0026-895X/00/061204-09$3.00/0
MOLECULAR PHARMACOLOGY
Copyright © 2000 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 58:1204–1212, 2000

Vol. 58, No. 6
305/866275
Printed in U.S.A.

Modulation of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid Receptor Desensitization by Extracellular Protons

EVA C. IHLE and DORIS K. PATNEAU

Committee on Neurobiology and Department of Neurobiology, Pharmacology and Physiology, University of Chicago, Chicago, Illinois

Received May 24, 2000; accepted August 28, 2000

This paper is available online at http://www.molpharm.org

ABSTRACT

The interstitial milieu of the brain is buffered to an average pH of 7.3, but synaptic activation produces a temporal sequence of events that can affect pH in the synaptic cleft. Furthermore, pathophysiological processes such as ischemia and seizures produce global and prolonged acidification of interstitial pH. Changes in pH, in turn, can affect neuronal excitability by modulating receptors and channels. Patch-clamp recordings were made from cultured rat hippocampal neurons to determine whether physiologically relevant changes in interstitial pH (6.5–7.8) significantly affect AMPA receptor function. Acidic pH, such as that typically associated with ischemia (pH 6.5), significantly inhibited AMPA receptor-mediated responses in neurons. The effect of pH was agonist-dependent, with 2-fold greater inhibition of responses evoked by the strongly desensitizing agonists glutamate and quisqualate than the weakly desensitizing agonist kainate. Additional experiments tested the hypothesis that protons modulate AMPA receptor desensitization. In the presence of drugs that block AMPA receptor desensitization, pH 6.5 had no effect on glutamate-evoked responses. In neuronal macropatches, protons increased equilibrium desensitization without affecting macroscopic desensitization or deactivation kinetics. The mechanisms and molecular determinants of pH-mediated effects were further investigated using human embryonic kidney 293 cells expressing recombinant AMPA receptors. Inhibition of kainate-evoked responses varied with subunit and isoform composition, ranging from 10% to >40%. *Flop* isoforms, which exhibit faster and more extensive desensitization, were most strongly inhibited. These findings suggest that interstitial acidification can modulate AMPA receptor-mediated synaptic transmission and that differences in receptor sensitivity to proton modulation may underlie the selective vulnerability of certain neuronal populations to ischemia.

Synaptic activity induces significant temporal and spatial fluctuations in interstitial pH of the brain (Chesler, 1990; Chesler and Kaila, 1992). Gross changes lasting milliseconds to minutes have been measured both in vivo and in vitro using pH-sensitive microelectrodes (Kraig et al., 1983; Chen and Chesler, 1992) and optical methods (Krishtal et al., 1987; Gottfried and Chesler, 1996). Local pH fluctuations in the synaptic cleft are believed to be both larger and briefer (Chesler and Kaila, 1992). Furthermore, pathophysiological insults such as spreading depression and seizures reduce brain pH by approximately 0.4 pH units (Kraig et al., 1983; Somjen, 1984; Siesjö et al., 1985), whereas ischemia or hypoxia can shift interstitial pH to 6.5 and below for prolonged periods (Siemkowicz and Hansen, 1981; Siesjö, 1988).

Many ligand-gated channels, including those gated by glutamate, are sensitive to extracellular pH. Previous studies examining modulation of mammalian glutamate receptors by extracellular pH have focused primarily on *N*-methyl-D-aspartate (NMDA) receptors, which are inhibited significantly even at pH 7.3 (Tang et al., 1990; Vyklicky et al., 1990; Traynelis and Cull-Candy, 1991). The inhibition of non-NMDA receptors by extracellular protons observed in these studies was modest in comparison, and was not considered physiologically relevant (Traynelis and Cull-Candy, 1991). However, because activation of a typical glutamatergic synapse produces < 200 μV depolarization (Andersen, 1990), a high degree of temporal and/or spatial summation is necessary to produce an action potential, and even small pH-mediated changes in AMPA receptor-mediated postsynaptic potentials could affect the efficacy of synaptic transmission in the central nervous system. Transient changes in pH in the synaptic cleft sufficient to significantly affect the function of postsynaptic AMPA receptors are likely to occur, particularly with high-frequency synaptic activity (Chesler, 1990).

Molecular receptor composition has previously been shown to affect proton modulation of NMDA and γ-aminobutyric acid (GABA) receptors (Traynelis and Cull-Candy, 1995;

This work was supported by grants from the American Heart Association and Brain Research Foundation to D.K.P. and the Women's Council of the Brain Research Foundation to E.C.I.

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; GABA, γ-aminobutyric acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; HEK, human embryonic kidney; GluR, glutamate receptor; BES, *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic; MK-801, (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Krishek et al., 1996). Differences in the proton sensitivity of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors of varying composition has not been examined, but could be important for understanding the vulnerability of some neurons to excitotoxicity. Certain neuronal populations are selectively vulnerable to ischemia, including principal neurons in the hippocampus, striatum and cerebellum, although in these same regions interneurons may be spared (Cervos-Navarro and Diemer, 1991). This selective vulnerability may be explained by the particular subtypes of AMPA receptors that the different cells express. For example, the selective vulnerability of Purkinje cells may result from expression of AMPA receptors that exhibit less complete desensitization (Brorson et al., 1995) because they contain the *flip* isoforms of AMPA receptor subunits (Tomiyama et al., 1999).

AMPA receptor antagonists are neuroprotective in in vivo models of ischemia (Sheardown et al., 1990; Gill, 1994) and are also potent anticonvulsants (Chapman et al., 1991), suggesting that AMPA receptors play an important role in both ischemia and seizures, two pathophysiological conditions accompanied by significant acidification in brain. NMDA antagonists are less neuroprotective in models of ischemia (Buchan et al., 1991), presumably because NMDA receptors are already inhibited by acidic pH (Giffard et al., 1990; Tombaugh and Sapolsky, 1990). It was previously assumed that acidification would also protect against AMPA receptor-mediated neurotoxicity. In a recent article, however, McDonald et al. (1998) found that neurotoxicity mediated by AMPA receptors is enhanced rather than inhibited at acidic pH. Thus, modulation of AMPA receptors by protons seems to have significant consequences. A more detailed analysis is indicated, to identify the mechanisms responsible for the effects of protons on AMPA receptor function and to determine whether the molecular composition of AMPA receptors affects their sensitivity to proton modulation.

The experiments described below demonstrate significant modulation of AMPA receptors by pH changes typically associated with ischemia, and focus on identification of the mechanisms and molecular determinants of this modulation. The results identify several factors affecting the magnitude of proton-mediated modulation of AMPA receptors, including agonist properties and the molecular composition of receptors, and suggest that protons selectively interact with a desensitized state of the receptor.

Experimental Procedures

Cell Culture. Primary cultures of hippocampal neurons were prepared according to established methods (Patneau et al., 1993). Briefly, the hippocampi were dissected from the brains of newborn (P0–1) Sprague-Dawley rats, incubated in papain (Worthington Biochemical Corp., Lakewood, NJ) and dissociated by trituration with a glass pipette. The cells were suspended in 90% Dulbecco's modified Eagle's medium/10% FBS supplemented with 2 mM Glutamax (Life Technologies, Rockville, MD) and 1% penicillin/streptomycin, plated onto glass coverslips precoated with collagen and poly-L-lysine, and maintained at 37°C in a humidified 10% CO_2 incubator. Under these conditions glia proliferate and few neurons survive. After the glial monolayer became confluent, 33 μg/ml uridine and 13 μg/ml 5′-fluorodeoxyuridine was added to the culture medium to inhibit mitosis. Neurons from a second dissociation were plated onto the glial monolayer and maintained in 94% Dulbecco's modified Eagle's medium/5% horse serum/1% FBS, supplemented with 2 mM Glutamax, 5′-fluorodeoxyuridine, and a neuronal supplement. Experiments were performed at room temperature (23–25°C) using neurons after 2 to 15 days in culture.

For expression of recombinant AMPA receptors, human embryonic kidney (HEK) 293 cells (American Type Culture Collection, Manassas, VA) were grown at 37°C in a humidified 5% CO_2 incubator in 90% minimal essential medium plus Earle's salts/10% FBS, supplemented with 2 mM L-glutamine. Plasmids containing cDNA for the glutamate receptor (GluR) subunits GluR-A, -B, -C and -D, in both *flip* (*i*) and *flop* (*o*) isoforms, were gifts from Dr. P. Seeburg (Heidelberg, Germany). The plasmids were grown in *Escherichia coli* and purified by 2× CsCl density centrifugation or using a plasmid kit (Qiagen Inc., Valencia, CA). Cells were transfected with cDNA (10 μg/60 mm dish) using a modified $CaPO_4$ precipitation method (Chen and Okayama, 1987). After 8 to 10 h, the medium containing precipitate was removed and the transfected HEK 293 cells were replated onto collagen/poly-L-lysine-treated coverslips in growth medium that contained 0.5 mM kynurenate to minimize excitotoxicity. Electrophysiological recordings were made 18 to 48 h later.

Recording Solutions. Extracellular saline for neuronal experiments contained 166 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM BES (or HEPES), 10 mM glucose, and 0.01 mg/ml phenol red; the osmolarity was adjusted to 325 mOsM, and the pH titrated with NaOH to the desired value. When varying pH, the buffer species was selected to maintain optimal buffering capacity over the pH range examined and was held constant in each experiment because significant effects of varying buffer were observed in preliminary experiments (data not shown). Thus HEPES (pK_a = 7.48) buffered the solutions for experiments examining the effects of alkalinization, whereas BES (pK_a = 7.09) buffered solutions for all other experiments. Tetrodotoxin (400 nM; Calbiochem, La Jolla, CA), bicuculline (5 μM), and (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801; 500 nM) were added to block voltage-gated sodium channels, GABA receptors, and NMDA receptors, respectively. MK-801 was also added to neuronal cultures in the incubator to allow spontaneous synaptic activity to effect the essentially irreversible activity-dependent block of NMDA receptors before recording. The standard intracellular solution for neurons contained 125 mM $CsMeSO_3$ (Aldrich Chemical Co., Milwaukee, WI), 15 mM CsCl, 10 mM HEPES, 5 mM Cs_4BAPTA (Molecular Probes, Eugene, OR), 0.5 mM $CaCl_2$, 3 mM $MgCl_2$ and 2 mM Na_2ATP; for recording from nucleated macropatches, 10 mM CsF replaced 10 mM $CsMeSO_3$. The osmolarity was adjusted to 305 to 310 mOsM, and the pH was titrated to 7.2 with CsOH. In some experiments, 35 mM $CsMeSO_3$ was replaced with 20 mM creatine phosphate (di-Tris salt) and an additional 2 mM Na_2ATP and 50 U/ml creatine phosphokinase were included (to regenerate ATP).

Extracellular saline for recording from HEK 293 cells contained 145 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM BES, and 0.01 mg/ml phenol red. The osmolarity was adjusted to 295 mOsm and the pH to 6.5 or 7.3 with NaOH. In some experiments, the $[Ca^{2+}]$ was lowered to 0.2 mM to reduce rundown of responses. Patch pipettes were filled with intracellular solution containing 135 mM CsCl, 10 mM CsF, 3 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM Cs_4BAPTA, 10 mM HEPES and 2 mM Na_2ATP. Osmolarity was adjusted to 295 mOsM and pH to 7.2 with CsOH. In some experiments, the intracellular solution was ATP-regenerating (see above).

Electrophysiology. The recording chamber was continuously perfused with control extracellular saline at 0.2 to 0.5 ml/min. Recording electrodes were pulled from borosilicate glass, coated with Sylgard, and fire-polished; typical electrode resistance was 2 to 4 MΩ when filled with intracellular solution. An isolated neuron or HEK 293 cell was voltage-clamped in the whole-cell configuration at −60 mV using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA) and standard techniques. In experiments to examine the kinetics of desensitization, nucleated outside-out patches (macropatches) were used (Sather et al., 1992; Patneau et al., 1993). Resis-

tance in series with the cell or macropatch was typically 4 to 8 MΩ and was compensated by 60 to 90%. Data for on-line recording were filtered at 1 to 5 kHz and sampled at 2 to 20 kHz using pClamp (Axon Instruments).

Perfusion Techniques. For whole-cell recording, rapid agonist application was achieved using a glass flowpipe array (6–8 parallel barrels, each 400 μm in diameter) mounted on a 'bimorph' ceramic wafer. The flowpipe array was placed near a voltage-clamped cell. Solutions were driven by a peristaltic pump (Minipuls3; Gilson, Middleton, WI) through three-way Isolatch valves (Parker Hannifin, Fairfield, NJ). Control solution (at the desired pH) from one barrel of the flowpipe continuously bathed the cell and its processes until agonist application was initiated; at this time the bimorph was charged, which rapidly moved an adjacent barrel containing agonist solution in front of the cell. Then, simultaneously, the valve regulating control solution flow was closed and the valve for agonist flow was opened. This system achieves solution exchange around a small neuron and its processes within approximately 10 ms. Other cells in the recording chamber were partially protected from pre-exposure to agonists by the inclusion of 0.5 mM kynurenate in the bath solution.

For fast perfusion of macropatches, solutions were gravity-fed through four-barreled square glass tubing pulled to a width of approximately 100 μM per barrel. Recording salines at pH 7.3 were in one horizontal pair of barrels (control and agonist), salines at pH 6.5 in the second pair. The tubing was mounted on a piezo translator driven by a 100 V power supply (PZ 100; Burleigh Instruments Inc., Fishers, NY). A macropatch was positioned in the control solution stream, near the interface between control and agonist-containing solutions. Upon charging or discharging of the piezo element, the interface between solutions was rapidly moved across the macropatch. This system achieves solution exchange on a nucleated macropatch in $\leq$ 300 μs, as determined from the 10 to 90% rise- and decay-time for a sodium concentration change in the presence of kainate. Vertical displacement of the recording pipette moved the macropatch between solutions at pH 7.3 and pH 6.5. At the end of each recording, to verify the correct positioning of the macropatch for optimal solution exchange, the macropatch was disrupted and junction potentials at the pipette tip were recorded ($[Na^+]$ was 6 mM lower in the control solutions; see Fig. 3 for examples). Data were excluded if the junction potentials indicated the macropatch was not optimally positioned.

Data Analysis. Peak and steady-state amplitude and kinetic measurements were based on single or the average of a few (3–5) agonist-evoked responses. The majority of experiments examined the effects of protons on steady-state responses. Peak response amplitudes and kinetic measurements were only analyzed for macropatches, because the exchange time for whole-cell perfusion was too slow (see above) to make peak measurements reliable. Control responses preceded and followed an experimental manipulation. To correct for rundown, the experimental observation was normalized relative to the average of the two control responses. Numbers and statistical analyses in the text are based on these corrected responses. However, both control responses are illustrated in some bar graphs to demonstrate recovery (see Fig. 1). Because substantial rundown can indicate a compromised recording, data exhibiting more than a 15% (neurons) or 25% (macropatches and HEK 293 cells) decline in control responses were excluded from analysis.

The kinetics of desensitization and deactivation were determined from exponential fits to responses from macropatches using a Chebyshev (pClamp) or Simplex (Kaleidagraph; Synergy Software, Reading, PA) fitting algorithm. Significance of pH-mediated effects was evaluated with paired-differences or Student's *t* tests. All data in the text are reported as mean ± S.D. unless noted otherwise. Bar graphs show mean ± S.E.M.

Materials. Stock solutions of most drugs were prepared in the appropriate intracellular or extracellular solution, stored frozen, and added to experimental solutions immediately before use. When a very high final concentration ($\geq$ 3 mM) of drug was required, the stock solution was made in NaCl-reduced saline to keep the final Na^+ concentration constant across experimental solutions. Cyclothiazide was dissolved in dimethyl sulfoxide at 20 mM before dilution with extracellular solution, and an equivalent final concentration of dimethyl sulfoxide (0.5%) was added to all experimental and control solutions. The pK_a values of the carboxylic acid group of glutamate, quisqualate, homocysteate, kainate, and AMPA were determined by standard pH titration methods, with pK_a values of 4.41, 4.30, 2.79, 4.32, and 5.28, respectively. Thus, the concentrations of the unprotonated form of the first four of these agonists in solution at pH 6.5 and 7.3 were negligibly different and did not require adjustment; AMPA was not used because it would be partially protonated at pH 6.5. AMPA, bicuculline, kynurenate, MK-801, and quisqualate were purchased from Tocris Cookson (Ballwin, MO). Other reagents were obtained from Sigma Chemical (St. Louis, MO) unless noted otherwise.

Results

Modulation of AMPA Receptors by Protons Is Agonist-Dependent. Initial experiments focused on proton-mediated modulation of kainate- or glutamate-evoked responses in hippocampal neurons within a physiologically relevant pH range (pH 6.5–7.8). Alkaline pH (7.8) did not affect the amplitude of AMPA receptor-mediated responses to kainate or glutamate (data not shown). Because pathophysiological pro-

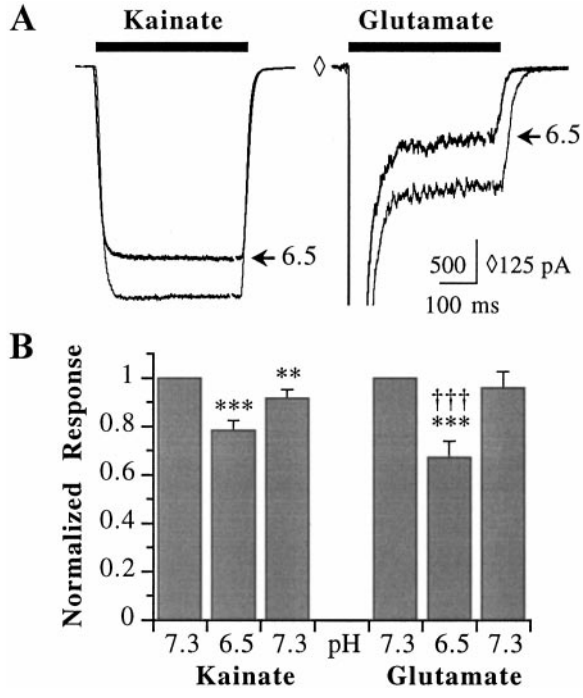

Fig. 1. Agonist-dependent modulation of AMPA receptors by protons. A, responses recorded from a hippocampal neuron to kainate (300 μM) and glutamate (1 mM) were inhibited by 19% and 44%, respectively, at pH 6.5 relative to pH 7.3. The solid bars above the traces indicate agonist application. The artifacts from the voltage-command switching the bimorph have been blanked (as indicated by gaps in the current traces) and the peak response to glutamate was truncated to better illustrate the change in the steady-state response. B, the bar graph compares the mean effect of acidic pH on responses to kainate and glutamate in the same neurons (n = 14). Responses at pH 6.5 were bracketed by control responses at pH 7.3 and data were normalized relative to the first response at pH 7.3. Responses to glutamate and kainate at pH 6.5 were significantly inhibited relative to both control responses (***$P < .001$), and responses to glutamate were inhibited significantly more than responses to kainate (††† $P < .001$). The decline in kainate-evoked control responses, which reflects rundown, was significant (**$P < .01$).

cesses rarely alkalinize the interstitial milieu of the brain (Chesler, 1990) pH values greater that 7.8 were not tested. However, acidic pH (pH 6.8 or 6.5) did significantly affect responses to kainate and glutamate. This modulation was concentration-dependent, with larger effects observed at pH 6.5 than pH 6.8. The amplitudes of glutamate-evoked responses at pH 6.8 and pH 6.5 were 90 ± 3% and 72 ± 4% ($P < .01$, $n = 6$) of those at pH 7.3, respectively. Kainate-evoked responses at pH 6.8 and pH 6.5 were significantly reduced to 95 ± 2% and 86 ± 4% ($P < .01$, $n = 10$) of those at pH 7.3, respectively. To maximize the effect of protons while reproducing pathophysiologic conditions associated with ischemia, all further experiments compared agonist-evoked responses recorded at pH 7.3 (control) and pH 6.5.

The difference in the magnitude of inhibition of glutamate- and kainate-evoked responses in the initial experiments suggested that modulation by protons might be agonist-dependent. To test this hypothesis, the effects of pH 6.5 on glutamate- and kainate-evoked responses from single cells were compared (Fig. 1). Responses to glutamate were inhibited by 32 ± 6%, which was significantly greater than the 18 ± 4% inhibition of responses to kainate (paired differences t test, $P < .001$, $n = 14$). Similar results were obtained in experiments comparing proton-mediated inhibition of responses evoked by quisqualate and kainate. Responses evoked by kainate were inhibited by 19 ± 3% at pH 6.5 relative to pH 7.3 ($P < .001$), whereas responses evoked by quisqualate were inhibited by 35 ± 11% ($P < .01$), a significant difference ($P < .05$, $n = 5$). Thus, the differential effects of protons could be ascribed to agonist properties, with responses evoked by the strongly desensitizing agonists glutamate and quisqualate, more susceptible to inhibition than those evoked by the weakly desensitizing agonist, kainate.

Modulators of AMPA Receptor Desensitization Block Proton-Mediated Inhibition. Because kainate and glutamate differ in their desensitization properties (Patneau et al., 1993), the agonist-dependence of proton modulation suggested that protons may affect desensitization. One way in which protons could modulate desensitization is selective interaction with the desensitized state of the receptor. To determine whether modulation of AMPA receptors by protons was state-dependent, extracellular pH was varied as glutamate was applied to neurons in the presence of trichlormethiazide or cyclothiazide, benzothiadiazine modulators that block desensitization of AMPA receptors by glutamate (Patneau et al., 1993; Yamada and Tang, 1993).

When glutamate was applied to hippocampal neurons in the presence of trichlormethiazide, no effect of protons was observed (Fig. 2A). Responses to 1 mM glutamate at pH 6.5 averaged 99 ± 6% ($n = 6$) of those evoked at pH 7.3 (Fig. 2B). Similar results were obtained for glutamate-evoked currents in the presence of 100 μM cyclothiazide; responses at pH 6.5 were 97 ± 2% ($n = 8$) of those at pH 7.3. Thus, modulators that block AMPA receptor transitions to the desensitized state prevent inhibition of glutamate-evoked responses by protons.

Protons Do Not Affect AMPA Receptor Desensitization or Deactivation Kinetics. The results of experiments described above suggested that protons modulate AMPA receptor desensitization. The effects of protons on the kinetics of desensitization were examined using macropatches pulled from hippocampal neurons and a piezo-based perfusion system to achieve submillisecond solution exchange. Responses to applications of three agonists, L-glutamate (3 mM), L-homocysteate (10 mM), and kainate (3 mM), were analyzed. Homocysteate is a strongly desensitizing full agonist with desensitization onset kinetics similar to those for glutamate, but with much lower affinity for the receptor (steady-state EC_{50} value of 447 μM versus 19 μM for glutamate; Patneau and Mayer, 1990, 1991). Kainate is a weakly desensitizing partial agonist with desensitization kinetics that are significantly faster than glutamate and an intermediate affinity for the receptor (142 μM; Patneau et al., 1993).

To determine whether protons affect AMPA receptor desensitization, responses to 40 ms (kainate) or 100 ms (glutamate and homocysteate) applications were recorded at pH 7.3 and 6.5 (Fig. 3A). The onset of desensitization, as reflected in the decay of the response to its steady-state amplitude in the presence of agonist, was then fitted with one (kainate) or the sum of two (glutamate and homocysteate) exponentials. There was no significant effect of pH on the kinetics of desensitization onset for any of the agonists, nor in the relative weights of the fast and slow components for responses to glutamate and homocysteate (Fig. 3C).

Receptor deactivation kinetics, as exhibited by the decay of the response after cessation of agonist application, were also examined to determine whether protons affect the rate of channel closing. For the strongly desensitizing agonists glutamate and homocysteate, a 1-ms pulse isolates deactivation from receptor desensitization (Fig. 3B). Because kainate-evoked responses are incompletely desensitized, sufficient steady-state current remained during a 40-ms pulse to determine deactivation kinetics from the decay of the response after removal of kainate. For all three agonists, decay of the current after removal of agonist was well-fit with a single exponential function at both pH 7.3 and pH 6.5, with time constants as illustrated in Fig. 3D. Deactivation time constants for glutamate at pH 7.3 (1.7 ± 0.4 ms, $n = 9$) were three times slower than for the low affinity agonist homo-

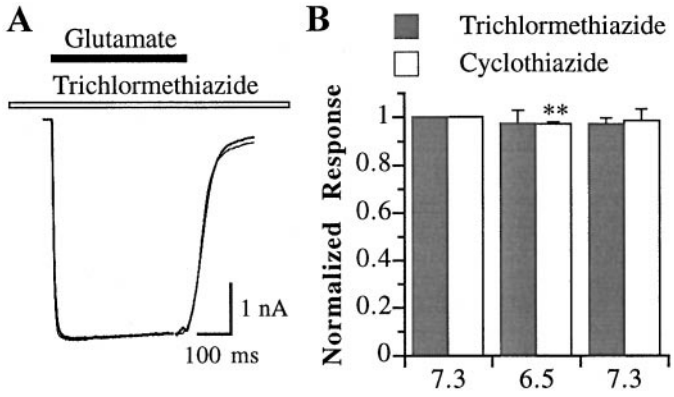

Fig. 2. Drugs that prevent AMPA receptor desensitization block proton-mediated inhibition. A, nondesensitizing responses to 1 mM glutamate (solid bar) recorded from a hippocampal neuron in the presence of 500 μM trichlormethiazide (open bar) are unaffected by pH. The traces recorded at pH 7.3 (thin trace) and pH 6.5 (thick trace) almost superimpose. B, the bar graph shows the mean effect of acidic pH on responses to glutamate recorded in the presence of 500 μM trichlormethiazide ($n = 6$) or 100 μM cyclothiazide ($n = 8$). Steady-state responses at pH 6.5 were bracketed by control responses and normalized relative to the first response at pH 7.3. There was no significant difference between responses at pH 6.5 and 7.3 in the presence of trichlormethiazide. The slight (3%) decline in responses at pH 6.5 in the presence of cyclothiazide was significant relative to the first control response (**$P = 0.01$), but not the second.

cysteate (0.56 ± 0.12 ms, $n = 7$), indicating that the deactivation kinetics for glutamate primarily reflect agonist dissociation rather than channel closing. The mean deactivation time constant for kainate (1.3 ± 0.6 ms, $n = 11$) was consistent with its intermediate affinity for AMPA receptors. There was no significant difference between the deactivation time constants at pH 7.3 and pH 6.5 for any agonist, suggesting that neither channel closing nor agonist dissociation rates are significantly affected by protons.

Although protons did not alter the kinetics of desensitization onset, they could affect receptor desensitization by changing the rate of recovery from desensitization. This possibility was tested with a paired-pulse protocol, in which a pulse of 3 mM glutamate was followed by a second pulse at

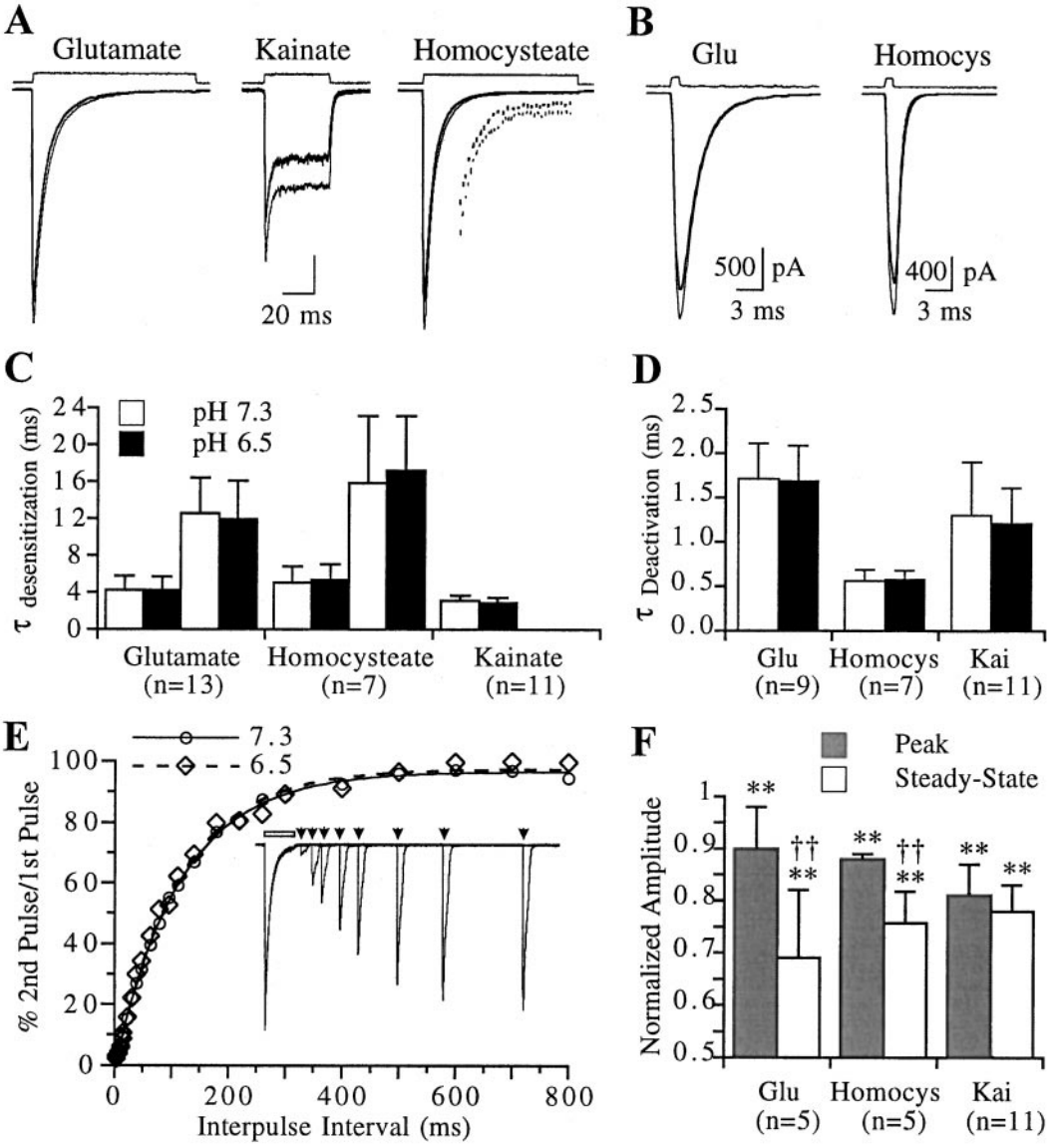

Fig. 3. Modulation by protons does not affect the kinetics of AMPA receptor desensitization or deactivation. A, examples of desensitizing responses from hippocampal neuron macropatches evoked by 100-ms applications of glutamate (3 mM) and homocysteate (10 mM) or 40-ms application of kainate (3 mM) at pH 7.3 (thin traces) and 6.5 (thick traces). Agonist application in A and B is denoted by the junction potentials obtained after disrupting the macropatch. Amplitude scale bar denotes 300 pA (glutamate), 150 pA (kainate), or 400 pA (homocysteate). Steady-state responses to homocysteate at 10× amplification are also illustrated (indicated by the dashed traces). B, responses to 1-ms applications of glutamate and homocysteate were used to isolate deactivation from receptor desensitization. Note the very rapid deactivation of responses to the low affinity agonist homocysteate. C, desensitization onset was unaffected by acidic pH. The bar graph shows the mean time constants determined by fitting the decay of responses in the presence of agonist, as illustrated in A, with a single (kainate) or the sum of two exponentials (glutamate and homocysteate). D, deactivation kinetics were not affected by a change in extracellular pH for any of the three agonists. The bar graph shows mean time constants determined from single exponential fits to the decay of responses after removal of agonist, after a 1-ms application of glutamate or homocysteate (B), or after cessation of a 40-ms application of kainate (A). E, protons did not affect the kinetics of recovery from desensitization. The rate of recovery from desensitization after a 50-ms application of glutamate (open bar) was assessed with a paired-pulse protocol as illustrated in the inset. The arrows above the overlaid current traces for eight different intervals indicate 10-ms glutamate pulses. The graph is data from a single macropatch for paired-pulse intervals from 4 to 800 ms. The resulting recovery curves were fit with single exponentials, with time constants of 116 ms at pH 7.3 and 113 ms at pH 6.5. F, comparisons between inhibition of steady-state and peak amplitudes at pH 6.5 relative to pH 7.3 were made for agonist-evoked responses as illustrated in A. Only macropatches for which the steady-state amplitude of glutamate- and homocysteate-evoked responses at pH 7.3 was ≥ 10 pA were included in this analysis. Acidic pH significantly inhibited both the peak and steady-state amplitudes for all three agonists (**$P <$ .01), but steady-state responses to glutamate and homocysteate were inhibited significantly more than peak responses (††$P <$ .01).

intervals of 2 to 800 ms. To estimate the potential effects of protons on synaptic responses, 1-ms pulses that mimic the time course of glutamate in the synaptic cleft were used in the initial analysis. The ratio of the peak amplitude of the second response to that of the first response was plotted against interpulse-interval, and the data were fitted with a single exponential function. The peak amplitudes of the first pulses were significantly inhibited, by 10 ± 3%, at pH 6.5 relative to pH 7.3 (n = 7, P < .001). But recovery from desensitization was not affected by pH, with time constants of 68 ± 11 ms and 64 ± 7 at pH 7.3 and 6.5, respectively. Because it is possible that occupancy of different conformational states may result from longer agonist applications that fully desensitize the receptor, we also examined recovery after 50 ms applications of glutamate, which produce almost complete receptor desensitization (see inset in Fig. 3E). Although the mean time constants for recovery from desensitization were slower than after 1-ms pulses (95 ± 12 ms at pH 7.3 and 90 ± 12 ms at pH 6.5, n = 3), there was no significant effect of pH (Fig. 3E).

Protons Increase Steady-State Desensitization of AMPA Receptors. The rapid solution exchange possible on macropatches permitted comparison of the effects of protons on peak and steady-state responses. This analysis included all macropatches exposed to kainate. However, only macropatches with control steady-state responses to glutamate and homocysteate ≥ 10 pA were included because changes in the amplitude of smaller responses could not be distinguished from background noise. The peak amplitudes of responses to all three agonists were significantly inhibited at pH 6.5 relative to pH 7.3 (Fig. 3F). Inhibition of steady-state responses to glutamate (31 ± 13%) and kainate (22 ± 5%) in macropatches was comparable with that observed in whole-cell recording. Peak responses to glutamate and homocysteate were inhibited by approximately 10%, significantly less than steady-state responses (Fig. 3D); this indicates that the conformational states of the receptor occupied during instantaneous (peak) and equilibrium (steady-state) responses to glutamate are differentially sensitive to protons. The difference between inhibition of peak and steady-state responses to kainate at pH 6.5 was not significant.

However, examination of the data for the 11 macropatches exposed to kainate revealed a strong correlation (r = 0.89) between steady-state desensitization and proton-mediated inhibition. As shown in Fig. 4A, there was substantial variability in the amount of desensitization in kainate-evoked responses, which varied from 20 to 50%, and in proton-mediated inhibition of those responses, which varied from 17 to 32%. Kainate-evoked responses exhibiting the most desensitization were also most strongly inhibited at pH 6.5 (Fig. 4B). Because steady-state desensitization in kainate-evoked responses is affected by membrane voltage (Patneau et al., 1993), the relationship between desensitization and modulation by protons could also be examined within a single macropatch (Fig. 4C). Steady-state desensitization was strongly voltage-dependent at both pH values, with desensitization at +80 mV approximately 20% less than at −80 mV. But at all voltages, more extensive desensitization of kainate-evoked responses was observed at pH 6.5 than at pH 7.3 (Fig. 4D). Thus, protons seem to increase equilibrium desensitization, but by a mechanism that does not affect the macroscopic kinetics of desensitization.

Proton-Mediated Modulation Varies with AMPA Receptor Composition. Molecular composition has previously been shown to affect proton modulation of NMDA and GABA receptors (Traynelis et al., 1995; Krishek et al., 1996). The

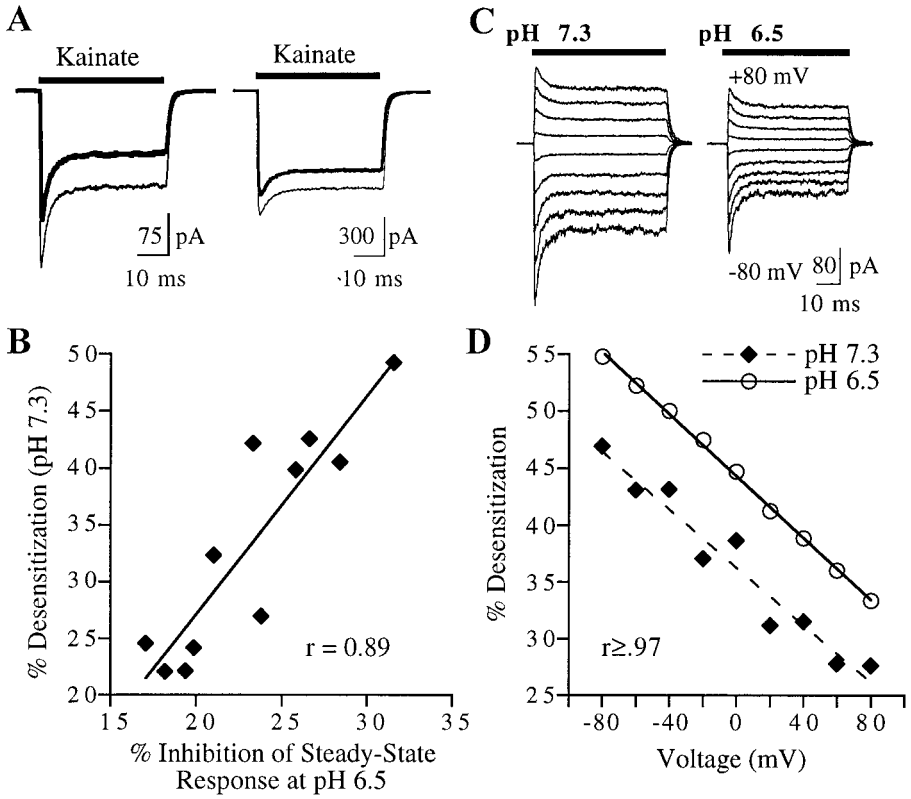

Fig. 4. Proton-mediated inhibition and desensitization of kainate-evoked responses covary. A, responses to 3 mM kainate recorded from two macropatches illustrate variability in the magnitude of desensitization between patches. Note that the responses on the left exhibit both more extensive kainate-evoked desensitization and greater proton-mediated inhibition at pH 6.5. B, percentage desensitization is plotted versus percentage inhibition of steady-state responses evoked by kainate for 11 macropatches. The solid line is a linear regression fit to the data (r = 0.89). C, leak-subtracted data for current-voltage determinations of kainate-evoked responses in a macropatch at pH 7.3 and 6.5 recorded at voltages from −80 to +80 mV. Note the apparent voltage-dependence of receptor desensitization but voltage-independence of proton-mediated inhibition. D, percentage desensitization of responses illustrated in C is plotted versus voltage. At all voltages kainate-evoked responses exhibited almost 10% more desensitization at pH 6.5 than at pH 7.3. Solid lines are linear regressions fit to the data with a correlation of r ≥.97 for each fit.

experiments described above suggest that AMPA receptor desensitization and proton modulation interact. Subunit (GluR-A to -D or 1–4; Hollmann and Heinemann, 1994) and isoform (*flip* or *flop*) composition of recombinant AMPA receptors determine desensitization time constants and the magnitude of steady-state desensitization of glutamate-evoked responses (Mosbacher et al., 1994; Partin et al., 1994). The *flip* (*i*) isoforms exhibit slower rates of desensitization and less extensive steady-state desensitization, whereas the *flop* (*o*) isoforms, and particularly D*o*, desensitize very rapidly (τ<1 ms) to a steady-state response that is $\ll$1% of the peak. We therefore tested the hypothesis that proton-mediated modulation would vary with the desensitization properties of recombinant AMPA receptors. HEK 293 cells were transiently transfected with either *flip* or *flop* isoforms of two subunits, and agonist-evoked responses were recorded at pH 7.3 and pH 6.5.

Glutamate-evoked responses for many *flop*-containing receptors exhibited steady-state responses that were too small to be distinguished from background noise, but responses to kainate (1 mM) had amplitudes large enough to reliably measure proton-mediated effects. The magnitude of inhibition of kainate-evoked responses at pH 6.5 varied with both subunit and isoform composition, ranging from 10% to > 40%. Inhibition in receptors composed of *flop* isoforms (on average 30 ± 8%) was greater than in receptors composed of *flip* isoforms (on average 14 ± 5%). The specific compositions tested, and the percentage inhibition for each, were: A*i*B*i*, 11 ± 4 (n = 7); A*i*D*i*, 14 ± 6, (n = 10); B*i*C*i*, 18 ± 4 (n = 4); A*o*B*o*, 26 ± 8 (n = 8); A*o*D*o*, 41 ± 2 (n = 7); A*o*C*o*, 30 ± 3 (n = 8); and B*o*C*o*, 24 ± 1 (n = 4). Figure 5 illustrates the effect of pH 6.5 on kainate-evoked responses for *flip* and *flop* isoforms in cells expressing heterodimeric receptors composed of GluR-A and -B or GluR-A and -D subunits. Note that receptors containing the subunit exhibiting the fastest desensitization, D*o* (Mosbacher et al., 1994), were most sensitive to proton-mediated inhibition.

Discussion

These experiments have produced three novel and significant findings. First, proton-mediated inhibition of AMPA receptors is dependent on the type of agonist. Second, protons seem to specifically interact with receptor desensitization but do not affect the macroscopic kinetics of desensitization. Finally, molecular receptor composition determines the magnitude of proton-mediated inhibition.

Previous research on proton-mediated modulation of non-NMDA receptors concluded that protons increase the closed time of the channel (Christensen and Hida, 1990; Traynelis and Cull-Candy, 1991). However, these studies did not determine whether protons increased closed time by directly affecting the transitions between open and closed states or, more indirectly, by affecting receptor desensitization. The experiments described above indicate that protons affect receptor desensitization. However, because protons do not affect desensitization kinetics or the affinity of the desensitized state, our results are inconsistent with a simple model of AMPA receptor gating that includes a single open state accessible from a nondesensitized state (e.g., Patneau and Mayer, 1991). One way in which protons could affect steady-state receptor desensitization without affecting the macroscopic kinetics of desensitization is through modulation of a second open state accessible from a desensitized state. Inclusion of such an open state has been proposed previously by Raman and Trussell (1992) in their model for AMPA receptor gating in chick cochlear nucleus.

This cyclic model for AMPA receptor gating and desensitization is based on one originally proposed by Patneau and Mayer (1991), but includes a second open state that is accessible from the desensitized state. A represents the agonist; R and R_D represent the receptor in its unoccupied resting and desensitized states, respectively; A_2R is the agonist-bound, active (closed) state; A_2R_D is the agonist-bound, desensitized state; d_1 and d_{-1} represent the rate constants for entry into and recovery from the desensitized state, respectively; β and α designate rate constants of channel opening and closing for the first open state (Open), whereas β' and α' are the equivalent rate constants for the second open state (Open′).

$$\begin{array}{ccccccc} A + R & \rightleftharpoons & A + AR & \rightleftharpoons & A_2R & \overset{\beta}{\underset{\alpha}{\rightleftharpoons}} & \text{Open} \\ \uparrow\downarrow & & \uparrow\downarrow & & d_{-1}\uparrow\downarrow d_1 & & \\ R_D & \rightleftharpoons & A + AR_D & \rightleftharpoons & A_2R_D & \overset{\beta'}{\underset{\alpha'}{\rightleftharpoons}} & \text{Open}' \end{array}$$

Support for protons specifically modulating a "desensitized" open state comes from several findings. First, the smaller effect of protons on responses to the weakly desensitized agonist kainate, compared with the strongly desensitizing agonist glutamate, suggest that the receptor is more susceptible to proton-mediated modulation when it spends more time in the desensitized state. Second, benzothiadiazine drugs that prevent AMPA receptor desensitization also block proton-mediated modulation. Because protons do not affect the rate of entry into or recovery from desensitization, this suggests that protons affect another open state that is not available when desensitization is blocked. Third, inhibition of kainate-evoked responses in macropatches was correlated with the magnitude of desensitization, and protons increased equilibrium desensitization without affecting the macroscopic kinetics of desensitization. Fourth, in transfected cells, receptor compositions previously shown to exhibit the fastest and most extensive desensitization were most sensitive to modulation. Finally, the model is supported by the findings that protons did not affect the kinetics of receptor deactivation for any of the three agonists examined and that protons inhibited peak responses to glutamate and homocysteate significantly less than steady-state responses. If there were only a single open state, and protons increased its closed time, receptor deactivation should have been affected. Although the kinetics of deactivation after removal of glutamate may reflect multiple openings with agonist still bound (see Fig. 3B), the very rapid deactivation observed after removal of the low-affinity agonist homocysteate would seem to accurately reflect the true channel-closing rate, which was not altered by protons. This result therefore suggests that the conformational states of the receptor underlying the instantaneous (peak) and equilibrium (steady-state) responses to glutamate are differentially sensitive to protons.

Our experiments indicate that modulation of AMPA recep-

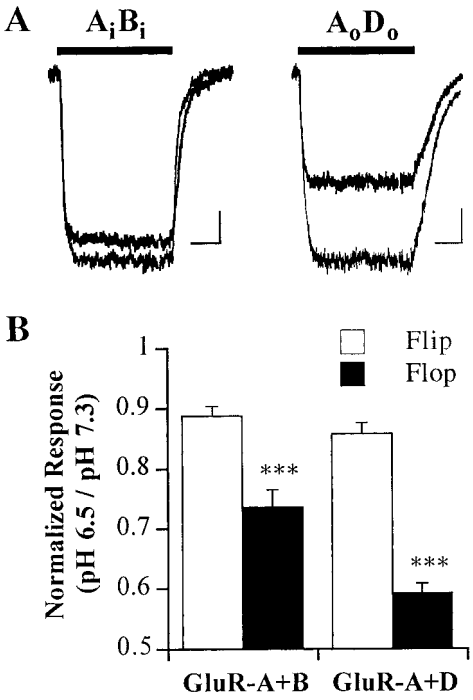

Fig. 5. The molecular composition of AMPA receptors determines their sensitivity to proton-mediated modulation. HEK 293 cells were transfected with cDNA for heterodimeric receptors varying in both subunit and isoform composition. A, kainate-evoked responses at pH 7.3 (thinner traces) and 6.5 (thicker traces) for the two compositions least (A*i*B*i*) and most (A*o*D*o*) sensitive to proton-mediated inhibition. Scale bars are 50 ms, 30 pA (A*i*B*i*); and 75 ms, 20 pA (A*o*D*o*). The bimorph switching artifacts have been blanked, as indicated by gaps in the current traces. B, mean ratios of responses evoked by kainate at pH 6.5 relative to pH 7.3 for HEK 293 cells transfected with the glutamate receptor subunits/isoforms GluR A*i*B*i* ($n = 7$), A*o*B*o* ($n = 8$), A*i*D*i* ($n = 10$), or A*o*D*o* ($n = 7$). Note the greater sensitivity to modulation by protons of *flop* isoforms (***$P < .001$), and that the receptor composition including the subunit exhibiting the fastest rate of onset of desensitization (Mosbacher et al., 1994), A*o*D*o*, was most strongly inhibited.

tors by protons in vivo will depend on both receptor composition and the magnitude of the pH change, whether the acidification is global and persistent, as in ischemia, or local and transient, as may occur in the cleft during synaptic transmission. Although local fluctuations in pH at the synapse have not been directly quantified, the processes that may contribute are known. Glutamatergic vesicles of hippocampal neurons are more acidic than the interstitial milieu (pH 5.67; Miesenbock et al., 1998). Thus, a rapid acidic transient is predicted to occur simultaneously with the release of neurotransmitter (Krishtal et al., 1987) and could be further amplified by deprotonation of glutamate upon release into the less acidic cleft. In addition, proton ATPases from synaptic vesicles may be incorporated in the presynaptic membrane in vesicle fusion and could transport protons from the presynaptic terminal into the cleft (Krishtal et al., 1987). The receptors directly under the site of release should see the largest acidic transient.

The temporal and spatial dispersion of acidic transients will be determined by the kinetics and capacity of the endogenous CO_2/HCO_3^- buffering system. Physiological levels of bicarbonate in the brain confer a high buffer capacity, but substantial experimental evidence suggests that synaptic activation produces transient changes in interstitial pH that are not rapidly buffered (Chesler, 1990). Thus, rapid acidic transients in the synaptic cleft sufficient to significantly affect the function of postsynaptic AMPA receptors are likely to occur, in particular with high frequency synaptic activity or at synapses with multivesicular release.

In animal models of ischemia, significant protection from excitotoxic cell death is conferred by AMPA receptor antagonists (Sheardown et al., 1990; Gill, 1994). The global acidification of the brain that occurs during ischemia is neuroprotective for NMDA receptor-mediated cell death, and it was assumed that it would also reduce AMPA receptor-mediated cell death (Christensen and Hida, 1990; Giffard et al., 1990). In contrast, McDonald et al. (1998) found that acidification similar to that occurring during ischemia (pH 6.6) significantly potentiated AMPA receptor-mediated neurotoxicity in neuronal cultures.

Although our findings do not provide an explanation for the results of McDonald et al. (1998), they suggest a mechanism for the selective vulnerability of principal neurons to ischemia (Cervos-Navarro and Diemer, 1991). Brorson et al. (1995) found that AMPA receptor-mediated responses of Purkinje cells, which are among those cell types most vulnerable to ischemia, exhibit less complete desensitization than those of other cerebellar neurons. This difference in desensitization properties has recently been related to higher levels of expression of *flip* isoforms of AMPA receptor subunits (Tomiyama et al., 1999). Similarly, cortical pyramidal cells exhibit slower and less extensive desensitization than interneurons (Hestrin, 1993). The desensitization properties of pyramidal neurons are also highly correlated with expression of AMPA receptor *flip* isoforms (Lambolez et al., 1996). Thus, AMPA receptors that exhibit less steady-state desensitization (e.g., *flip*-containing receptors) will disproportionately contribute to the neuronal depolarization resulting from tonic glutamate release and will also be less sensitive to the potentially inhibitory effects of interstitial protons. Neurons expressing these AMPA receptors are predicted to be more vulnerable to glutamate-mediated excitotoxicity in ischemia.

References

Andersen P (1990) Synaptic integration in hippocampal CA1 pyramids. *Prog Brain Res* **83:**215–222.

Brorson JR, Manzolillo PA, Gibbons SJ and Miller RJ (1995) AMPA receptor desensitization predicts the selective vulnerability of cerebellar Purkinje cells to excitotoxicity. *J Neurosci* **15:**4515–4524.

Buchan A, Li H and Pulsinelli WA (1991) The *N*-methyl-D-aspartate antagonist, MK-801, fails to protect against neuronal damage caused by transient, severe forebrain ischemia in adult rats. *J Neurosci* **11:**1049–1456.

Cervos-Navarro J and Diemer NH (1991) Selective vulnerability in brain hypoxia. *Crit Rev Neurobiol* **6:**149–182.

Chapman AG, Smith SE and Meldrum BS (1991) The anticonvulsant effect of the non-NMDA antagonists, NBQX and GYKI 52466, in mice. *Epilepsy Res* **9:**92–96.

Chen JC and Chesler M (1992) Modulation of extracellular pH by glutamate and GABA in rat hippocampal slices. *J Neurophysiol* **67:**29–36.

Chen C and Okayama H (1987) High-efficiency transformation of mammalian cells by plasmid DNA. *Mol Cell Biol* **7:**2745–2752.

Chesler M (1990) The regulation and modulation of pH in the nervous system. *Prog Neurobiol* **34:**401–427.

Chesler M and Kaila K (1992) Modulation of pH by neuronal activity. *Trends Neurosci* **15:**396–402.

Christensen BN and Hida E (1990) Protonation of histidine groups inhibits gating of the quisqualate/kainate channel protein in isolated catfish cone horizontal cells. *Neuron* **5:**471–478.

Giffard RG, Monyer H, Christine CW and Choi DW (1990) Acidosis reduces NMDA receptor activation, glutamate neurotoxicity, and oxygen-glucose deprivation neuronal injury in cortical cultures. *Brain Res* **506:**339–342.

Gill R (1994) The pharmacology of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate antagonists and their role in cerebral ischemia. *Cerebrovasc Brain Metab Rev* **6:**225–256.

Gottfried JA and Chesler M (1996) Temporal resolution of activity-dependent pH shifts in rat hippocampal slices. *J Neurophysiol* **76:**2804–2807.

Hestrin S (1993) Different glutamate receptor channels mediate fast excitatory

synaptic currents in inhibitory and excitatory cortical neurons. *Neuron* **11:**1083–91.
Hollmann M and Heinemann S (1994) Cloned glutamate receptors. *Annu Rev Neurosci* **17:**31–108.
Kraig RP, Ferreira-Filho CR and Nicholson C (1983) Alkaline and acid transients in cerebellar microenvironment. *J Neurophysiol* **49:**831–850.
Krishek BJ, Amato A, Connolly CN, Moss SJ and Smart TG (1996) Proton sensitivity of the $GABA_A$ receptor is associated with the receptor subunit composition. *J Physiol Lond* **492:**431–443.
Krishtal OA, Osipchuk YV, Shelest TN and Smirnoff SV (1987) Rapid extracellular pH transients related to synaptic transmission in rat hippocampal slices. *Brain Res* **436:**352–356.
Lambolez B, Ropert N, Perrais D, Rossier J and Hestrin S (1996) Correlation between kinetics and RNA splicing of α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid receptors in neocortical neurons. *Proc Natl Acad Sci USA* **93:**1797–1802.
McDonald JW, Bhattacharyya T, Sensi SL, Lobner D, Ying HS, Canzoniero LM and Choi DW (1998) Extracellular acidity potentiates AMPA receptor-mediated cortical neuronal death. *J Neurosci* **18:**6290–6299.
Miesenbock G, De Angelis DA and Rothman JE (1998) Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. *Nature (Lond)* **394:**192–195.
Mosbacher J, Schoepfer R, Monyer H, Burnashev N, Seeburg PH and Ruppersberg JP (1994) A molecular determinant for submillisecond desensitization in glutamate receptors. *Science (Wash DC)* **266:**1059–1062.
Partin KM, Patneau DK and Mayer ML (1994) Cyclothiazide differentially modulates desensitization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor splice variants. *Mol Pharmacol* **46:**129–138.
Patneau DK and Mayer ML (1991) Kinetic analysis of interactions between kainate and AMPA: Evidence for activation of a single receptor in mouse hippocampal neurons. *Neuron* **6:**785–798.
Patneau DK and Mayer ML (1990) Structure-activity relationships for amino acid transmitter candidates acting at *N*-methyl-D-aspartate and quisqualate receptors. *J Neurosci* **10:**2385–2399.
Patneau DK, Vyklicky L and Mayer ML (1993) Hippocampal neurons exhibit cyclothiazide-sensitive rapidly desensitizing responses to kainate. *J Neurosci* **13:**3496–3509.
Raman IM and Trussell LO (1992) The kinetics of the response to glutamate and kainate in neurons of the avian cochlear nucleus. *Neuron* **9:**173–186.
Sather W, Dieudonne S, MacDonald JF and Ascher P (1992) Activation and desensitization of *N*-methyl-D-aspartate receptors in nucleated outside-out patches from mouse neurones. *J Physiol Lond* **450:**643–672.
Sheardown MJ, Nielsen EO, Hansen AJ, Jacobsen P and Honore T (1990) 2,3,-Dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline: A neuroprotectant for cerebral ischemia. *Science (Wash DC)* **247:**571–574.
Siemkowicz E and Hansen AJ (1981) Brain extracellular ion composition and EEG activity following 10 minute ischemia in normo- and hyperglycemic rats. *Stroke* **12:**236–240.
Siesjö BK (1988) Mechanisms of ischemic brain damage. *Crit Care Med* **16:**954–963.
Siesjö BK, von Hanwehr R, Nergelius G, Nevander G and Ingvar M (1985) Extra- and intracellular pH in the brain during seizures and in the recovery period following the arrest of seizure activity. *J Cereb Blood Flow Metab* **5:**47–57.
Somjen GG (1984) Acidification of interstitial fluid in hippocampal formation caused by seizures and spreading depression. *Brain Res* **311:**186–188.
Tang C-M, Dichter M and Morad M (1990) Modulation of the *N*-methyl-D-aspartate channel by extracellular H^+. *Proc Natl Acad Sci USA* **87:**6445–6449.
Tombaugh GC and Sapolsky RM (1990) Mild acidosis protects hippocampal neurons from injury induced by oxygen and glucose deprivation. *Brain Res* **506:**343–345.
Tomiyama M, Palacios JM, Cortes R and Mengod G (1999) Flip and flop variants of AMPA receptor subunits in the human cerebellum: Implications for the selective vulnerability of Purkinje cells. *Synapse* **31:**163–167.
Traynelis SF and Cull-Candy SG (1991) Pharmacological properties and H^+ sensitivity of excitatory amino acid receptor channels in rat cerebellar granule neurones. *J Physiol Lond* **433:**727–763.
Traynelis SF, Hartley M and Heinemann SF (1995) Control of proton sensitivity of the NMDA r4eceptor by RNA splicing and polyamines. *Science (Wash DC)* **268:**873–876.
Vyklicky L Jr, Vlachova V and Krusek J (1990) The effect of external pH changes on responses to excitatory amino acids in mouse hippocampal neurones. *J Physiol Lond* **430:**497–517.
Yamada KA and Tang CM (1993) Benzothiadiazides inhibit rapid glutamate receptor desensitization and enhance glutamatergic synaptic currents. *J Neurosci* **13:**3904–3915

Send reprint requests to: Dr. Doris K. Patneau, 947 East 58th Street, Chicago, IL 60637. E-mail: doris@drugs.bsd.uchicago.edu