NMDA Receptor Inhibition Prevents Intracellular Sodium Elevations Induced by the Sodium Ionophore Monensin in Human Olfactory Neuroepithelial Precursors Derived from Bipolar Patients

Rif S. **El-Mallakh**, MD,[1] Yonglin **Gao**, MD,[1] Aaron **Mack**, MD,[1] Nick **Delamere**, PhD,[2]
[1]Department of Psychiatry, University of Louisville School of Medicine, Louisville, Kentucky
[2]Head, Department of Physiology, University of Arizona, Tucson, Arizona

INTRODUCTION

- The life-time prevalence of comorbid alcohol use disorder in patients with bipolar illness exceeds 60%
- We have previously reported that ionic disturbances caused by glutamate in neuronal cells derived from subjects with bipolar disorder are corrected by lithium or ethanol (EtOH), but only in cells from bipolar individuals and not in cells from non-bipolar controls
- We examined NMDA receptor function in these cells in response to a non-glutamate ionic stress.

METHODS

- Olfactory neuroepithelial precursors (ONPs) were obtained by biopsy from type I bipolar patients and non-bipolar controls matched for age, gender, and passage number (n=3 and n=6, respectively).
- ONPs were cultured in MEM, gentamycin 0.1mg/mL and FBS 10%, in 5% CO2. ONPs in culture produce neurons, glia, and undifferentiated neural precursors cells. ONPs were treated with, 1 µM monensin (sodium ionophore) for 6 hours, 0.1mM AP_5 (NMDA glutamate binding site receptor antagonist) for 6 hours, Or pretreated AP5 30 minutes followed by monensin for 6 hours.
- Intracellular sodium ($[Na]_i$) was measured with flame spectroscopy and expressed as concentration per protein as measured by Lowery.

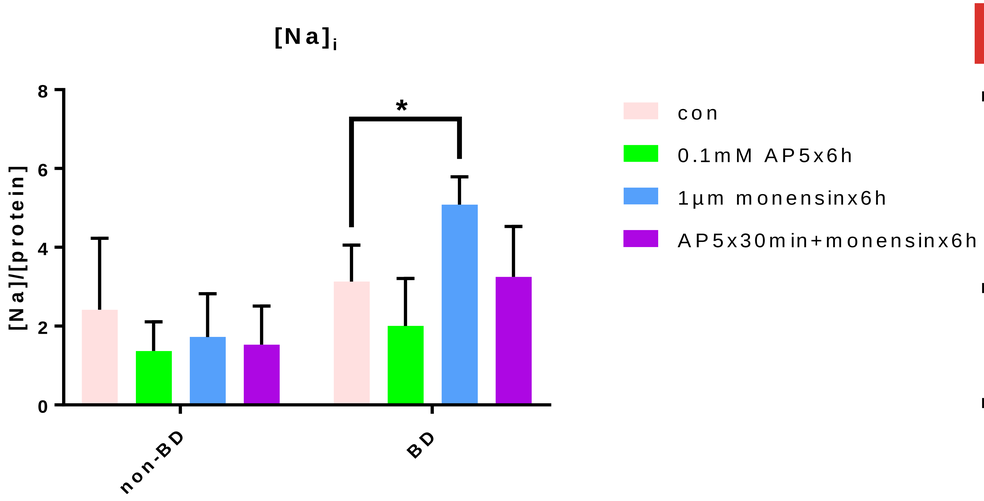

Figure 1: Intracellular sodium concentration significantly increased in BD-ONPs with monensin 1 µm for 6 hours (* P<0.05). AP5 pretreated 30 minutes followed by monensin for 6 hours treatment normalize the intracellular sodium concentration

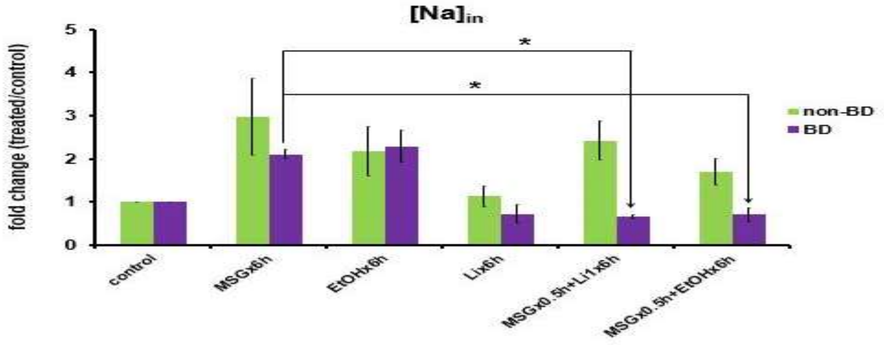

Figure 2: Both EtOH and lithium normalize elevated intracellular sodium caused by glutamate in ONPs obtained from bipolar individuals but not from cells obtained from non-bipolar subjects

RESULTS

- Monensin alone significantly increased $[Na]_i$ in ONPs from bipolar individuals (5.08 ± 0.71 vs baseline 3.13 ± 0.93, P =0.03) and AP_5 had no effect (2.0 ± 1.2 vs baseline 3.13 ± 0.93, P =0.27).
- The combination of AP_5 and monensin resulted in normalization of $[Na]_i$ (3.25 ± 1.28 vs baseline 3.13 ± 0.93, P =0.89).
- This effect was not observed in cells from non-bipolar individuals (monensin alone, 1.72 ± 1.10 vs baseline 2.42 ± 1.80, P =0.25; AP_5 alone 2.10 ± 1.10 , AP_5 combined with monensin, 1.53 ± 0.98 vs baseline 2.42 ± 1.80, P =0.31).

DISCUSSION & CONCLUSION

- ONPs derived from bipolar individuals are more susceptible to $[Na]_i$ elevations induced by monensin compared to controls
- Blockade of NMDA glutamate binding site with AP_5 prevents elevations of $[Na]_i$ and normalizes response to sodium ionophore monensin
- NMDA receptor appears necessary for bipolar-specific sodium ion dysregulation

Gao Y, Lohano K, Delamere NA, Lei Z, El-Mallakh RS. Ethanol normalizes glutamate-induced elevation of intracellular sodium in olfactory neuroepithelial progenitors from subjects with bipolar illness but not nonbipolar controls: Biologic evidence for the self-medication hypothesis. *Bipolar Disorders* **21**(2):179-181, 2019. doi: 10.1111/bdi.12737