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ALTERED EXPRESSION OF $GABA_A$ AND $GABA_B$ RECEPTOR SUBUNIT mRNAs IN THE HIPPOCAMPUS AFTER KINDLING AND ELECTRICALLY INDUCED STATUS EPILEPTICUS

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Abstract—Epilepsy may result from altered transmission of the principal inhibitory transmitter GABA in the brain. Using *in situ* hybridization in two animal models of epileptogenesis, we investigated changes in the expression of nine major $GABA_A$ receptor subunits (α1, α2, α4, α5, β1-β3, γ2 and δ) and of the $GABA_B$ receptor species $GABA_BR1a$, $GABA_BR1b$ and $GABA_BR2$ in 1) hippocampal kindling and 2) epilepsy following electrically-induced status epilepticus (SE). Hippocampal kindling triggers a decrease in seizure threshold without producing spontaneous seizures and hippocampal damage, whereas the SE model is characterized by spontaneous seizures and hippocampal damage. Changes in the expression of $GABA_A$ and $GABA_B$ receptor mRNAs were observed in both models, and compared with those seen in other models and in human temporal lobe epilepsy.

The most prominent changes were a relatively fast (24 h after kindling and electrically-induced SE) and lasting (7 and 30 days after termination of kindling and SE, respectively) reduction of $GABA_A$ receptor subunit δ mRNA levels (by 43–78%) in dentate granule cells, accompanied by increases in mRNA levels of all three β-subunits (by 8–79%) and subunit γ2 (by 11–43%). Levels of the minor subunit α4 were increased by up to 60% in dentate granule cells in both animal models, whereas those of subunit α5 were decreased 24 h and 30 days after SE, but not after kindling. In cornu ammonis 3 pyramidal cells, downregulation of subunits α2, α4, α5, and β1–3 was observed in the ventral hippocampus and of α2, α5, β3 and γ2 in its dorsal extension 24 h after SE. Similar but less pronounced changes were seen in sector cornu ammonis 1. Persistent decreases in subunit α2, α4 and β2 transcript levels were presumably related to SE-induced cell loss. $GABA_B$ receptor expression was characterized by increases in $GABA_BR2$ mRNA levels at all intervals after kindling and SE.

The observed changes suggest substantial and cell specific rearrangement of GABA receptors. Lasting downregulation of subunits δ and α5 in granule cells and transient decreases in subunit α2 and β1–3 mRNA levels in cornu ammonis 3 pyramidal cells are suggestive of impaired $GABA_A$ receptor-mediated inhibition. Persistent upregulation of subunits β1–3 and γ2 of the $GABA_A$ receptor and of $GABA_BR2$ mRNA in granule cells, however, may result in activation of compensatory anticonvulsant mechanisms. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: seizures, hippocampus, inhibitory neurotransmission, temporal lobe epilepsy.

GABA is the major inhibitory neurotransmitter in the mammalian brain and exerts its actions through two classes of receptors, ionotropic $GABA_A$ receptors and the metabotropic $GABA_B$ receptors ($GABA_BR$) (Bowery, 1993; Mody et al., 1994; Sieghart, 1995). $GABA_A$ receptors mediate a fast hyperpolarizing action of GABA by ligand-operated chloride channels constituted of five subunits. Several gene families encode these subunits (α1–α6, β1–β3, γ1–γ3, δ, ε, π, θ). Typically two α-, two β-, and a γ-, δ-, ε-, π, or θ-subunit participate in the pentameric structure (Schofield et al., 1987; Mehta and Ticku, 1999; Pirker et al., 2000; Sieghart and Sperk, 2002). Considering the numerous possible combinations of these subunits, the existence of a variety of $GABA_A$ receptor subtypes with presumably varying physiological properties has been postulated and also demonstrated (Tretter et al., 1997; Jechlinger et al., 1998; Sieghart and Sperk, 2002).

$GABA_BR$ are G-protein-coupled receptors located at pre- and postsynaptic sites (Bowery, 1993; Bettler et al., 1998; Bowery and Enna, 2000; Couve et al., 2000). So far two different genes have been identified encoding two splice variants of $GABA_B$ receptor-1 ($GABA_BR1$) ($GABA_BR1a$ and 1b) and $GABA_B$ receptor-2 ($GABA_BR2$). $GABA_BR1$ and $GABA_BR2$ form a functionally active heterodimeric complex in the membrane (Kaupmann et al., 1998; White et al., 1998). Stimulation of postsynaptic $GABA_BR$ causes an increased K^+ conductance generating late IPSPs (Gahwiler and Brown, 1985). Presynaptic $GABA_BR$ mediate a suppression of neurotransmitter release by inhibiting voltage-sensitive Ca^{++} channels (Klapstein and Colmers, 1992; Takahashi et al., 1998). Depending on their specific neuronal localization, they may similarly suppress the release of glutamate or of GABA resulting in decreased excitation or inhibition, respectively.

There is now strong evidence that impaired GABAergic transmission mediated by $GABA_A$ and/or $GABA_BR$ can cause seizures. Thus, blocking $GABA_A$ receptors with bicuculline or picrotoxin results in severe convulsions

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Abbreviations: CA, cornu ammonis; $GABA_BR$, $GABA_B$ receptor; $GABA_BR1$, $GABA_B$ receptor-1; $GABA_BR2$, $GABA_B$ receptor-2; GAD, glutamic acid decarboxylase; ROD, relative optical density; SE, status epilepticus; TLE, temporal lobe epilepsy.

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(Wood, 1975). Deletion of certain subunits of the $GABA_A$ receptor complex, e.g. subunits $\alpha1$, $\beta1$, $\beta3$ and $\gamma2$ results in increased seizure susceptibility or limbic seizures in rodents (DeLorey et al., 1998; Karle et al., 2001; Nusser et al., 2001; Prosser et al., 2001; Kralic et al., 2002). Similarly, mice lacking $GABA_BR1$ are more susceptible to seizures (Schuler et al., 2001).

Recent genetic studies of familial epilepsy syndromes revealed associations with defects of $GABA_A$ and $GABA_BR$ (Noebels, 2003). A rare form of juvenile myoclonic epilepsy is related to a missense mutation of the $GABA_A$ receptor $\alpha1$-subunit gene (Cossette et al., 2002), and deletion of the $GABA_A$ receptor $\beta3$-subunit gene in the Angelman syndrome is associated with seizures (Sugimoto et al., 1992). Point mutations of the $\gamma2$-subunit gene were linked to pedigrees with childhood generalized tonic–clonic epilepsy with febrile seizures (Baulac et al., 2001) or generalized absence epilepsy and febrile seizures (Wallace et al., 2001), and truncation of the $\gamma2$-subunit gene causes different epilepsy syndromes (Harkin et al., 2002; Kananura et al., 2002). A polymorphism of the $GABA_BR1$ gene was linked to a human temporal lobe epilepsy (TLE) phenotype (Gambardella et al., 2003).

Animal models of TLE revealed strong evidence for altered expression of $GABA_A$ and $GABA_BR$ in response to seizure activity or epileptogenesis (Sperk et al., 2003). Pronounced changes in mRNA and protein levels were observed after kainic acid-induced status epilepticus (SE) and subsequent epileptogenesis (Friedman et al., 1994; Schwarzer et al., 1997; Tsunashima et al., 1997; Fritschy et al., 1999; Furtinger et al., 2003a; Straessle et al., 2003), pilocarpine-induced seizures (Rice et al., 1996; Kapur and MacDonald, 1997; Brooks-Kayal et al., 1998; Chandler et al., 2003; Houser and Esclapez, 2003; Peng et al., 2004; Zhang et al., 2004), and electrically induced SE (Kokaia et al., 1994; Kokaia and Kokaia, 2001). Pronounced changes in the expression of both $GABA_A$ and of $GABA_BR$ were observed in the human hippocampus of TLE patients (Wolf et al., 1994; Loup et al., 2000; Munoz et al., 2002; Furtinger et al., 2003b; Pirker et al., 2003; Princivalle et al., 2003). The functional relevance of these changes in seizures and epileptogenesis and the associated neurodegenerative events are still unknown. To address this issue, it is essential to provide a comprehensive description of the changes in the GABA-ergic system using animal models that involve different mechanisms of epileptogenesis and extents of neurodegeneration.

We therefore investigated changes in the expression of mRNAs encoding for nine major $GABA_A$ receptor subunits and the $GABA_BR$ in two animal models of TLE in rats: 1) electrical stimulation of the ventral hippocampus inducing self-sustained SE (referred to as “SE rats”) and 2) the classical hippocampal kindling protocol (“kindled rats”). Whereas kindling induces a reduction in seizure threshold with minimal neuronal damage and no spontaneous seizures, the SE model is characterized by late spontaneous seizures and widespread nerve cell loss. For differentiating acute from chronic changes in GABA receptor subunits, we investigated early (24 h) and late time intervals (7 and 30 days, respectively) after completion of kindling or induction of SE.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (250–280 g, Charles River, Calco, Italy) were housed at constant temperature (23 °C) and relative humidity (60%) with fixed 12 h light/dark cycle and free access to food and water. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with international laws and policies (European Ethics Committees Council Directive 86/609, OJ L 358, 1, 12 December 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Hippocampal kindling

Electrodes were implanted into the dorsal hippocampus under Equithesin anesthesia (9.7 mg/ml sodium pentobarbital in saline, 42.6 mg/ml chloral hydrate in propylenglycol and 21.2 mg/ml $MgSO_4$ in ethanol; 3.5 ml/kg i.p.), at the following coordinates: from bregma (mm), AP −3.5; L ±2.3; H 2.9 below the dura mater. The nosebar was set at 2.5 mm below the interaural line. EEG recordings were made using bilateral cortical and hippocampal electrodes in unaesthetized, freely moving rats. Kindling was started 7 days after implantation of electrodes when the animals showed no behavioral signs of discomfort or pain (Gobbi et al., 1998). Rats were allowed to acclimatize in a Plexiglas cage and an EEG recording was acquired for at least 10 min to assess the baseline EEG pattern. Before electrical stimulation, animals were randomly assigned to three groups: controls (implanted, but not stimulated) and two groups of rats evaluated at two different time points after kindling completion (24 h and 7 days, respectively).

Constant current stimuli were delivered unilaterally to the dorsal hippocampus through a bipolar electrode (recording electrode) twice daily for 5 days per week at intervals of at least 6 h. Stimulation parameters were 50 Hz, 2 ms monophasic rectangular wave pulses for 1 s. The current intensity ranged from 80 to 200 μA. Behavior was observed and duration of afterdischarge was measured in the stimulated hippocampus after each stimulation for every animal. Rats received an average of 28±3 stimuli to reach a fully kindled state (three consecutive stage 5 seizures according to Racine, 1972).

Self-sustained limbic SE

Rats underwent “continuous” hippocampal stimulation as previously described (Schwarzer et al., 1995; De Simoni et al., 2000). Electrodes were implanted at the following coordinates: from bregma (mm), AP −3.6; L ±4.9; H 5.0 below the dura. The nosebar was set at 5 mm below the interaural line. EEG recordings were made in the same way as for classical kindling. Before electrical stimulation, animals were randomly assigned to three groups for controls (implanted, but unstimulated) and two timepoints after SE. Animals were entered into the study only if their afterdischarge thresholds were ≤250 μA. The stimulus intensity was set to 400 μA (50 HZ, 1 ms biphasic square waves in 10 s-trains applied every 11 s) to override postictal refractoriness. Animals were then exposed to a “continuous” electrical stimulation protocol lasting 90 min. The subsequently developed SE abated within 24 h (Bertram et al., 1990).

Brain sections. Rats were killed by decapitation at 24 h (n=7) or 7 days (n=7) following kindling completion (at least three consecutive stage 5 seizures), and 24 h (n=10) or 30 days (n=8) after SE. Age-matched control rats were killed at each interval