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Cytoplasmic Phospholipase A₂ Modulation of Adolescent Rat Ethanol-Induced Protein Kinase C Translocation and Behavior

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Abstract

Ethanol consumption typically begins during adolescence, a developmental period which exhibits many age-dependent differences in ethanol behavioral sensitivity. Protein kinase C (PKC) activity is largely implicated in ethanol-behaviors, and our previous work indicates that regulation of novel PKC isoforms likely contributes to decreased high-dose ethanol sensitivity during adolescence. The cytoplasmic Phospholipase A₂ (cPLA₂) signaling cascade selectivity modulates novel and atypical PKC isoform activity, as well as adolescent ethanol hypnotic sensitivity. Therefore, the current study was designed to ascertain adolescent cPLA₂ activity both basally and in response to ethanol, as well as its involvement in ethanol-induced PKC isoform translocation patterns. cPLA₂ expression was elevated during adolescence, and activity was increased only in adolescents following high-dose ethanol administration. Novel, but not atypical PKC isoforms translocate to cytosolic regions following high-dose ethanol administration. Inhibiting cPLA₂ with AACOCF₃ blocked ethanol-induced PKC cytosolic translocation. Finally, inhibition of novel, but not atypical, PKC isoforms when cPLA₂ activity was elevated, modulated adolescent high-dose ethanol-sensitivity. These data suggest that the cPLA₂/PKC pathway contributes to the acute behavioral effects of ethanol during adolescence.

Keywords

Adolescence; Ethanol; Cytoplasmic phospholipase A₂; PKC δ ; PKC ϵ ; Arachidonic acid

Introduction

Adolescence is a critical period of development during which the brain undergoes dramatic reorganization [1]. Further, ethanol exposure during this timeframe impacts behavioral responding and the normal developmental trajectory of the central nervous system (CNS). Ethanol consumption primarily begins during adolescence, and exposure before the age of 15 leads to an increased propensity for alcohol use disorders in adulthood [2]. Interestingly, adolescents display differential behavioral responses following ethanol consumption

compared to adults, such as reduced sensitivity to ethanol's sedative/hypnotic effects [3]. This is problematic, as decreased soporific sensitivity to ethanol likely lengthens consumption periods and further amplifies detrimental CNS effects, such as decreased neurogenesis and increased levels of cellular death [4, 5]. Thus, it is imperative to understand the molecular underpinnings underlying reduced ethanol-sensitivity during adolescence as they likely contribute to alcohol use disorders later in life.

Our recent work indicates that protein kinases have a prominent role in adolescent ethanol-induced behavioral responses, particularly PKC [6–8]. The PKC family is made up of 10 isoforms subdivided into three subfamilies: conventional (c), novel (n) and atypical (a) PKCs [9]. Classically, PKC activation is unique for each subfamily. For instance, phospholipase C (PLC) liberates diacylglycerol (DAG) as well as inositol triphosphate (IP₃) for release of Ca²⁺, both of which traditionally are required to activate cPKC isoforms. However, only DAG is required to activate nPKC isoforms near the membrane. aPKC isoforms require neither DAG nor Ca²⁺ for activation [9]. Interestingly, our recent work demonstrated decreased adolescent n- and aPKC isoforms in P2 preparations (see methods) following high-dose ethanol-administration, an effect that was not observed in adults [7, 8, 10]. Further, these effects appear to be isoform selective, as cPKC isoforms at both ages were unaltered following acute ethanol exposure. The selectivity of ethanol-induced alterations in PKC signaling make it unlikely that alterations in the classical DAG-pathway of PKC activation account for the observed differences across age.

A second method of PKC activation may account for age-related differences in n- and aPKC isoform expression following ethanol administration. The cPLA₂ family, in particular group IV cPLA₂, is a primary mediator of arachidonic acid (AA) generation that is commonly involved in pro-inflammatory responses [11, 12]. Interestingly, the cPLA₂ cascade, in particular AA, has been shown to preferentially activate n- and aPKC isoforms [13]. In fact, we recently reported that inhibiting both PKC and cPLA₂ increased adolescent, but not adult sensitivity to the soporific effects of ethanol. Given these findings, we hypothesized that elevated cPLA₂ activity during adolescence accounts for ethanol-induced alterations in PKC isoform translocation, as well as reductions in adolescent ethanol-sensitivity. In the present study, we investigated whether there are age-dependent, as well as ethanol-induced alterations in cPLA₂ expression and activation via phosphorylation. We further assessed whether ethanol modulates PKC isoform cytosolic translocation, and if so, whether pharmacological inhibition of cPLA₂ activity prevented these changes. Finally, we behaviorally examined which select PKC isoforms may predominantly underlie differential ethanol-sensitivity during the adolescent period.

Experimental Procedures

Animals

Experiments were conducted in accordance with guidelines from the National Institute of Health under the Institutional Animal Care and Use Committee approval at Binghamton University, State University of New York. Adolescent (PD 35) and adult (PD 75) male Sprague–Dawley rats were bred at Binghamton University. Rats were maintained on a standard 12 h light–dark schedule with lights on at 7:00 a.m. Animals had ad libitum access

to rat chow and water. Rats that underwent intracerebroventricular (i.c.v.) surgery, described below, were subsequently housed individually. Non-surgerized adult rats were pair-housed, while adolescents were group housed with 3–4 other rats. All subjects had environmental enrichment in the form of crinkled paper.

Intracerebroventricular Surgery

Stereotactic surgeries were performed to implant guide cannula directed toward the lateral ventricles. Briefly, rats were anesthetized with 3.0 % isoflurane and subsequently placed into a stereotactic frame. Guide cannula (PlasticsOne, Roanoke, VA, USA) were implanted unilaterally into the lateral cerebral ventricle at coordinates AP -0.5 mm, L ± 1.2 mm from bregma, and DV -2.5 [14]. Cannula were secured to the skull using stainless steel screws and dental cement, with the skin surrounding the surgical site sutured closed. Cannula integrity was protected with an internal guide and cap. Buprenex was administered for postoperative care immediately following surgery as well as 24 h following cannulation. Animals were given a 1-week recovery period prior to experimental use, with cannula patency being assessed periodically during routine handling. Following sacrifice, India ink was used to determine i.c.v. cannula placements. Only animals with a positive indication of ink in their ventricles (98.9 %) were used for subsequent analysis.

Pharmacological Agents

Ethanol (20 % v/v in saline) was purchased from Pharmco (Brookfield, CT, USA). The cPLA₂ inhibitor, arachidonyl trifluoromethyl ketone (AACOCF₃) was purchased from Enzo Life Sciences (Farmingdale, NY, USA) and dissolved in artificial cerebral spinal fluid (aCSF) at a dose sufficient to inhibit AA production (5 nmol/rat) as noted elsewhere and as we have done previously [7, 15]. PKC ϵ inhibitory peptide (PKC ϵ -I; 5 nmol/rat) [16] was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX) and dissolved in aCSF, while PKC δ inhibitory peptide (PKC δ -I; 2.5 μ mol/rat) [17] was purchased from AnaSpec, Inc. (Fremont, CA), and dissolved in DMSO. Zeta inhibitory peptide (ZIP; 50 nmol/rat) [18] was purchased from Tocris Bioscience (Minneapolis, MN, USA) and dissolved in aCSF.

Tissue Collection

For analysis of PKC isoform translocation following ethanol administration, rats were injected with ethanol (3.5 g/kg) or saline, and sacrificed at predetermined time points (30 and 60 min). Animals involved in studies assessing alterations in cPLA₂ expression and phosphorylation were injected with ethanol (3.5 g/kg) or saline, and sacrificed 15 min post injection. This time was utilized as we anticipate changes in cPLA₂ to occur prior to observed changes in PKC at 30 and 60 min [7]. For studies examining modulation of PKC translocation following cPLA₂ inhibition, animals were administered AACOCF₃ intracerebroventricularly 8 h prior to ethanol (3.5 g/kg, i.p.), and sacrificed 60 min post ethanol-injection, consistent with our previous work [7]. For all studies, the brain was rapidly removed from the skull, flash frozen, and stored at -80 °C until further use.

Sample Preparations

For all samples, cortical tissue was utilized in order to gain a better perspective in regions associated with loss of righting reflex/drug-induced loss of consciousness [19, 20]. Phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) was added to tissue collected for phosphorylation analysis. All tissue underwent subcellular fractionation for localization of PKC isoforms as we have done previously [7, 8]. Briefly, cerebral cortical samples were homogenized in 0.32 M sucrose/PBS solution, and subjected to low speed centrifugation (1000×g) followed by centrifugating the resulting supernatant at 12,000×g for 20 min. The remaining pellet (P2 fraction) was suspended in buffered PBS. The subcellular fractionation procedure, as characterized by Gray and Whittaker [21], results in a crude P2 fraction that is particularly enriched in synaptosomes, but is an incomplete membrane fraction as other membrane portions precipitate out with the P1. The resultant supernatant was used for cytosolic assessments. Protein concentrations of all samples were quantified using a bicinchoninic acid method.

Western Blot Analysis

Protein samples were subjected to sodium-dodecyl-sulfate polyacrylamide gel electrophoresis using Novex Tris–Glycine gels (8–16 %) and transferred to polyvinylidene difluoride membranes (Invitrogen, Carlsbad, CA, USA). For assessment of cPLA₂, 80 µg protein per sample was used, whereas 50 µg protein was used for PKC isoforms. Each sample represented a single animal. Membranes were probed with antibodies for the following proteins: PKCε (610086), PKCδ (610397), (BD Biosciences, San Jose, CA, USA), cPLA₂ (sc-454), p-cPLA₂ (ser505) (sc-34391) and PKCζ (sc-216) (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) for simultaneous detection of PKMζ and PKCι/λ. All primary blots were subsequently re-exposed to a second primary antibody directed against β-actin (MAB1501, Millipore) to verify equivalent protein loading and transfer. Samples were run in duplicate or triplicate and averaged. All bands were detected by enhanced chemiluminescence (GE Healthcare, Piscataway, NJ, USA) and exposed to autoradiography film under non-saturating conditions. Data were quantified using NIH Image J.

LORR Paradigm

To measure the effect of PKC isoform modulation on ethanol-induced LORR, adolescent (P35) rats were administered PKCδ-I, PKCε-I, ZIP or respective controls at predetermined time points prior to a hypnotic dose of ethanol [4.0 g/kg, intraperitoneally (i.p.)], as we have done previously [6, 7]. Administration of PKCδ-I, PKCε-I and ZIP were based on experimental protocols described elsewhere [16–18]. All i.c.v. injections were done at a flow rate of 1 µL/min. Following completion of drug delivery, injection needles were left in place for an additional minute to mitigate backflow into the cannula. Following ethanol administration, rats were observed until they exhibited LORR via placement in a supine position in V-shaped troughs (90° angle). Animals remained in the supine position until they regained their righting reflex as assessed by the ability to right three times in a 60-s period. LORR duration was calculated by subtracting the time of LORR onset from the time of recovery. Tail blood samples were taken immediately after rats regained their righting reflex

and analyzed using an AM5 Alcohol Analyzer (Analox Instruments, Lunenburg, MA, USA). PKC δ -I, PKC ϵ -I and ZIP do not elicit hypnotic activity in the absence of ethanol. With the exception of PKC δ -I treated rats, 2 rats per group failed to lose their righting reflex. Rats that failed to lose their righting reflex were excluded from LORR duration analyses.

Statistical Analysis

For Western blots, all comparisons were made within blots. Ethanol exposure time dependent studies were compared to saline controls run in parallel. Analyses were conducted using one-way ANOVA with *Dunnnett's* post hoc test when appropriate. For cPLA₂ expression/phosphorylation and AACOCF₃ translocation studies, data was assessed using a two-way ANOVA with Fisher LSD post hoc analysis. Student's *t* test was employed for LORR studies, and data was calculated as percent control. Due to significant variability in LORR procedure room temperature across testing days, LORR data are reported as percent of controls run within the same day. For all experiments, $p < 0.05$ ($\alpha = 0.05$) was considered significant. Chi squared analyses were conducted to ascertain significance of animals which failed to lose their righting reflex.

Results

Adolescents Display Basal Elevations in cPLA₂ Expression and Ethanol-Induced Increases in cPLA₂ Activity Compared to Adults

In order to test the hypothesis that adolescent translocation of novel isoforms is mediated in part through cPLA₂, we assessed cPLA₂ expression and activity basally and following ethanol administration. For cytosolic cPLA₂ expression, analysis revealed a main effect of age ($F_{1,27} = 31.76$, $p < 0.01$), but not ethanol or an interaction. Further analysis indicated that cPLA₂ is elevated by approximately 58 % during adolescence relative to adulthood (Fig. 1a). For cPLA₂ activity as measured by phosphorylation of Serine 550 (ser550), there was a main effect of age ($F_{1,27} = 14.36$, $p < 0.01$), ethanol administration ($F_{1,27} = 27.19$, $p < 0.001$), and an interaction of both ($F_{1,27} = 16.27$, $p < 0.01$). Further analysis revealed that ethanol administration increased cPLA₂ activity by 141.6 % only in adolescents (Fig. 1b).

Adolescent Novel PKC Isoforms Translocate to Cytosolic Localizations Following High-Dose Ethanol Administration

We previously demonstrated that adolescent n- and a-, but not cPKC isoforms are decreased in P2 fractions following acute ethanol exposure [7]. However, it is unclear whether n- and aPKC isoforms translocate to cytosolic regions, where cPLA₂ is localized following ethanol administration. Therefore, cytosolic samples were analyzed at similar time points to our previous P2 fraction analysis following 3.5 g/kg ethanol [7]. For PKC δ , an effect of time post ethanol was observed ($F_{2,21} = 6.495$, $p < 0.01$). Further analysis revealed that PKC δ was increased at 60 min following ethanol exposure by 22.7 % (Fig. 2a). An effect of time post ethanol was similarly observed for PKC ϵ ($F_{2,21} = 3.711$, $p < 0.05$), with further analysis revealed that PKC ϵ was increased 30 min following ethanol exposure by 36.4 % (Fig. 2b). Cytosolic atypical isoforms PKC ι/λ and PKM did not differ (Fig. 2c, d).

cPLA₂ Inhibition Prevents Adolescent Novel PKC Isoform Translocation

Given that cPLA₂ activity is increased following ethanol administration, coupled with cytosolic translocation of nPKC's only in adolescents, we next set out to ascertain whether cPLA₂ activity modulated adolescent nPKC translocation. In order to ascertain successful inhibition of cPLA₂ by AACOCF₃, we probed a subset of samples for cPLA₂ phosphorylation activity 1-h following ethanol administration. Analysis of phosphorylated cPLA₂ analysis revealed a main effect of AACOCF₃ treatment ($F_{1,19} = 4.867, p < 0.05$), ethanol exposure ($F_{1,19} = 21.913, p < 0.001$), and an interaction of both ($F_{1,19} = 12.586, p < 0.01$) (Fig. 3a). Post-hoc analysis revealed an increase in aCSF treated animals following ethanol exposure ($p < 0.001$), but that AACOCF₃ reduced phosphorylated cPLA₂ compared to ethanol exposed aCSF controls ($p < 0.01$). Phosphorylated cPLA₂ did not differ between AACOCF₃ treated saline and ethanol exposed animals. For total cytosolic cPLA₂ expression, only a main effect of AACOCF₃ treatment was observed ($F_{1,19} = 7.806, p < 0.05$) (Fig. 3b), such that cPLA₂ expression was reduced following AACOCF₃ administration. Taken together, these results (1) extend the time course of adolescent ethanol-induced increases in activated cPLA₂, and (2) that AACOCF₃ sufficiently reverses this effect.

We next determined whether cPLA₂ inhibition adolescent ethanol-induced nPKC translocation. For PKC δ in the P2 fraction, we observed a main effect of AACOCF₃ treatment ($F_{1,25} = 7.445, p < 0.05$) (Fig. 4a). For cytosolic PKC δ , analysis revealed a main effect of AACOCF₃ treatment ($F_{1,25} = 8.762, p < 0.01$), of ethanol exposure ($F_{1,25} = 5.308, p < 0.05$), and an interaction of both ($F_{1,25} = 5.000, p < 0.05$) (Fig. 4b). Post-hoc analysis indicated that PKC δ was a significantly increased in aCSF-treated animals following ethanol exposure ($p < 0.05$), and that ethanol-induced increases were prevented in AACOCF₃ exposed animals ($p < 0.01$). Interestingly, no differences were observed for PKC ϵ demonstrated no changes in either the P2 or cytosolic preparations (Fig. 4c, d).

δ and ϵ PKC Isoforms Differentially Modulate Adolescent High-Dose Ethanol Sensitivity

Finally, we wanted to assess which nPKC isoform was responsible for cPLA₂-modulation of adolescent behavioral responses to ethanol. As isoform-specific activators are not available, we instead selectively inactivated PKC isoforms with specific peptide inhibitors while leaving adolescent ethanol-induced increases in cPLA₂ activity intact. Inhibition of PKC δ decreased LORR duration by 16 % ($p < 0.05$; Fig. 5). Interestingly, 40 % of animals in the PKC δ condition failed to lose their righting reflex, which approached significance ($\chi^2 = p < 0.07$). Conversely, PKC δ -I subjects had a significantly higher BEC upon recovery ($p < 0.05$, *data not shown*). Importantly, it should be noted that we observed the exact opposite results with rottlerin (*not shown*); however, it has been shown to be ineffective as a PKC δ inhibitor [22] and is therefore likely eliciting its behavioral response through as of yet unknown mechanisms. Following PKC ϵ inhibition animals on average lost their righting reflex for 37.1 % longer than control animals ($p < 0.05$), and displayed lower BEC's ($p < 0.05$, *data not shown*). To rule out the possibility that aPKCs were involved, ethanol sedative/hypnotic responses were assessed in the presence of ZIP, which has selectivity for PKC ι/λ and PKM ζ [23] Inhibition of ZIP-sensitive kinases did not alter behavior ($p = 0.27$) or BEC's ($p = 0.59$, *data not shown*).

Discussion

The present study demonstrates that cPLA₂ activity modulates nPKC isoform translocation patterns that may contribute to reduced adolescent ethanol-sensitivity. Given that the cPLA₂ cascade has previously been shown to be involved in PKC translocation patterns, we chose to investigate basal and ethanol-induced alterations in cPLA₂ expression and activity. Interestingly, we found that adolescent cPLA₂ expression is basally elevated relative to adults, and only adolescents exhibited a robust increase in cPLA₂ activity following high-dose ethanol administration. Consistent with our previous decrements in P2 fraction levels, we found that only nPKC isoforms exhibited cytosolic translocation following high-dose ethanol administration. Results further demonstrated that ethanol-induced alterations in the nPKC isoform, particularly PKC δ , were ablated following administration of AACOCF₃ at time points related to our initial decreases. Finally, due to the specificity of PKC isoform translocation, we investigated whether specific PKC isoforms modulated ethanol's sedative/hypnotic sensitivity. We found that PKC δ and ϵ inhibition decreased and increased adolescent high-dose ethanol-sensitivity, respectively, whereas modulation of ZIP-sensitive kinases had no effect on behavioral responses.

Our lab previously demonstrated decreased n- and aPKC isoform expression in the P2 fraction following a soporific ethanol exposure in adolescents, but not adults [7, 8]. Effects appear to be PKC isoform specific, as cPKC did not differ post-ethanol exposure in either age. Consistent with our previous report, data from the current study further demonstrate that adolescent nPKC isoforms translocate away from synaptosomal regions to cytosolic fractions following ethanol-administration. Interestingly, the aPKC isoforms, PKC ι/λ and PKM ζ , are not altered in the cytosol, potentially suggesting either enzymatic degradation or nuclear translocation [24, 25].

As the cPLA₂/AA pathway preferentially activates nPKC isoforms in cytosolic regions, we chose to assess ethanol-induced changes in cPLA₂ signaling. Not only was cPLA₂ expression elevated during adolescence, but following ethanol exposure, only adolescent cPLA₂ activity was enhanced. cPLA₂ activity was assessed through measurement of ser505 phosphorylation. Evidence suggests that phosphorylation of cPLA₂ on serine505 (ser505) is required for cPLA₂ catalytic activity, which in turn is necessary for AA production [26]. Consequently, quantification of this residue likely acts as an indirect measure of AA generation. AA is of primary interest as it is at present thought to activate PKC [13]. This result also agrees with our prior report demonstrating that cPLA₂ inactivation increased adolescent, but not adult, ethanol-hypnotic sensitivity [7]. While our previous work is the first study to behaviorally assess the role of cPLA₂ in ethanol-related behaviors, much of our current results are in agreement with previously published work. Our observed increases in adolescent cPLA₂ activity following an *acute* dose of ethanol parallel adult up-regulation of cPLA₂ activity following *chronic* exposure [27, 28]. This is intriguing, as behaviorally; adolescent's display decreased hypnotic sensitivity analogous to adult chronic ethanol-exposure. Taken together, a leftward shift in cPLA₂ activity may underlie aspects of acute high-dose ethanol-sensitivity, particularly in both adolescents and chronic alcoholics.

It is important to note that our translocation and behavioral data do not take into account the activational state of PKC isoforms. Due to these factors, two additional interpretations have emerged regarding ethanol-induced PKC translocation. First, in an active state, PKC translocation can have a regulatory effect on synaptosomal neurotransmitter systems, particularly post-synaptic receptor function. For instance, changes in the cellular distribution of PKC isoforms is involved in regulating γ -aminobutyric acid receptor (GABA_AR) function and expression [29, 30]. Of specific interest to this study, PKC δ has been shown to potentiate δ subunit containing GABA_AR's following ethanol administration [29]. Therefore, it is conceivable that adolescent translocation of active PKC δ to cytosolic regions following ethanol-administration may result in decreased ethanol-induced tonic inhibition, thereby contributing to the reduced sensitivity to high-dose ethanol-administration seen during the adolescent period. In support of this, AACOCF₃ increased P2 localization of PKC δ , likely contributing to the increased loss of righting reflex duration observed in our prior studies [7] and current behavioral data demonstrating reduced high-dose ethanol sensitivity following PKC δ inhibition. It must be acknowledged that PKC δ inhibition only decreases ethanol-induced sleep time rather modestly. However, this is likely attributable to a sensitivity threshold, as 40 % of adolescents in the PKC δ -I condition failed to lose their righting reflex, with comparable BEC levels at synonymous time-points.

A second possibility relates to PKC being in an inactive state. Specifically, cytosolic translocation of inactive PKC isoforms would exert minimal effects on synaptically localized proteins. However, if cytosolically activated by cPLA₂, PKC could interact with numerous non-synaptic proteins. For instance, PKC ϵ has been shown to interact with the GABA_AR γ_2 subunit, whereby phosphorylation acts to decrease ethanol potentiation [31]. Recent reports indicate that GABA_AR γ_2 subunits exist extrasynaptically in regions likely not captured in our P2 preparations [32], which may be a primary target of cytosolic PKC ϵ . Further, cytosolically localized PKC may decrease the probability of GABA release at post-synaptic terminals through interactions with endoplasmic reticulum Ca²⁺ release [33]. Thus, PKC ϵ in cytosolic regions may act to decrease ethanol-sensitivity through both extrasynaptic receptor post-translational modification or release probability. Further, as AACOCF₃ maintains minimally active PKC ϵ expression near the membrane, potentiation of GABA_AR γ_2 containing-subunits would likely be enhanced following ethanol-exposure, thereby also explaining the behavioral effects of AACOCF₃ administration. Relatedly, cytosolic activation of PKC δ may have troublesome implications for proper neuronal function. In fact, cellular localization of PKC δ , particularly cytosolic and nuclear locations, is involved in apoptotic functions [34, 35]. Thus, cytosolic action of PKC δ and $-\epsilon$ following ethanol-exposure may have divergent consequences for proper neuronal function and development. In either case, without the availability of PKC isoform selective activators to restore isoform function following AACOCF₃ inhibition, further work delineating the downstream role of PKC δ and $-\epsilon$ in adolescent ethanol behavioral responses is limited.

While we did not definitively show AACOCF₃ prevented PKC ϵ translocation, it is likely attributable to time points utilized, as decreases in P2 fraction PKC ϵ levels occurs at earlier time points. Conversely, it's possible that animals that underwent surgical procedures, do not exhibit similar alterations in PKC ϵ distribution following ethanol-exposure. This is not surprising, as the anesthetic utilized in our study, isoflurane has been shown to activate

PKC ϵ but not δ in synaptosomal regions [36]. Along the same line, we surprisingly observed an increase in PKC γ cytosolic translocation, which was also suppressed following administration of AACOCF₃. Conversely, stress from single-housing following surgical procedures may contribute to differences in our biochemical analysis [37]. Future studies are needed to further assess PKC contribution to anesthetic- and stress-related ethanol interactions during adolescence.

Observed increases in cPLA₂ activity correspond with previous literature demonstrating increased cellular death [4] and decreased neurogenesis [5] following adolescent exposure to ethanol. Chronic ethanol is known to induce a multitude of pro-inflammatory markers and contribute to CNS degradation, particularly during critical periods of development such as adolescence [38]. Further, chronic alcohol abuse leads to increased brain edema and glial swelling that subsequently induces neuronal cPLA₂ activity [11]. By itself, cPLA₂ activity promotes oxidative-stress-induced inflammation and neuronal cell death [39, 40], analogous to that seen following chronic ethanol exposure. Such effects are even more intriguing as cPLA₂ knockout mice are resistant to ischemia-induced brain edema [41]. In addition, ethanol-induced increases in cPLA₂ activity during earlier developmental periods may have persistent consequences, as adults exposed to ethanol in utero exhibit decreased cPLA₂ in both frontal cortex and hippocampus that associate with long-lasting cognitive deficits [42]. Thus, the cPLA₂ pathway may contribute to cellular death following adolescent ethanol exposure. Future studies are needed to directly assess the role of cPLA₂ in adolescent ethanol-induced oxidative stress.

Overall, our data highlight a potential signaling cascade in adolescent ethanol-action, such that activation of the cPLA₂/AA pathway following high-dose ethanol-administration causes translocation of nPKC isoforms away from the cell membrane to cytosolic localizations. These experiments may impact future lines of investigation, as this pathway may have dual consequences on adolescent development, as it is involved in decreased ethanol sensitivity and increased ethanol-induced neurotoxicity. Ultimately, this cascade may not only allow adolescents to imbibe increased levels of ethanol, but also contributes to greater levels of ethanol-related cell death. Importantly, such results will lay the foundation for future studies designed to further assess cPLA₂ and PKC involvement in adolescent ethanol responses.

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Fig. 1.

Adolescents display basal elevations in cPLA₂ expression and ethanol-induced increases in cPLA₂ activity compared to adults. Representative *blots* are shown (a) and quantifications of cPLA₂ (b) and phosphorylated cPLA₂ (c) are depicted. Data represent mean $\pm$ SEM *main effect of age $p < 0.05$, [^] $p < 0.05$ compared to age-matched control, [#] $p < 0.05$ compared to adolescent ethanol, $n = 7-8$ /group

Fig. 2.

Adolescent novel PKC isoforms translocate to cytosolic localizations following high-dose ethanol administration. Representative blots are shown for PKC δ (a), PKC ϵ (b), PKC ι/λ (c) and PKM ζ (d). Data represent mean $\pm$ SEM * p < 0.05 compared to vehicle control, n = 7–8/group

Fig. 3. Infusion of AACOCF₃ successfully inhibits cPLA₂ activity and expression. Representative blots are shown (a) and graphs are depicted for phosphorylated cPLA₂ (b) and cPLA₂ (c). Data represent mean ± SEM *main effect of AACOCF₃ $p < 0.05$, [^] $p < 0.05$ compared to vehicle/vehicle, [#] $p < 0.05$ compared to vehicle/ethanol, $n = 5-6$ /group

Fig. 4. cPLA₂ inhibition prevents adolescent nPKC isoform translocation. Representative *blots* are shown for PKC δ in P2 (**a**) and cytosolic regions (**b**) and PKC ϵ in P2 (**c**) and cytosolic preparations (**d**). Data represent mean $\pm$ SEM *main effect of AACOCF₃, $p < 0.05$, ^ $p < 0.05$ compared to vehicle/vehicle, # $p < 0.05$ compared to vehicle/ethanol, $n = 7-8$ /group

Fig. 5.

Novel, but not atypical PKC isoforms, differentially modulates adolescent high-dose ethanol sensitivity. Intracerebroventricular infusion of PKC δ -I decreased sleep time, while PKC ϵ -I increased sleep time in adolescent rats. ZIP had no effects ($n = 6-7/\text{group}$). * $p < 0.05$, compared to age-matched controls