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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

This report details our progress during the first year of a three-year proposal. The proposal's overall goal is to uncover biochemical mechanisms that underlie learning and memory deficits resulting from fetal alcohol exposure (FAE). We have found that adult rats that were exposed to alcohol prenatally display a decrement in learned fear. One and twenty-four hours following fear conditioning this learning deficit is associated with altered brain signal transduction mechanisms that are dependent on an enzyme termed phosphatidylinositol-specific phospholipase C-β1a (PLC-β1a). Tissue has been collected, and will be analyzed during the second year of the proposal, at additional times following fear conditioning. We have initiated studies aimed at elucidating the biochemical basis of the effects of FAE on PLC-β1a enzyme activity. Our preliminary results indicate that brain PLC-β1a forms a tight complex with protein kinase C, which has been implicated as playing an essential role in learning and memory. In the coming year, we will assess the effects of prenatal alcohol exposure on this association. The next phase of these studies are expected to yield important information about the neurochemical mechanisms that underlie fear and stress, and, consequently, may provide insight into the neurochemical basis of posttraumatic stress syndrome.

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INTRODUCTION

Maternal consumption of moderate levels of ethanol causes subtle cognitive and behavioral impairments in her offspring that often become apparent only under stressful conditions. The overall aim of the funded proposal is to uncover biochemical mechanisms that underlie these learning and memory failures, using a rat model of moderate fetal alcohol exposure (FAE) and a behavioral technique termed fear conditioning. Specifically, the funded studies investigate the relationships between FAE, fear-conditioned learning, and an enzyme termed phosphatidylinositol-specific phospholipase C-β1a (PLC-β1a). The approved goals of the research are: 1) To determine the time course of one trial fear conditioning-induced changes in PLC-β1a subcellular distribution and catalytic activity in adult rats who were exposed, or not, to moderate levels of ethanol throughout gestation; and, 2) To determine whether one trial fear conditioning alters the phosphorylation state of PLC-β1a and, if so, whether fetal alcohol exposure modifies the changes in PLC-β1a phosphorylation.

BODY

There were two goals listed in the approved Statement of Work.

GOAL 1: To determine the time course of one trial fear conditioning-induced changes in PLC-β1a subcellular distribution and catalytic activity in adult rats who were exposed, or not, to moderate levels of ethanol throughout gestation.

GOAL 2: To determine whether one trial fear conditioning alters the phosphorylation state of PLC-β1a and, if so, whether fetal alcohol exposure modifies the changes in PLC-β1a phosphorylation.

Progress made toward GOAL 1 is as follows. We have confirmed the preliminary studies presented in the original grant application showing that FAE is associated with altered hippocampal formation and medial frontal cortical PLC-β1a responses to fear conditioning (data not shown). The results of these studies were presented at the 2001 Research Society on Alcoholism meeting in Montreal, Canada and the 2001 Neurobehavioral Teratology Society in Montreal, Canada (please see Reportable Outcomes below and abstracts contained in Appendix).

We have collected hippocampal formation, medial frontal cortical and amygdalar tissue and prepared subcellular fractions, as described in the approved grant proposal, for three (specifically, 30 minutes, 60 minutes, and three hours) of the proposed eight time points in each of the three experimental groups (i.e., fear-conditioned, unpaired controls and unhandled rats). The PLC-β1a enzyme activity measurements and determinations of PLC-β1a protein levels in the collected tissues will be conducted during the next 12 months. Collection, processing and biochemical characterization of tissue for the remaining time points will be completed during the remaining 24 months of the grant period.

Progress made toward GOAL 2 is as follows. We have initiated studies aimed at elucidating the biochemical basis of the effects of FAE on fear conditioning-dependent changes in PLC-β1a enzyme activity. Figures 1 and 2 demonstrate that rat brain PLC-β1a forms a tight complex with protein kinase C-α (PKC-α). PKC isozymes have been reported to play an important role in learning and memory (Colombo et al. 1997; Fordyce et al. 1995).

In the study presented in Figure 1A – 1C (see next page), anti-PLC-β1a immunoprecipitates were prepared from subcellular fractions derived from the amygdala (Figure 1A), medial frontal cortex (Figure 1B) and hippocampal formation (Figure 1C) naïve rats and probed initially for anti-PKC-α (Mr ≅ 80,000 daltons) immunoreactivity, and, subsequently probed without stripping the membrane of the anti-PKC-α antibody, for anti-PLC-β1(Mr ≅ 150,000 daltons) immunoreactivity. The presence of the anti-PLC-β1a immunopositive band in each sample confirms that the immunoprecipitation procedures was successful. Of significance is the demonstration that each of the anti-PLC-β1a immunoprecipitates contained a protein of Mr ~

80,000 daltons, the appropriate Mr for PKC-α, that was recognized by an anti-PKC-α antibody. We have confirmed this result using an anti-PKC-α antibody from two other commercial suppliers (Chemicon and Santa Cruz Biotechnology). These results demonstrate that PKC-α and PLC-β1a can be co-immunoprecipitated from amygdalar, hippocampal formation and medial frontal cortical subcellular fractions, indicating that the two proteins interact, either directly or indirectly, *in vivo*. The results presented in Figure 2 support this conclusion. Anti-PKC-α immunoprecipitates prepared from hippocampal formation membrane fractions were found to contain PLC-β1a immunoreactivity,

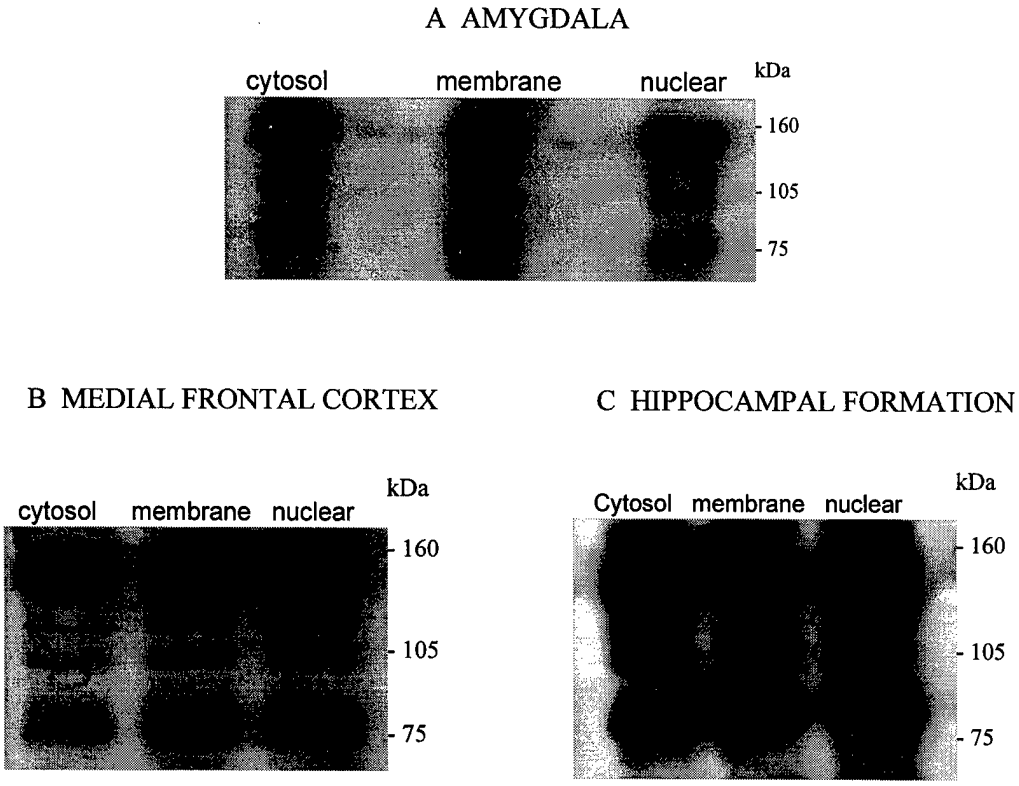

FIGURE 1 *Co-immunoprecipitation of PLC-β1a and PKC-α from amygdalar (A), medial frontal cortical (B) and hippocampal formation (C) subcellular fractions.* The amygdala, medial frontal cortex and hippocampal formation were isolated from a naïve rat, and subcellular fractions (nuclear, cytosolic and membrane) were prepared as described in the Methods section of the funded grant proposal. PLC-β1a was immunoprecipitated from either 100μg (amygdala and medial frontal cortex) or 150 μg (hippocampal formation) subcellular fraction total protein, the immunecomplex was resolved by SDS-PAGE, transferred to PVDF and probed for anti-PKC-α immunoreactivity as described in the original proposal. Subsequently, the PVDF membrane was re-probed for anti-PLC-β1a immunoreactivity as described. The immunopositive band migrating at approximately 80,000 daltons was detected by the anti-PKC-α antibody, while the Mr 150,000 dalton band was detected by the anti-PLC-β1a antibody.

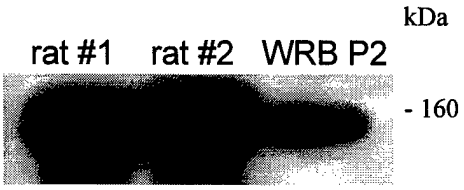

FIGURE 2 *PLC-β1a immunoreactivity is contained in anti-PKC-α immunecomplexes isolated from hippocampal formation membrane subcellular fractions.* Hippocampal formation subcellular fractions (200 μg protein) isolated from two (rats #1 and #2) naïve rats were treated with 5 μg anti-PKC-α (BD Transduction Labs) antibody. Immunecomplexes were isolated, resolved by SDS-PAGE, transferred to PVDF and probed for anti-PLC-β1a immunoreactivity as described in the grant application. Ten micrograms of a postnuclear fraction isolated from whole rat brain (WRB P2) was also included on the SDS-PAGE gel to show the relative migration of PLC-β1a.

These results are consistent with a recent publication by Xu et al. (2001), who demonstrated that insulin-like growth factor I (IGF-I) treatment of *in vitro* cultured fibroblasts induces the association of PLC-β1 and PKC-α in the cell nucleus. In addition, Xu et al. confirmed previous work by Litosch (1996), who demonstrated that PKC-α-dependent phosphorylation of PLC-β1 decreases PLC-β1 enzyme activity. It should be noted that Xu et al. performed their studies using cell cultures over-expressing PLC-β1, while our studies were performed using tissue derived from standard (i.e., commercially available) rats. Thus, PKC-α is our lead candidate as the protein kinase responsible for the down-regulation, a putative phosphorylation-dependent mechanism, of PLC-β1a enzyme activity that we have observed following fear conditioning.

Finally, in preliminary studies, we have found that PLC-β1a in the nuclear fraction derived from the medial frontal cortex, but nor the hippocampal formation, of naïve rats can be immunoprecipitated with an anti-phosphotyrosine antibody (Figure 3). This indicates that medial frontal cortical nuclear PLC-β1a is either phosphorylated on tyrosine *in vivo* or associates with a phosphotyrosine-containing protein.

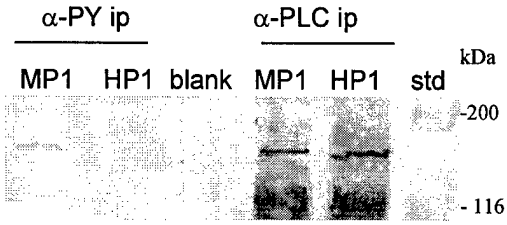

FIGURE 3 *PLC-β1a is immunoprecipitated from medial frontal cortical nuclear, but not hippocampal formation nuclear, fractions by an anti-phosphotyrosine antibody.* Nuclear (P1) fractions were isolated from the medial frontal cortex (M) and hippocampal formation (H) from a naïve rat, as described in the grant proposal. MP1 (26 μg) or HP1 (44 μg) fractions were treated with either an anti-phosphotyrosine (α-PY) or an anti-PLC-β1a α-PLC) antibody and immunecomplexes were isolated as described. The immunecomplexes were resolved by SDS-PAGE, transferred to PVDF and probed for anti-PLC-β1a immunoreactivity as described in the grant application. PLC-β1a immunoreactivity was present in the anti-phosphotyrosine immunoprecipitate isolated from the MP1 fraction but not the HP1 fraction. This difference was apparently not due to differences in the amount of PLC-β1a present in MP1 and HP1 preparations, since similar amounts of anti-PLC-β1a immunoreactivity could be immunoprecipitated from the two preparations using an anti-PLC-β1a antibody.

In the coming year, we will assess the effects of prenatal alcohol exposure and fear conditioning on the interaction of PLC-β1a with PKC-α, as well as determine whether there is a correlation between this association and the phosphorylation state of PLC-β1a. In addition, we will pursue the possibility that medial frontal cortical nuclear PLC-β1a is phosphorylated on tyrosine residues.

KEY RESEARCH ACCOMPLISHMENTS

- We have collected and tissue and prepared subcellular fractions for three-eighths of the studies proposed in GOAL1.
- As part of the GOAL 2 studies, we have identified PKC-α as a lead candidate for the protein kinase responsible for the down-regulation, of PLC-β1a enzyme activity that we have observed following fear conditioning.
- As part of the GOAL 2 studies, we have identified tyrosine phosphorylation in the medial frontal cortical nuclear fraction as a means to regulate PLC-β1a enzyme activity.

REPORTABLE OUTCOMES

Caldwell, K.K., Weeber, E.J., Buckley, C.T. Savage, D.D. and Sutherland, R.J. (2001) Fear conditioning-induced alterations of phospholipase C-β1a protein level and enzyme activity in rat hippocampal formation and medial frontal cortex. Alcohol. Clin. Exp. Res. 25 (Supplement to issue no. 5) 58A.

Savage, D.D., Caldwell, K.K., Perrone-Bizzozero, N.I. and Sutherland, R.J.: Learning deficits in prenatal ethanol-exposed offspring: Integrating behavioral, physiological and biochemical analyses of neural plasticity in hippocampal formation. Neurobehavioral Teratology Soc. Abstracts 2001.

CONCLUSIONS

During the first year of this three year proposal, we have made important progress toward achieving goal 1. First, we have confirmed our preliminary studies. Second, and more importantly, we have positioned ourselves to make significant advances in the coming year by having bred, behaviorally challenged (or not), and prepared tissue form rats for approximately one-half of the studies proposed in this goal. In regard to our progress toward achieving goal 2, we are particularly excited about the recent discovery that PLC-β1a and PKC-α interact in rat brain. This observation provides us with a lead candidate (i.e., PKC-α) for our studies on the phosphorylation-dependent regulation of PLC-β1a enzyme activity. In addition, preliminary evidence indicates that tyrosine phosphorylation may be an important mechanism for the regulation of medial frontal cortical nuclear PLC-β1a enzyme activity.

The next phase of these studies are expected to yield important information about the neurochemical mechanisms that underlie fear and stress, and, consequently, may provide insight into the neurochemical basis of posttraumatic stress syndrome.

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Xu, A., Wang, Y., Xu, L.Y., and Gilmour, R.S. (2001) Protein kinase C α-mediated negative feedback regulation is responsible for the termination of insulin-like growth factor I-induced activation of nuclear phospholipase C β1 in Swiss 3T3 cells. J. Biol. Chem. 276, 14980-14986.

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POTENTIATION BY PHORBOL ESTER OF 5-HT$_{3A}$ RECEPTOR REDUCED THE RECEPTOR'S SENSITIVITY TO ETHANOL. E. M. Moradel, J. Schoenebeck, A. Ziglari, L. Zhang and F. F. Weight. Lab. of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8115.

Ethanol potentiates 5-HT$_3$ receptor-mediated currents in various neurons. Recent clinical evidence suggests that the 5-HT$_3$ receptor may be one of the alcohol targets involved in alcoholism. However, the molecular mechanism by which ethanol potentiates 5-HT$_3$ receptor function is not clear. Our laboratory previously reported that phorbol ester (PMA), a protein kinase C (PKC) activator, and ethanol potentiate the function of 5-HT$_{3A}$ receptors expressed in *Xenopus* oocytes (*NeuroReport*, 6:1464-1468, 1995; *Mol. Pharmcol.* 50:1010-1016, 1996). Both effects of PMA and ethanol showed a dependence on agonist concentration. To address whether or not the receptor sensitivity to ethanol is modulated by PMA potentiation of receptor function, we examined ethanol potentiation of 5-HT$_{3A}$ receptor function before and after the application of PMA. The 5-HT$_{3A}$ receptors were expressed in *Xenopus* oocytes and their sensitivity to ethanol and PMA was examined using two-electrode voltage-clamp. Consistent with our previous findings, treatment with PMA (10 nM) for 10 minutes potentiated 5-HT$_{3A}$ receptor-mediated currents. The potentiation lasted for longer than 40 minutes and reached the maximal amplitude at 10-15 minutes after PMA application. On the other hand, ethanol (100 mM) increased the current amplitude activated by 5-HT by 44 ± 8 % before the PMA treatment, however, the potentiation by ethanol was 19 ± 6 % (n = 5) at 20 min. after the PMA treatment. Thus, these results suggest that PMA potentiation of the 5-HT$_{3A}$ receptor reduced the amplitude of the 5-HT$_{3A}$ receptor's response to ethanol.
Supported by the Intramural Research Program of NIAAA, NIH.

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DELETIONAL ANALYSIS OF THE ROLE OF THE LARGE INTRACELLULAR LOOP IN THE PHORBOL ESTER POTENTIATION OF THE 5-HT$_3$ RECEPTOR AND THE RECEPTOR'S SENSITIVITY TO ETHANOL. H. Sun, J. Schoenebeck, E. Moradel, F. F. Weight and L. Zhang. Lab. of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8115.

The 5-HT$_3$ receptor is a member of the ligand-gated membrane ion channel superfamily that includes GABA$_A$ and glycine receptors. One of the features of the topology of these receptors is a large intracellular loop (LIL). It has been proposed that the LIL is involved in modulating the function of receptors in this superfamily of ligand-gated channels. However, whether or not the LIL is involved in modulating phorbol Ester (PMA) potentiation of 5-HT$_3$ receptor function or the receptor's sensitivity to ethanol has not been established. In this study, we sequentially deleted segments of the 5-HT$_3$ receptor LIL and examined the function of the wild type and mutant receptors, and their sensitivity to PMA and ethanol. Ten subdomains, each contained 10-20 amino acids, of the LIL were separately deleted, and the cRNAs of the mutant or wild type receptors were injected into the *Xenopus* oocytes. In these cells, the EC$_{50}$ values of the 5-HT concentration-response curves for most of the mutant of the receptors, except for two mutant receptors that did not exhibit a response to 5-HT, were similar to that of the wild type receptor. PMA (10 nM) or ethanol (100-200 mM) potentiated all mutant and wild type receptor-mediated currents at low agonist concentrations (EC$_5$). However, further quantitative analysis of each individual mutant receptor revealed a differential sensitivity of the receptors to PMA and ethanol, suggesting that modulation by PMA and ethanol of 5-HT$_{3A}$ receptor function may not be mediated thru the same molecular sites. Supported by the Intramural Research Program of NIAAA, NIH.

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DIRECT ACTIVATION OF THE 5-HT$_{3A}$ RECEPTOR BY ETHANOL CORRELATES WITH THE CHANNEL SPONTANEOUS OPENING THRU SINGLE AMINO ACID MUTATIONS AT 245. L. Zhang, M. Hosoi, M. Fukuzawa, E. Moradel, H. Sun and F. F. Weight. Labs. of Mol. & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892.

In a previous study, we reported that substitution of an arginine with alanine at 245 in the N-terminal domain of the 5-HT$_{3A}$ receptor altered the ethanol sensitivity and apparent agonist affinity of receptor (*Soc. Neurosci. Abst.* 28:1819, 1998). Here, we extended our previous investigation by replacing R245 with a number of amino acids. In *Xenopus* oocytes previously injected with cRNAs of the mutant or wild type (WT) receptors, the EC$_{50}$ values of the 5-HT concentration response curves for the mutant receptors, except for R245K, were 10 - 100 fold less than that of the wild type receptor. In the cells expressing the mutant receptors substituted with F, I, Q, D, and G at 245, pharmacologically relevant concentrations of ethanol (10 - 100 mM) directly activated inward currents in a concentration dependent manner. However, R243A, R245E/K/T/A/N and wild type receptors were less or insensitive to ethanol in the absence of agonist. Both MDL 72222 (MDL) and LY 278, 584 (LY), selective antagonists of 5-HT$_3$ receptors, inhibited the currents activated by ethanol. The IC$_{50s}$ of MDL and LY inhibition were 6.7 ± 0.3 nM and 11 ± 1 nM, respectively. For the mutant receptors, MDL (100 nM) induced outward currents. The amplitudes of the outward current activated by MDL correlated with the amplitude of the current directly activated by ethanol for all of the receptors tested (R = 0.94, $p<0.0001$, nonparametric statistics, STATISTICA). Our results suggest that mutation of arginine at 245 of the 5-HT$_{3A}$ receptor can alter the sensitivity of the receptor to ethanol. In addition, the direct activation of the receptor by ethanol correlates with the spontaneous opening of the mutant receptor.

C. Adenosine

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310 ANTAGONISM OF ACUTE ETHANOL/CANNABINOID-INDUCED MOTOR INCOORDINATION WITH ADENOSINE A$_1$ RECEPTOR ANTISENSE IN MICE. M. S. Dar, S. J. Mustafa, Department of Pharmacology, Brody Medical School at East Carolina University, Greenville, NC 27858.

Previous reports from first author's laboratory demonstrate that ethanol- and Δ^9-THC-induced ataxia is modulated by cerebellar adenosine A$_1$ receptor because intracerebellar (ICB) adenosine A$_1$ agonists potentiated and A$_1$ antagonists attenuated motor impairment by ethanol (i.p.) and Δ^9-THC (ICB). In this study, the novel approach involving pretreatment with adenosine A$_1$ antisense oligodeoxynucleotide via multiple routes provided further direct evidence of cerebellar A$_1$ modulation. Animal groups were pretreated orally (3.12, 6.25, 12.5, 50μg/12h; 3 treatments), i.p. (3.12, 5, 10, 50 μg/12h;3 treatments), ICB (2μg/12h; 3 treatments) with A$_1$ antisense and its mismatch. Marked antagonism to ethanol (2g/kg; i.p.) and Δ^9-THC (15μg; ICB)-induced motor incoordination as well as its accentuation by A$_1$-selective agonist, CHA (4ng; ICB) was observed 12 h after the last antisense treatment based on our standard rotorod test. The A$_1$ receptor mismatch pretreatment was without any effect. The antagonism due to pretreatment with antisense was dose-dependent. No change in the normal motor coordination was observed when the animals were pretreated with antisense or its mismatch and vehicle. Results of western blotting using commercially available antibodies in cerebellar membranes from various animal groups given antisense and its mismatch via three routes confirmed significant decrease in the A$_1$ adenosine receptor protein. These results, perhaps for the first time, show the oral and systemic effectiveness of A$_1$ antisense towards adenosine receptors in the central nervous system.

D. Second Messengers and G-Proteins

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DIFFERENCES IN CANNABINOID RECEPTOR AGONIST-STIMULATED [^{35}S]GTPγS BINDING IN THE BRAIN OF C57BL/6 AND DBA/2 MICE SELECTED FOR THEIR DIFFERENCES IN VOLUNTARY ETHANOL CONSUMPTION. B.S. Basavarajappa, T.B. Cooper and B. L. Hungund NYS Psychiatric Institute at NKI and Department of Psychiatry, college of Physicians and Surgeons, Columbia University, New York, NY 10032.

Two inbred strains of mice C57BL/6 (alcohol-preferring) and DBA/2 (alcohol- avoiding) have been shown to differ significantly in their preference for alcohol (EtOH). The differences in the density and the affinity of cannabinoid (CB1) receptors in the brains of the two inbred C57BL/6 and DBA/2 strains of mice have previously been reported. In the present study, we investigated the CB1 receptor agonist stimulated guanosine-5'-0- (3-[^{35}S] thio)-triphosphate ([^{35}S]GTPγS) binding in plasma membranes from C57BL/6 and DBA/2 mice. The results indicate that the net CP55,940-stimulated [^{35}S]GTPγS binding was increased with increasing concentrations of CB1 receptor agonists and GDP. The net CB1 receptor agonist (WIN55,212-2 or HU-210 or CP55,940) -stimulated [^{35}S]GTPγS binding was reduced significantly ($P < 0.05$) in PM from DBA/2 mice; no significant differences were observed in basal [^{35}S]GTPγS binding among these strains. Non-linear regression analysis of net CP55,940-stimulated [^{35}S]GTPγS binding showed that the B_{max} of cannabinoid agonist-stimulated binding was significantly reduced (- 24 %) in DBA/2 mice (B_{max} = 12.43 ± 0.64 for C57BL/6 and 9.46 ± 0.98 pmol/mg protein for DBA/2: $P < 0.05$) without any significant changes in the G-protein affinity. The pharmacological specificity of CP55,940-stimulated [^{35}S]GTPγS binding was examined with CB1 receptor antagonist SR141716A, and these studies indicated that CP55,940-stimulated [^{35}S]GTPγS binding was blocked by SR141716A with decrease in the IC_{50} values in the PM from DBA/2 mouse strain. These results suggest that a signal transduction pathway (s) downstream from the CB1 receptor system may play an important role in controlling the voluntary EtOH consumption by these strains of mice.

312 FEAR CONDITIONING-INDUCED ALTERATIONS OF PHOSPHOLIPASE C-β1a PROTEIN LEVEL AND ENZYME ACTIVITY IN RAT HIPPOCAMPAL FORMATION AND MEDIAL FRONTAL CORTEX. K.K. Caldwell, E.J. Weeber, C.T. Buckley, D.D. Savage and R.J. Sutherland University of New Mexico, Albuquerque, NM, 87131-5223

We investigated the effects of one-trial fear conditioning on phospholipase C-β1a (PLC-β1a) catalytic activity and protein level in hippocampal formation (HPF) and medial frontal cortex (MFC) of untreated control rats and rats prenatally exposed to ethanol. One hour following fear conditioning of untreated control rats, PLC-β1a protein level was increased in the HPF cytosolic fraction and decreased in the HPF membrane, MFC cytosolic and MFC membrane fractions. Twenty-four hours after fear conditioning, PLC-β1a protein level was reduced in the HPF cytosolic fraction and elevated in the MFC nuclear fraction; in addition, 24 hours after conditioning, PLC-β1a activity in the MFC cytosolic fraction was increased. Rats that were exposed prenatally to ethanol displayed attenuated contextual fear conditioning, whereas conditioning to the acoustic conditioned stimulus was not different from controls. In certain cases, prenatal ethanol exposure modified the relationship between fear conditioning and changes in PLC-β1a protein level and/or activity. The majority of these effects occurred one hour, rather than 24 hours, after fear conditioning. Multi-variate analysis of variance revealed interactions between fear conditioning, subcellular fraction and prenatal ethanol exposure for measures of PLC-β1a protein level in HPF and PLC-β1a enzyme activity in MFC. In the majority of cases, fear conditioning-induced changes in HPF PLC-β1a protein level were augmented in rats prenatally exposed to ethanol. In contrast, fear conditioning-induced changes in MFC PLC-β1a activity were, often, in opposite directions in prenatal ethanol-exposed compared to diet control rats. We speculate that alterations in subcellular PLC-β1a catalytic activity and protein level contribute to contextual fear conditioning and that learning deficits observed in rats exposed prenatally to ethanol result, in part, from dysfunctions in PLC-β1a signal transduction. This research was supported by National Institute on Alcohol Abuse and Alcoholism Grants AA12635 (KKC) and AA06548 (DDS) and U.S. Department of Army Award #DAMD17-00-1-0582 (KKC).

SAVAGE, D.D., CALDWELL, K.K., PERRONE-BIZZOZERO, N.I. and SUTHERLAND, R.J. Departments of Neurosciences and Psychology, University of New Mexico, Albuquerque, New Mexico.
Learning deficits in prenatal ethanol-exposed offspring: Integrating behavioral, physiological and biochemical analyses of neural plasticity in hippocampal formation.

We have utilized a one-trial contextual fear conditioning paradigm in conjunction with a moderate prenatal ethanol exposure paradigm to assess the behavioral, physiological and biochemical effects of prenatal ethanol exposure on hippocampal function in adult offspring. Offspring whose mothers consumed a 5% ethanol liquid diet during gestation exhibited less behavioral freezing to context twenty hours after one-trial fear conditioning than offspring whose mothers consumed either rat chow *ad libitum* or were pair-fed a 0% ethanol liquid diet throughout gestation.

Concurrently, we have observed that hippocampal AMPA receptor binding, phospholipase C activation, GAP-43 phosphorylation and evoked amino acid release are elevated 24 hours after fear conditioning in *ad lib* chow and pair-fed control offspring. By contrast, prenatal ethanol-exposed rats display diminished neurochemical changes in response to fear conditioning. We conclude that one-trial fear conditioning can be used to study the biochemical machinery involved in synaptic strengthening, along with the hippocampal formation's role in contextual-based learning. Further, the impact of moderate prenatal ethanol exposure on hippocampal function is more apparent when examining behavioral activation states than states of relative inactivity.

Supported by (AA06548, AA12400, AA11333, AA11336, AA12635 and DAMD17-00-1-0582).