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Impact of receptor changes on treatment of status epilepticus

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Neurotransmitter receptors play a key role in the pathogenesis and current treatment strategies for status epilepticus (SE). Benzodiazepines are commonly used to treat SE exert their anticonvulsant action by stimulating γ -aminobutyric type A receptors (GABA_A receptors). These drugs are quite effective in terminating SE in approximately 55–66% of patients, but there is a residual group of patients with seizures resistant to benzodiazepines. The mechanisms underlying initiation and maintenance of prolonged SE were explored.

Methods

Studies were carried out in hippocampal slices removed from male Sprague–Dawley rats that had undergone SE induced by a combination of lithium and pilocarpine and age-matched controls. SE animals were studied 10–60 min after first grade 5 (Racine Score) seizures, when seizures are diazepam-resistant. GABAergic synaptic and tonic currents were recorded from hippocampal dentate granule cells (DGCs) by patch clamp electrophysiology after blocking excitatory transmission. Surface expression of GABA_A receptors in hippocampal slices was studied by means of a biotinylation pull down assay. GABA_A receptor trafficking was also studied by using an antibody-feeding technique in hippocampal neuronal cultures (14–18 days in vitro), dissociated from E 18 rats, exposed to high extracellular potassium.

Results

Mechanism of seizure initiation

In order to understand initiation of SE, we studied the effects of organophosphate paraoxon. This is an analog of organophosphate insecticides parathion and malathion, which cause SE in humans. Various doses of paraoxon (ranging from 100 nM to 3 μ M) were injected into the hippocampus and electrographic activity was recorded by electrodes implanted in the contralateral hippocampus and cortex and behavioral seizures were visually analyzed. These studies revealed that 150 nM paraoxon infused into the hippocampus reliably leads to prolonged SE.

We studied the effect of organophosphate paraoxon on glutamatergic synaptic transmission using patch clamp electrophysiology. Voltage clamp recordings of spontaneous excitatory postsynaptic currents were obtained from hippocampal dentate granule cells and after obtaining a baseline recording 3 μ M paraoxon was applied. This dramatically increased the frequency of sEPSCs (200% of baseline). The finding was confirmed in 4 cells. To further characterize this effect, the paraoxon concentration, sEPSC frequency increase relationship was studied. Paraoxon was applied at 0.1, 0.3, and 1 μ M concentrations and it increased sEPSC frequency in a concentration-dependent fashion. We further tested whether paraoxon increased action potential independent release of glutamate by recording its effects on action potential

independent miniature EPSCs. Preliminary experiments to date suggest that paraoxon increases action potential-independent release. We further tested whether OP cholinesterase inhibitor increases glutamate release via mechanisms dependant upon muscarinic or nicotinic receptor activation. Muscarinic agonist carbachol (50 μ M) increased the frequency of sEPSCs and thus mimicked the action of paraoxon. Carbachol action was blocked by preapplication of atropine. Furthermore, atropine markedly (almost completely) attenuated the paraoxon-induced enhancement sEPSC frequency. Nicotine also increased the frequency of sEPSC. However, nicotinic antagonist α Bungarotoxin itself increased sEPSCc frequency. The nicotinic mechanisms are under active investigation.

Mechanisms of benzodiazepine resistance

GABAergic synaptic transmission on DGCs in hippocampal slices from rats subjected to SE (SE DGCs) was compared with that on DGCs from control animals (control DGCs). The mIPSCs recorded from SE DGCs were smaller in amplitude and tended to be less frequent than those recorded from control DGCs. Activity of extrasynaptic receptors on control and SE DGCs was compared by response to the competitive GABA_A receptor antagonists bicuculline and GABAzine and to the open channel blocker penicillin. Tonic inhibition was similar in both groups of cells. The surface expression of $\gamma 2$ and $\beta 2/3$ subunits, which are expressed in receptors present at GABAergic synapses, was compared in hippocampal slices from SE-treated and controls animals. Expression of these subunits was diminished in slices from SE-treated animals. By contrast, the surface expression δ subunit, which is expressed in perisynaptic membrane, was similar in SE-treated and control slices.

Reduced surface expression of GABA_A receptors could result from excessive extracellular GABA or due to increased excitation and activity during SE. Hippocampal neuronal cultures were incubated in 100 μ M GABA for 60 min and the surface expression of $\gamma 2$ subunit containing (synaptic) receptors remained unchanged. In contrast, increased neuronal activity produced by high potassium reduced surface expression of $\gamma 2$ subunit—containing (synaptic) receptors but not the δ subunit.

Conclusions

These studies demonstrate that cholinergic stimulation can initiate SE by enhancing glutamate release from presynaptic terminals. Furthermore, prolonged seizures of SE compromise synaptic inhibition by promoting internalization of benzodiazepine-sensitive synaptic receptors. However, the internalization of extrasynaptic receptors was not stimulated by seizures and tonic inhibition mediated by these receptors was preserved.

These studies have therapeutic implications. Early SE can perhaps be terminated by drugs that diminish GABA release from presynaptic terminals. Among the candidate molecules are neuropeptides such as galanin, somatostatin, neuropeptide Y, and others such as adenosine. Furthermore, extrasynaptic receptors may be targeted for the treatment of benzodiazepine refractory SE.

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