Proceedings of the 24th American Peptide Symposium
Ved Srivastava, Andrei Yudin, and Michal Lebl (Editors)
American Peptide Society, 2015 *http://dx.doi.org/10.17952/24APS.2015.101*

Alcohol Abrogates Intracellular Ca^{2+} Elevation by Angiotensin II and ATP in Cultured Rat Astrocytes

Steve Wu[1], Zhiqian Wu[2], and Desuo Wang[2]
[1]PharmD Candidate PCOM School of Pharmacy Class 2017; [2] Department of Pharmaceutical Sciences, Philadelphia College of Osteopathic Medicine School of Pharmacy, Suwanee, GA, 30024, USA

Introduction

Astrocytes have a robust and dynamic intracellular Ca^{2+} activity in response to stimulation by various neurotransmitters and neuromodulators such as angiotensin II, glutamate, and ATP [1-4]. ATP evokes Ca^{2+} bursts in astrocytes by activation of purinergic receptors [4] and angiotensin II (AngII) by stimulation of AT1 receptors [2]. Intracellular Ca^{2+} mobilization by the peptide and high energy purine signaling molecules enhances astrocyte cycling excitatory neurotransmitter glutamate, which governs the social behavior and cognitive ability [5-7]. Acute alcohol overdose alters users' social behavior and affects abusers' cognition [8,9]. We hypothesize that alcohol impairs the intracellular Ca^{2+} handling in astrocytes particularly in response to neurotransmitter/neuromodulator stimulation, which may play an important role in understanding alcohol intoxication. The impairment may relate to the development of alcohol addiction, dependence and tolerance. In this work, we studied the alteration of intracellular Ca^{2+} signaling by alcohol treatment in cultured rat hippocampal astrocytes. The findings of this study enrich our understanding of ethanol intoxication and may lead to a new treatment target for alcoholism.

Results and Discussion

Rat hippocampus astrocytes (GFAP immunocytofluorescence positive) from Creative Bioarray (CSC-C8055L) were used to measure intracellular Ca^{2+} transients at 33±0.5ºC. The cells were continuously perfused with Hank's solution containing 2.0 mM $CaCl_2$ (pH=7.4) and loaded with 1 µM Fura-2 AM (Thermo Fisher Scientific, Life Technologies, F-1201) in 0.1 mM Ca^{2+} Hank's solution containing 1 nM pluronic acid at 37 ºC for 30 min. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured as a ratio of 340- over 380-nm wavelength fluorescence signals and digitized and recorded using an IonOptix PMT system.

In control experiments (without alcohol treatment), both Ang II and ATP concentration-dependently elevated $[Ca^{2+}]_i$ in the cultured astrocytes. The responsive Ca^{2+} signals occurred reliably in almost every single cell tested (n>20) and the elevation of $[Ca^{2+}]_i$ was repeatable in the same cell when it was challenged second time with Ang II or ATP as shown in Figure 1.

Peptidergic neurotransmitter angiotensin II produced a peak and a prolonged plateau elevation in $[Ca^{2+}]_i$. In comparison, purinergic neuromodulator ATP caused a brief and steeper Ca^{2+} peak with a much faster recovery phase (i.e., lack of prolonged plateau elevation of $[Ca^{2+}]_i$. These results confirmed our previous work and were consistent with the data published by other investigators [2, 10,11]. The commercially available primary culture of glial fibrillary acidic protein (GFAP)-positive astrocytes is

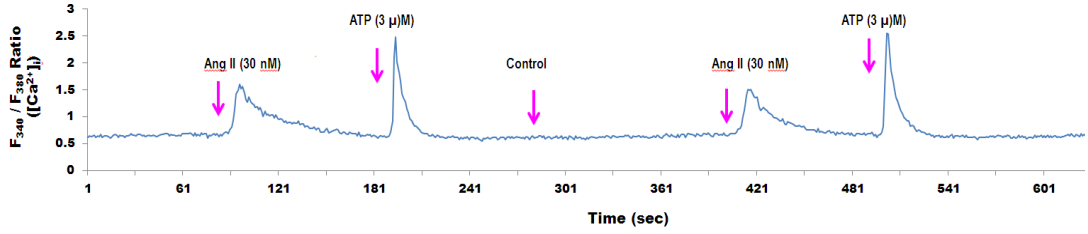

Fig. 1. Puff-application of Ang II (30 nM) or ATP (3 µM) or control vehicle caused increase in $[Ca^{2+}]_i$ in an astrocytes (a typical control experiment). The vertical axis is the ratio of fluorescence signal (F_{340}/F_{380}), an up-deflection indirectly indicates an increase in $[Ca^{2+}]_i$ relative to baseline. The horizontal axis is the real time elapsed after the experiment begins. Arrows indicate the puff-application of the test agents.

a convenient and reliable experimental model for studying the regulation of $[Ca^{2+}]_i$ by neurotransmitters and neuromodulators. Brain ACE/Ang II/AT1 axis plays an important role in stimulating the elevation of $[Ca^{2+}]_i$. When the cells were challenged with another angiotensin peptide Ang(1-7), there was no intracellular Ca^{2+} mobilization (data not shown). This indicates that the ACE2/Ang(1-7)/mas axis does not alter intracellular Ca^{2+} homeostasis in astrocytes [12].

We noticed that puff-application of ATP at micromolar concentration (1 to 100 µM) is a dependable positive control for measurement of astroglial $[Ca^{2+}]_i$. In this cell culture model, astrocytes that responded to ATP stimulation also faithfully answered the challenge by Ang II. However, it is worth to point out that although peptidic Ang II and non-peptidic ATP stimulation both can activate astrocytes by elevation of $[Ca^{2+}]_i$, the Ca^{2+} transient signals elicited by these two stimuli are distinguishable by their peak amplitude and duration (Figure 1).

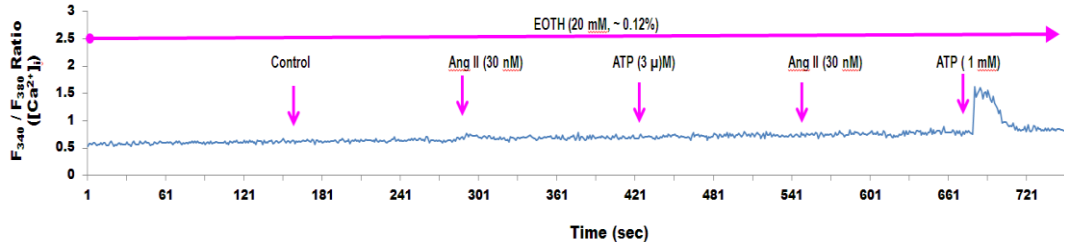

Fig. 2. Alcohol (EOTH) treatment abolishes intracellular Ca^{2+}signal triggered by both Ang II and AT. Same results were recorded from three cells (n=3). The horizontal arrow indicates the length of the EOTH application. The vertical arrows mark the puff of test agents.

The spatial and temporal differences in Ca^{2+} mobilization in the same cell demonstrate the diversity in the regulation of $[Ca^{2+}]_i$ even though activation of AT1 receptor by Ang II and P2Y receptor by ATP both are coupled to Gq-PLC-IP3 signaling transduction pathway [2,4]. What is/are the underlying mechanism(s) for these differences are not clear and it is worth to be elucidated in future studies.

Alcohol treatment (for 5-10 min) markedly affects ACE/Ang II/AT1 Ca^{2+} signaling in astrocytes (Figure 2). Ethanol (EOTH) itself did not cause any dynamic $[Ca^{2+}]_i$ fluctuation. However, in the presence of alcohol (20 mM, approximately equal to 0.12% plasma alcohol level), both the peak and the plateau phases of $[Ca^{2+}]_i$ elevation by angiotensin II (30 nM) were completely abolished (Figure 2 and 3). Application of higher concentration of Ang II (300 nM) did not counteract EOTH inhibition. The impact of alcohol on ATP-stimulated Ca^{2+} signal is more complicated. In one group of cells, EOTH abrogated physiologically relevant micromolar ATP-triggered $[Ca^{2+}]_i$ elevation [13] and only blunted $[Ca^{2+}]_i$ elevation when cells were challenged with much higher concentration of ATP (1 mM, Figure 2). In another group of cells, EOTH treatment only abolished peptidergic Ang II signal but had no impact on micromolar purinergic ATP-stimulated Ca^{2+} signaling (Figure 3). It is interesting

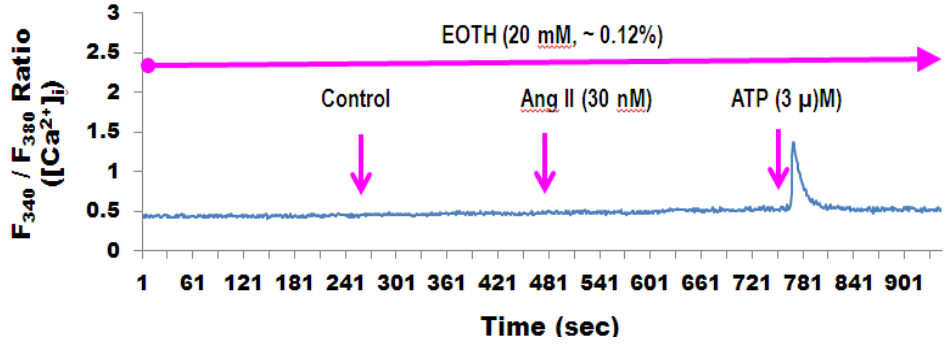

Fig. 3. Ethanol (EOTH) treatment abolishes intracellular Ca^{2+} signal triggered by Ang II. However, in this group of cells, EOTH exposure does not affect the effect of ATP on Ca^{2+} mobilization. Same results were recorded from three cells (n=3). The horizontal arrow indicates the length of the EOTH application. The vertical arrows mark the puff of test agents.

to find out the mechanism(s) that underlie the distinguishing influence of ethanol intoxication on Ang II-induced Ca^{2+} signaling and ATP-stimulated Ca^{2+} elevation in astrocytes in future study.

We noticed that EOTH (20 mM) treatment seemed to slightly elevate the baseline $[Ca^{2+}]_i$ in the time course of experimental recordings (Figure 2 and 3). This could be due to the non-specific impact of ethanol on lipid membrane or other Ca^{2+} transporting mechanisms in astrocytes. The small elevation of baseline $[Ca^{2+}]_i$ does not affect the dynamic Ca^{2+} signaling as shown in Figure 3.

The concentration of ethanol (20 mM) used in this study is approximately equal to 0.12% plasma alcohol level, which is relevant to alcohol intoxication. When blood alcohol content is in the range of 0.06-0.15%, drinkers may have moderate memory impairments, significant impairments in driving skills, impaired speech, balance, coordination and attention [14]. How the alteration of intracellular Ca^{2+} handling in astrocytes is correlated to these social behavioral and cognitive symptoms of alcohol intoxication is not clear. We suggest that acute ethanol exposure not only impairs the function of neurons but also disrupts the regulation of $[Ca^{2+}]_i$ by neurotransmitters and neuromodulators in astrocytes of the brain [15,16]. The later may play a significant role in alcoholism. As suggested by Agulhon, et al. [17], astrocytic G_q GPCR-mediated Ca^{2+} elevations may trigger the release of gliotransmitters through unresolved pathways. Astrocyte-released gliotransmitters may then signal back to neurons by activating presynaptic or postsynaptic (extrasynaptic) neuronal receptors to modulate synaptic transmission. Ethanol abrogates G_q GPCR-mediated Ca^{2+} elevations (panel B of Figure 4) and disrupts the normal signal communication between neurons and astrocytes.

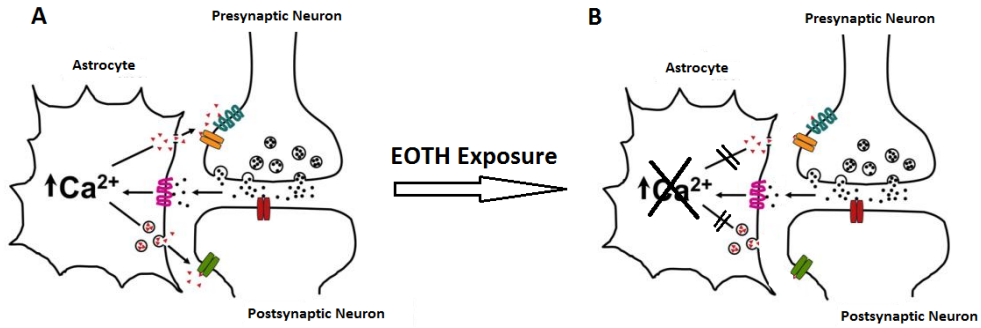

Fig. 4. Schematic depiction of the impact of EOTH exposure on neuro-astrocyte interaction (modified from Agulhon et al 2008).

In summary, our results are the first to demonstrate that acute alcohol exposure affects G_q GPCR-mediated Ca^{2+} elevations by Ang II and ATP (at least in some cells for the latter). The alcohol-caused alteration in the regulation of $[Ca^{2+}]_i$ by neurotransmitters and neuromodulators in astrocytes may have a significant role in alcohol intoxication. These study findings enrich our understanding of ethanol intoxication and may open up new treatment modalities for alcoholism.

Acknowledgments

SW was supported by the federal work study program. This work is supported by a research grant funded by the Research Division of Chief Science Officer in Philadelphia College of Osteopathic Medicine (Georgia Campus).

References

1. *Neural-Immune Interactions in Brain Function and Alcohol Related Disorders*, Cui, C., Grandison, L., Noronha, A. (Eds.), Springer, 2013, http://dx.doi.org/10.1007/978-1-4614-4729-0
2. Wang, D., et al. *J. Neurochem.* **67**, 996-1004 (1996), http://dx.doi.org/10.1046/j.1471-4159.1996.67030996.x
3. Shigetomi, E., et al. *Nat. Neurosci.* **13**, 759-766 (2010), http://dx.doi.org/10.1038/nn.2557
4. Franke, H., et al. *Purinergic Signal.* **8**, 629-657 (2012), http://dx.doi.org/10.1007/s11302-012-9300-0
5. Lee, L., et al. *J. Alzheimers Dis.* **38**, 49-62 (2014), http://dx.doi.org/10.3233/JAD-130740
6. Yadav, R., et al. *PLoS One* **7**, e32969 (2012), http://dx.doi.org/10.1371/journal.pone.0032969
7. Gupta, S.C., et al. *Neuropharmacology* **93**, 274-284 (2015), http://dx.doi.org/10.1016/j.neuropharm.2015.02.013
8. Cash, C., et al. *J. Psychopharmacol.* **29**, 436-446 (2015), http://dx.doi.org/10.1177/0269881115570080
9. Archimi, A., Kuntsche E. *Addict. Behav.* **39**, 713-716 (2014), http://doi/10.1016/j.addbeh.2013.11.011

10. Hamilton, N. *Glia.* **56**, 734-749 (2008), http://doi/10.1002/glia.20649
11. Park, H., et al. *J. Neurosci.* **29**, 13063-13073 (2009), http://doi/10.1523/JNEUROSCI.3193-09.2009
12. Ohishi, M., et al. *Curr. Pharm. Des.* **19**, 3060-3064 (2013), http://doi/10.2174/1381612811319170013
13. Yegutkin, G.G., et al. *Mol. Biol. Cell.* **17**, 3378-3385 (2006), http://doi/10.1091/mbc.E05-10-0993
14. http://pubs.niaaa.nih.gov/publications/AlcoholOverdoseFactsheet/Overdosefact.htm
15. Muñoz, G., et al. *Neurobiol. Aging* **36**, 845-856 (2015), http://doi/10.1016/j.neurobiolaging.2014.10.017
16. Llorente, J., et al. *Mol. Pharmacol.* **84**, 252-260 (2013), http://doi/10.1124/mol.113.085936
17. Agulhon, C., et al. *Neuron.* **59**, 932-946 (2008), http://doi/10.1016/j.neuron.2008.09.004