Selective Alteration of GABA<sub>A</sub> Receptor Associated Protein (GABARAP) in Thalamus in a Rat Model of Acquired Absence Epilepsy

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GABA<sub>A</sub> receptor associated protein (GABARAP) has been identified as a unique protein that plays a role in the targeting and transport of the γ<sub>2</sub> subunit to the GABA<sub>A</sub>R at the cytoplasmic membrane [1]. GABARAP binds to the γ<sub>2</sub> subunit of the GABA<sub>A</sub>R via its N-terminal domain and tubulin through its C-terminal domain, thereby acting as an adaptor between the GABA<sub>A</sub>R and the microtubular network [1]. GABARAP has a functional effect that promotes γ<sub>2</sub> subunit trafficking and expression in neurons [2]. Therefore, GABARAP is important for GABA<sub>A</sub>R integrity, particularly γ<sub>2</sub> subunit trafficking, targeting and fusion to the cytoplasmic membrane, which makes it an interesting candidate for the study of the mechanisms underlying the deficient GABA<sub>A</sub>R γ<sub>2</sub> subunit expression. Our previous studies revealed a reduction in the expression of the GABA<sub>A</sub>R γ<sub>2</sub> subunit selectively in the nRt but not cortex in a Cholesterol Synthesis Inhibition (CSI) rodent model, which may underlie the generation of thalamocortical SWDs [3]. To test the hypothesis that deficient γ<sub>2</sub> subunit trafficking and expression in the CSI model may appreciably increase GABARAP expression, we investigated protein expression patterns of GABARAP in the nRt and cortex in both CSI model and control rats.

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Barrow Neurological Institute. The CSI model was established as previously described [3,4]. Western blot and immunohistochemistry experiments were performed as previously described [5].

EEG recordings were performed on all animals used in this study, and all AY-9944-treated animals showed SWDs (Figure 1A). Western blot analyses revealed changes in expression of GABARAP in control (n = 5) versus CSI model rats (n = 6) and compared expression patterns in the nRt to somatosensory cortex. In the nRt, a significant increase in relative GABARAP protein expression (compared to β-actin) was identified in CSI compared to control rats (0.41 ± 0.03 vs. 0.21 ± 0.03, respectively, p < 0.01; Figure 1A,B), whereas in cortex there was no significant difference (0.53 ± 0.02 vs. 0.56 ± 0.06, p = 0.85, Figure 1A,B). In order to evaluate the specific alterations in GABARAP expression between nRt and cortex, we performed immunohistochemistry experiments using GABARAP-specific antibody. As shown in Figure 2, the nRt exhibited significantly stronger GABARAP immunoreactivity as measured by counts of GABARAP positive cells in tissue sections from CSI model compared to control rats (control nRt: 38 ± 9 vs. CSI model nRt: 134 ± 15, p < 0.01; Figure 2A,C). Consistent with the Western blot results, immunostaining intensities in sections of cortex was not significantly different between control and CSI model rats (control cortex: 133 ± 15 vs. CSI model cortex: 127 ± 23, p = 0.57; Figure 2A,C). However, the numbers of neurons in both nRt and cortex, as measured by treatment-blinded counts of cells marked by Nissl staining, were same (control cortex: 243 ± 27 vs. CSI model cortex: 245 ± 21; control nRt: 216 ± 14 vs. CSI model nRt: 207 ± 25; Figure 2D).

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2B,D). These results demonstrate that there is increased GABARAP expression in tissue sections from the nRt of CSI models compared to controls, suggesting that GABARAP expression is selectively increased in the nRt but not in cortex in CSI models.

The mechanism by which cholesterol synthesis inhibition in newborn rat brain leads to alteration in brain physiology remains unclear. Our previous studies have shown that early-life block of cholesterol synthesis results in a significantly higher occurrence of EEG SWDs and decreased GABA \(_R\) \(\gamma_2\) subunit protein expression with unchanged \(\gamma_2\) subunit mRNA levels in the nRt. The discrepancy between the unchanged mRNA levels by RT-PCR and decreased protein expression suggests a post-translational modification of \(\gamma_2\) subunit expression [6,7]. GABARAP binds to and plays an important role in the intracellular trafficking and/or postsynaptic clustering of the GABA \(_R\) \(\gamma_2\) subunit. A compensative increase in expression of GABARAP might be the post-translational mechanism underlying lower \(\gamma_2\) subunit expression.

The present study intended to examine changes in GABARAP expression in the CSI model in order to investigate whether a homeostatic mechanism may be responsible for the observed lower \(\gamma_2\) subunit expression in the nRt following early-life block of cholesterol synthesis. Both Western blot and immunostaining results demonstrate that the nRt has a significantly higher level of expression of GABARAP but possesses the same number of cells when comparing the nRt and cortex in CSI model rats, which supports the conclusion that alteration in GABARAP expression is likely a homeostatic consequence of reduced \(\gamma_2\) subunit expression.

This is the first time to demonstrate an alteration of GABARAP in a specific brain area (nRt) in an absence epilepsy model. It will be highly interesting in further evaluation of the impact of this GABARAP change in epileptogenesis and therapeutics.

References