CURRENT LITERATURE

INSIGHT INTO MOLECULAR MECHANISMS OF CATAMENIAL EPILEPSY

Diminished Allopregnanolone Enhancement of $GABA_A$-Receptor Currents in a Rat Model of Chronic Temporal Lobe Epilepsy

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1. Neurosteroid modulation of γ-aminobutyric acid $(GABA)_A$ receptors present on dentate granule cells (DGCs) acutely isolated from epileptic (epileptic DGCs) or control rats (control DGCs) was studied by application of GABA with or without the modulators and by measuring the amplitude of peak whole-cell currents.
2. In epileptic DGCs, GABA efficacy ($1{,}394 \pm 277$ pA) was greater than that in control DGCs (765 ± 38 pA).
3. Allopregnanolone enhanced GABA-evoked currents less potently in epileptic DGCs ($EC_{50} = 92.7 \pm 13.4$ n*M*) than in control DGCs ($EC_{50} = 12.9 \pm 2.3$ n*M*).
4. Pregnenolone sulfate inhibited GABA-evoked currents with similar potency and efficacy in control and epileptic DGCs.
5. Diazepam enhanced GABA-evoked currents less potently in epileptic ($EC_{50} = 69 \pm 14$ n*M*) compared with the control DGCs ($EC_{50} = 29.9 \pm 5.7$ n*M*).
6. Two different patterns of zolpidem modulation of $GABA_A$ receptor currents were found in the epileptic DGCs. In one group, zolpidem enhanced $GABA_A$-receptor currents but with reduced potency compared with the control DGCs ($EC_{50} = 134 \pm 20$ n*M* *vs.* 52 ± 13 n*M*). In the second group of epileptic DGCs, zolpidem inhibited $GABA_A$-receptor currents, an effect not observed in control DGCs.
7. Epileptic DGCs were more sensitive to Zn^{2+} inhibition of $GABA_A$-receptor currents ($IC_{50} = 19 \pm 6$ μM) compared with control ($IC_{50} = 94.7 \pm 7.9$ μM).
8. This study demonstrates significant differences between epileptic and control DGCs. We conclude that (a) diminished sensitivity of $GABA_A$ receptors of epileptic DGCs to allopregnanolone can increase susceptibility to seizures; (b) reduced sensitivity to diazepam and zolpidem and increased sensitivity to Zn^{2+} indicate that loss of allopregnanolone sensitivity is likely to be owing to altered subunit expression of postsynaptic $GABA_A$ receptors present on epileptic DGCs; and (c) an inverse effect of zolpidem in some epileptic DGCs demonstrates the heterogeneity of $GABA_A$ receptors present on epileptic DGCs.

COMMENTARY

Changes in cell number and neural circuits in the epileptic dentate gyrus have garnered much attention over the last decades, and deservedly so. It has been known for years that cell loss (e.g., within the dentate hilus), has a potentially important relation to epilepsy (1). More recently, seizure-induced neurogenesis has shown that increased granule cell numbers may occur and also influence seizures (2,24). Synaptic reorganization also is quite likely to play a role in the pathophysiology of the epileptic dentate gyrus (3).

In addition to these changes, studies that have taken advantage of modern molecular methods elucidated the alterations in dentate granule cell γ-aminobutyric acid (GABA) receptors after seizures, and showed time and again that these changes are likely to have functional importance.

For example, changes in GABA-receptor subunits during status epilepticus may explain decreased benzodiazepine efficacy as status progresses (4). Other studies using similar methods have shown why GABAergic inhibition may appear strong in the epileptic dentate gyrus, but actually be susceptible to collapse (5,6). The changes in GABA receptors are multifaceted (7), and although they may have functional implications, data from different models and human epileptic tissue do not always agree (3,8).

New data now expand the ways in which changes of GABA receptors alter the epileptic brain, and in this case potentially elucidate the pathophysiology of the female brain in catamenial epilepsy.

Mtchedlishvili et al. (9) examined dissociated granule cells from the epileptic brain of rats that were stimulated unilaterally in the ventral hippocampus to induce status epilepticus and subsequently, spontaneous recurrent seizures (10). Many weeks after status, during the period of recurrent seizures, animals were killed and granule cells were immediately dissociated.

Currents evoked by GABA application were compared with currents evoked by GABA and neurosteroids. The two neurosteroids that were chosen already are known modulate physiologic function, although studies to date have not used epileptic tissue. These neurosteroids are allopregnanolone and pregnenolone sulfate.

Pregnenolone is the precursor to progesterone, and allopregnanolone is a metabolite of progesterone. Allopregnanolone and pregnenolone sulfate have potent effects at the $GABA_A$ receptor normally (11). Allopregnanolone is thought to mediate anticonvulsant effects of progesterone (12). In contrast, pregnenolone sulfate appears to increase the susceptibility to seizures (13).

Mtchedlishvili et al. showed that dentate granule cells isolated from rats with recurrent seizures ("epileptic DGCs") had distinct pharmacology compared with DGCs of control rats. Notably, epileptic DGCs were far less sensitive to allopregnanolone, whereas effects of pregnenolone sulfate appeared to be similar.

These results suggest a molecular explanation for the increased seizure susceptibility in epileptic female brain, although admittedly the studies were conducted in male rats, and only DGCs were studied. Still, if granule cell inhibition is the barrier to seizure propagation in the hippocampus, as proposed (14,15), and similarity is found between female and male DGCs, the results are potentially very important.

These data add to a literature that has already pointed out the proepileptic changes involving altered GABA-receptor subunits occur in the normal female brain and are likely to be relevant to catamenial epilepsy.

Thus, during the normal menstrual cycle, the time when progesterone declines (the perimenstrual period) has been associated with increased seizures, and attributed to a "withdrawal" from the inhibitory effects of allopregnanolone (16,17). Studies by Smith et al. (18) showed that changes in GABA receptors subunits in a rodent model of progesterone withdrawal may be the reason for increased seizure susceptibility. Thus, both a decline in the concentration of allopregnanolone and an altered sensitivity of GABA receptors to allopregnanolone are likely to be important.

Studies in nonepileptic animals have shown that sensitivity to allopregnanolone was reduced when $\alpha 4$ (18) or δ subunits (19) were expressed. Although potentially relevant to altered seizure susceptibility after progesterone withdrawal, the relevance to chronic epilepsy was unclear. In the studies of Mtchedlivishili et al., epileptic DGCs appeared to be less sensitive to modulators of $\alpha 4$ or δ subunits. Thus, changes in $\alpha 4$ or δ subunit expression may underlie the altered sensitivity to allopregnanolone in epileptic DGCs. Increased expression of these receptors has been previously shown in epileptic DGCS by using another animal model of epilepsy (20), but whether this was the case in the experiments of Mtchedvilshili et al. was not explored.

The $\alpha 1$ subunit also was studied because this subunit appears to be influenced by allopregnanolone normally (21), and $\alpha 1$ subunits changes after seizures (20,22). Mixed results were obtained when the $\alpha 1$-subunit modulator zolpidem was used to probe potential changes in $\alpha 1$ subunit function. This may reflect that some changes in DGCs are not homogeneous, making epileptic DGC function more complex than other results would predict.

One could argue that isolated neurons are not as useful as intact neurons to understand epilepsy, because of potential alterations in receptors during the acute dissociation procedures, but the authors point out that both control and epileptic tissue were treated in the same way. Therefore, differences exist, although the relative contribution of synaptic versus extrasynaptic receptors may not yet be clear. Indeed one would hope that even more studies of this kind could be done to answer this question. In addition, expansion of the techniques to other cell types could potentially identify functional changes in receptors on both neurons and glia in the epileptic brain. For example, the $\alpha 1$ subunit is strongly expressed in some GABA neurons (23); what changes occur after seizures, and how does that influence granule cell inhibition? Such molecular studies would be an ideal complement to the more "intact" approaches that are presently defining changes in cell numbers and synaptic circuitry.

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