Effects of Blocking Brain Estrogen Synthesis on Expression of Extracellular Matrix Genes in the Mouse Cortex

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Abstract

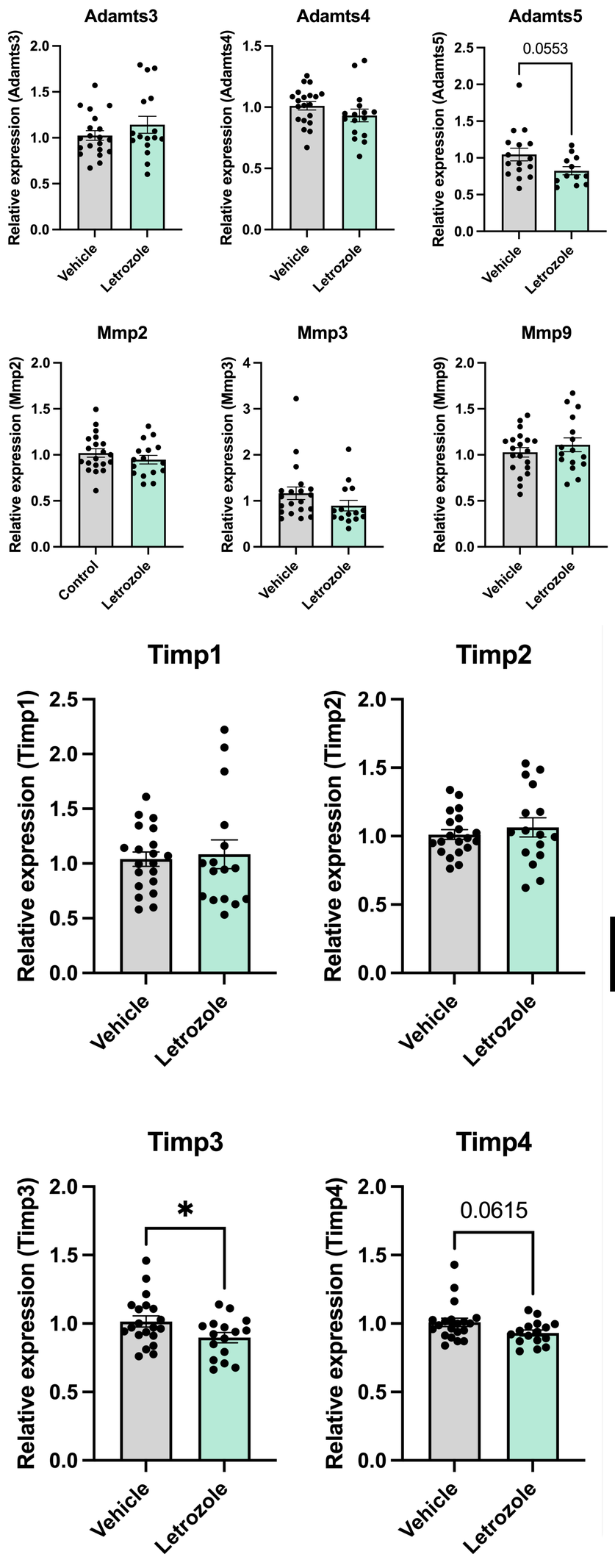
Alcohol Use Disorder (AUD) is defined by the inability of individuals to control or stop alcohol use despite profound consequences on individual health, relationships, and daily life. Compulsive drinking is a key symptom in the development of AUD treatment due to its resistance to conventional treatment methods. Previous research suggests that extracellular matrix formations known as perineuronal nets (PNNs) found in the insular cortex of the brain could be responsible for elevated aversion-resistant alcohol consumption and compulsive drinking in mice. Female mice have been found to have elevated levels of PNNs in their insular cortex, as well as higher levels of aversion-resistant drinking than their male counterparts. To determine the cause of the sex difference between PNN intensity, female mice were treated with an estrogen-blocking drug called Letrozole to see what impact estrogen has on the formation of

Methods

- RNA was extracted from Insular cortex tissue from 36 female mice that had ovariectomies performed, 16 of which were treated with Letrozole
- The extracted RNA was then turned into complimentary DNA (cDNA)
- The cDNA was used in qPCR testing to determine the presence of extracellular matrix genes
- The genes being tested for where: Timp1, Timp2, Timp3, Timp4, Mmp2, Mmp3, Mmp9, Adamts3, Adamts4, Adamts5
- PCR was also performed for housekeeping genes: Gusb, Hprt and RpI13
- The PCR test consisted of 0.5µL of gene respective primers combined with 5µL of SYBR Green
- This mixture was combined with 5µL of each sample and then PCR tested

Results

- The mice treated with Letrozole have lower relative gene expression compared to the control group
- The only statistically significant difference can be found in the gene Timp3 ($p<0.05$) while the Adamts5 ($p=0.0553$) and Timp4 ($p=0.0615$) show trends toward significant decrease



Discussion

- The Adamts3-5 genes and the Mmp2,3 and 9 genes are all responsible for degrading PNN structures
- The lower expression of these genes could mean that mice treated with Letrozole have higher levels of PNNs in their insular cortex
- The other genes that were significant were Timp3-4 which are responsible for offsetting the degradation process of the previously mentioned genes
- These lowered levels could just be the body trying to re-establish homeostasis and lowering these genes as a result of the lowered degradation genes
- In conclusion, estrogen may modulate the levels of mRNA in the insula that encodes enzymes and their inhibitors that are involved in PNN degradation
- More experiments and brain staining would need to be done in order to pinpoint exact PNN levels and determine the significance of the difference

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