GABA$_A$ Receptor Trafficking during Status Epilepticus

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Disclosure

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Status Epilepticus: A Common Neurological Emergency

- Bimodal distribution
- Mortality rates as high as ~25%

Slide not available.
Status Epilepticus: A Dynamic, Evolving Syndrome

Modified from Lothman. Neurology, 1990
The benzodiazepines have been the drugs of first choice since the 1960’s

  - We consider that [diazepam] was outstanding for the reliability and rapidity of its action which together make it a more effective drug than others we have used in the past . .

  - Our data . . . encourage us to recommend [diazepam] as the drug of choice in this difficult therapeutic problem.”


- Leppik et al. *Double-blind study of lorazepam and diazepam in status epilepticus*. JAMA, 1983

Despite efficacy in the early stages of SE, the BDZs often fail to control SE in its later stages.

- **San Francisco**: Retrospective analysis of seizures of at least 10 min in duration. 31% of seizures were refractory to a combination of a BDZ and either PHT, FOS, or PB.

- **VA Cooperative Study**: First treatment was successful in 55.5% of patients with overt SE and 14.9% of patients with subtle SE.
Despite efficacy in the early stages of SE, the BDZs often fail to control SE in its later stages. 

Duration of SE prior to Diazepam treatment and Response to Treatment for Each of the Four EEG Pattern Groups

<table>
<thead>
<tr>
<th>EEG group</th>
<th>Duration of SE prior to treatment (min) $^a$</th>
<th>All seizures stop $^b$</th>
<th>Convert to sublc or electrographic SE</th>
<th>Overt SE continues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrete seizures</td>
<td>7.3 ± 2.57</td>
<td>6/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Waxing and waning</td>
<td>16.2 ± 5.05</td>
<td>3/6</td>
<td>2/6</td>
<td>1/6</td>
</tr>
<tr>
<td>Continuous spiking</td>
<td>36.7 ± 15.51</td>
<td>1/6</td>
<td>2/6</td>
<td>3/6</td>
</tr>
<tr>
<td>PEDs</td>
<td>127.0 ± 10.34</td>
<td>1/6</td>
<td>5/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

$^a$ Values are means ± SD.

$^b$ No further behavioral or electrical seizure activity.
The function of a subset of GABA$_A$ receptors is enhanced by BDZs

- Postsynaptic
- Heteropentameric glycoprotein
- $2\alpha,2\beta,\gamma$ or $2\alpha,2\beta,\delta$

The function of a subset of GABA$_A$ receptors is enhanced by BDZs

- Allosteric regulators: benzodiazepines, ethanol, neurosteroids, anesthetics
- Pharmacological properties are subunit dependent

Saxena and Macdonald. J Neurosci, 1994;
GABA_A receptors mediate synaptic and tonic inhibition

- Cellular localization (synaptic vs. extrasynaptic) is subunit dependent.

Modified from Semyanov et al. TINS, 2004; Mangan et al. Mol Pharmacology, 2005
SE results in a rapid modification of the postsynaptic GABA$_A$ receptor population.

GABA-mediated synaptic inhibition in hippocampal slices was reduced after SE

GABA$_A$ receptors undergo a continuous cycle of internalization and insertion into the cell membrane.

- Internalization of surface GABA$_A$ receptors correlates with a reduced response to GABA
- Inhibition of internalization results in an increase in the amplitude of synaptic GABA$_A$ receptor currents.

GABA_A receptors undergo a continuous cycle of internalization and insertion into the cell membrane.

- Internalization of surface GABA_A receptors correlates with a reduced response to GABA
- Inhibition of internalization results in an increase in the amplitude of synaptic GABA_A receptor currents.
- A rapid-activity dependent increase in the intracellular accumulation of synaptic GABA_A receptors results in the reduction in GABA-mediated inhibition that occurs during status epilepticus

Measurement of GABA<sub>A</sub> receptor internalization in cultured hippocampal neurons

Step 1: anti-β2/3
4° C for 1h

Step 2: 37° C for 0 to 60min

Step 3: Fixation
1<sup>st</sup> 2ndary Antibody

Step 4: Permeabilization
2<sup>nd</sup> 2ndary antibody

Mangan et al. Mol Pharm, 2005; Goodkin et al. J Neurosci, 2005
GABA\textsubscript{A} receptor in cultured hippocampal neurons undergo rapid, constitutive intracellular accumulation

Mangan et al. Mol Pharm, 2005; Goodkin et al. J Neurosci, 2005
The intracellular accumulation of GABA$_A$ receptors was increased during bursting.

Goodkin et al., J Neurosci, 2005
Surface expression of the γ2 subunit was reduced in hippocampal slices after SE

Sulfonated biotin

Biotinylated surface receptors

Unlabelled receptors

Tonic inhibition and the surface expression of δ subunit-containing GABA$_A$ receptors was preserved during SE

Cell surface stability of $\text{GABA}_A$ receptors is dependent on the phosphorylation of AP2 binding sites

Jacob, Moss, and Jurd, 2008
Status Epilepticus decreased the phosphorylation of the β3 subunit

- Decrease in PKC activity and its association with the β3 subunit
- PKC activation in SE-treated slices produced a sustained increase in surface expression of GABA_A receptors

Jacob, Moss, and Jurd, 2008
The surface expression of the $\gamma 2$ subunit is ligand-independent.
Summary: Pathogenesis of SE

- A self-reinforcing condition in which there is a progressive, use-dependent reduction in GABA-mediated synaptic inhibition.
- During SE, activity-dependent, subunit-dependent trafficking of GABA_A receptors results in a reduction in the surface expression of β2/3 and γ2-containing GABA_A receptors but not δ-containing receptors.
- The reduction in the surface expression is the result of a ligand-independent process.
- The reduction in the surface expression of the benzodiazepine-sensitive γ2-containing GABA_A receptors is a potential mechanism to partially explain the development of benzodiazepine pharmacoresistance.
Treatment implications

• Treat SE early
• Therapies that target the endocytic machinery

Fig. 1. Time from seizure onset to seizure treatment.

Pellock et al. Epilepsy Behav, 2004
Treatment implications

- Therapies that target the benzodiazepine-insensitive $\delta$ subunit-containing GABA$_A$ receptors
  - Neurosteroids
  - Early use of anesthetics

Proposed accelerated protocol:

- Seizing:
  - Lorazepam 0.05-0.1 mg/kg plus phenytoin or fosphenytoin 18-20 mg/kg repeat once

- Still seizing:
  - Intubate and ventilate, keep $\leq 37^\circ$C
  - Consider vecuronium 0.1 mg/kg
  - Midazolam 0.2 mg/kg then 1.2 mg/kg/min or propofol 1 mg/kg then 1-5 mg/kg/hr or pentobarbital 5-15 mg/kg, then 0.5-5 mg/kg/hr
  - Admit to ICU, early EEG monitoring titrate to burst suppression

- Coma:
  - Intubate & anesthesia

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Goodkin et al., J Neurosci, 2005