Published in final edited form as:

Biol Psychiatry. 2016 March 15; 79(6): 425–426. doi:10.1016/j.biopsych.2015.12.013.

Binge Drinking With Protein Kinase C Epsilon: A Role for Mammalian Target of Rapamycin Complex 2?

Angelo Blasio and Robert O. Messing

Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, Texas

In the United States, the 2010 total estimated cost of excessive drinking was \$249.0 billion; binge drinking accounted for 76.7% of this amount (1). Binge drinking is associated with substantial lost productivity, medical complications (including fetal alcohol syndrome), and legal costs. Understanding the underlying neurobiology of binge drinking could inform treatment strategies with a major impact on human health.

In this issue of *Biological Psychiatry*, Cozzoli *et al.* (2) investigate the role of the enzyme protein kinase C epsilon (PKC ϵ) in binge-like ethanol consumption using two models: a modified drinking in the dark (DID) procedure and a modified scheduled high alcohol consumption procedure. Their findings add to a growing literature, much of it from our laboratory, that indicates an important role for PKC ϵ in ethanol consumption and reward (3). The PKC ϵ enzyme is a member of the PKC family of serine-threonine kinases, which transduce signals carried by lipid second messengers. PKC ϵ knockout mice drink substantially less ethanol than wild-type mice in a continuous access, two-bottle choice procedure and when trained in operant self-administration of ethanol. They also show heightened aversion to ethanol and greater signs of ethanol intoxication secondary to impaired acute functional tolerance. These behaviors are not the result of developmental changes, as inducible transgenic expression of PKC ϵ in the amygdala and striatum restores normal sensitivity to intoxication and increases drinking in PKC ϵ knockout mice to levels of wild-type mice, whereas knockdown of PKC ϵ in the amygdala reduces binge ethanol consumption in adult wild-type mice.

Alcohol acutely inhibits glutamate signaling, and repeated ethanol exposure induces a hyperglutamatergic state that is thought to contribute to excessive ethanol consumption (4). Cozzoli *et al.* explored mechanisms by which ethanol and glutamate signaling converge to regulate binge drinking via PKC ϵ . They first showed that in C57BL/6J mice, 30 days of repeated 2-hour episodes of DID followed by 24 hours of withdrawal increased PKC ϵ phosphorylation at Ser729 in the nucleus accumbens (NAc) and central amygdala (CeA), two brain regions important for self-administration of abused drugs. The authors repeated the experiment using a modified scheduled high alcohol consumption procedure with similar

Address correspondence to Robert O. Messing, M.D., UT Austin College of Pharmacy, C0875, 107 West Dean Keeton, BME 6.116A, Austin, TX 78712; romessing@austin.utexas.edu.

Disclosures

AB reports no biomedical financial interests or potential conflicts of interest.

findings in NAc. The mammalian target of rapamycin complex 2 (mTORC2) phosphorylates PKC ϵ at S729, and this post-translational processing event is required for full kinase activity (5). Therefore, these findings suggest that binge drinking stimulates mTORC2 activity in the NAc and CeA, leading to PKC ϵ phosphorylation at Ser729, which increases the capacity for PKC ϵ activation by lipid second messengers. Mechanisms by which ethanol stimulates mTORC2 kinase activity have been most extensively studied in C2C12 mouse myoblasts, where ethanol increases the expression of several mTORC2 components, while impairing interactions with negative regulators (6). Nothing is known about how ethanol activates mTORC2 in the brain, making this a new area ripe for future study.

Cozzoli *et al.* next explored the role of glutamate signaling in binge drinking. Previous work from our laboratory indicated a relationship between PKC ϵ and signaling through group 1 metabotropic glutamate receptors (mGluRs); we found that the mGluR5 antagonist 6-methyl-2-(phenylethynyl)pyridine administered into the NAc of *Prkce*^{-/-} mice failed to reduce their ethanol consumption, whereas activation of group 1 mGluRs increased phosphorylation of PKC ϵ at S729 in the NAc of wild-type mice (7). Cozzoli *et al.* further investigated the relationship between group 1 mGluR signaling and PKC ϵ in the DID procedure. Delivery of the PKC ϵ inhibitor peptide into the CeA reduced DID, and coadministration of an mGluR5 or a phospholipase C inhibitor further reduced ethanol intake, whereas an mGluR1 inhibitor did not. These results suggested that the effect of mGluR1 in the CeA is PKC ϵ dependent, whereas the effect of mGluR5 is partly PKC ϵ independent. In the NAc, the PKC ϵ inhibitor peptide also reduced DID, but coadministration of an mGluR5, mGluR1, phospholipase C, or phosphatidylinositol-3-kinase (PI3K) inhibitor attenuated this effect. Although the authors did not test the effect of mGluR1, mGluR5, phospholipase C, or PI3K inhibitors alone, their result in the NAc resembles that of Gass and Olive (8), who found that administering the PKC ϵ inhibitor peptide into the NAc reverses the ability of an mGluR5 antagonist to reduce ethanol self-administration in rats. PKC phosphorylation is known to downregulate type 1 mGluR function by promoting rapid receptor desensitization and by reducing receptor trafficking to the cell surface (9). Such processes could provide mechanisms by which inhibiting PKC ϵ —and upregulating type 1 mGluR function—diminishes the effectiveness of type 1 mGluR antagonists. Further work is needed to determine if PKC ϵ is the PKC isozyme that specifically phosphorylates and downregulates these receptors.

However, before discussing these results further, certain caveats should be mentioned. First, these drinking data were analyzed by analysis of variance followed by a least significant difference test. The low stringency of this post hoc test raises concern that some results may be false positives and need further validation. Second, the authors tested only single concentrations of inhibitors; therefore, we do not know for certain if the doses chosen provide maximal inhibition of their pharmacologic targets. Third, the peptide inhibitor of PKC ϵ was developed to inhibit the interaction of PKC ϵ with the Golgi protein β 'COP, also known as RACK2 or ϵ RACK (3). Although this peptide is commonly used as a PKC ϵ inhibitor, one must interpret results with some caution because it is not known if it inhibits PKC ϵ interactions with other anchoring proteins important for the phenotypes under study or if it mislocalizes activated PKC ϵ and promotes aberrant PKC ϵ phosphorylation in the cell that contributes to these findings. Therefore, it will be important to confirm findings in this

report using additional approaches to manipulate PKC ϵ , such as kinase inhibition, gene targeting, or RNA interference.

In a final set of experiments, Cozzoli *et al.* explored mechanisms by which mGluRs regulate PKC ϵ phosphorylation at S729 and binge drinking, focusing on mGluR5 and Homer2 because they previously found in mice that binge drinking upregulates mGluR5-Homer2-PI3K signaling in the NAc and that inhibition or knockdown of these proteins in NAc decreases binge drinking (10). In this article, they report that mGluR5^{F1128R} transgenic mice, which have impaired coupling of Homer2 to mGluR5, show reduced PKC ϵ phosphorylation at S729 in the NAc, consistent with mTORC2 activation being downstream of mGluR5 and Homer2. They also report that PKC ϵ inhibition in the NAc or CeA does not reduce binge drinking by Homer2 knockout mice. The latter result is difficult to reconcile with a simple model that places PKC ϵ as a downstream effector of mGluR5-Homer2 signaling because by such a model, one would predict that PKC ϵ inhibition should reduce ethanol drinking in the absence of Homer2. An alternative possibility is that mGluR5-Homer2 signaling is required for mTORC2-mediated processing of PKC ϵ , and when this processing is deficient, the capacity for lipid activation of PKC ϵ through mGluR5 activation of phospholipase C is reduced to such an extent that PKC ϵ is essentially “offline,” making the PKC ϵ inhibitor no longer effective. Such a model predicts that PKC ϵ phosphorylation at S729 should be reduced in the Homer2 knockout, but this was not tested.

In conclusion, Cozzoli *et al.* provide additional evidence demonstrating that PKC ϵ drives ethanol consumption and that mGluRs promote PKC ϵ signaling by increasing phosphorylation of PKC ϵ at a substrate site for mTORC2. This work opens the door for future studies aimed at understanding how ethanol activates mTORC2 in the NAc and CeA and whether mGluR1/5 signaling through Homer2 plays a role in that process, particularly after repeated ethanol exposure, when increased glutamate signaling plays an important role in excessive drinking.

Acknowledgments

This work was supported by Public Health Service Grant Nos. AA013588 and AA017072 (ROM).

ROM is an inventor on US Patent No. US 8,785,648 B1 entitled “PKC-Epsilon Inhibitors,” awarded July 22, 2014.

References

1. Sacks JJ, Gonzales KR, Bouchery EE, Tomedi LE, Brewer RD. 2010 National and state costs of excessive alcohol consumption. *Am J Prev Med.* 2015; 49:e73–e79. [PubMed: 26477807]
2. Cozzoli DK, Courson J, Rostock C, Campbell RR, Wroten MG, McGregor H, et al. Protein kinase C epsilon activity in the nucleus accumbens and central nucleus of the amygdala mediates binge alcohol consumption. *Biol Psychiatry.* 2016; 79:443–451. [PubMed: 25861702]
3. Ron D, Messing RO. Signaling pathways mediating alcohol effects. *Curr Top Behav Neurosci.* 2013; 13:87–126. [PubMed: 21877259]
4. Spanagel R. Alcoholism: A systems approach from molecular physiology to addictive behavior. *Physiol Rev.* 2009; 89:649–705. [PubMed: 19342616]
5. Cameron AJ, Linch MD, Saurin AT, Escribano C, Parker PJ. mTORC2 targets AGC kinases through Sin1-dependent recruitment. *Biochem J.* 2011; 439:287–297. [PubMed: 21806543]

6. Hong-Brown LQ, Brown CR, Navaratnarajah M, Huber DS, Lang CH. Alcohol-induced modulation of rictor and mTORC2 activity in C2C12 myoblasts. *Alcohol Clin Exp Res*. 2011; 35:1445–1453. [PubMed: 21438886]
7. Olive MF, McGeehan AJ, Kinder JR, McMahon T, Hodge CW, Janak PH, et al. The mGluR5 antagonist 6-methyl-2-(phenylethynyl) pyridine decreases ethanol consumption via a protein kinase C epsilon-dependent mechanism. *Mol Pharmacol*. 2005; 67:349–355. [PubMed: 15548766]
8. Gass JT, Olive MF. Role of protein kinase C epsilon (PKC ϵ) in the reduction of ethanol reinforcement due to mGluR5 antagonism in the nucleus accumbens shell. *Psychopharmacology (Berl)*. 2009; 204:587–597. [PubMed: 19225761]
9. Mao L-M, Liu X-Y, Zhang G-C, Chu X-P, Fibuch EE, Wang LS, et al. Phosphorylation of group I metabotropic glutamate receptors (mGluR1/5) in vitro and in vivo. *Neuropharmacology*. 2008; 55:403–408. [PubMed: 18585398]
10. Cozzoli DK, Courson J, Caruana AL, Miller BW, Greentree DI, Thompson AB, et al. Nucleus accumbens mGluR5-associated signaling regulates binge alcohol drinking under drinking-in-the-dark procedures. *Alcohol Clin Exp Res*. 2012; 36:1623–1633. [PubMed: 22432643]