Enhancement of AMPA-Mediated Current after Traumatic Injury in Cortical Neurons

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Overactivation of ionotropic glutamate receptors has been implicated in the pathophysiology of traumatic brain injury. Using an *in vitro* cell injury model, we examined the effects of stretch-induced traumatic injury on the AMPA subtype of ionotropic glutamate receptors in cultured neonatal cortical neurons. Recordings made using the whole-cell patch-clamp technique revealed that a subpopulation of injured neurons exhibited an increased current in response to AMPA. The current–voltage relationship of these injured neurons showed an increased slope conductance but no change in reversal potential compared with uninjured neurons. Additionally, the EC_{50} values of uninjured and injured neurons were nearly identical. Thus, current potentiation was not caused by changes in the voltage-dependence, ion selectivity, or apparent agonist affinity of the AMPA channel. AMPA-elicited current could also be fully inhibited

by the application of selective AMPA receptor antagonists, thereby excluding the possibility that current potentiation in injured neurons was caused by the activation of other, non-desensitizing receptors. The difference in current densities between control and injured neurons was abolished when AMPA receptor desensitization was inhibited by the coapplication of AMPA and cyclothiazide or by the use of kainate as an agonist, suggesting that mechanical injury alters AMPA receptor desensitization. Reduction of AMPA receptor desensitization after brain injury would be expected to further exacerbate the effects of increased postinjury extracellular glutamate and contribute to trauma-related cell loss and dysfunctional synaptic information processing.

Key words: glutamate; traumatic brain injury; AMPA receptor; desensitization; excitotoxicity; cortex

It is commonly accepted that secondary or delayed neuronal damage after traumatic brain injury (TBI) is triggered, in part, by a disruption of ionic homeostasis. Substantial evidence implicates the activation of ionotropic glutamate receptors as a component of the biochemical cascade leading to abnormal neuronal function and secondary neuronal damage (Hayes et al., 1988; Faden et al., 1989; Bernert and Turski, 1996; Turski et al., 1998). Activation of ionotropic glutamate receptor channels, classified as NMDA receptors, kainate receptors, and AMPA receptors (Mayer and Westbrook, 1987; Collingridge and Lester, 1989), results in Na^+ and K^+ flux through all three receptor–channel subtypes, as well as Ca^{2+} influx via NMDA channels and certain subtypes of AMPA channels (Iino et al., 1990). Overactivation of NMDA and non-NMDA glutamate receptors results in neuronal injury and cell death *in vitro* (Rothman and Olney, 1986; Choi, 1987; Choi et al., 1987; Koh et al., 1990). Thus, excitotoxicity has been suggested as a contributing factor in the secondary pathology of TBI. This is supported by evidence demonstrating that extracellular glutamate levels are elevated after neurotrauma *in vivo* (Katayama et al., 1990; Palmer et al., 1993; Zauner et al., 1996), which would provide an abundant source of stimulatory agonist with which to activate these receptors. Furthermore, the administra-

tion of glutamate receptor antagonists before or after injury has been found to be neuroprotective in different models of neurotrauma, further supporting the excitotoxicity hypothesis (Hayes et al., 1988; Faden et al., 1989; Bernert and Turski, 1996; Turski et al., 1998).

In addition to the possibility that trauma-induced elevations in excitatory amino acids may excessively stimulate glutamate receptors, we previously found that mechanical deformation of cells can also directly alter the properties of glutamate receptors (Zhang et al., 1996). Using a unique *in vitro* cell injury model (Ellis et al., 1995), we found a reduction of the voltage-dependent Mg^{2+} blockade of NMDA channels in mechanically injured neurons, which in turn led to elevated intracellular $[Ca^{2+}]_i$ levels when these cells were challenged with exogenous NMDA (Zhang et al., 1996). In the current study, we used this cell injury model to examine the effects of mechanical injury on AMPA receptors. We report that mechanical injury also directly altered the AMPA receptors of cultured neonatal neurons, producing an enhancement of AMPA-mediated current that appears to be caused by decreased AMPA receptor desensitization. As for stretch-induced changes in NMDA receptors, this alteration would be expected to exacerbate the activation of glutamatergic receptors.

MATERIALS AND METHODS

Cell culture. Primary cultures of neuronal plus glial cells were prepared as described by McKinney et al. (1996) and used for all experiments. After decapitation, neocortices were isolated from 1- to 2-d-old Sprague Dawley rats (Zivic-Miller, Allison Park, PA). The neocortices were minced in saline, trypsinized (0.125%) for 10 min at 37°C, and then transferred to culture medium (DMEM containing 4.5 gm/l glucose supplemented with 10% FBS, 100 U/ml penicillin, 100 μ g/l streptomycin, and 2 mM, L-glutamine. The tissue was washed and dispersed by a series of triturations through Pasteur pipettes of decreasing diameter. The suspension

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was centrifuged for 10 min at $200 \times g$. The pellet was removed, culture media was added, and the suspension was triturated twice. The final suspension was filtered using an 88 μm nylon sieve and diluted with DMEM. Cells were plated at a density of 10^6 per well on collagen-coated SILASTIC (Dow Corning, Midland, MI) membranes that formed the bottom of a six-well Flex plate (Flexcell International, McKeesport, PA). Cell cultures were incubated at 37°C in 95% air–5% CO_2 . After 2–3 d, culture medium was replaced with growth medium (DMEM, 30 mM glucose, 100 U/ml, 100 $\mu\text{g}/\text{ml}$ penicillin/streptomycin, and 5% horse serum; no mitotic inhibitors were added). Cells were fed twice weekly and used after 10–16 d *in vitro* (DIV). The cultures consisted of neuronal and glial cells, which formed a confluent layer at 10–16 DIV.

Cell injury. Cells bathed in growth medium were injured as described by Ellis et al. (1995) with a model 94A cell injury controller (Commonwealth Biotechnology, Richmond, VA) at room temperature. A 50 msec pulse of compressed nitrogen deformed the SILASTIC membrane by 5.7 mm corresponding to a 31% stretch of the membrane and attached cells. This perturbation simulated mild, sublethal injury. After injury, cells were washed three times, and growth medium was replaced with external recording solution (see below). Control cells were treated identically with the exception that no injury was delivered. Experiments were performed from 10 min to 7 hr after injury, with the majority of recordings occurring between 30 min and 3.5 hr after injury.

Electrophysiology. Whole-cell voltage-clamp recordings were made with an Axopatch-1D amplifier (Axon Instruments, Foster City, CA). Patch electrodes (4–12 M Ω) were pulled from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) using a Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA). Pyramidal neurons were visualized using an Olympus Opticals (Tokyo, Japan) IMT-2 inverted microscope. Currents were digitized using a Macintosh Centris 800 (Apple Computer, Cupertino, CA) or Motorola (Phoenix, AZ) StarMax 3000/180 equipped with an Instrutech (Great Neck, NY) ITC-16 computer interface and Pulse Control (Herrington and Bookman, 1994) and Igor (Wavemetrics, Lake Oswego, OR) software. Recordings were filtered at 1 kHz and digitized at 2–5 kHz. Data were stored on video cassette recorder tape using an Instrutech VR-10-B digital data recorder. Statistics are expressed as mean $\pm$ SEM. During voltage-clamp experiments, cells were maintained at a holding potential of -40 mV (-47 mV with correction for the liquid junction potential), and current-voltage (I - V) relationships were generated using a voltage ramp (-100 to $+40$ mV; 22.6 mV/sec). Measurements of membrane potential have been corrected for the liquid junction potential (7 mV). Capacitance was calculated by integrating the transient current response to a small hyperpolarizing voltage step. For voltage-clamp experiments, patch electrodes were filled with a solution containing (in mM): CsAsp 135, KCl 4, NaCl 2, EGTA 10, CaCl_2 0.2, MgATP 2, NaGTP 0.6, and HEPES 10, pH 7.2. The external recording solution contained (in mM): NaCl 130, KCl 4, CaCl_2 3, MgCl_2 2, HEPES 10, glucose 11, and TTX 0.0005, pH 7.3. The osmolality of these solutions was 285 mOsm. For current-clamp experiments, patch electrodes were filled with a solution containing (in mM): Kasp 135, KCl 5, NaCl 2, MgCl_2 2, EGTA 1, CaCl_2 0.2, and HEPES 10, pH 7.2. The external recording solution contained (in mM): NaCl 130, KCl 5, CaCl_2 3, MgCl_2 2, and HEPES 10, pH 7.2. The osmolality of these solutions was 291 mOsm. Drugs used were AMPA, kainate, L-glutamic acid, and D(-)-2-amino-5-phosphonopentanoic acid (D-APV) (Research Biochemicals, Natick, MA), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (Tocris Cookson, Ballwin, MO), cyclothiazide (CTZ) (Sigma, St. Louis, MO), and LY303070 (gift from Eli Lilly, Indianapolis, IN). AMPA, kainate, glutamate, and D-APV were dissolved in saline. CNQX, CTZ, and LY303070 were dissolved in dimethylsulfoxide (final concentration 0.1 or 0.5%). All drugs were prepared as stock solutions (5–30 mM) and stored frozen. Solution changes were made with the use of a SF-77 Perfusion Fast-Step (Warner Instruments, Hamden, CT). Three adjacent square glass capillary tubes (600 μm width) were mounted on a manipulator and positioned within 100 μm of the neuronal cell body. Control and drug solutions were perfused through adjacent capillaries from independent reservoirs. Solutions were exchanged by laterally shifting the capillary tubes over the cell. Cells were continuously perfused with bath-applied standard external solution at a rate of 2.5 ml/min. All recordings were performed at room temperature.

RESULTS

A cell injury device delivered mechanical strain to 10- to 16-d-old rat cortical neurons cultured on a deformable SILASTIC membrane. A 50 msec pulse of compressed air deformed the mem-

Figure 1. Mechanical injury potentiates AMPA-elicited whole-cell current. *a*, Representative patch-clamp recordings of whole-cell currents elicited by the application of $100 \mu\text{M}$ AMPA (filled bar) for an uninjured neuron (left) and a stretch-injured neuron (right). Cells were voltage-clamped at -40 mV. Note differences in the scales used. The control neuron displayed both desensitizing and steady-state current components. In contrast, the injured neuron displayed increased current amplitude, as well as an apparent loss of desensitization. *b*, A subpopulation of injured neurons exhibit altered AMPA channel function in response to mechanical injury. Whole-cell currents were normalized by cell capacitance and expressed as current densities. Amplitude histograms of AMPA-elicited whole-cell steady-state current densities in uninjured neurons (left) and stretch-injured neurons (right). Mean steady-state current densities were 3.6 ± 0.3 pA/pF ($n = 39$) for uninjured neurons and 6.8 ± 0.6 pA/pF ($n = 59$) for injured neurons ($p < 0.0001$; Student's *t* test). A subpopulation of stretch-injured neurons possessed steady-state current densities of 8 pA/pF; this is greater than two SDs from the mean steady-state current density of control neurons.

brane 5.7 mm, which corresponds to 31% stretch and simulates mild-to-moderate sublethal injury (Ellis et al., 1995). Using the whole-cell patch-clamp technique (Hamill et al., 1981), agonist-activated currents were recorded from control and injured pyramidal neurons. Drugs were locally applied using a rapid application system. Under these conditions, sustained application of $100 \mu\text{M}$ AMPA elicited whole-cell currents with both desensitizing and steady-state components in uninjured neurons (Fig. 1*a*). Rapid and profound desensitization is a characteristic of AMPA receptors and has been demonstrated in a variety of cell types after application of selective agonists (Kiskin et al., 1986; Trussell et al., 1988; Tang et al., 1989). After mechanical injury, 33.9% of the injured neurons displayed a marked increase in peak and steady-state AMPA current amplitude and either an apparent reduction or a complete loss of AMPA receptor desensitization (Fig. 1*a*). Mean steady-state current amplitudes increased from 180.8 ± 20.7 pA ($n = 39$) to 411.7 ± 43.8 pA ($n = 59$; $p < 0.0001$) pA for control and injured neurons, respectively. To determine whether these differences were caused by differences in cell size,

currents were normalized by cell capacitance and expressed as current densities. Mean cell capacitance did not differ significantly between control and injured neurons: 49.8 ± 2.9 ($n = 39$) versus 57.0 ± 3.0 ($n = 59$; $p > 0.05$) pF. However, mean steady-state current density nearly doubled after mechanical injury: 6.8 ± 0.6 ($n = 59$) versus 3.6 ± 0.3 ($n = 39$; $p < 0.0001$) pA/pF.

As shown in amplitude histograms of the steady-state AMPA current densities measured in control and injured cells (Fig. 1*b*), there was an enhancement of AMPA-activated current in a subpopulation of neurons after injury. We identified potentiated neurons as injured neurons possessing a steady-state current density of 8 pA/pF. This current density is greater than two SDs from the mean steady-state current density of control neurons. Potentiated injured neurons did not display any striking morphological differences at the light microscope level when compared with control or nonpotentiated injured neurons having control-type AMPA channels. The expression of AMPA channel alteration also did not appear to be influenced by the location of the cell on the SILASTIC substrate or by cell size. The mean cell capacitance of potentiated injured neurons (58.4 ± 4.4 pF; $n = 20$) did not differ significantly from either nonpotentiated injured neurons (56.3 ± 3.6 pF; $n = 39$) or control neurons (49.8 ± 2.9 pF; $n = 39$; $p > 0.05$; ANOVA). There was no correlation between cell capacitance and steady-state AMPA current densities. The development of AMPA channel alteration did not appear to be time-dependent because there was no striking correlation between the time elapsed after injury and whether a cell displayed potentiated AMPA-elicited currents.

We next examined whether the potentiation of AMPA-elicited currents in injured neurons was mediated exclusively by AMPA receptors or whether it could reflect an enhanced contribution of non-AMPA receptor channels to the whole-cell current after injury. Although AMPA is mostly selective for the AMPA receptor, it may also act as a weak agonist for kainate receptors (Herb et al., 1992). We thus tested the sensitivity of AMPA-elicited currents to LY303070, a highly selective noncompetitive AMPA receptor antagonist to discriminate between AMPA and kainate receptor-mediated responses to AMPA (Bleakman et al., 1996). As shown in Figure 2, 30 μ M LY303070 completely inhibited whole-cell steady-state currents activated by 100 μ M AMPA in both control and injured neurons. Similar results were obtained with 50 μ M CNQX, a competitive antagonist of non-NMDA ionotropic glutamate receptors (Honoré et al., 1988), which produced 86–100% inhibition of AMPA-elicited currents. These findings show that the steady-state currents activated by AMPA in cortical neurons are primarily, if not entirely, caused by the activation of AMPA receptors and not a separate pool of non-desensitizing receptors, including kainate receptors.

We next investigated whether stretch injury increased the affinity of the AMPA receptor for AMPA, which could conceivably account for the enhanced current density observed after injury. As expected, concentration–response relationships for control and potentiated injured neurons revealed that the maximal steady-state current density, elicited by a saturating concentration of AMPA (100 μ M), increased from 2.7 ± 0.7 pA/pF ($n = 11$) for control neurons to 12.5 ± 1.2 pA/pF ($n = 8$; $p < 0.0001$) for potentiated injured neurons (Fig. 3). However, there was no significant difference in the AMPA concentration eliciting a half-maximal response in either the control or potentiated stretch-injured neurons. Thus, the EC_{50} for AMPA was 3.3 ± 0.7 μ M ($n = 11$) for control neurons and 3.1 ± 0.4 μ M ($n = 8$; $p > 0.50$) for stretch-injured neurons. This suggests that the apparent af-

Figure 2. Potentiated current is completely inhibited by AMPA receptor antagonists. Representative patch-clamp recordings of whole-cell currents elicited by the application of 100 μ M AMPA (filled bar), followed by 100 μ M AMPA and 30 μ M LY303070 (open bar) in an uninjured neuron (left) and potentiated stretch-injured neuron (right). Cells were voltage-clamped to -40 mV. LY303070 (30 μ M) completely inhibited AMPA-mediated currents in both the uninjured and injured neurons.

Figure 3. Mechanical injury does not alter the EC_{50} for AMPA. Concentration–response curves for uninjured (filled circles) and potentiated stretch-injured neurons (filled triangles) constructed from measurements of whole-cell steady-state current densities in response to AMPA concentrations ranging from 0.1 to 100 μ M. Maximal steady-state current density increased from 2.7 ± 0.7 pA/pF for uninjured neurons ($n = 11$) to 12.5 ± 1.2 pA/pF for injured neurons ($n = 8$; $p < 0.0001$; unpaired Student's t test). The EC_{50} values did not differ significantly between uninjured neurons (3.3 ± 0.7 μ M) and injured neurons (3.1 ± 0.4 μ M; $p > 0.50$; unpaired Student's t test). *Inset*, Concentration–response relationships have been normalized by the maximal response for each cell. Plotted are the means $\pm$ SEM.

finity of active AMPA receptors for AMPA is unaltered by stretch injury and cannot account for the increased current density observed after injury.

An additional possibility was that stretch injury increased steady-state current by altering the voltage dependence or ion selectivity of AMPA receptor channels. However, as shown in

Figure 4. Mechanical injury does not alter the I - V of the AMPA receptor channel. Representative I - V curves of AMPA-mediated whole-cell currents in uninjured neurons (left) and potentiated stretch-injured neurons (right). Current amplitudes were measured in the presence and absence of $100 \mu\text{M}$ AMPA as membrane voltage was ramped from -100 to $+40$ mV (23 mV/sec). AMPA difference currents were normalized with respect to the current measured at -100 mV. Stretch injury did not alter the reversal potential (-0.6 ± 3.2 mV; $n = 6$; vs -0.5 ± 1.4 mV; $n = 6$; $p > 0.1$; control vs injured, respectively) or rectification properties of AMPA-mediated current.

Figure 4, the I - V relationships of control and potentiated injured cells obtained under voltage clamp did not change significantly with injury. Both curves exhibited similar nonrectifying properties, and the mean reversal potential obtained for control neurons (-0.6 ± 3.2 mV; $n = 6$) did not differ significantly from that for potentiated injured neurons (-0.5 ± 1.4 mV; $n = 6$; $p > 0.10$). These results suggest that voltage dependence and ion selectivity are not strikingly altered by stretch injury, but that the whole-cell conductance activated by AMPA is increased by injury.

We hypothesized that a change in receptor kinetics, namely a decrease in receptor desensitization, might underlie the potentiation of whole-cell currents in stretch-injured neurons. However, although the rate of AMPA receptor desensitization is known to be extremely rapid, reportedly ranging from 2 to 40 msec in neurons (Kiskin et al., 1986; Trussell et al., 1988; Tang et al., 1989), the speed with which we could apply agonists to our single neurons was limited (>50 msec). Thus, to further explore the role of desensitization using the whole-cell approach, we eliminated fast desensitization by the coapplication of $100 \mu\text{M}$ AMPA and $100 \mu\text{M}$ CTZ, a drug that selectively blocks AMPA receptor desensitization (Bertolino et al., 1993). When CTZ was coapplied with AMPA, current density did not differ between control neurons (31.4 ± 6.2 pA/pF; $n = 10$) and potentiated injured neurons (35.7 ± 6.9 pA/pF; $n = 9$; $p > 0.50$). Measurements of whole-cell currents in response to $100 \mu\text{M}$ AMPA alone, before the addition of CTZ, were recorded in 5 of the 10 control neurons and all 9 injured neurons (Fig. 5). In these cells, $100 \mu\text{M}$ AMPA elicited significantly larger currents in injured neurons (10.6 ± 0.5 pA/pF) compared with control neurons (4.4 ± 0.7 pA/pF; $p < 0.0001$). However, coapplication of AMPA and CTZ to the same cells abolished the difference observed with AMPA alone, producing current densities of 41.6 ± 10.2 pA/pF ($n = 5$) and 35.7 ± 6.9 pA/pF ($n = 9$; $p > 0.50$) for control and injured neurons, respectively. Similar results were obtained when kainate was used as an agonist to activate AMPA receptors. Previous reports have suggested that kainate, at concentrations similar to that used for this

Figure 5. Blockade of AMPA receptor desensitization abolishes injury-induced current potentiation. *a*, Representative patch-clamp recordings of whole-cell currents activated by the application of $100 \mu\text{M}$ AMPA (filled bar) plus $100 \mu\text{M}$ CTZ (open bar) in an uninjured neuron (top left) and potentiated injured neuron (top right) or application of $200 \mu\text{M}$ kainate (filled bar) in an uninjured neuron (bottom left) and potentiated injured neuron (bottom right) show a lack of fast desensitization. Cells were voltage-clamped to -40 mV. *b*, Blockade of receptor desensitization by the coapplication of $100 \mu\text{M}$ AMPA plus $100 \mu\text{M}$ CTZ or the application of $200 \mu\text{M}$ kainate elicited whole-cell currents that did not differ between uninjured neurons and injured neurons displaying potentiated AMPA-elicited current. First four columns, Mean current density in response to $100 \mu\text{M}$ AMPA was 4.4 ± 0.7 ($n = 5$) and 10.6 ± 0.5 ($n = 9$) pA/pF for uninjured and injured cells, respectively ($p < 0.0001$; unpaired Student's t test). In the same cells, mean current density in response to AMPA-CTZ application was 41.7 ± 10.2 ($n = 5$) and 35.7 ± 6.9 ($n = 9$) pA/pF for uninjured and injured neurons, respectively ($p > 0.50$; unpaired Student's t test). Last four columns, Mean current density in response to $100 \mu\text{M}$ AMPA was 4.2 ± 0.6 ($n = 11$) and 12.5 ± 2.3 pA/pF ($n = 6$) for uninjured neurons and injured neurons, respectively ($p < 0.001$; unpaired Student's t test). In the same cells, mean current density in response to kainate application was 19.2 ± 4.6 and 17.5 ± 4.3 pA/pF for uninjured and injured cells, respectively ($p > 0.05$; unpaired Student's t test).

study (200 μM), activates but does not desensitize AMPA receptors (Kiskin et al., 1986; Trussell et al., 1988; Tang et al., 1989; Patneau and Mayer, 1991; Jonas and Sakmann, 1992; Raman and Trussell, 1992). Kainate (200 μM) elicited whole-cell currents with minimal detectable desensitization, and the amplitudes of these currents did not differ significantly between control and injured neurons that did exhibit current potentiation in response to AMPA only (Fig. 5). Mean current density activated by 100 μM AMPA was 4.2 ± 0.6 pA/pF ($n = 11$) for control neurons and 12.5 ± 2.3 pA/pF ($n = 6$; $p < .001$), for injured neurons. The mean current densities activated by 200 μM kainate in the same cells were 19.2 ± 4.6 ($n = 11$) and 17.5 ± 4.3 ($n = 6$, $p > 0.10$) pA/pF, for control and injured neurons, respectively. Kainate-activated currents were also fully blocked by 30 μM LY303070, indicating the current was mediated by AMPA and not kainate receptors. Because the potentiation caused by stretch injury was abolished by conditions that minimized desensitization, the data *in toto* support the hypothesis that mechanical injury potentiates AMPA-mediated current by decreasing AMPA receptor desensitization.

To test whether the changes in AMPA-elicited currents that we observed in voltage clamp were functionally relevant, we tested whether the application of the physiological AMPA agonist L-glutamate to control versus injured neurons differentially affected their electrical activity. Voltage responses elicited by 1 and 2.5 μM glutamate were recorded from control and injured neurons in current clamp (Fig. 6*a*). External APV (20 μM) was included to block the possible contribution of NMDA receptors, whereas the contribution of AMPA receptor current to these voltage responses was quantified as the amount of depolarization that was LY303070 (30 μM) blockable. To distinguish potentiated from nonpotentiated injured neurons, whole-cell AMPA currents were measured in voltage clamp at the end of each experiment. LY303070-sensitive responses were then compared between control, potentiated, and nonpotentiated injured neurons (Fig. 6*b*).

The resting membrane potential of the cortical neurons before glutamate application was not significantly different between control cells (-58.4 ± 1.3 mV; $n = 6$), nonpotentiated injured cells (-57.7 ± 0.4 mV; $n = 7$), and potentiated injured cells (-59.1 ± 1.5 mV; $n = 5$; $p > 0.50$; ANOVA). As shown in Figure 6, the rapid application of 2.5 μM glutamate to injured neurons having potentiated AMPA currents produced pronounced membrane depolarization and prominent cell firing. In contrast, control or nonpotentiated neurons typically had much smaller responses to glutamate. The mean AMPA antagonist-sensitive membrane depolarization caused by 2.5 μM glutamate was 20.6 ± 3.3 mV ($n = 5$) for potentiated injured neurons, which was significantly larger than for control (4.1 ± 1.3 mV; $n = 6$) or nonpotentiated injured neurons (5.7 ± 2.7 ; $n = 7$; $p < 0.01$; ANOVA). Responses to 1 μM glutamate were minimal and did not differ significantly between the three groups.

Thus, the decreased desensitization and concomitant potentiation of AMPA-mediated current that we observed after mechanical injury resulted in the potentiation of glutamate-induced membrane depolarization and cell firing. These functional correlates may be important because CSF glutamate concentration is known to be elevated after *in vivo* TBI. (Katayama et al., 1990; Palmer et al., 1993; Zauner et al., 1996). Thus, the alterations we report here may contribute to increased glutamate excitotoxicity via the activation of potentiated AMPA receptors, if this mechanism is indeed operative *in vivo*.

Figure 6. Electrical responses are larger in neurons having potentiated AMPA currents. *a*, Measurements of membrane potential in an uninjured neuron (top trace) and potentiated injured neuron (bottom trace) in response to the application of 2.5 μM L-glutamate, followed by L-glutamate plus 30 μM LY303070, the selective AMPA receptor antagonist. APV (20 μM) was added to solutions to block the contribution of NMDA receptors. The resting membrane potential of the uninjured and injured neurons were -61.8 and -63.2 mV, respectively. For the control neuron, application of glutamate produced a smaller membrane depolarization (10.1 mV) that was relatively insensitive to LY303070 (2.6 mV blocked) and in this case no firing. For the injured neuron, glutamate produced a much larger depolarization (31.2 mV) that triggered prominent action potentials, and this depolarization was almost completely inhibited by LY303070 (27.9 mV blocked). *b*, Mean AMPA receptor-mediated depolarization for uninjured, nonpotentiated injured, and potentiated injured neurons. Membrane depolarization was significantly larger for potentiated injured neurons (20.6 ± 3.3 mV; $n = 5$) compared with control (4.1 ± 1.3 mV; $n = 6$) and nonpotentiated injured neurons (5.7 ± 2.7 mV; $n = 7$; $p < 0.01$; ANOVA).

DISCUSSION

The potentiation of AMPA current amplitude and a concomitant prolongation of AMPA receptor activation after mechanical injury would be expected to lead to elevated ionic influx in the face of increased glutamate concentration in the brain. This would be expected to produce ionic imbalances that may contribute to the secondary pathophysiology of TBI. Elevated cation influx would be expected to produce cell swelling and/or membrane depolarization with consequent Ca^{2+} entry via voltage-sensitive Ca^{2+} channels (VSCCs). Calcium influx via VSCCs or Ca^{2+} -permeable AMPA channels may lead to elevated $[\text{Ca}^{2+}]_i$, which has been linked to neuronal dysfunction and death (McIntosh et al., 1998). The neurotoxic effects observed after excessive glutamate exposure and overactivation of NMDA and non-NMDA receptors are well documented (Rothman and Olney, 1986; Choi, 1987; Choi et al., 1987; Koh et al., 1990). Enhanced AMPA channel activity after mechanical injury would thus be expected to exacerbate the effects of the sustained elevation of extracellular glutamate observed after TBI or secondary ischemia (Benveniste et al., 1984; Katayama et al., 1990; Palmer et al., 1993; Zauner et al., 1996). In support of this, studies have shown that blockade of AMPA receptor desensitization via wheat germ agglutinin or CTZ application augments AMPA-mediated neurotoxicity in hippocampal, neocortical, and cerebellar Purkinje neurons (Zorumski et al., 1990; Jensen et al., 1998; Ohno et al., 1998).

Additionally, there is evidence suggesting that TBI results in compromised cellular metabolism (Vink et al., 1988; Dietrich et al., 1994), and, in our *in vitro* model, we have found that stretch injury causes an inhibition of the Na^+/K^+ ATPase because of an apparent decrease in metabolic energy (Tavalin et al., 1997). A reduction in Na^+/K^+ ATPase function would further amplify the ionic imbalance caused by increased Na^+ influx via altered AMPA receptors, leading to a reduction in transmembrane ion gradients, cell swelling, and membrane depolarization, even after abnormal levels of extracellular glutamate are cleared.

Potentiation of AMPA receptor function could also result in dysfunctional neuronal synaptic communication under conditions of high extracellular glutamate and even after a return to normal glutamate levels. Although it remains unresolved whether AMPA receptor desensitization influences the amplitude and time course of individual synaptic events at all synapses (Kiskin et al., 1986; Trussell et al., 1988; Tang et al., 1989; Colquhoun et al., 1992; Silver et al., 1996; Arai and Lynch, 1998a), receptor desensitization has been shown to attenuate the responsiveness of AMPA receptors after repetitive stimulation (Arai and Lynch, 1998b). Additionally, *in vitro* inhibition of AMPA receptor desensitization by pharmacological agents enhances excitatory postsynaptic potentials—currents in a variety of neuronal preparations (Vyklícký et al., 1991; Pelletier and Hablitz, 1994; Boxall and Garthwaite, 1995). An indiscriminate increase in synaptic efficacy caused by the reduction of AMPA receptor desensitization could thus disrupt neuronal signaling and could conceivably contribute to the behavioral and cognitive deficits that develop after neurotrauma (McIntosh et al., 1989; Lyeth et al., 1990; Hamm et al., 1992).

How might mechanical injury modulate AMPA desensitization? Although we are actively pursuing studies designed to shed light on this question, such alteration could conceivably result from either a direct effect of mechanical deformation on the AMPA channel itself or through an indirect mechanism involving intracellular signaling molecules, which in turn target the channel.

As for the former class of mechanism, physical strain in this scenario would be directly transferred to the AMPA receptor channel itself and conceivably alter receptor desensitization. Because AMPA channels are known to be multimeric receptors (for review, see Hollmann and Heinemann, 1994; Dingledine et al., 1999) and because the rate of AMPA receptor desensitization appears to be determined at least in part by the particular subunit composition of the receptor and by specific extracellular domains (for review, see Dingledine et al., 1999), strain transferred during stretch injury might directly and irreversibly alter AMPA receptor kinetics by either disrupting the physical interactions between different AMPA receptor subunits or by distorting the microdomains within single subunits that are involved in mediating desensitization. In general support of this concept, it is widely held that mechanical deformation can activate or modulate certain ion channels, including, for example, stretch-activated ion channels (for review, see Hamill and McBride, 1996) and NMDA channels (Paoletti and Asher, 1994).

Additionally, it is possible that the neuronal cytoskeleton may serve as the major strain detector in mechanical injury, and, in this case, alterations in the linkages between the cytoskeleton and AMPA channels or associated proteins could conceivably change AMPA receptor function. Although we have not yet specifically investigated whether cytoskeletal integrity is compromised in the *in vitro* model system we used, there is ample evidence that the neuronal cytoskeleton is altered after TBI *in vivo* (Yaghai and Povlishock, 1992; Povlishock and Pettus, 1996; Saatman et al., 1998).

Alternatively, mechanical stretch may indirectly alter AMPA receptor function by selectively activating intracellular second messenger systems, resulting in changes in the phosphorylation state of the AMPA channel. There is substantial evidence that AMPA receptors are targets for several protein kinases, including calcium/calmodulin-dependent protein kinase II (Barria et al., 1997), PKC (Wang et al., 1994), and PKA (Greengard et al., 1991; Wang et al., 1991). Interestingly, protein kinase A, which potentiates the AMPA currents of cultured neurons through increases in channel open time and probability of opening (Greengard et al., 1991; Wang et al., 1991), has also been shown to reduce the desensitization of AMPA channels in horizontal cells of the perch retina (Hatt, 1999). However, it is not known whether PKA activity is altered after brain trauma.

Evidence in support of an indirect mechanism involving PKC activation was previously proposed by our laboratory to account for stretch-induced modulation of NMDA receptors in this same injury model (Zhang et al., 1996). Thus, we found that pretreatment of cortical neurons with the PKC inhibitor calphostin C partially abrogated injury-induced loss of Mg^{2+} block of the NMDA channel. This result was consistent with earlier reports that, in nodose neurons, Mg^{2+} block of NMDA receptors was decreased by PKC stimulation (Chen and Huang, 1992) and that mechanical strain activates specific PKC isoforms (Persson et al., 1995). Thus, it would seem feasible that mechanical injury could indirectly alter AMPA receptor desensitization through changes in channel phosphorylation. Because Weber et al. (1997) reported that intracellular $[\text{Ca}^{2+}]$ is elevated immediately after mechanical stretch of our mixed neuron–glia cultures, an early rise in cytoplasmic calcium could be an initiating signal in these cortical neurons or glia.

In summary, as previously reported for the NMDA subtype of ionotropic glutamate receptors, we have found that a substantial fraction of fast-desensitizing AMPA receptors are altered by

mechanical injury in cortical neurons. This alteration is suggested to result in further overstimulation of brain excitatory pathways and increased vulnerability to neuronal dysfunction and neuronal death after mechanical trauma to the brain.

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