Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 10499–10503, November 1992
Neurobiology

Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats

(kainate/α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/excitotoxicity)

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Contributed by Michael V. L. Bennett, July 20, 1992

ABSTRACT **Severe, transient global ischemia of the brain induces delayed damage to specific neuronal populations. Sustained Ca^{2+} influx through glutamate receptor channels is thought to play a critical role in postischemic cell death. Although most kainate-type glutamate receptors are Ca^{2+}-impermeable, Ca^{2+}-permeable kainate receptors have been reported in specific kinds of neurons and glia. Recombinant receptors assembled from GluR1 and/or GluR3 subunits in exogenous expression systems are permeable to Ca^{2+}; heteromeric channels containing GluR2 subunits are Ca^{2+}-impermeable. Thus, altered expression of GluR2 in development or following a neurological insult or injury to the brain can act as a switch to modify Ca^{2+} permeability. To investigate the molecular mechanism underlying delayed postischemic cell death, GluR1, GluR2, and GluR3 gene expression was examined by *in situ* hybridization in postischemic rats. Following severe, transient forebrain ischemia GluR2 gene expression was preferentially reduced in CA1 hippocampal neurons at a time point that preceded their degeneration. The switch in expression of kainate/AMPA receptor subunits coincided with the previously reported increase in Ca^{2+} influx into CA1 cells. Timing of the switch indicates that it may play a causal role in postischemic cell death.**

Transient but severe global or forebrain ischemia, observed in patients successfully resuscitated from cardiac arrest or induced experimentally in animals, damages specific populations of neurons. Pyramidal cells in the CA1 subfield of the hippocampus are particularly vulnerable and become irreversibly damaged after a few minutes of ischemia. However, histological signs of degeneration in these neurons are not apparent until 48–72 hr after circulation has been restored (1, 2). Glutamate and aspartate are released in excess during cerebral ischemia (3) and are thought to play a role in the pathological mechanism responsible for this damage. Glutamate receptors are enriched in brain regions susceptible to ischemic injury (4), and their antagonists are neuroprotective in ischemic models. Specific blockers of the *N*-methyl-D-aspartate (NMDA) receptor are protective in moderate (5) but not severe (6, 7) ischemia, whereas systemic administration of the relatively selective kainate/α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor blocker, 2,3-dihydro-6-nitro-7-sulfamoylbenzoquinoxaline (NBQX), is effective in preventing delayed CA1 cell death following severe global ischemia in gerbils (8) and in rats (9), even when given 12 hr after the ischemic challenge (W.A.P., unpublished data).

Sustained Ca^{2+} influx through glutamate receptors is thought to play an important role in neuronal degeneration resulting from a number of pathological states, including ischemia, epilepsy, Huntington chorea, and Alzheimer disease (10, 11). Until recently, the NMDA receptor was considered to be unique among glutamate receptors in that it is Ca^{2+}-permeable (12). It is now established that kainate receptors in specific types of neurons (13, 14) and glia (15, 16) are Ca^{2+}-permeable. Furthermore, receptors formed from GluR1 and/or GluR3 subunits expressed exogenously are also permeable to Ca^{2+} (17, 18). Coexpression of GluR2 with GluR1 and/or GluR3 results in Ca^{2+}-impermeable channels; the GluR2 subunit governs divalent permeability of the expressed receptors. We examined the possibility that a modification in expression of kainate/AMPA receptor subunits leading to increased Ca^{2+} influx might be a mechanism underlying delayed necrosis of CA1 neurons caused by transient global ischemia.

MATERIALS AND METHODS

Induction of Ischemia in Rats. Transient, severe forebrain ischemia was induced in male Wistar rats (175–250 g) by a modified four-vessel occlusion method (6); cerebral circulation was restored after 10 min. The animals' body temperature was maintained by means of a heat lamp and rectal thermistor at 37.5°C ± 0.5°C throughout the ischemic period and for 6–8 hr after cerebral reperfusion. At the end of 10 min of ischemia the animals remained unresponsive to stimuli and had dilated pupils. Ischemic rats were sacrificed at 1, 6, 12, 18, and 24 hr after reperfusion, times preceding histologically detectable cell death in CA1. Control rats were sham-operated and sacrificed 24 hr later.

***In Situ* Hybridization.** *In situ* hybridization was performed in coronal brain sections with uridine 5′-[α-[^{35}S]thio]triphosphate (UTP[^{35}S])-labeled antisense RNA probes complementary to GluR1, GluR2, and GluR3 mRNAs (19) by one of us (D.E.P.-G.), who was blind to the experimental treatment. Sections were apposed to Kodak XAR-5 film for 24 hr and then dipped in Kodak NTB-2 nuclear emulsion, developed after 8 days, and counterstained with hematoxylin/eosin. Sense RNA probes did not label, and pretreatment with RNase A (100 μg/ml) prior to hybridization prevented labeling. Specificity of GluR1, GluR2, and GluR3 probes was assessed by competition experiments using a 10-fold excess of unlabeled cRNAs (19); hybridization to any given probe was blocked nearly completely by preincubation of sections with an excess of the corresponding unlabeled cRNA but was

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NBQX, 2,3-dihydro-6-nitro-7-sulfamoylbenzoquinoxaline; NMDA, *N*-methyl-D-aspartate.

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not blocked by preincubation with the other cRNAs. Conditions were assumed to be of sufficiently high stringency as to rule out cross-hybridization to GluR4, which is virtually absent from regions of rat brain other than cerebellum (20). Other glutamate receptor-related subunits (GluR5, GluR6, GluR7, and KA-1) exhibit <40% homology with GluR1 through GluR4 (21) and should not have been labeled. The "flip" and "flop" splice variants do not differ in their Ca^{2+}-permeability properties (22); we used "pan" probes (23) to label both splice variants.

Quantitative Analysis. The rationale of the quantitative analysis was based on the constant relationship between densities in film autoradiograms and number of grains overlying single neurons in emulsion-dipped sections. Optical densities and number of grains per cell in each region were essentially constant among sections from the same animal. The concentration of RNA probe used (10^6 cpm/80 μl per section) was saturating and afforded the maximal signal-to-noise ratio for GluR1, GluR2, and GluR3. Preexposure of films and analysis of UTP[^{35}S]-labeled brain paste standards indicated that exposure times were in the linear response range of the film. Corresponding brain sections of different postischemic times (but always including control brain) were cut in the same experimental session, incubated with the same solutions of GluR1, GluR2, and GluR3 probes, and apposed to the same sheet of film. Autoradiograms were analyzed with a Molecular Dynamics model 300A computing densitometer (Sunnyvale, CA). Optical densities of pixels overlying regions of interest from a minimum of four different sections per rat were corrected for background (optical density of the film), averaged, and normalized to the values of the corresponding regions in control sections for a given film. In Fig. 2 values are reported as normalized means ± SEM. Statistical analysis (analysis of variance followed by Tukey's *w* test for multiple comparisons) was performed on logit-transformed normalized percent optical densities. In Fig. 3, GluR1, GluR2, and GluR3 mRNA optical densities for each time point were normalized to the control value for each mRNA in each region. Percent optical densities of control were used to compute the ratio (GluR1 + GluR3)/GluR2 for each animal. In control rats labeling of GluR1, GluR2, and GluR3 mRNAs in each of the regions examined (CA1, CA3, and dentate gyrus) was at comparable levels, as determined by *in situ* hybridization (19) or Northern blotting (24). Since the control mRNA level for each region examined was set at 100%, the control ratio (GluR1 + GluR3)/GluR2 was 2.

RESULTS

Autoradiograms of coronal sections of control rat brain at the level of the hippocampus showed characteristic distributions of GluR1, GluR2, and GluR3 mRNAs in confirmation of earlier studies (19, 20). In hippocampus, all three transcripts were expressed at high levels in the granule cell layer, in pyramidal cell layers of the CA1, CA3, and CA4 subfields, and in subiculum (Fig. 1 *A*, *C*, and *E*).

Forebrain ischemia caused a striking change in kainate/AMPA receptor mRNA expression in CA1, the hippocampal area most sensitive to ischemic damage (Fig. 1). At 24 hr after ischemia, GluR2 expression was dramatically reduced in CA1 (Fig. 1 *C* and *D*), whereas GluR3 was reduced but somewhat less so (Fig. 1 *E* and *F*) and GluR1 was only slightly reduced (Fig. 1 *A* and *B*). Quantitative analysis of film autoradiograms revealed that for all probes the decrease in hybridization within the CA1 region developed between 6 and 12 hr postischemia ($P < 0.05$ for GluR2 at 12 hr vs. 1 hr) (Fig. 2*A*). Whereas GluR1 and GluR3 did not significantly change between 12 and 24 hr, GluR2 continued to decrease ($P < 0.05$ at 18 hr vs. 1 hr or 6 hr) for at least 24 hr ($P < 0.05$ vs. 18 hr, $P < 0.01$ vs. 12 hr). At 24 hr after ischemia GluR2 expression was reduced in CA1 by 69% ($P < 0.05$ vs. 18 hr and $P < 0.01$ vs. 12 hr), GluR3 by 50% ($P < 0.05$ vs. 1 hr, $P < 0.01$ vs. 6 hr), and GluR1 by only 24% (not significant vs. earlier time points because of the large variance) (Fig. 2*A*). GluR2 in CA1 was also significantly more depressed below control values than GluR1 ($P < 0.01$) and GluR3 ($P < 0.05$) at 24 hr. Modest

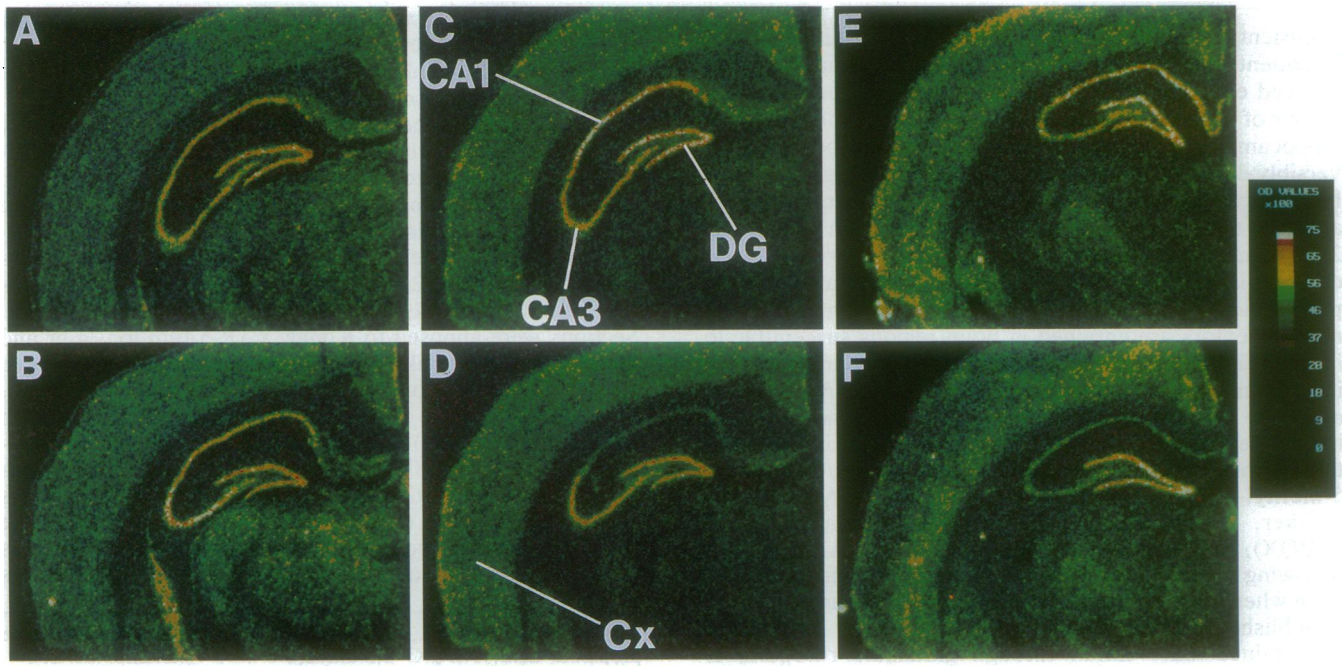

FIG. 1. Pseudo-color display of density of autoradiograms of GluR1, GluR2, and GluR3 mRNAs in coronal sections of control and postischemic rat brain at the level of the hippocampus. (*A*) GluR1 expression in control (sham-operated) brain. (*B*) GluR1 expression in ischemic rats 24 hr after 10 min of global ischemia. (*C*) GluR2 expression in control. (*D*) GluR2 expression 24 hr postischemia, showing dramatic and selective reduction in CA1 labeling. (*E*) GluR3 expression in control brain. (*F*) GluR3 expression 24 hr postischemia, showing reduction in CA1 that is not as marked as in *D*. Other experiments did not show as great a decline in CA3 as seen in *F*. DG, dentate gyrus; Cx, neocortex. (×7.)

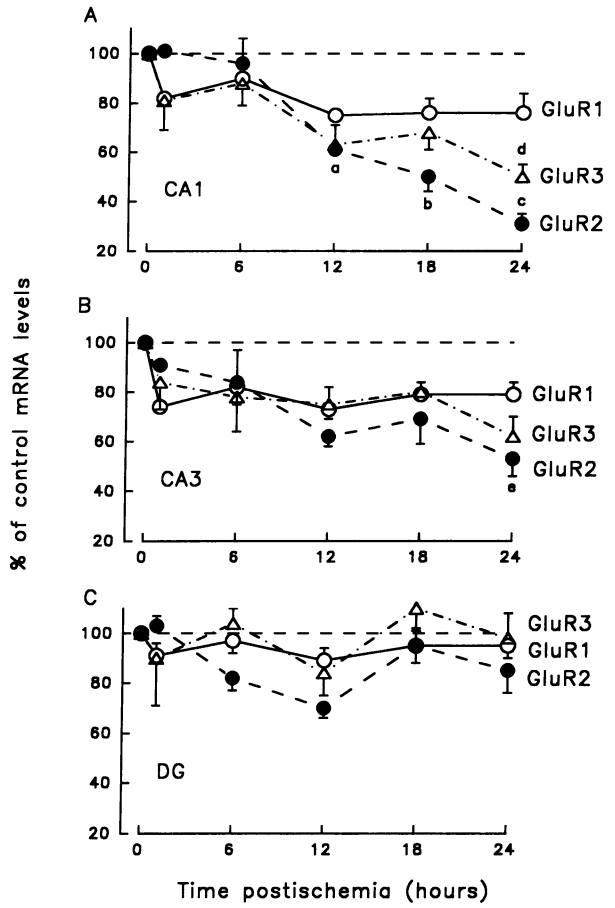

FIG. 2. Expression of GluR1, GluR2, and GluR3 mRNAs as a function of time postischemia in CA1, CA3, and dentate gyrus. In each panel, values are plotted as percent (± SEM) of control density measured at 24 hr (which is, however, plotted at time 0). (*A*) Hybridization levels in CA1 decrease for all probes between 6 and 12 hr. GluR1 and GluR3 did not significantly change between 12 and 24 hr, whereas GluR2 continued to decrease. At 24 hr GluR2 was significantly different from GluR1 and GluR3. (*B*) Hybridization levels in CA3. Modest decreases were observed for all probes. GluR2 at 24 hr is significantly different from earlier time points but not from GluR1 or GluR3. (*C*) Hybridization levels in dentate gyrus (DG). None of the probes is significantly different from controls at any time point. Statistical analysis was performed on logit-transformed normalized percent optical densities by analysis of variance and Tukey's *w* test for multiple comparisons. a = $P < 0.05$ vs. 1 hr; b = $P < 0.05$ vs. 1 hr or 6 hr; c = $P < 0.05$ vs. 18 hr and vs. GluR3 at 24 hr and $P < 0.01$ vs. 12 hr and vs. GluR1 at 24 hr; d = $P < 0.05$ vs. 1 hr and $P < 0.01$ vs. 6 hr. $n = 3$ (1 hr and 6 hr); $n = 4$ (controls, 12 hr, 18 hr, and 24 hr).

decreases in all three mRNAs were observed in CA3; their ratios did not differ significantly from those of controls at any time examined (Fig. 2*B*). In several other regions, including the dentate gyrus (Fig. 2*C*) and neocortex (data not illustrated), GluR1, GluR2, and GluR3 mRNAs were not significantly different from control values.

The efficacy of labeling, rates of subunit and channel formation as a function of mRNA levels, and the subunit composition of kainate/AMPA receptors in the brain are all unknown. However, as a first estimate of Ca^{2+} permeability, normalized levels of mRNAs encoding GluR1 and GluR3 (which form Ca^{2+}-permeable channels) were compared with that of mRNA encoding GluR2 (which when assembled with GluR1 and/or GluR3 forms Ca^{2+}-impermeable channels) in various hippocampal regions (Fig. 3). A larger value of the ratio (GluR1 + GluR3)/GluR2 is likely to be associated with greater Ca^{2+} permeability through kainate/AMPA channels.

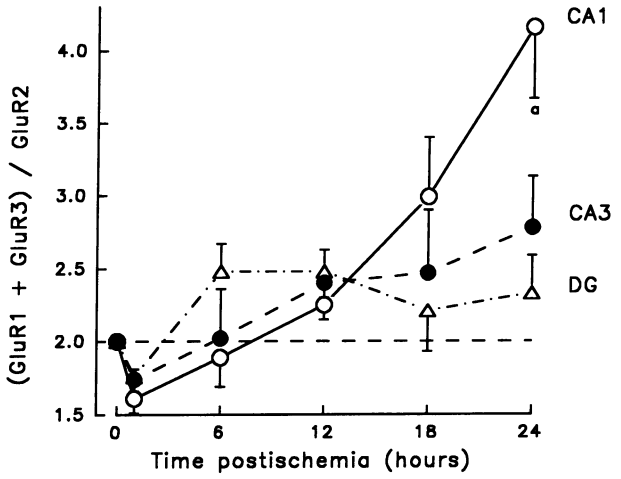

FIG. 3. Relative labeling of GluR1 and GluR3 compared to that of GluR2 in CA1, CA3, and dentate gyrus (DG) following transient global ischemia. Larger values for the ratio (GluR1 + GluR3)/GluR2 suggest that an increased number of Ca^{2+}-permeable kainate/AMPA channels will be formed (see text). Only the ratio for CA1 at 24 hr is significantly increased relative to earlier time points. Also, the ratio for CA1 is significantly increased relative to CA3 and DG values at 24 hr. Values for each group were analyzed statistically as indicated in the legend to Fig. 2. a = $P < 0.05$ vs. CA3 at 24 hr and $P < 0.01$ vs. CA1 at 1 hr, 6 hr, or 12 hr and vs. dentate gyrus at 24 hr. Vertical bars represent SEM.

At 24 hr postischemia the ratio was increased significantly, relative to earlier time point values, only in the CA1 region (Fig. 3, $P < 0.01$ vs. 1 hr, 6 hr, or 12 hr; $P < 0.05$ vs. CA3, $P < 0.01$ vs. dentate gyrus at 24 hr).

The dramatic and selective decrease in GluR2 expression in CA1 neurons at 24 hr postischemia was confirmed by microscopic observation of emulsion-dipped sections (Fig. 4). Parallel histological studies (see also ref. 1) and examination of counterstained emulsion-dipped sections revealed that neurodegeneration was not apparent at any postischemic interval examined in this study. In control brains silver grains overlay virtually all pyramidal cells of the CA1 after GluR2 hybridization (Fig. 4*G*); at 24 hr postischemia, labeling of individual CA1 neurons was greatly reduced or, in some cases, virtually absent (Fig. 4 *D* and *H*). In contrast, neurons of the adjacent CA3 region were still well-labeled (Fig. 4 *C* and *D*). These findings indicate that the decrease in CA1 GluR2 determined by densitometric analysis of film autoradiograms represents a reduction in the amount of mRNA per neuron. In CA1 pyramidal cells of control brain, GluR1 and GluR2 were labeled at comparable levels (Fig. 4 *E* and *G*). In two of four ischemic animals studied at 24 hr all CA1 pyramidal cells examined expressed levels of GluR1 mRNA that were not obviously different from controls (Fig. 4 *B* and *F*); in emulsion-dipped sections from the other two animals, GluR1 was somewhat below control levels (not shown).

DISCUSSION

Our results demonstrate that GluR1, GluR2, and GluR3 mRNAs are differentially regulated following a transient but severe global ischemic insult. This regulation may occur at the level of transcription. Another possibility is that GluR1 and GluR3 mRNAs are more stable than GluR2 mRNA, which degrades rapidly. Then as CA1 cells became "sick" and no new mRNA was synthesized, the amount of GluR2 mRNA would become small relative to that of GluR1 and GluR3. [This explanation cannot account for the decrease in GluR2 entirely, because synthesis of certain other mRNAs is increased just prior to cell loss (25–27).] It should be empha-

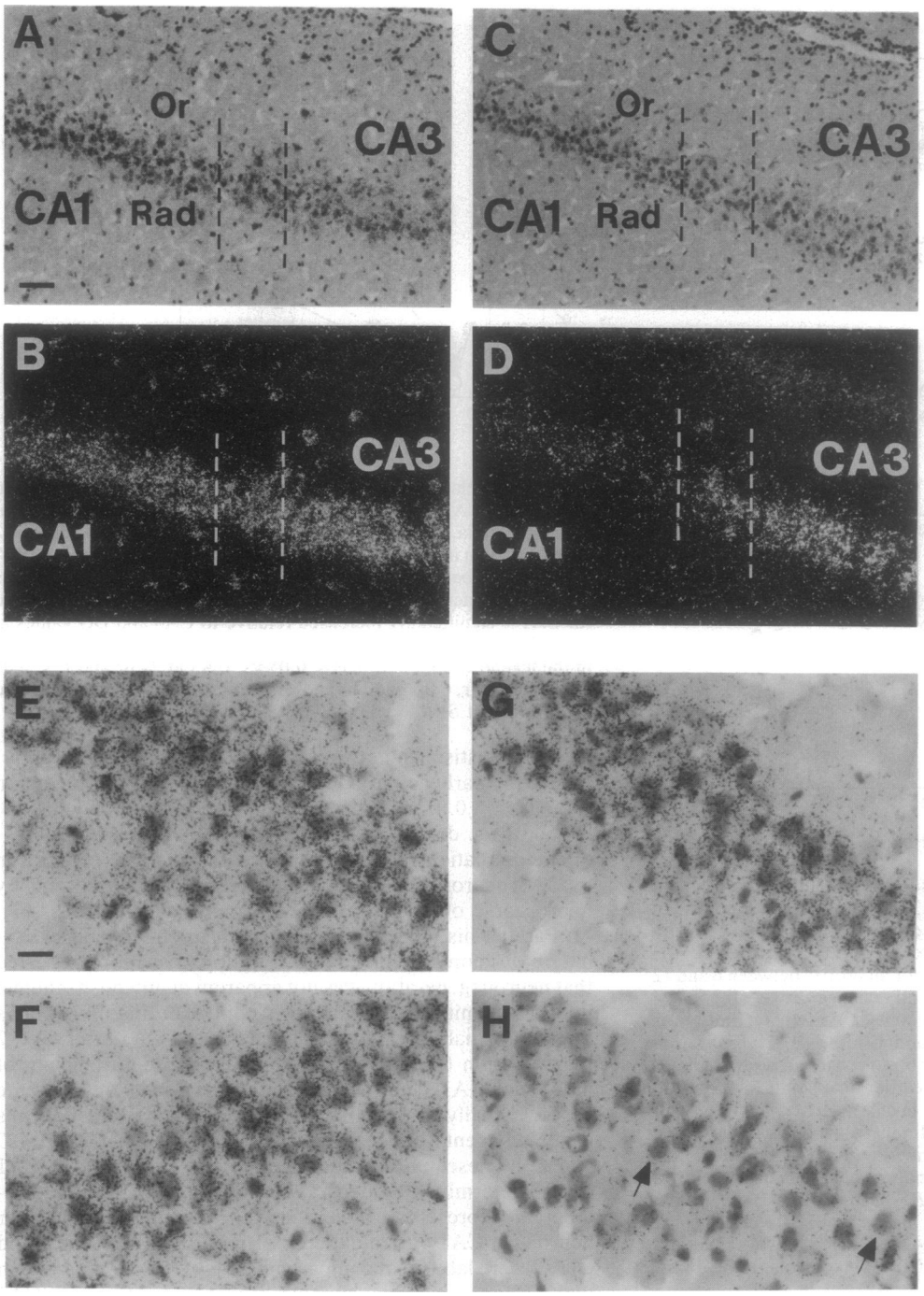

FIG. 4. Emulsion-dipped slides showing *in situ* hybridization grains over hippocampal pyramidal neurons counterstained with hematoxylin/eosin. (*A* and *B*) Bright-field and dark-field photomicrographs of GluR1 mRNA in hippocampus 24 hr postischemia. The CA1 and CA3 region are both abundantly labeled. Rad, stratum radiatum; Or, stratum oriens. (*C* and *D*) Bright-field and dark-field photomicrographs of GluR2 mRNA in hippocampus 24 hr postischemia. There is a large amount of GluR2 mRNA in the CA3 region but very low levels in CA1. Dashed lines mark the transition zone between CA1 (densely packed, small cells) and CA3 (loosely packed, large cells). (*E–H*) Higher magnification. (*E*) GluR1 hybridization grains overlying individual CA1 pyramidal cells in control (sham-operated) brain. All cells express GluR1 mRNA. (*F*) GluR1 hybridization in the same region is equally intense 24 hr postischemia. (*G*) GluR2 hybridization in control CA1 is comparable to control GluR1 levels. (*H*) GluR2 mRNA levels are dramatically reduced in the same cells 24 hr postischemia. Arrows depict individual pyramidal cells showing GluR2 hybridization that approaches background. (*A–D* bar = 100 μm; *E–H* bar = 25 μm.)

sized that the decreases in GluR2 and GluR3 mRNAs seen at 24 hr are before any loss of neurons, and emulsion-dipped sections show decreases in GluR2 and GluR3 expression per neuron.

The most striking observation of our study is the much larger reduction in GluR2 relative to GluR1 mRNA levels in postischemic CA1 pyramidal cells. In functional expression systems, GluR1 and GluR3 form channels that are Ca^{2+}-permeable (and inwardly rectifying; refs. 17, 18, 28). GluR2, whether expressed alone or in combination with GluR1 or GluR3, forms channels that are Ca^{2+}-impermeable (and electrically linear). These observations, together with data from *in situ* hybridization (19, 20) and electrophysiological experiments (29), indicate that native kainate/AMPA receptors in CA1 and CA3 pyramidal cells are heterooligomers containing the GluR2 subunit. Although it is not established that rates of formation of GluR protein subunits are directly proportional to mRNA levels, the findings of the present study suggest that, following global ischemia, reduction in GluR2 expression would lead to increased Ca^{2+} permeability through kainate/AMPA receptors in neurons of the CA1. In support of this prediction, measurements of $^{45}Ca^{2+}$ uptake or direct physical measurements of Ca^{2+} content show that in the CA1 of gerbils (30) and rats (31, 32) Ca^{2+} levels are maximal at 24–72 hr postischemia.

The switch in expression of mRNAs encoding kainate/AMPA receptor subunits occurred at a time that clearly preceded CA1 pyramidal cell necrosis as determined histologically in the four-vessel occlusion model (1). The subunit switch was not observed in regions, such as CA3 or the dentate gyrus, that are known to be resistant to ischemic injury. If the alterations in kainate/AMPA mRNA expression observed in the present study are translated into functional membrane receptors, then our results suggest that the late

flux of Ca^{2+} ions through GluR2-deficient receptor/channel complexes may contribute importantly to delayed ischemic necrosis of CA1 hippocampal neurons. The observation that the kainate/AMPA receptor blocker NBQX, but not antagonists of NMDA receptors, prevents this lesion when administered even after the ischemic insult (8, 9) further supports this concept. NBQX might be protecting CA1 pyramidal cells by blocking modified kainate/AMPA receptors or by preventing the modification we describe. Analysis of the mechanism of NBQX protection will require additional information on its kinetics and metabolism.

In situ hybridization enables localization and quantitation of specific messages within individual cells (33). This technique has allowed us to demonstrate that 24 hr after global ischemia individual CA1 pyramidal cells exhibit a greatly reduced level of GluR2 mRNA, whereas GluR1 expression is at near control levels (Fig. 4). Immunocytochemistry and functional studies will be required to correlate changes in mRNA content with altered subunit composition and permeability of CA1 kainate/AMPA receptors.

The mechanism triggering the differential decrease in GluR2 expression is presently unknown. Further information may come from studies of other brain areas vulnerable to transient severe global ischemia or of regulation of gene expression by transcription factors. In any case, differential expression of kainate/AMPA receptor genes caused by transient global ischemia may be responsible for the vulnerability of specific cell types and lead to the observed pathological changes. Further elucidation of this excitotoxic mechanism may suggest effective pharmacological interventions for patients who survive cardiac arrest.

We thank Dr. John A. Kessler for helpful discussion and criticism of the manuscript, Dr. Martin Lesser for assistance with the statistical analysis of the data, and Dr. Lucy L. Brown for help in preparation of the color-coded images. This work was supported by National Institutes of Health Grants NS 20752 (R.S.Z.) and NS 03346 (W.A.P.). M.V.L.B. is the Sylvia and Robert S. Olnick Professor of Neuroscience.

1. Pulsinelli, W. A., Brierley, J. B. & Plum, F. (1982) *Ann. Neurol.* **11**, 491–498.
2. Kirino, T. (1982) *Brain Res.* **239**, 57–69.
3. Benveniste, H., Drejer, J., Schousboe, A. & Diemer, N. (1984) *J. Neurochem.* **43**, 1369–1374.
4. Cotman, C. W., Monaghan, D. T., Ottersen, O. P. & Storm-Mathisen, J. (1987) *Trends Neurosci.* **10**, 273–280.
5. Swan, J. H., Evans, M. C. & Meldrum, B. S. (1988) *J. Cereb. Blood Flow Metab.* **8**, 64–78.
6. Buchan, A., Li, H. & Pulsinelli, W. A. (1991) *J. Neurosci.* **11**, 1049–1056.
7. Wieloch, T., Gustafson, I. & Westerberg, E. (1988) in *Neurology and Neurobiology: Frontiers of Excitatory Amino Acid Research*, eds. Turski, L., Lehmann, E. & Cavalheiro, E. (Liss, New York), pp. 715–722.
8. Sheardown, M. J., Nielsen, E. O., Hansen, A. J., Jacobsen, P. & Honoré, T. (1990) *Science* **247**, 571–574.
9. Buchan, A. M., Li, H., Cho, S. & Pulsinelli, W. A. (1991) *Neurosci. Lett.* **132**, 255–258.
10. Siesjö, B. K. & Bengtsson, F. (1989) *J. Cereb. Blood Flow Metab.* **9**, 127–140.
11. Choi, D. W. (1988) *Neuron* **1**, 623–634.
12. Mayer, M. L. & Miller, R. J. (1990) *Trends Pharmacol. Sci.* **11**, 254–260.
13. Murphy, S. N. & Miller, R. J. (1989) *J. Pharmacol. Exp. Ther.* **249**, 184–193.
14. Iino, J. M., Ozawa, S. & Tsuzuki, K. (1990) *J. Physiol. (London)* **424**, 151–165.
15. Muller, T., Moller, T., Berger, T., Schnitzer, J. & Kettenmann, H. (1992) *Science* **256**, 1563–1566.
16. Burnashev, N., Khodorova, A., Jonas, P., Helm, P. J., Wisden, W., Monyer, H., Seeburg, P. H. & Sakmann, B. (1992) *Science* **256**, 1566–1570.
17. Hollmann, M., Hartley, M. & Heinemann, S. (1991) *Science* **252**, 851–853.
18. Burnashev, N., Monyer, H., Seeburg, P. H. & Sakmann, B. (1992) *Neuron* **6**, 189–198.
19. Pellegrini-Giampietro, D. E., Bennett, M. V. L. & Zukin, R. S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4157–4161.
20. Keinänen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T. A., Sakmann, B. & Seeburg, P. (1990) *Science* **249**, 556–560.
21. Bettler, B., Egebjerg, J., Sharma, G., Pecht, G., Hermans-Borgmeyer, I., Moll, C., Stevens, C. F. & Heinemann, S. (1992) *Neuron* **8**, 257–265.
22. McGurk, J. F., Roginski, R. S., Zukin, R. S. & Bennett, M. V. L. (1991) *Soc. Neurosci. Abstr.* **17**, 335.
23. Sommer, B., Keinänen, K., Verdoorn, T. A., Wisden, W., Burnashev, N., Herb, A., Köhler, M., Takagi, T., Sakmann, B. & Seeburg, P. (1990) *Science* **249**, 1580–1585.
24. Durand, G. M., Bennett, M. V. L. & Zukin, R. S. (1991) *Soc. Neurosci. Abstr.* **17**, 794.
25. Nowak, T. S., Jr. (1991) *J. Cereb. Blood Flow Metab.* **11**, 432–439.
26. Jorgensen, M. B., Johansen, F. F. & Diemer, N. H. (1991) *Acta Neurol. Scand.* **84**, 352–356.
27. Muller, M., Cleef, M., Röhn, G., Bonnekoh, G. P., Pajunen, A. E., Bernstein, H.-G. & Paschen, W. (1991) *Acta Neuropathol.* **83**, 39–45.
28. Verdoorn, T., Burnashev, N., Monyer, H., Seeburg, P. H. & Sakmann, B. (1991) *Science* **252**, 1715–1718.
29. Jonas, P. & Sakmann, B. (1991) *J. Physiol. (London)* **438**, 321P.
30. Dux, E., Mies, G., Hossmann, K.-A. & Siklós, L. (1987) *Neurosci. Lett.* **78**, 295–300.
31. Dienel, G. A. (1984) *J. Neurochem.* **43**, 913–925.
32. Deshpande, J. K., Siesjö, B. K. & Wieloch, T. (1987) *J. Cereb. Blood Flow Metab.* **7**, 89–95.
33. Uhl, G. R. (1986) *In Situ Hybridization in Brain* (Plenum, New York).