8$) with a Hill coefficient of 1.60 ± 0.07 ($n = 8$).

**Gd$^{3+}$ increases the potency of glutamate**

We next examined whether or not low concentrations of Gd$^{3+}$ would enhance glutamate potency. Gd$^{3+}$ (10 μM) shifted the peak current versus glutamate concentration curve to the left (control, $EC_{50} = 0.15 ± 0.03$ μM; Gd$^{3+}$, 0.09 ± 0.02, $n = 9$, $P < 0.01$, Fig. 2, A and B) without changing that for steady-state currents (control, $EC_{50} = 0.06 ± 0.01$ μM; Gd$^{3+}$, 0.05 ± 0.01, $n = 9$, $P > 0.05$, Fig. 2C).

![FIG. 2. Gd$^{3+}$ enhanced the potency of glutamate. A: glutamate currents from the same neuron in the absence (top) and presence (bottom) of Gd$^{3+}$ (10 μM). B: concentration-response curve constructed by plotting glutamate concentration versus the peak currents ($n = 9$). Note that Gd$^{3+}$ slightly but significantly shifted the curve to the left. C: concentration-response curve constructed by plotting glutamate concentration vs. the steady-state currents ($n = 9$). Note that Gd$^{3+}$ did not significantly shift the curve.](http://jn.physiology.org/)
To determine if the enhancement induced by Gd$^{3+}$ was voltage dependent, we examined its effect on AMPA receptors activated by three different agonists, glutamate, AMPA, and kainate, at different holding potentials. Gd$^{3+}$ (5 μM) increased the steady-state currents evoked by all three agonists at each of the holding potentials tested, suggesting that the effect of Gd$^{3+}$ was unlikely to be voltage dependent (Fig. 3, A–C). The lesser degree of enhancement at depolarized potentials likely reflects the voltage dependence of desensitization itself.

**Gd$^{3+}$ both inhibits AMPA receptor-mediated currents and reduces desensitization**

Our results suggest that Gd$^{3+}$ may have reduced AMPA receptor desensitization, and therefore we examined the rate of onset of desensitization of AMPA currents in recordings from outside-out patches using an ultra-fast perfusion system. The decay of the current evoked by the application of glutamate (10 mM, 100 ms, the onset of desensitization) was well fit by single exponential function. Gd$^{3+}$ (5 μM) increased the apparent time constant of desensitization (control, 8.2 ± 1.5 ms; Gd$^{3+}$, 12.5 ± 2.0 ms, n = 9, P < 0.01, Fig. 4, A and B), suggesting that Gd$^{3+}$ does indeed reduce AMPA receptor desensitization. The peak resolved by our ultra-fast perfusion likely represents the activity of a majority of nondesensitized receptors. In this respect, Gd$^{3+}$ inhibited these peak currents by 14.6 ± 2.5% (Fig. 4, Ad and B, n = 9, P < 0.01), suggesting that Gd$^{3+}$ may also block AMPA currents at low concentrations in addition to reducing desensitization. To confirm this possibility, we used cyclothiazide (CTZ) to block AMPA receptor desensitization and anticipated that a blockade would be revealed even at relatively low concentrations of Gd$^{3+}$. For example, a low concentration of Gd$^{3+}$ enhanced kainate-evoked currents (Fig. 4C, left) in the absence of CTZ. As anticipated, applications of CTZ to the same cells dramatically increased the kainate-evoked current by reducing desensitization (Fig. 4C, right). However, in the presence of CTZ applications of Gd$^{3+}$ consistently inhibited currents by 6.4 ± 0.6% (n = 5, P < 0.01, Fig. 4, C and D), confirming the inhibition of AMPA receptor currents.

One possible explanation for the enhancement of steady-state currents was that Gd$^{3+}$ increased the open probability of AMPA channels even though it can potentially block open channels. Therefore we examined the effect of Gd$^{3+}$ on the open probability and the single-channel conductance of AMPA channels at the peak of the response using outside-out patches and nonstationary variance analysis. Gd$^{3+}$ reduced the open probability at the peak (control, 0.69 ± 0.04; Gd$^{3+}$, 0.45 ± 0.09, n = 5, P < 0.05) without changing the single-channel conductance (control, 13.6 ± 4.7 pS; Gd$^{3+}$, 10.2 ± 2.8 pS, n = 5, P > 0.05; Fig. 5). The reduction in the open probability may have resulted from a Gd$^{3+}$-induced inhibition of channel gating or a slowing of activation kinetics (see following text).

Another possibility is that Gd$^{3+}$ enhances steady-state currents by increasing the rate of recovery from desensitization. Therefore we examined recovery in outside-out patches using a paired-pulse paradigm in which the first application of glutamate (10 mM for 100 ms) was followed at variable intervals ranging, from 20 to 420 ms, by a second application of glutamate (10 mM, 20 ms; Fig. 6A). The ratio of the amplitude of the second to the first response (P2/P1) was plotted against the interpulse interval thus providing the time course of recovery from desensitization (Fig. 6B). The time constant of recovery, estimated by a single exponential fit, was reduced by Gd$^{3+}$ (control, τ = 47.8 ± 4.9 ms; Gd$^{3+}$, τ = 30.4 ± 3.7 ms, n = 5, P < 0.05) demonstrating that Gd$^{3+}$ likely enhances the rate of recovery from desensitization.

**Gd$^{3+}$ reduces both the activation and deactivation of AMPA receptors**

Several modulators of AMPA receptor desensitization, such as cyclothiazide, aniracetam and thiocyanate, also modulate AMPA receptor deactivation (Partin et al. 1996). Therefore we next examined the effect of Gd$^{3+}$ on AMPA receptor deactivation using the ultra-fast perfusion system. In the presence of...
Gd$^{3+}$, the deactivation time constant was enhanced by 18.0 ± 4.3% (control, $\tau = 2.7 \pm 0.2$ ms; Gd$^{3+}$, $\tau = 3.2 \pm 0.2$ ms, $n = 7$, $P < 0.01$), indicating the deactivation process was slowed by Gd$^{3+}$. We also observed an increase in the 10–90% rise time in the presence of Gd$^{3+}$, suggesting that activation kinetics were also slowed (Fig. 7). Therefore Gd$^{3+}$ may slow both the binding and unbinding of agonist to the receptor.

Several important functions of AMPA receptors such as the determination of Ca$^{2+}$ permeability, sensitivity to a blockade by polyamines and relative rates of desensitization are dependent on the presence or absence of the GluR2 subunit in the AMPA receptor (Dingledine et al. 1999). Most AMPA receptors in CA1 neurons contain the GluR2 subunit and this subunit determines the permeability of the channels to Ca$^{2+}$. Therefore we considered the possibility that the Gd$^{3+}$-induced modulation of desensitization required the presence of this subunit. However, Gd$^{2+}$ enhanced glutamate-evoked currents in the hippocampal CA1 neurons isolated from GluR2-deficient mice (Jia et al. 1996) (Fig. 8). Neither was the potency of Gd$^{3+}$ changed (Fig. 8, A and B).
The kinetics of excitatory postsynaptic currents in individual cells are determined by a combination of receptor deactivation, desensitization, and the rate of recovery from desensitized state (Edmonds et al. 1995; Trussell and Otis 1996). Since Gd$^{3+}$ slowed both deactivation and desensitization kinetics and enhanced the rate of recovery from desensitization, we next examined whether or not Gd$^{3+}$ modulates the kinetics of mEPSCs recorded in cultured hippocampal neurons. Bath application of Gd$^{3+}$ (5 $\mu$M) significantly increased the frequency of AMPA mEPSCs (control, 0.6 ± 0.1 Hz; Gd$^{3+}$, 4.0 ± 0.4 Hz, $n = 8$, $P < 0.01$, Fig. 9), suggesting that Gd$^{3+}$ also had presynaptic actions. Peak mEPSC and the 10–90% rise times were not significantly altered by Gd$^{3+}$ (Fig. 9C). However, the time constants of decay were consistently enhanced by Gd$^{3+}$ (Fig. 9), suggesting that Gd$^{3+}$ also modulates postsynaptic AMPA receptors.

**FIG. 5.** Gd$^{3+}$ reduce the maximum open probability of AMPA receptors determined by nonstationary variance analysis. A and B: 5 macroscopic current responses recorded from an outside-out patch excised from cultured hippocampal neuron before (A) and during (B) the application of Gd$^{3+}$ (5 $\mu$M) are superimposed. C and D: the composite current-variance plots of 50 responses before (C) and during (D) the application of Gd$^{3+}$ from the same patch in A and B are shown (corrected to 0). E: the conductances ($\gamma$) from 5 patches before and during the application of Gd$^{3+}$ are plotted. Gd$^{3+}$ did not significantly change $\gamma$. F: the open probabilities at the peak ($P_{o,\text{peak}}$) from 5 patches before and during the application of Gd$^{3+}$ are plotted. Gd$^{3+}$ significantly reduce $P_{o,\text{peak}}$ (*$P < 0.05$).

Application of Gd$^{3+}$ (5 $\mu$M) significantly increased the frequency of AMPA mEPSCs (control, 0.6 ± 0.1 Hz; Gd$^{3+}$, 4.0 ± 0.4 Hz, $n = 8$, $P < 0.01$, Fig. 9), suggesting that Gd$^{3+}$ also had presynaptic actions. Peak mEPSC and the 10–90% rise times were not significantly altered by Gd$^{3+}$ (Fig. 9C). However, the time constants of decay were consistently enhanced by Gd$^{3+}$ (Fig. 9), suggesting that Gd$^{3+}$ also modulates postsynaptic AMPA receptors.
The time course and amplitude of the macroscopic AMPA receptor-mediated current reflects multiple concurrent processes including receptor activation, deactivation, and desensitization (Partin et al. 1996). The behavior of AMPA receptors is complex and has been modeled using various multi-state kinetic schemes. However, for simplicity, we characterized the effects of Gd$^{3+}$ on AMPA receptor function by describing changes in the peak and steady-state current. Nevertheless it is recognized that this simplistic process understates the complexity of AMPA receptor function. Our results demonstrate that Gd$^{3+}$ reduces AMPA receptor desensitization in hippocampal neurons since Gd$^{3+}$ increased the ratio of steady state to peak currents in whole cell recordings, slowed the activations and deactivations kinetics of AMPA receptors, and accelerated the recovery from desensitization.

**FIG. 6.** Gd$^{3+}$ enhanced the rate of recovery from desensitization. A: current responses evoked by a paired-pulse paradigm with different intervals from an outside-out patch before (top) and during (bottom) the application of Gd$^{3+}$ (5 μM). Note that Gd$^{3+}$ enhanced the rate of recovery from desensitization. B: recovery curves from experiments similar to those shown in A. Each data point represented the mean ± SE of the ratio P2/P1 where P1 and P2 were the peak current amplitudes of the first and second pulse, respectively (n = 5). Data points were fit to a single exponential function. Gd$^{3+}$ significantly increased the recovery from desensitization.

**FIG. 7.** Gd$^{3+}$ slowed the activation and deactivation kinetics. A: current responses evoked by glutamate (10 mM, 1 ms) from an outside-out patch before and during the application of Gd$^{3+}$ (5 μM, left). The junction potential was shown on the top. The 2 current responses were normalized to show the activation and deactivation kinetics (right). B: summarized data from 7 patches. Note that Gd$^{3+}$ increased the deactivation time constants and 10–90% rise time, suggesting that Gd$^{3+}$ slowed both the activation and deactivation kinetics.
onset of desensitization in outside-out patches, and enhanced the recovery from desensitization. The observation that the enhancement of kainate-evoked currents by Gd$^{3+}$ was blocked in the presence of cyclothiazide, which blocks or eliminates AMPA receptor desensitization, also suggests that Gd$^{3+}$ reduces AMPA receptor desensitization.

A small region (about 38 amino acids) of the extracellular M3–M4 loop of AMPA receptors is formed from an alternatively spliced exon termed the flip/flop domain (Bennett and Dingledine 1995; Hollmann et al. 1994; Sommer et al. 1990; Sommer et al. 1990; Sommer et al. 1990; Sommer et al. 1990; Sommer et al. 1990; Sommer et al. 1990). This region regulates the kinetics of deactivation (Partin et al. 1996), the onset of and recovery from desensitization (Lomeli et al. 1994; Mosbacher et al. 1994; Sommer et al. 1990) as well as the sensitivity to drugs such as cyclothiazide, aniracetam, and thiocyanate (Johansen et al. 1995; Partin et al. 1994, 1996). Gd$^{3+}$ may also bind to this region to reduce AMPA receptor deactivation and desensitization based on our observations that Gd$^{3+}$ slowed both deactivation and desensitization kinetics, Gd$^{3+}$ increased recovery from desensitization, and the Gd$^{3+}$-mediated enhancement of kainate-evoked current was occluded by cyclothiazide.

In addition to reducing AMPA receptor deactivation and desensitization, Gd$^{3+}$ also had inhibitory effects on AMPA currents. When the Gd$^{3+}$ concentration was low, the inhibition was masked by the enhanced currents caused by reduction of AMPA receptor desensitization. The inhibitory effect was revealed by cyclothiazide or by the use of the ultra-fast perfusion to more accurately resolve peak currents. We failed to detect any change in the peak of whole cell currents evoked by glutamate in response to Gd$^{3+}$. There are several possible explanations for this discrepancy. For example, the properties (i.e., Gd$^{3+}$ sensitivity) of the channels may have been altered in the outside-out patch configuration as reported by Tong and Jahr (1994). Alternatively and more likely, the rate of solution exchange was so much slower in whole cell recordings that the apparent peak current may simply have reflected a balance between enhanced desensitization and inhibition.

The time course of deactivation and desensitization that we
observed for glutamate-evoked currents were in the same range as reported from hippocampal neurons by other laboratories (Donevan and Rogawski 1998; Fleck et al. 1996; Patneau et al. 1993). However, they were slower than those reported for chick nucleus magnocellularis (nMAG) neurons (Raman and Trussell 1992), rat MNTB relay neurons, and Bergmann glia (Geiger et al. 1995) or homo-oligomeric AMPA receptors (Mosbacher et al. 1994; Partin et al. 1996). In contrast, the recovery from desensitization of AMPA receptors in hippocampal neurons (τ548 ms) was faster than that reported for homo-oligomeric GluR1 receptors (τ147 ms) (Partin et al. 1996) but slower than that for reported for nMAG neurons (τ16 ms) (Raman and Trussell 1992). These differences between recombinant and native receptors or among the native neurons of different origins may be caused by heterogeneity in subunit composition and/or by differential RNA splicing/editing of individual subunits (Koike et al. 2000; Lomeli et al. 1994; Partin et al. 1994; Sommer et al. 1990) or perhaps due to methodological differences such as the use of different enzymes for isolating cells.

Application of Gd3+ to the cultured hippocampal neurons significantly enhanced the frequency of mEPSCs, suggesting that Gd3+ enhances release of transmitter. This result is consistent with a previous observation (Capogna et al. 1996), although the mechanism by which Gd3+ increases transmitter release is unclear. Nevertheless we also observed an increase in the time constant of the decay of mEPSC; this is consistent with actions on postsynaptic AMPA receptors. It is unlikely that desensitization contributes to the decay of mEPSCs, and it is more likely that the Gd3+-induced slowing of AMPA receptors deactivation (Colquhoun et al. 1992; Jonas and Sakmann 1992) is responsible for the prolongation of decay of mEPSCs we observed.

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