Development and phenotypic characterization of GAD67 knockdown mice by using a Tet-off system

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GABA is a major inhibitory neurotransmitter in the mammalian brain and regulates emotional behaviors. GABA is synthesized from glutamate by glutamate decarboxylase (GAD) which exists as two isoforms, GAD65 and GAD67, encoded by separate genes. The function of GAD65 is well investigated by using global GAD65 knockout ($GAD65^{-/-}$) mice. However, the function of GAD67, particularly in the adult brain, is not well investigated because $GAD67^{-/-}$ mice died on the day of the birth. In order to resolve the function of GAD67, we developed novel GAD67 knockdown ($GAD67^{tTA/STOP\text{-}tetO}$) mice by using a Tet-off system and investigated the biological phenotypes in those mice. Approximately 30% of $GAD67^{tTA/STOP\text{-}tetO}$ mice survived to adulthood. Treatment with doxycycline (Dox) for 3 weeks in adulthood markedly decreased the protein levels of GAD67 in the frontal cortex, hippocampus and cerebellum of $GAD67^{tTA/STOP\text{-}tetO}$ mice. Metabolome analysis demonstrated that the GABA contents were significantly decreased in the frontal cortex, hippocampus and cerebellum of Dox-treated $GAD67^{tTA/STOP\text{-}tetO}$ mice compared to Dox-treated $GAD67^{+/+}$ mice. In addition, several other metabolites were significantly decreased in the frontal cortex, but not in the hippocampus or cerebellum, of Dox-treated $GAD67^{tTA/STOP\text{-}tetO}$ mice. On the other hand, no significant difference was observed in the metabolite levels, including GABA content, in the frontal cortex between $GAD65^{-/-}$ and $GAD65^{+/+}$ mice. These results suggest that GAD67 is the major enzyme of GABA synthesis and regulates metabolite levels in a brain region-dependent manner.