CURRENT LITERATURE

AN *IN VITRO* MODEL OF STROKE-INDUCED EPILEPTOGENESIS

Glutamate Injury-Induced Epileptogenesis in Hippocampal Neurons: An *In Vitro* Model of Stroke-Induced "Epilepsy"

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BACKGROUND AND PURPOSE: Stroke is the major cause of acquired epilepsy. The mechanisms of ischemia-induced epileptogenesis are not understood, but glutamate is associated with both ischemia-induced injury and epileptogenesis in several models. The objective of this study was to develop an in vitro model of epileptogenesis induced by glutamate injury in hippocampal neurons as observed during stroke.

METHODS: Primary hippocampal cultures were exposed to 5 micromol/L glutamate for various durations. Whole-cell current clamp electrophysiology was used to monitor the acute effects of glutamate on neurons and chronic alterations in neuronal excitability up to 8 days after glutamate exposure.

RESULTS: A single, 30-minute, 5-micromol/L glutamate exposure produced a subset of neurons that died and a larger population of injured neurons that survived. Neuronal injury was characterized by prolonged reversible membrane depolarization, loss of synaptic activity, and neuronal swelling. Surviving neurons manifested spontaneous, recurrent, epileptiform discharges in neural networks characterized by paroxysmal depolarizing shifts and high-frequency spike firing that persisted for the life of the culture.

CONCLUSIONS: This study demonstrates that glutamate injury produced a permanent epileptiform phenotype expressed as spontaneous, recurrent epileptiform discharges for the life of the hippocampal neuronal culture. These results suggest a novel *in vitro* model of glutamate injury-induced epileptogenesis that may help elucidate some of the mechanisms that underlie stroke-induced epilepsy.

COMMENTARY

Stroke continues to loom large as a cause of epilepsy, accounting for the majority (approximately 40%) of acquired epilepsy cases. Although head trauma as a cause of epilepsy seems to receive more attention, stroke ranks equally with severe head trauma as a potential predisposing cause of epilepsy, and stroke is vastly more prevalent than severe head trauma. The studies highlighted in the Clinical Science section of this issue concerning status epilepticus arising de novo in hospitalized patients (Delanty et al.), and status epilepticus cases after stroke (Velioglu et al.) suggest that the extent of neuronal injury is a more important trigger for hyperexcitability and epileptogenesis (i.e., more extensive damage is associated with higher seizure rates) than is the particular mechanism of injury. Thus, reductionist models that aim to study pathologic events common to stroke and other causes of neuronal damage are useful in the study of epileptogenesis and are important in our pursuit of developing therapeutic strategies to prevent and treat the acquired epilepsies.

Ischemia and anoxia during a stroke are known to cause a massive release of glutamate and an excessive activation of postsynaptic glutamate receptors, which via metabolic cascades involving calcium ultimately lead to neuronal injury. Additional injury and death may occur in the peri-infarct penumbra, but it is the surviving neurons within the penumbra that are the underlying substrates for ischemia-induced epileptogenesis. By using cultured hippocampal neurons, the authors have investigated glutamate injury-induced epileptogenesis.

After a single 30-minute exposure of cells to glutamate, simulating the massive release of transmitter following ischemia, some cells did undergo an excitotoxic process that resulted in cell death. When intracellular current clamp recordings were made in the 1- to 8-day period after glutamate exposure, the surviving neurons in culture demonstrated hyperexcitability, manifested by seizure-like activity. This activity was characterized by spontaneous, recurrent epileptiform discharges and was seen in 77% to 100% of neurons, with discharges averaging approximately 2 minutes in duration. Phenobarbital inhibited such discharges, but ethosuximide did not.

The neurophysiologic behavior of neurons in the model appears to mimic the behavior of neurons in epileptic foci, suggesting that the model could be of value in studies on the

mechanisms of epileptogenesis. Moreover, because glutamate release may be a source of neuronal excitotoxicity in other types of brain injury, such as severe head trauma, the model may also be relevant to other clinical situations. Nonetheless, enthusiasm must be tempered somewhat by considerations regarding the experimental preparation itself. The circuitry in hippocampal dissociated cultures may be inherently more excitable than normal hippocampus, and thus, the relevance of the model to pathology in the intact brain is uncertain.

The authors suggest that activation of NMDA receptors is important in the epileptogenic process and that alterations in NMDA receptor subunit expression may underlie the development of hyperexcitability. Unfortunately, they did not consider the probable contributions of AMPA and metabotropic glutamate receptors in the process. AMPA receptors have been implicated in other models as the receptors involved in the maintenance of the epileptic state. These studies draw from and implicate the process of long-term potentiation as being involved in epileptogenesis. (In long-term potentiation, induction is NMDA receptor dependent and maintenance is AMPA dependent.) In addition, prolonged glutamate exposure would surely have activated metabotropic receptors, if they are present. Robert Wong's group and others have previously shown that metabotropic glutamate receptors, which are engaged for brief periods, also lead to persistent hyperexcitable states manifested as widespread recurrent paroxysmal activity in hippocampal slices. Such possibilities should be considered as the authors continue to exploit their model.

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