Role of Munc13-1 and Munc13-2 proteins in alcoholism, and its potential clinical applications

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ABSTRACT

Munc13-1 and Munc13-2 are presynaptic proteins that are involved in the vesicular priming and subsequent release of glutamate neurotransmitter. Previous research has demonstrated some of the effects of ethanol in specific areas of the brain related to addiction, but the effects of ethanol on the expression of Munc13-1 and Munc13-2 and their glutamatergic effects in different brain regions is unknown. First, we performed immunocytochemistry (ICC) of Munc13-1 and Munc13-2 on ethanol-treated differentiated HT22 cells in-vitro and found that there was a significant change in the overall expression of Munc13-1 in a dose and time-dependent manner. Next, we harvested primary hippocampal neurons, treated them with ethanol in a dose and time-dependent manner, and then measured both Munc13-1 and Munc13-2 protein expression using Western-blot analysis. Here, our results showed that ethanol significantly upregulates the expression of both Munc13-1 and Munc13-2. We then tested the effects of ethanol on Munc13 proteins in-vivo on both wild-type (Wt) and heterozygous Munc13-1 knockout mice, using the drinking in the dark (DID) paradigm. In comparison to our previous ex-vivo data, these results showed that with alcohol exposure there was a significant increase in the expression of Munc13-1 in the hippocampus and cerebellum of Wt mice, but a decrease in the expression of Munc13-2 in the hippocampus, cerebellum and cortex. In the heterozygous Munc13-1 knockout mice, ethanol caused significant compensation for the loss of Munc13-1 in the hippocampus and cerebellum, and also an increase in Munc13-2 expression in the cerebellum and cortex. Since both Munc13-1 and Munc13-2 predominantly control glutamatergic synapses, their modulation by ethanol exposure could potentiate an increase in glutamate release from the pre-synaptic terminals of these regions and stimulate excitation of the Central Nervous System (CNS). This is analogous to the CNS excitation we see with chronic AUD patients experiencing alcohol withdrawal symptoms. By identifying these proteins as drug targets, we may be able to unfold a different mechanism of alleviating alcohol withdrawal symptoms in AUD patients, without the GABAergic adverse effects that usually present with the current mainstay treatment options.

BACKGROUND

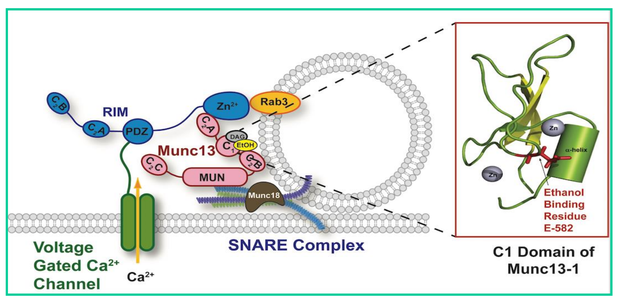


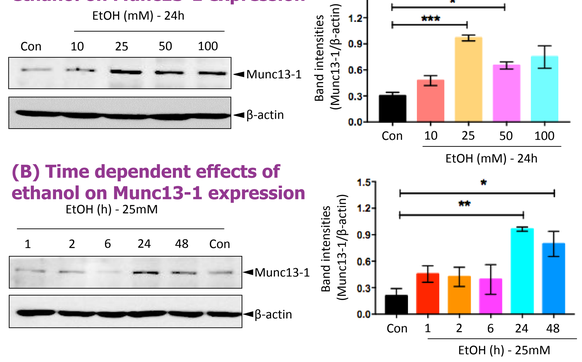
Fig. 1. Munc13-1 involved in active-zone priming of glutamate vesicles for release into the synapse

RESULTS

HT22 CELLS (*In-vitro*)

Fig 2. Ethanol upregulates Munc13-1 in differentiated HT22 cells

(A) Dose dependent effects of ethanol on Munc13-1 expression

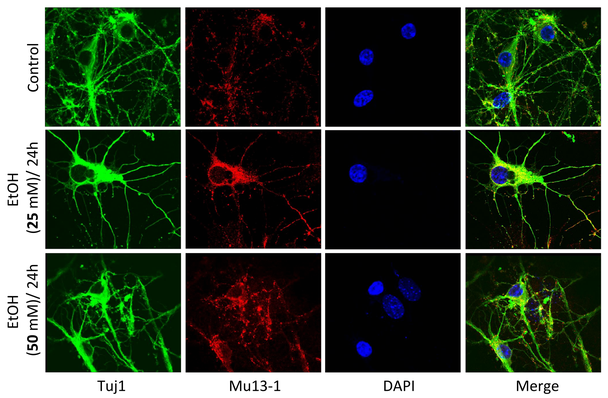


(B) Time dependent effects of ethanol on Munc13-1 expression

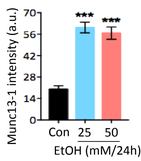
PRIMARY HIPPOCAMPAL NEURONS (*Ex-vivo*)

Fig. 3. Ethanol up-regulates the expression of Munc13-1 in primary hippocampal neurons

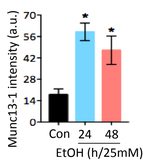
(A) Immunocytochemistry of Tuj1 and Munc13.1 (dose dependent)



(B) Munc13-1 intensity (dose dependent)



(D) Munc13-1 intensity (time dependent)



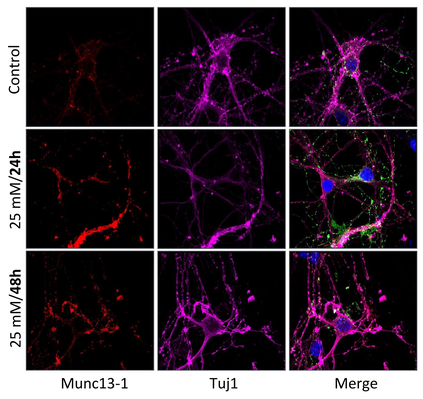
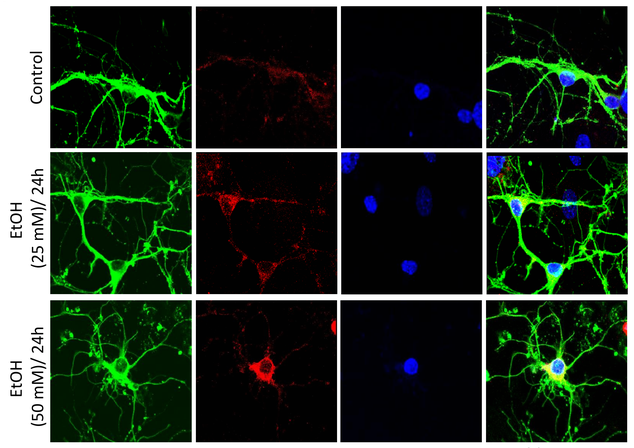
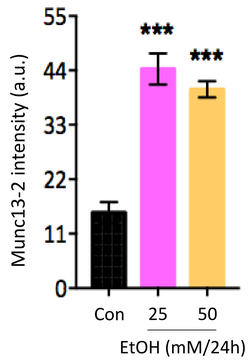


Fig. 4. Ethanol up-regulates the expression of Munc13-2 in primary hippocampal neurons

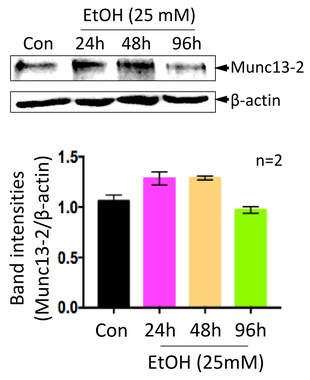
(A) Double labeled ICC of Tuj1 and Munc13-2 (dose-dependent) in primary hippocampal neurons



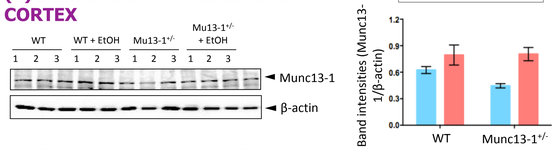
(B) Munc13-2 fluorescence intensity (dose dependent)



(C) Immunoblot & band intensity of Munc13-2 (time dependent)



(C) Immunoblot of Munc13-1 in CORTEX



(D) Immunoblot of Munc13-1 in STRIATUM

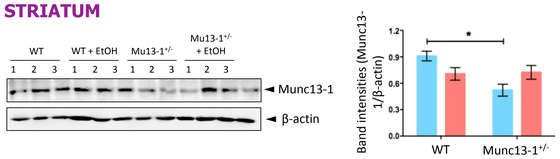
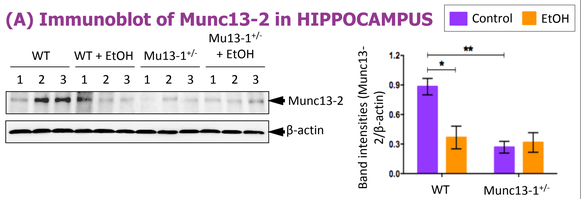
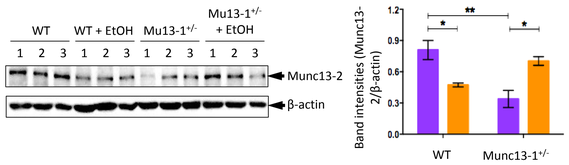


Fig. 6. Effects of ethanol on Munc13-2 expression in different regions of brain

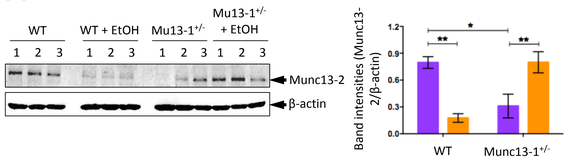
(A) Immunoblot of Munc13-2 in HIPPOCAMPUS



(B) Immunoblot of Munc13-2 in CEREBELLUM



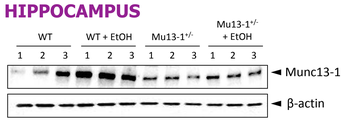
(C) Immunoblot of Munc13-2 in CORTEX



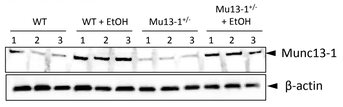
MICE (*In-vivo*)

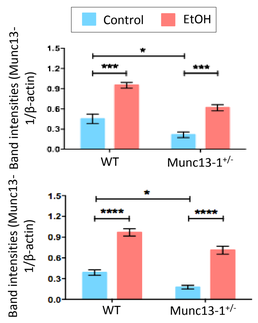
Fig. 5. Effects of ethanol on Munc13-1 expression in different regions of brain

(A) Immunoblot of Munc13-1 in HIPPOCAMPUS



(B) Immunoblot of Munc13-1 in CEREBELLUM





CONCLUSIONS

- ✦Ethanol upregulates the expression of Munc13-1 and downregulates the expression of Munc13-2 in chronically ethanol-treated mice.
- ✦This modulation of protein expression by ethanol may be responsible for the increased glutamatergic activity we see in AUD patients during alcohol withdrawals
- ✦Further studies using drugs that can inhibit Munc13-1 and/or enhance the effect of Munc13-2 could lend to the development of a glutamatergic therapeutic approach which differs from the conventional GABAergic drug therapies that are currently utilized.

Acknowledgement: NIH R01 grant (1R01AA022414-01A1)