Protein Kinase C Epsilon Activity in the Nucleus Accumbens and Central Nucleus of the Amygdala Mediates Binge Alcohol Consumption

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ABSTRACT
BACKGROUND: Protein kinase C epsilon (PKCe) is emerging as a potential target for the development of pharmacotherapies to treat alcohol use disorders, yet little is known regarding how a history of a highly prevalent form of drinking, binge alcohol intake, influences enzyme priming or the functional relevance of kinase activity for excessive alcohol intake.

METHODS: Immunoblotting was employed on tissue from subregions of the nucleus accumbens (NAc) and the amygdala to examine both idiopathic and binge drinking-induced changes in constitutive PKCe priming. The functional relevance of PKCe translocation for binge drinking and determination of potential upstream signaling pathways involved were investigated using neuropharmacologic approaches within the context of two distinct binge drinking procedures, drinking in the dark and scheduled high alcohol consumption.

RESULTS: Binge alcohol drinking elevated p(Ser729)-PKCe levels in both the NAc and the central nucleus of the amygdala (CeA). Moreover, immunoblotting studies of selectively bred and transgenic mouse lines revealed a positive correlation between the propensity to binge drink alcohol and constitutive p(Ser729)-PKCe levels in the NAc and CeA. Finally, neuropharmacologic inhibition of PKCe translocation within both regions reduced binge alcohol consumption in a manner requiring intact group 1 metabotropic glutamate receptors, Homer2, phospholipase C, and/or phosphotyidylinositol-3 kinase function.

CONCLUSIONS: Taken together, these data indicate that PKCe signaling in both the NAc and CeA is a major contributor to binge alcohol drinking and to the genetic propensity to consume excessive amounts of alcohol.

Keywords: Alcohol use disorders, Drinking in the dark, Glutamate, HDID-1 mice, Homer, Scheduled high alcohol consumption

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Globally, alcohol use is the leading cause of premature death and disability in persons aged 15 to 49 (1), with upward of 50% of alcohol-drinking individuals reporting consuming 4+ alcoholic drinks per occasion (binge drinking) (2,3). Despite the high prevalence of binge alcohol drinking worldwide (2), a knowledge gap exists regarding the neurobiological consequences of this pattern of excessive alcohol intake relevant for understanding the etiology and treatment of this form of alcohol use disorder (AUD). In two different murine models for binge-like drinking, drinking in the dark (DID) and scheduled high alcohol consumption (SHAC), a binge history sensitizes both presynaptic and postsynaptic aspects of glutamate signaling within the nucleus accumbens (NAc) and central nucleus of the amygdala (CeA) (4–7)—two mesocorticolimbic structures highly implicated in alcoholism psychopathology (6). Moreover, biochemical indices of a hyperglutamate state in these structures are biochemical traits associated with an excessive alcohol drinking phenotype in mice (5–7,9,10). Thus, pharmacotherapeutic strategies aimed at reducing glutamate hyperactivity may well serve as a rational treatment option for curbing binge drinking behavior. In support of this assertion, neuropharmacologic or transgenic manipulations that interfere with signaling through group 1 metabotropic glutamate receptors (mGlRs) in the NAc and/or CeA attenuate binge alcohol intake in murine models (6,7,11).

One emerging potential target for pharmacotherapeutic development for AUDs is protein kinase C epsilon (PKCe), a serine/threonine kinase that is diacylglycerol (DAG)-dependent (12,13). PKCe is activated by Gsα/11-coupled receptors, including group 1 mGlRs (14), following DAG formation from phosphatidylinositol 4,5-biphosphate after hydrolysis by phospholipase C (PLC) (15). Alcohol interacts directly with PKCe (16) and upregulates indices of PKCe phosphorylation, priming, and activity in brain (10,17–19). Activated PKCe can then, in turn, phosphorylate Ser-327 on the γ2 subunits of the γ-aminobutyric acid type A receptor (20) to mediate...
acutely to the intoxicating effects of alcohol. Additionally, null mutations of PKCε reduce alcohol reward-related behaviors in various paradigms as determined in studies of mice (13, 21–27), and PKCε knockdown in the CeA blunts binge alcohol intake by mice (28). Thus, an understanding of the potential initiators of PKCε signaling will provide significant insight into the molecular pathways influenced by alcohol that lead to pathological drinking.

One potential intermediary may be group 1 mGluRs. These receptors are specifically coupled to PKCε in certain brain areas (14). Alcohol intake, including binge drinking, is partly mediated by mGlu5 activity (29, 30) and the subsequent stimulation of PKCε- and/or phosphotyrosyl inositol-3 kinase (PI3K)-dependent transduction pathways (5–7, 11, 19, 27), which were suggested much earlier to involve the group 1 mGluR-associated scaffolding protein Homer (19). However, no study to date has directly investigated the links between group 1 mGluRs, Homer2, PI3K, and PKCε for effects on alcohol consumption. Thus, we employed a combination of immunoblotting and neuropharmacologic approaches to test the hypothesis that in both the NAc and the CeA, signaling from mGlu1/5-Homer2 to PKCε is critical for the manifestation of excessive alcohol drinking. Such findings further elucidate the intracellular signaling pathways influenced by alcohol that likely underpin anomalies in excitatory neurotransmission contributing to risky alcohol drinking behavior.

METHODS AND MATERIALS

Overview of the Experiments

A history of binge alcohol drinking using modified DID (31) and SHAC (32) models upregulates indices of group 1 mGluR/Homer2/PI3K signaling within the CeA and/or NAc of C57BL/6J (B6) mice (5–7, 11). Therefore, we employed immunoblotting procedures (10) to determine the effects of a history of binge alcohol drinking on PKCε levels and priming in both brain regions of the B6 mice assayed for the expression of putative upstream signaling molecules in our prior work (5–7). Across inbred mice, strain differences exist regarding PKCε phosphorylation of Ser729 in the NAc suggestive of a relation between higher levels of constitutive PKCε priming and alcohol intake (10). Thus, we also examined the relation between basal p(Ser729)-PKCε levels in the CeA and NAC and genetic propensity to binge drink by immunoblotting tissue from alcohol-naïve S15 HDID-1 [1st replicate line of mice selected for high blood alcohol concentrations (BACs) under DID procedures] vs. non-selected mice from a genetically heterogeneous HS/Npt stock (33). We also compared S4 SHAC (selected for high BACs under SHAC procedures) vs. SLAC (selected for low BACs under SHAC procedures) mice (5) and mGlul51128R transgenic (TG) mice, with a point mutation in the Homer binding site on mGlul5. TG mice exhibit low binge drinking under SHAC procedures versus their wild-type (WT) counterparts that binge drink with this paradigm (5).

We next employed established neuropharmacologic approaches (5–7, 11) to examine the influence of locally inhibiting PKCε translocation using a Tat-cV1-2 peptide (34), either alone or in tandem with inhibition of mGlul5, and PLC upon binge alcohol intake by B6 mice. Finally, as Homer2 is in position to mediate the effects of mGlul5/PKCε signaling (35, 36), we examined whether or not local inhibition of PKCε translocation reduced binge alcohol consumption in Homer2 knockout (KO) animals (37).

The details of the experimental procedures for these experiments are provided in Supplement 1.

RESULTS

Drinking in the Dark Upregulates the Relative Expression of p(Ser729)-PKCε

The average daily alcohol intake under DID procedures exhibited by the B6 mice was 4.8 ± .37 g/kg per 2 hours (6,7,11), which is predicted to result in a mean BAC ~ 100 mg % (31) that exceeds the National Institute on Alcohol Abuse and Alcoholism criterion for binge drinking (38). In the NAc, binge drinking elevated the relative expression of p(Ser729)-PKCε (PKCε ratio: t22 = −3.984, p = .001), and this reflected a nonsignificant reduction in total PKCε levels (t test: p > .05) and a significant 50% increase in p(Ser729)-PKCε in the CeA (Figure 1A) (t22 = −2.314, p = .03). These same B6 mice also exhibited an increase in the relative levels of p(Ser729)-PKCε in the CeA (Figure 1B) (t19 = 2.122, p = .047); this reflected a strong trend toward reduced total kinase levels in binge drinking mice (t test: p > .05), as p(Ser729)-PKCε levels were not different from water control mice (t test: p > .05). In contrast, no changes in total or phosphorylated PKCε were apparent in the NAc core or basolateral amygdala (t tests, p > .05; Table S1 in Supplement 1).

Basal PKCε Phosphorylation as a Correlate of Genetic Propensity to Binge Drink

We next determined whether or not basal p(Ser729)-PKCε expression is a biochemical correlate of a high binge alcohol drinking phenotype through a comparison of tissue from selectively bred HDID-1 and genetically heterogeneous HS/Npt control mice (33). We observed no genotypic differences in PKCε expression within either NAc subregion (t tests: ps > .05; Figure 2A and Table S2 in Supplement 1). While no genotypic differences in total PKCε levels were apparent in CeA (t test: p > .05), HDID-1 mice exhibited a robust increase in the absolute, as well as relative, levels of p(Ser729)-PKCε in this region (Figure 2B) (total: t26 = 3.25, p = .003; ratio: t26 = 3.306, p = .003). However, HDID-1 mice did not differ from HS/Npt control mice regarding the basal levels of Homer proteins, mGlul5/6, GluN2A/B, PI3K, or AKT phosphorylation (the latter serving to index PI3K activity) in this subregion (Table S3 in Supplement 1). Moreover, there were no genotypic differences in PKCε levels in basolateral amygdala (t tests: p > .05; Table S2); nor were differences observed for any other protein examined (t tests; ps > .05; Table S3 in Supplement 1).

PKCε: Signaling Within the NAc Is Necessary for Binge Drinking

We next assessed the role for PKCε in maintaining binge drinking under DID procedures via intra-NAc infusions of the PKCε translocation inhibitor Tat-cV1-2 and employed
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Figure 1. Thirty days of binge alcohol consumption under drinking in the dark (DID or D) procedures upregulates protein kinase C epsilon (PKCε) activity in the nucleus accumbens (NAC) shell and central nucleus of the amygdala (CeA). Summary of the change in protein levels of total PKCε, p-Ser729-PKCε, and their relative levels (PKCε ratio) in the NAC (A) and CeA (B) following 24-hour withdrawal from 30 days of alcohol drinking under drinking in the dark procedures. Two representative immunoblots for PKCε and p-Ser729-PKCε for each group are included as insets. After normalization for loading, the data are expressed as a percent of the average protein levels of water-drinking control animals (Water or W; n = 5–6/gel). Data represent the mean ± SEM of the number of animals indicated in the figure, *p < .05 (t tests).

co-infusion procedures to address whether or not the effects of PKCε inhibition were shared with those produced by infusion of maximally effective doses of mGlu1, mGlu5, PLC, and PI3K inhibitors (Figure 3). Before any drug infusions, the B6 mice exhibited an average alcohol intake of 4.56 ± 0.39 g/kg during the 2-hour drinking period, an intake correlated with BACs > 80 mg% (31). An intra-NAC infusion of Tat-eV1-2 alone, as well as the co-infusion of this inhibitor with 3 μg/side 3-((2-Methyl-4-thiazolyl)ethynyl)pyridine (MTEP), 15 pg/side JNJ-16259685, 5.8 fg/side U-73122, or .17 ng/side LY294002 reduced alcohol intake under DID procedures (Figure 3A) (F5,104 = 6.54, p < .001). Least significant difference post hoc analyses confirmed that all intra-NAC manipulations lowered alcohol intake below that exhibited by control mice infused with the scrambled Tat peptide (all ps < .05). Post hoc comparisons were made also between mice infused with the PKCε inhibitor alone and those co-infused with the pharmacologic agents. As illustrated in Figure 3A, co-infusion of 3 μg/side MTEP attenuated significantly the capacity of Tat-eV1-2 to inhibit binge drinking (p = .037), while this effect was also significant (albeit less robust) when animals were co-infused with 15 pg/side JNJ-16259685 (p = .049), 5.8 fg/side U-73122 (p = .047), or .17 ng/side LY294002 (p = .047). Representative placements of our microinjector tips in the NAC are presented in Figure 3C.

PKCε Signaling in the CeA Is Necessary for Binge Drinking

An intra-CeA infusion of Tat-eV1-2 or co-infusion of the PKCε inhibitor with 3 μg MTEP, 15 pg JNJ-16259685, or 5.8 fg U-73122 inhibited binge alcohol intake under our DID procedures in B6 mice (Figure 3B) (F4,74 = 14.61, p < .001). B6 mice infused intra-CeA with the scrambled control peptide consumed alcohol in excess of amounts predicted to result in BACs > 80 mg% (4.73 ± .28 g/kg) (31) and least significant difference post hoc analyses confirmed that alcohol drinking was attenuated by infusion of Tat-eV1-2 alone (p = .009), as well as by co-infusion of the inhibitor with MTEP (p < .001), with JNJ-16259685 (p = .002), or with U-73122 (p < .001) (Figure 3B). However, in contrast to the NAc (Figure 3A), the magnitude of the reduction in alcohol intake produced by an intra-CeA infusion of Tat-eV1-2 was enhanced by co-infusion with MTEP (p = .005) or U-73122 (p = .001). These data suggest that, at least within the CeA, mGlu5 and PLC operate to influence alcohol drinking through a pathway independent of PKCε. In contrast, the reduction in drinking produced by Tat-eV1-2 co-infusion with JNJ-16259685 was similar in magnitude to that produced by the PKCε inhibitor alone (p > .05), suggesting that mGlu1 and PKCε in the CeA operate to influence binge alcohol intake via a shared pathway. Representative placements of our microinjector tips in the CeA are presented in Figure 3D.

PKCε Signaling and Sucrose Intake

To examine potential nonspecific effects of our PKCε translocation inhibitor, we infused B6 mice with .25 μL/side Tat-eV1-2 into the NAc or CeA and measured 5% sucrose intake under our 2-hour DID procedures. As summarized in Table S4 in Supplement 1, PKCε inhibition did not alter sucrose intake in this model (t tests, p > .05).

Homer2 Is Necessary for the Anti-Drinking Effect of PKCε Inhibition

To test the hypothesis that Homer proteins play a role in regulating the functional interaction between mGlu5 and PKCε (19), we next examined the effects of Tat-eV1-2 infusions on
alcohol intake by Homer2 WT and KO mice. As observed in previous reports (6, 7, 11), no differences were found in baseline alcohol intake between Homer2 WT and KO mice (Figure 4).

The level of alcohol intake exhibited by the mice was 3.31 ± 0.35 (NAC study) and 4.19 ± 0.27 (CeA study) g/kg per 2 hours. The effects of infusing Tat-v1-2 either into the NAc or into the CeA varied as a function of genotype but not of region (genotype × pretreatment: F(1,31) = 5.43, p = 0.03; no main effect of or interactions with region, p > 0.05). Post hoc comparisons confirmed that Tat-v1-2 inhibited alcohol intake by WT mice when infused into the NAC (Figure 4A) (t6 = 3.079, p = 0.02) or CeA (Figure 4B) (t6 = 3.0, p = 0.03). In contrast, the inhibitor had no effect in KO animals (for NAC: p = 0.97; for CeA: p = 0.15).

**Interactions between PKCε and Alcohol Intake Under SHAC Procedures**

A final series of experiments were designed to determine whether or not the interactions between NAC PKCε activity and alcohol drinking observed under DID procedures generalized to binge drinking under SHAC procedures. Thus, we examined relative p(Ser729)-PKCε levels in tissue from the entire NAC obtained from mice with a history of repeated bouts of binge alcohol drinking under SHAC procedures (average daily intake = 1.6 ± 0.2 g/kg per 30 minutes; resultant average BAC = 109.5 ± 37.3 mg%), which had been processed previously for binge drinking induced changes in putative upstream signaling molecules (5). Although binge drinking under SHAC procedures did not significantly affect either total or p(Ser729)-PKCε levels in the entire NAC (t tests, ps > 0.05), there was an increase in the relative expression of p(Ser729)-PKCε in this study (Figure 5A) (t(22) = 2.44, p = 0.02). We also immunoblotted for the relative levels of p(Ser729)-PKCε in the entire NAC of mice selectively bred for high versus low alcohol intake under SHAC procedures (SHAC vs. SLAC lines, respectively), which had been processed previously for line differences in glutamate receptor/Homer/PI3K expression (5). As indicated in Figure 5B, SHAC line mice exhibited higher relative levels of p(Ser729)-PKCε versus their low-drinking SLAC counterparts (t(17) = 2.966, p = 0.013), and this effect reflected both an increase in p(Ser729)-PKCε expression (t(37) = 2.029, p = 0.06) and a modest, albeit nonsignificant, reduction in total kinase levels (t test: p > 0.05).

We next asked whether or not the converse might be true and assayed for genotypic differences in basal NAC PKCε phosphorylation between low SHAC-intake mGlu5 versus their WT littermates (5). While total PKCε levels did not differ between genotypes (p > 0.05), TG mice exhibited 40% lower p(Ser729)-PKCε levels (t(11) = 3.374, p = 0.002), as well as significantly lower relative levels of phosphorylated versus nonphosphorylated kinase (PKCε ratio; t(17) = 2.513, p = 0.022), compared with WT animals (Figure 5C).

We also examined the ability of the Tat-v1-2 translocation inhibitor to reduce alcohol intake under SHAC procedures when infused into the NAc of B6 mice. Indeed, Tat-v1-2 infusion significantly reduced consumption of 5% alcohol, relative to mice infused with a scrambled control peptide (Figure 5D) (t(10) = 4.28, p = 0.002). Importantly, the alcohol intake of the control mice in this study correlated with BACs > 80 mg%, while that of Tat-v1-2-infused animals was lower than that predicted to meet the criterion for binge drinking (5). Thus, PKCε inhibition in the NAc is sufficient to prevent binge alcohol drinking under SHAC procedures. Finally, intra-NAc inhibition of PKCε did not influence the consumption of 5% sucrose under SHAC procedures (p > 0.05; Table S4 in Supplement 1), providing further evidence that interfering with PKCε activity selectively affects alcohol intake.
DISCUSSION

Herein, alcohol consumption upregulated PKCε phosphorylation on Ser729 in both the NAc and the CeA and basal p(Ser729)-PKCε levels in these regions were a correlate of the genetic propensity to binge drink. These data implicate either idiopathic or alcohol-induced increases in PKCε priming in NAc and CeA in the etiology of excessive alcohol drinking. Furthering this hypothesis, inhibition of PKCε translocation in the NAc or CeA reduced alcohol intake under our DID and SHAC procedures, and experiments conducted in Homer2 KO mice provided evidence that this scaffolding molecule is integrally involved. The neuropharmacologic studies suggested that PKCε in the NAc regulates binge alcohol drinking via signaling pathways involving mGlu1, mGlu5, PLC, and PI3K, while PKCε in the CeA operates in a signaling pathway involving mGlu1. These results extend current knowledge, derived from studies using constitutive PKCε KO mice (13) and short hairpin RNA-PKCε approaches (28), by demonstrating that PKCε operates in both the NAc and CeA to facilitate voluntary alcohol consumption through distinct group 1 mGluR-associated signaling pathways.

In Vivo Regulation of PKCε by Alcohol and Relation to Alcoholism Vulnerability

In vitro, alcohol stimulates PKCε translocation (39), and chronic alcohol exposure increases PKCε immunoreactivity and PKCε-mediated phosphorylation (17). The precise neuroanatomical loci of alcohol-PKCε interactions are less well...
described and were examined herein using phosphorylation of PKCε at Ser729 as an indicator of isozyme priming (40). Binge alcohol drinking increased p(Ser729)-PKCε specifically in NAc and amygdala subregions that are components of the extended amygdala circuit highly implicated in alcoholism neurobiology (41,42). Moreover, this effect was observed within the same tissue reported previously by our group to exhibit increases in group 1 mGluR, Homer2a/b levels, and/or PI3K activation (5–7). Such observations indicate that elevated PKCε priming in these brain regions, presumably via pathways involving group 1 mGluRs and their Homer2 scaffolds, may be central to the manifestation of excessive alcohol intake.

In support of this assertion, a susceptibility locus for alcohol dependence is indicated on human chromosome 2p21, where the gene for PKCε is mapped (43,44), and genotypic differences exist for basal p(Ser729)-PKCε between inbred mouse strains with divergent alcohol drinking phenotypes (10). Herein, we extend these prior data to selectively bred and transgenic lines of mice varying in binge-drinking behavior. When assayed in the entire NAc, SHAC line mice selectively bred for high BACs under SHAC procedures exhibited higher p(Ser729)-PKCε levels compared with SLAC line animals selected for low BACs under SHAC procedures. Conversely, mGlu5(F1128R) TG mice that manifest low binge alcohol drinking (5) exhibit lower constitutive p(Ser729)-PKCε levels in the NAc than WT littermates that exhibit binge-drinking behavior (5). As the mGlu5(F1128R) TG mice employed in the present study exhibited reduced Homer binding to mGlu5 (5), these later results provide novel evidence in support of an important role for mGlu5-Homer interactions in regulating constitutive PKCε priming in vivo, as suggested previously (19). However, while HDID-1 mice, selected for high BAC under 4-hour DID procedures, exhibited higher indices of CeA p(Ser729)-PKCε than their genetically heterogeneous HS/Npt control mice, no differences were observed in putative upstream signaling molecules in amygdala, nor were differences present for p(Ser729)-PKCε levels in the NAc. As strain differences exist regarding basal NAc levels of p(Ser729)-PKCε (10) (Figure 5A), the failure to detect differences in PKCε priming between HDID-1 and HS/Npt control mice could simply relate to the genetic heterogeneity of the control line employed in this study. Alternatively, the use of more sensitive mass spectrometry procedures to examine the phosphorylation of Ser729 or of other sites more reflective of enzyme activation [e.g., Thr566 or Ser710 (40)] might provide more information pertaining to the link between idopathic or alcohol-induced changes in PKCε activity and alcohol drinking. Further preclinical exploration of this potential link is warranted, particularly considering evidence indicating the functional relevance for NAc group 1 mGluR/Homer2/PI3K/PKCε signaling in excessive alcohol drinking (present study; (5–7,11)).

**PKCε Translocation and Binge Alcohol Consumption**

The correlational nature of the aforementioned immunoblotting studies cannot inform us as to cause-effect relations between PKCε activity and binge drinking. However, earlier data from studies of constitutive PKCε KO mice argued an active and necessary role for this isozyme in alcoholism-related behaviors (13,25), and herein, the inhibition of PKCε translocation in either the NAc or the CeA by the local infusion of a Tat-eV1-2 peptide (34) was sufficient to reduce binge alcohol consumption in two distinct murine models. To the best of our knowledge, these data are the first demonstration that PKCε translocation specifically in the NAc modulates binge alcohol intake and further support the CeA as an anatomical site of action of PKCε for excessive alcohol consumption (28). This demonstration of a causal relation between PKCε translocation in at least two AUD-relevant brain regions and binge alcohol intake not only highlights the significance of both our immunoblotting results ([10]; present study) and those of the genetic mapping studies in humans (43,44), but also provides neuroanatomical insight into the facilitatory role for PKCε in excessive alcohol drinking.

**Potential Upstream Modulators of PKCε Translocation and Binge Alcohol Consumption**

Previously, a signaling pathway initiated upon mGlu5 stimulation that involved Homers and PI3K activity was proposed to play an important role in the activation of PKCε underpinning voluntary alcohol intake (19). In partial validation of this hypothesis, we demonstrated previously a critical role for group 1 mGluR/Homer2-mediated activation of both Gαq/11 and Gβγ signaling in the NAc and the CeA in maintaining binge alcohol drinking (5–7,11). Herein, an intra-NAc infusion of Tat-eV1-2, while effective at reducing alcohol intake in WT
mice, did not inhibit alcohol intake in Homer2 KO mice, implicating Homer2 as an important regulator of both PKCe priming and the facilitation of binge alcohol drinking elicited by PKCe translocation. In the NAc, the inhibitory effects of co-infusion of the Tat-eV1-2 inhibitor with mGlu1, mGlu5, PLC, or PI3K antagonists were not different from those produced by PKCe inhibition alone, indicating that at least in this striatal subregion, mGlu1/5, PLC, PI3K, and PKCe operate within shared intracellular signaling pathways, perhaps scaffolded by Homer2. While there is no evidence that Homer proteins interact directly with any PKC, our prior work indicated that intact Homer2 expression or Homer coupling to mGlu5 is required to observe an attenuation of binge drinking by mGlu1/5 receptor antagonists and inhibitors of PLC and PI3K (6,7,11). Moreover, the present observation of decreased constitutive PKCe priming in the NAC of mGlu5F1128R mice with reduced Homer binding supports some functional relation between mGluR-Homer complexes and PKCe. Thus, the coincident upregulation in mGlu1/5, Homer2, and PKCe expression, the activational state of PI3K, and PKCe priming observed in the NAc of binge-drinking animals or in animals with a high genetic propensity to binge drink ([5,6,11]; present study) likely reflects a shared signaling pathway that is permissive to excessive alcohol consumption.

Although binge alcohol drinking upregulates p(Ser729)-PKCe expression in the CeA and higher constitutive PKCe priming in the CeA is a genetic correlate of excessive alcohol drinking, the specific signaling pathway(s) operating in the CeA to regulate binge alcohol intake appear to be distinct from those operating within the NAc. For one, PI3K inhibition within the CeA does not affect binge alcohol drinking ([5,6] vs. [7]), arguing that Akt/PI3K hyperactivity in the CeA is not a major mediator of binge alcohol intake and likely not a major contributor to alcohol-induced increases in PKCe priming observed within this region. Moreover, mGlu1 and PLC operate independently within the CeA to influence binge drinking (7), and herein, the effect of an intra-CeA co-infusion of Tat-eV1-2 with an mGlu5 antagonist was greater than that produced by the antagonist alone. Such a result argues against a specific role for CeA mGlu5 in PKCe translocation facilitating alcohol intake. However, the inhibitory effect of an intra-CeA co-infusion of Tat-eV1-2 and an mGlu1 antagonist

Figure 5. Role for protein kinase C epsilon (PKCe) signaling in the nucleus accumbens (NAc) in binge alcohol consumption under scheduled high alcohol consumption procedures. (A) Summary of the change in protein levels of total PKCe, p-Ser729-PKCe, and their relative levels (PKCe ratio) in the entire NAc following 24-hour withdrawal from six bouts of alcohol drinking under scheduled high alcohol consumption (SHAC or S) procedures and water drinking control animals (Water or W; 5–6 gel). Representative immunoblots are included as insets. (B) Summary of the line differences in protein levels in the entire NAc of S4 mice selectively bred for high versus low alcohol intake under SHAC procedures (SLAC line or L—scheduled low alcohol consumption line; SHAC line or H—scheduled high alcohol consumption line), at 3 months after their final 30-minute alcohol-drinking session. The data are expressed as a percent of average levels of the SLAC line (5–6 SLAC mice/gel). Representative immunoblots are included as insets. (C) Summary of the line differences in protein levels in the NAc of mGlu5F1128R transgenic mice (mGlu5R TG or T) and wild-type littermates (mGlu5R WT or W), expressed as a percent of average levels of WT animals (5–6 WT mice/gel). Representative immunoblots are included as insets. (D) Summary of the effects of an intra-NAc shell infusion of the PKCe inhibitor Tat-eV1-2 or control peptide (Scrambled) on 5% alcohol intake exhibited by C57BL/6J mice under SHAC procedures. Data represent the mean ± SEM of the number of animals indicated in the figure. *p < .05 (t tests).
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was not different from that produced by the mGlu1 alone, arguing that PKCε operates in the CeA, via some mGluR1-dependent pathway, to influence binge alcohol intake. This mGlu1/PKCε pathway does not involve Gαq/11-mediated stimulation of PLC, as the attenuation of binge drinking by co-infusion of PLC and PKCε inhibitors was greater than that produced by PKCε inhibition alone. Thus, the capacity of PKCε priming to regulate binge drinking may be entirely independent of the generation of DAG by PLC. Although evidence indicates that PI3K can regulate PKCε priming (19), our failure to observe alcohol-induced increased PI3K activity in the CeA, coupled with no effect of intra-CeA infusions of different PI3K inhibitors upon binge drinking (7), argue that alcohol-induced or idiopathic increases in PKCε priming observed in this region likely do not involve βγ-mediated activation of PI3K upon mGlu1 stimulation (7).

What then is the pathway involved in mGlu1/PKCε-dependent signaling that regulates binge drinking in the CeA? mGlu1 activation can elicit cyclic adenosine monophosphate formation in vitro (45), raising the possibility that mGlu1-PKCε signaling in the CeA may involve this second messenger. Indeed, PKCε activation can be regulated by Gαq-coupled corticotrophin-releasing factor receptor 1 receptors (34) and there is cross-talk between protein kinase A and PKC in opioid-withdrawn animals (46), supporting this possibility. Alternatively, mammalian target of rapamycin complex 2 (mTORC2) is an important upstream regulator of PKCε priming (47). While not yet assessed in brain, mTORC2 activity can be upregulated by alcohol when assayed in myocytes (48), group 1 mGluR-mediated inhibition of tuberous sclerosis complexes can increase mTORC activation (49), and the capacity of group 1 mGluRs to augment mTORC activity depend upon constitutively expressed Homer proteins (50). Thus, either alcohol-induced or idiopathic increases in mGluR1/PKCε signaling in CeA may involve mTORC1 and/or mTORC2 as an intermediary, and this possibility requires systematic investigation. Alternatively, both mGlu1 and PKCε can regulate the function of N-type calcium channels, and Homer proteins have been implicated in mGlu1 regulation of channel function (51). While this latter mechanism cannot account for how mGlu1 stimulation elicits PKCε translocation in the CeA, genetic or pharmacologic interruption of mGlu1, Homer2, N-type calcium channels, or PKCε all produce an alcohol-avoiding phenotype in preclinical animal models (5–7,11,23,52). Thus, N-type calcium channels may be an important point of signaling convergence between mGlu1 and PKCε to be explored in future studies.

Conclusion

Using a combination of immunoblotting and neuropharmacologic approaches, the results of the present study support idiopathic or alcohol-induced increases in PKCε priming/translocation in the NAC and CeA as an important signaling event in the etiology of alcoholism and provide experimental support for the potential utility of targeting PKCε for the pharmacotherapeutic intervention of AUDs.

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ARTICLE INFORMATION

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