GABA<sub>A</sub> receptor β<sub>1-3</sub> subunit gene expression in the hippocampus of kindled rats

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Abstract
The effect of Schaffer collateral/commissural fiber kindling on the expression levels of GABA<sub>A</sub> receptor β<sub>1</sub>, β<sub>2</sub>, and β<sub>3</sub> subunit mRNA in the pyramidal and granular neurons of the rat dorsal hippocampus was studied, using semi-quantitative in situ hybridization. In pyramidal neurons of CA1 and CA3, only small changes (10-15%) were found. In dentate granule neurons, the expression level of GABA<sub>A</sub>R-β<sub>3</sub> mRNA was significantly, enhanced, bilaterally, in animals that were partial or fully kindled. At long-term, 4 weeks after the last convulsion no significant changes were found in pyramidal or granular neurons.

Key words: Kindling; GABA<sub>A</sub> receptor β subunit; γ-amino butyric acid; mRNA; Hybridization, in situ; Hippocampus; Fascia dentata; Epileptogenesis

The repeated application of a high-frequency electrical stimulation to discrete regions of the brain results in the occurrence of epileptiform afterdischarges of increasing duration and intensity and in the gradual appearance of behavioural epileptiform seizure activity [4]. This process of epileptogenesis, called 'kindling', leads to a long-lasting increased seizure susceptibility and has been extensively studied as an experimental model of focal epilepsy [8,12].

In previous work we have reported several changes that take place in the GABAergic inhibitory system in the hippocampus as a result of kindling stimulations. In CA1 area, a reduction of the inhibition takes place as evidenced by a reduced paired-pulse inhibition of local evoked field potentials [7,9,27], a decreased sensitivity of the pyramidal neurons for iontophoretically applied GABA [6], and a reduced binding of the GABA<sub>A</sub> receptor agonist [3H]muscimol [22]. In contrast to the decreased inhibition in CA1, in the fascia dentata paired-pulse inhibition is strengthened [7,23,28], together with a robust increase in [3H]muscimol binding in this area [14,18,22].

Structurally, the GABA<sub>A</sub>-receptor is a hetero-oligomeric complex composed of several subunits (α, β, γ, δ, ρ), each of which exists in the brain in different variants [11,16]. In recombinant expression studies, the association of different cloned subunit variants leads to a functional diversity of the GABA<sub>A</sub> receptor complexes with regard to GABA-activated chloride currents, benzodiazepine pharmacology and sensitivity for agonists [11,16,20]. In purified preparations of GABA<sub>A</sub> receptors of the brain, the binding of [3H]muscimol shows a preference for bands that co-migrate with those that stain with α subunit-specific antibody [1,2,3,15]. Therefore, we have investigated whether the observed alterations in [3H]muscimol binding in the two hippocampal areas may be related to a modified expression of the genes that encode for the three identified variants of the β-class [10,24,26]. To allow a differential measurement of the GABA<sub>A</sub>R-β<sub>1</sub>, -β<sub>2</sub>, and -β<sub>3</sub> subunit mRNA levels in the hippocampal subregions, we used the technique of in situ hybridization.

Male Wistar rats (n = 54) were used in this study. Stainless-steel electrodes were implanted in the CA1 area of the left dorsal hippocampus of rats under pentobarbital anaesthesia. The stimulation bundle was placed in the Schaffer-collateral/commissural fiber pathway and the...
The value obtained in the control group was used as reference and set at 100%. Statistical comparisons with respect to controls were carried out on the determined extinction values using the Student’s t-test. *P < 0.05; **P < 0.03; ***P < 0.01; ****P < 0.002 (two tailed).

Table 1
Relative changes in mean extinction values of the in situ hybridization autoradiograms of GABA<sub>A</sub> receptor β<sub>1</sub>, β<sub>2</sub>, and β<sub>3</sub> in the different kindled groups (% ± S.E.M.) and the pooled animals of 6-AD, 14-AD and FK group (kindle-pooled)

<table>
<thead>
<tr>
<th></th>
<th>Kindle-pooled (n = 21)</th>
<th>6-AD (n = 7)</th>
<th>14-AD (n = 6)</th>
<th>FK (n = 8)</th>
<th>LK (n = 8)</th>
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<tbody>
<tr>
<td>CA1</td>
<td></td>
<td></td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β&lt;sub&gt;1&lt;/sub&gt;</td>
<td>9.2 ± 2.1****</td>
<td>4.3 ± 2.8</td>
<td>9.6 ± 4.5</td>
<td>9.4 ± 3.0**</td>
<td>2.6 ± 2.6</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-7.5 ± 2.1*</td>
<td>-8.8 ± 3.4</td>
<td>-9.8 ± 2.8</td>
<td>-2.5 ± 2.9</td>
<td>-9.9 ± 3.9</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3.4 ± 2.5</td>
<td>4.1 ± 5.7</td>
<td>-1.2 ± 4.0</td>
<td>7.2 ± 2.4*</td>
<td>0.4 ± 4.8</td>
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<tr>
<td>CA3</td>
<td></td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5.6 ± 1.5*</td>
<td>0.5 ± 2.2</td>
<td>6.2 ± 1.8</td>
<td>7.1 ± 3.4</td>
<td>-2.3 ± 1.8</td>
</tr>
<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β&lt;sub&gt;2&lt;/sub&gt;</td>
<td>-11.4 ± 2.6***</td>
<td>-12.6 ± 2.7</td>
<td>-14.3 ± 3.1</td>
<td>-6.4 ± 5.3</td>
<td>6.6 ± 4.6</td>
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<tr>
<td>GABA&lt;sub&gt;A&lt;/sub&gt;β&lt;sub&gt;3&lt;/sub&gt;</td>
<td>7.4 ± 2.1**</td>
<td>5.9 ± 3.7</td>
<td>2.3 ± 2.4</td>
<td>14.5 ± 2.9***</td>
<td>-2.2 ± 4.2</td>
</tr>
</tbody>
</table>

| Fascia dentata |            |             |              |            |
| GABA<sub>A</sub>β<sub>1</sub> | 11.6 ± 3.5**| 3.2 ± 4.4   | 9.1 ± 6.0    | 6.6 ± 5.6  | 9.7 ± 5.2  |
| GABA<sub>A</sub>β<sub>2</sub> | 6.3 ± 3.2   | -4.2 ± 2.0  | 0.8 ± 7.7    | 21.1 ± 6.6**| -0.2 ± 4.8 |
| GABA<sub>A</sub>β<sub>3</sub> | 29.9 ± 3.0****| 26.7 ± 6.1****| 31.0 ± 5.7****| 29.9 ± 4.2****| 4.5 ± 6.0 |

The value obtained in the control group was used as reference and set at 100%. Statistical comparisons with respect to controls were carried out on the determined extinction values using the Student’s t-test. *P < 0.05; **P < 0.03; ***P < 0.01; ****P < 0.002 (two tailed).
In the LK group no significant changes were detected. In order to confirm the increased expression of GABAA,R-β3 in all principal neurons of the hippocampus in fully kindled animals, we studied the mRNA levels in a second series of fully kindled animals. Animals were kindled in a comparable way as described above, and sacrificed 24 h (n = 6) after the last generalized seizure. A significantly enhanced expression level of GABAA,R-β3 mRNA in all hippocampal areas was found in comparison with controls (n = 6). The relative increase was: in CA1: + 17.7 ± 5.2% (P ≤ 0.05), in CA3: + 23.5 ± 4.7% (P ≤ 0.002), and in fascia dentata: + 31.9 ± 5.3% (P ≤ 0.004). In an additional group of kindled animals, sacrificed 28 days (n = 6) after the last class 5 seizure, again no changes in GABAA,R-β3 mRNA levels were found that persisted as long as 4 weeks.

Kindling of the Schaffer collateral/commissural path-way leads to a gradual increase of the GABAergic inhibition in the fascia dentata [7,23,28] whereas in CA1 inhibition decreases [6,7,9,27]. These opposite changes in GABAergic inhibition are accompanied by a bilateral increased binding of GABA_A-receptor agonist [3H]muscimol in the fascia dentata [14,18,22] and a decreased binding in CA1 [22]. The absence of changes restricted to the stimulated hemisphere is probably due to the rapid spread of the afterdischarge activity from the site of stimulation to the contralateral hippocampus already occurring with the first kindling session.

Based on the observations described here, we conclude that kindling stimulations result only in small (10–15%) changes of GABAA,R-β subunit expression in CA1 and CA3 areas, but in opposite directions; a decrease of GABAA,R-β2 and an increase of GABAA,R-β1 mRNA levels. The decreased [3H]muscimol binding in CA1 in fully kindled animals cannot be related in a straightforward way to an altered expression of the GABAA,R-β subunit encoding genes. It may be possible that changes in the phosphorylation level of the GABAA,R-subunits rather than changes in receptor density or composition are primarily responsible for the reduction in GABAergic inhibition in this brain area [13,19,21]. In the fascia dentata the GABAA,R-β mRNA levels were clearly increased bilaterally in the course of kindling, most prominently the β1 levels. We hypothesize, that the enhanced expression of the GABAA,R-β genes in this area underlies the robust increase of [3H]muscimol binding sites in the fascia dentata in fully kindled animals which most likely subserves the observed increase of recurrent inhibition [7,23,28]. Such an alteration would counterbalance changes in the same area that result in enhanced glutamatergic excitatory synaptic transmission [5]. Obviously, further quantification of the receptor complex proteins, using subunit specific antibodies, will be needed to substantiate this conclusion and to establish the precise time course and persistence of the long-term changes.

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