ABSTRACT

Glutamate receptors are the most common excitatory neurotransmitter receptors in the central nervous system (CNS). Excessive stimulation of glutamate receptors, including receptors of the AMPA and kainate families, is believed to contribute to the neurodegeneration encountered in stroke, CNS trauma, and several degenerative neurologic disorders. Animal models also suggest that AMPA and kainate receptors participate in the killing of oligodendrocytes, which raises the possibility that these receptors may be important in the pathogenesis of multiple sclerosis. Animal models in which kainate-induced seizures produce delayed neurodegeneration in hippocampal CA1 and CA3 regions suggest that one subunit of AMPA receptors, known as GluR2, is particularly important in the development of delayed neurodegeneration. Recent discovery of a silencer mechanism that regulates GluR2 expression raises the possibility of novel therapeutic interventions that specifically target mechanisms of neurodegeneration common to a number of disorders of the CNS.

in the presence of protons. In addition, AMPA and kainate receptors may each be assembled from several possible combinations of glutamate receptor subunits. Of particular relevance to oligodendrocytes, a subpopulation of AMPA receptors that lacks the GluR2 receptor subunit has been identified. These variations in glutamate receptor structure can have considerable physiologic significance; it has been noted that AMPA receptors that lack the GluR2 subunit have unusually high permeability to extracellular Ca2+ and may therefore increase the susceptibility of cells to glutamate-mediated neurotoxicity. It is also possible for these different AMPA receptor subtypes to exist within the same neuron. Finally, the distribution of AMPA receptors on the cell membrane is not constant; AMPA receptors are added to and removed from the postsynaptic membrane dynamically in response to changes in the extracellular environment. The entry of Ca2+ into the cell (or possibly the liberation of Ca2+ from intracellular stores) triggers an increase in the expression of AMPA receptors to the postsynaptic membrane.

Evidence from animal model studies suggests that AMPA and kainate receptors may be important in the pathogenesis of multiple sclerosis. For example, Sanchez-Gomez and colleagues demonstrated that in oligodendrocytes, both AMPA and kainate receptors trigger Ca2+ influx and cell death. These investigators evaluated the mechanisms by which excitotoxicity kills cells in cultured oligodendrocytes derived from rat optic nerves. Stimulation of either AMPA or kainate receptors caused a marked increase in intracellular Ca2+ concentration, generation of oxygen free radicals, and the release of the protein cytochrome c, an initiator of apoptotic cell death. However, selective stimulation of AMPA or kainate receptors each activated independent intracellular molecular pathways that eventually culminate in the death of oligodendrocytes by distinct mechanisms.

**GluR2 Hypothesis**

Researchers who have examined the pathophysiologic mechanisms that underlie stroke, epilepsy, and other CNS disorders have proposed what has been called the “GluR2 hypothesis.” These investigators have suggested that the downregulation of the GluR2 subunit triggers a process of slowly developing neural injury. It has been noted that in animal epilepsy models, GluR2 downregulation occurs as the result of processes that are initiated by status epilepticus; GluR2 mRNA and protein are decreased before histologic signs of damage in conditions such as seizures or CNS ischemia.

The effects of experimentally induced seizures on GluR2 expression were examined in an animal epilepsy model in which sustained seizure activity was produced by the systemic administration of kainate. These investigators examined the effects of kainate-induced status epilepticus on GluR2 expression and neuronal death in the pyramidal cells of hippocampal CA1 and CA3 regions, which are particularly susceptible to neurologic injury. Kainate-induced seizures produced delayed neurodegeneration in these regions, which began to appear approximately 24 hours after the induction of status epilepticus and continued to develop over a period of several days. Neuronal injury was preceded by a significant downregulation of GluR2 mRNA (beginning about 12 to 16 hours after kainate administration; Figure) and GluR2 protein (measured 16 hours after kainate exposure). These findings are consistent with the notion that status epilepticus triggers the downregulation of GluR2 subunit, which increases Ca2+ conductance, triggering calcium-dependent processes of cell injury and death.

**Figure. Significant Downregulation of GluR2 mRNA Preceding Neuronal Injury**

Kainic acid-induced status epilepticus induced the downregulation of GluR2 mRNA in hippocampal CA1 and CA3 regions in adult rats. GluR2 expression remained stable in the dentate gyrus (DG). Status epilepticus did not affect GluR1 expression (not shown).

Adapted with permission from Grooms et al. Status epilepticus decreases glutamate 2 mRNA and protein expression in hippocampal pyramidal cells before neuronal death. Proc Natl Acad Sci U S A. 2000;97(7):3631-3636. Copyright 2000 National Academy of Sciences, USA.
How does status epilepticus decrease the expression of GluR2? This has been examined by studying the mechanisms by which the GluR2 gene is regulated. One mechanism that may be important in the effects of neurologic injury on GluR2 expression is the activity of a "silencer" element found on many glutamate receptor genes. This silencer element binds to a neuron-specific protein known as REST. REST engages histone deacetylation enzymes (HDAC), which in turn suppress the activity of the GluR2 promoter region of the GluR2 gene. Thus, the binding of REST to the GluR2 silencer element decreases the transcription of the GluR2 receptor gene. The activity of this suppressor mechanism may be significant in some forms of neurologic injury. In an animal model in which status epilepticus was induced by pilocarpine administration, seizure activity was associated with an increase in deacetylated histones of the GluR2 promoter and decreased GluR2 gene expression.

Modulating GluR2 expression may provide a novel approach to intervene in some types of neurologic injury. In principal, modifying GluR2 expression provides a more direct method to reduce Ca\(^{2+}\) conductance and neural injury than the use of receptor antagonists. Can this system be manipulated to reduce neurologic injury? The results of studies from animal epilepsy models that have examined the anticonvulsant valproate suggest that this might be possible. Valproate is an inhibitor of HDAC, and some studies that were performed in experimental animal modes of epilepsy have suggested that valproate may protect neurons from injury following status epilepticus. In one study, rats were treated with valproate approximately 1 hour before the induction of status epilepticus, and then again after status epilepticus repeatedly over a period of 3 days. Pretreatment with valproate was intended to inhibit HDAC, but the half-life of valproate in rats is about 20 minutes, so at the time that status epilepticus was induced, the plasma valproate level should have decreased to a sub-anticonvulsant level. The induction of status epilepticus caused significant neuronal degeneration in the hippocampus, including significant thinning of the pyramidal layer. In valproate-treated animals with hyperacetylated brain histones, less thinning was observed. This suggests that blocking HDAC may be an effective strategy to reduce seizure-related neurologic injury. Although valproate did not appear to attenuate seizures in this protocol, the identification of compounds that block histone deacetylases but do not have anticonvulsant properties is necessary to clearly demonstrate that the neuroprotective effect observed is unrelated to its anticonvulsant effect.

These results raise the possibility that increasing GluR2 gene transcription might produce significant neuroprotective effects. However, the translation of GluR2 mRNA is regulated by neurons, as suggested by the observation that some neurons have mRNA for GluR2 but no protein. Manipulating GluR2 translation may provide another mechanism to intervene in CNS injury.

**Implications of Glutamate Receptor Regulation for MS**

AMPA and kainate glutamate receptors are physiologically distinct: they are encoded by different genes, composed of different sets of subunits, and differ in their anatomic distribution and function in the CNS. A growing body of evidence demonstrates that these receptors are important in the pathophysiologic processes that cause many different neurologic conditions. There are several important unresolved issues about the roles that these receptors play in the pathogenesis of MS.

Does activation of kainate rather than AMPA receptors dominate the excitotoxicity in oligodendrocytes? Oligodendrocytes express kainate receptors, and kainate-receptor activation has been shown to kill these cells. Some of the purported AMPA receptor antagonists that have been studied in experimental allergic encephalomyelitis (EAE) are actually relatively nonspecific and block both AMPA and kainate receptors at the concentrations used. This is true of several of the most commonly studied agents, including NBQX, MPQX, and GYKI-52466. However, studies conducted using the highly specific AMPA receptor antagonist CP-465022 have demonstrated reduction of neurologic injury in EAE, suggesting that at least some of the neurotoxicity observed in this model is the result of activation of AMPA receptors. Few compounds have been described that specifically block kainate receptors. If kainate receptors prove to be particularly important in neurodegeneration, this could provide a significant advantage in designing neuroprotective treatment strategies. These receptors are not distributed as widely in the CNS as AMPA receptors, and kainate-receptor antagonists may therefore provide neuroprotection with few adverse effects on other CNS systems that rely on AMPA recep-
tors for normal neurotransmission. A second unresolved issue is the role of kainate-receptor activation in the death of motor neurons. Finally, does inflammation trigger the downregulation of GluR2 subunits? This could occur as the result of glutamate release from microglia. The role of inflammatory changes in GluR2 expression has not been well characterized.

SUMMARY AND CONCLUSIONS

AMPA and kainate receptors are structurally and functionally distinct, but stimulation of these receptors produces injury to neurons and oligodendrocytes that is believed to be important in the pathogenesis of many neurologic disorders, including MS. The downregulation of the GluR2 subunit of AMPA receptors precedes some types of neurologic injury and may predispose neurons to delayed neurodegeneration. Understanding the genetic mechanisms by which GluR2 expression is regulated may suggest therapeutic approaches to a number of related CNS disorders.

REFERENCES