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Excitotoxicity

Experimental Correlates to Human Epilepsy

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Abstract

Neurochemical observations on cortical biopsies form 48 patients under surgical treatment for pharmacoresistant partial epilepsy showed a 70–80% increase in glutamate concentration when expressed in relation to neuron specific enolase. Intraperitoneal administration of one of its receptor agonists, kainic acid (KA), to the rat led to increased epileptogenic activity of the limbic type in a dose-dependent fashion. The KA injection also led to a neuronal cell death and a gliosis, closely correlated to the extent of seizure activity. In biopsies from human epileptogenic cortex, the concentration of neuron specific enolase correlated inversely to that of glial fibrillary acidic protein, a marker for astrocytic glial cells.

Stimulation of the KA receptor decreased the extent of phosphorylation of the largest subunit of neurofilaments (NF-H) that have consequences for structural stability and axonal transport. Phosphorylated NF-H decreased also in human epileptic cortex, indicating either an overactivity of excitatory neurotransmitters or a loss of axonal compartments.

Index Entries: Epilepsy; excitotoxicity; glutamate; glial fibrillary acidic protein; neurofilament; phosphorylation.

Introduction

One property of glutamate and related excitatory amino acids (EAAs) on central neurons is their neurotoxicity, i.e., their property to kill neurons when present at high extracellular concentrations. Several lines of evidence suggest a critical role of this property in the pathogenesis of central neuronal cell loss in sustained epilepsy *(1)*:

1. A net buildup of extracellular concentrations of glutamate and aspartate in the vicinity of vulnerable neurons is an early event during seizures *(2–4)*, probably caused by an increased synaptic release and/or decreased cellular uptake.
2. The regional pattern and ultrastructural appearance of the brain damage caused by intracerebral injection of EAAs resemble those found in the postmortem studies of human cases with status epilepticus *(5)*.
3. EAA receptors are enriched in brain regions susceptible to injury during seizures, e.g., the CA3 region of the hippocampus. The number of NMDA receptors may increase in an epileptic focus.
4. Transection of glutamatergic excitatory afferents to vulnerable regions can prevent brain damage caused by seizures.
5. The crucial observation linking excitotoxicity and acute epileptic brain damage is the effect of EAA receptor antagonism *(6)*.

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Table 1
Tissue Glutamate Levels Per Neuronal Specific Enolase in Brain Speicimens Form Patients With Epilepsy

Group	No. of sample	Glutamate/neuronal specific enolase
Normal	8	4.06 ± 0.76
Mild cortical dysplasia	9	7.49 ± 0.72[a]
Gliosis	8	6.85 ± 0.69[b]

Data are mean ± SEM. Asterisks indicate a significant difference from normal.
[a] p 0.05.
[b] $p < 0.01$.

A number of findings from both in vitro (cell culture and brain slices) and in vivo studies have indeed shown that numerous EAA antagonists are effective in ameliorating the neuronal damage in a large variety of acute brain damage models *(1)*.

The histopathology of the epileptic cortex is characterized by gliosis of varying intensity. Reactive astrocytes display a marked enhancement in glial fibrillary acidic protein (GFA). In posttraumatic epilepsy, the formation of a glial scar may cause a functional impairment that greatly exceeds that of the primary injury. Here we report that the severity of kainic acid (KA)-induced seizure activity correlates with the extent of gliosis during limbic epilepsy in the rat.

Several of the effects of EAA stimulation appear to be exerted via elevation of intracellular Ca^{2+} levels. Stimulation of EAA receptors may result in dephosphorylation of DARPP-32 (32 kDa dopamine and cAMP-regulated phosphoprotein) *(7)* and the microtubule-associated protein MAP2 *(8)*, via activation of a neuronal protein phosphatase, presumably the calcium-dependent phosphatase calcineurin. This indicates that the effects of EAA are mediated, at least partly, by modulation of phosphorylation systems. Neurofilaments (NFs) are major structural components of neuronal processes and contain many heavily phosphorylated proteins. We have characterized the changes in the state of phosphorylation of the high molecular weight subunit of neurofilament (NF-H) in the KA-induced seizure model and in brain specimens from patients with epilepsy.

Results

Glutamate Levels in Human Epileptic Cortex

Cortical samples were obtained from 48 patients under surgical treatment of pharmacoresistant partial epilepsy *(9)*. The mean concentration of glutamate in epileptic cortices were higher than in a nonepileptic group, when based on the level of neuron specific enolase (NSE) (Table 1). The value in the MCD (mild cortical dysplasia) group of epileptic cortices was 185% of the nonepileptic group. The corresponding value in the gliosis groups of epilepsy was 169%.

Cellular Alterations in Experimental and Human Epilepsy

We have used a solid-phase radioimmunoassay *(10)* to measure neuron- and astroglia-localized marker proteins in homogenates prepared from the rat and human brains. Three days after ip injection of KA (2.5–10 mg/kg) to rats, an increase of GFA concentrations was observed in the amygdala/pyriform cortex. The concentration correlated with the clinical seizure rating (Fig. 1). The lowest dose of KA, 2.5 mg/kg, did not produce overt seizures and had no effects on the GFA concentration. Rats exhibiting higher seizure ratings (3 and 4) showed twice as high concentration of GFA as control rats. The NSE concentrations decreased inversely (Fig. 2A). Such an inverse relationship between NSE and GFA was also found in specimens from the cortex of patients with epilepsy (Fig. 2B).

Alterations of the State of Neurofilament Phosphorylation in Experimental and Human Epilepsy

The phosphorylation state of NF-H was monitored with a monoclonal antibody against a phosphorylation dependent epitope on NF-H in an immunoassay *(10)*. The degree of phosphorylation was significantly decreased in cortical specimens from patients with epilepsy, in rat brains after KA (ip) induced seizures, as well as in rats after a local injection of KA into the hippocampal structure (Fig. 3).

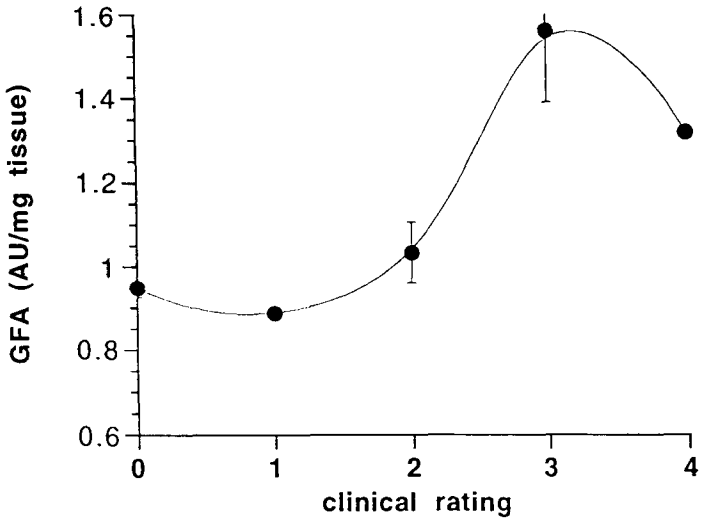

Fig. 1. Changes of GFA in the rat brain 3 d after KA injection in relation to clinical seizure rating. Data are drawn as mean values ± SEM (n = 4–6). The values at clinical rating 3 and 4 were significantly different from controls ($p < 0.01$, Student's t-test).

Discussion

Human hippocampal or mesial-basal limbic epilepsy comprises 78–80% of the temporal epilepsies and is commonly combined with amygdala epilepsy. These seizure prone areas in the human disease are also preferentially affected in the brain of rats subjected to KA-induced seizures *(11)*. The most common neuropathological observation in humans is incisural or hippocampal sclerosis. Our classical concept of progressive degeneration of nervous tissue in the epileptic brain is based primarily on the structural studies of Penfield et al. *(12)* and of Alexander and Woodhall *(13)*. The authors describe loss of neurons, proliferation of glial cells, and increased numbers of blood vessels. In our human epileptic material, a decrease of the neuronal marker NSE correlated with an increase of the astroglial marker GFA. In the KA-induced seizure model in the rat, GFA increased in a dose-dependent way as well as correlated with the clinical seizure rating but was inversely related to the concentration of neuronal cell markers *(11)*. A concept of a protective role of glial cells in containing seizures was developed by Woodbury *(14)*. Experimentally, seizures increase the number of glial cells as well as their HCO_3-ATPase, Na^+K^+ATPase, and carbonic anhydrase enzymatic activities. Glial cells may thereby increase their capacity to regulate the cation and anion microenvironment and can thus contain the spread of seizures. Aging, which causes gliosis and enhances carbonic anhydrase activity, also decreases seizure susceptibility *(14)*.

There is growing evidence that failure in glial protective mechanisms may signal ictal transformation and secondary generalization of a discharge. Reduced capacity of glial membranes to control extracellular potassium levels may play a role in ictal transformation. Enhanced neuronal firing elevates extracellular potassium and enhances glial oxygen consumption, glutamate, and glucose uptake and ATP levels. Furthermore, it elevates glycogen synthesis and pyruvate kinase activity in glial cells *(15)*. We showed that protein synthesis and aggregation of intermediate filaments in glial cells also increased in close relation to the seizure activity induced by extensive EAA receptor stimulation.

In this work glutamate concentrations in human epileptic cortex were given on an NSE basis in order to test whether the glutamate concentration per "average" neuron differed. On a total protein basis, decreased or nonaffected levels of glutamate and aspartate have been reported in human epileptic brain tissue *(4)*. In agreement, our data show no difference between epileptic and nonepileptic tissue on a protein basis. However, on an NSE basis, epileptic cortex of both MCD and gliosis types had a 70–80% higher glutamate concentration than the nonepileptic cortex. This favors the hypothesis that EAAs are involved in human epileptic disease.

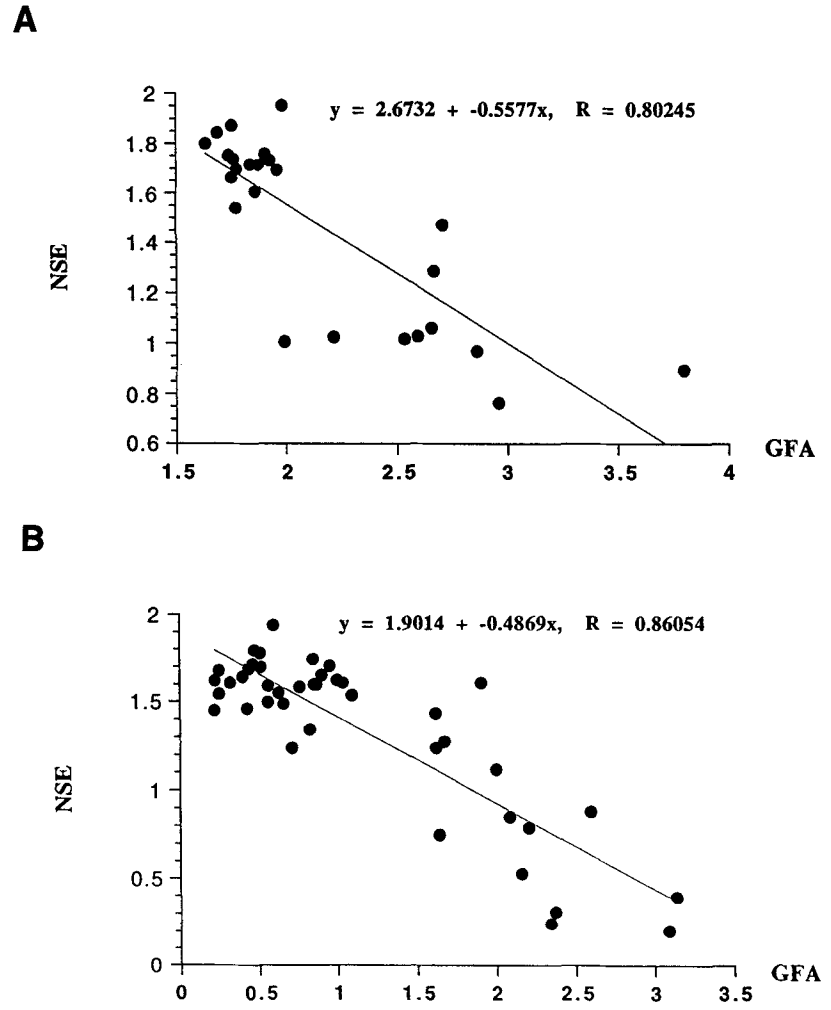

Fig. 2. Correlation analyses between NSE and GFA. **(A)** Rats with KA-induced seizures; **(B)** patients with epilepsy.

NP-H exists *in situ* in both phosphorylated and nonphosphorylated forms *(16–18)*. Phosphorylated NF-H is preferentially localized in the axon *(18)*, accounts for its structural stability *(19)*, and retards the transport rate of the filament, allowing them to form and maintain interactions with other cytoskeletal elements. Removal of phosphate groups from NFs may induce alterations in the conformation of NF proteins and change their number of potential binding sites for other cellular elements *(20)*. Abnormal accumulation of phosphorylated NF-H in neuronal somata has been implicated in the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases and amyotrophic lateral sclerosis *(21)*. Experimentally, abnormal accumulation of NF-H is caused by β,β'-iminodiproprionitrile (IDPN), acrylamide, aluminum, and 2,5-hexanedione *(22)*. Immunolabeling of ventral horn neurons by an antibody to phosphorylated NF-H has been demonstrated in the rat spinal cord after lumbar injection of KA *(23)*. Immunochemical methods do not reveal changes of NF-H in somata. The decrease in phosphorylation state of NF-H in this study may be preferentially in axons in view of the axonal localization of phosphorylated NF-H. Furthermore, the KA receptors are presynaptic, possibly also axonal *(24)*. A progressive pathology of dendrites mainly as a loss of spines is seen in neurons in the hippocampus of human epileptics *(25)*. Here we show that phosphorylated NF-H decreased also in cortical specimens from human epileptics, indicating either overactivity of excitatory neurotransmitters or a loss of axonal compartments.

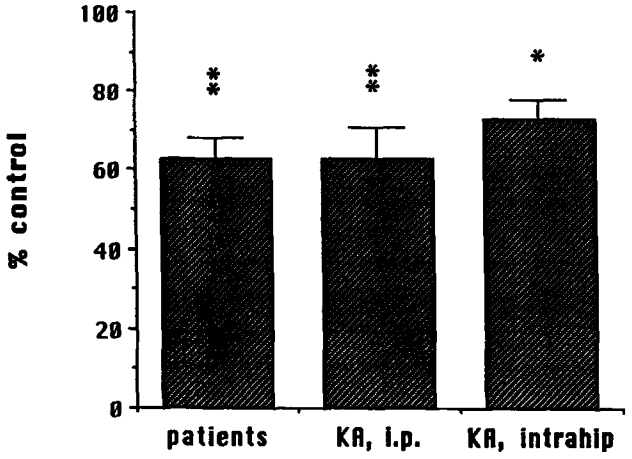

Fig. 3. Changes in the concentration of phosphorylated NF-H after epilepsy. NF-H was quantified with the monoclonal antibody FE3 to a phosphorylation-dependent epitope. Patients: cortical specimens from patients with epilepsy (n = 17). KA, ip: the hippocampus rats received an ip injection of 10 mg/kg of kainic acid (n = 7). KA, intrahip: the hippocampus formation of rats received a local injection of 8 nmol of kainic acid into the hippocampal structure (n = 7). The values from nonepileptic human or saline-injected rat. Controls were set at 100%. Asterisks indicate a significant decrease in comparison with the controls: $^{*}p < 0.05$; $^{**}p < 0.01$. Error bars represent SEM.

Acknowledgments

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