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Prenatal alcohol: Effect on Depression and Brain Derived Neurotrophic Factor (BDNF) levels

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Abstract

Fetal alcohol exposure poses a significant social problem. Conservative rates of Fetal alcohol syndrome and Alcohol-Related Neurodevelopmental Disorders (ARND) together have been estimated at 9.1/1000 live births (Sampson 1997). Prenatal ethanol exposure can produce subtle learning disabilities in children, which may not become apparent until a child is school-aged and can occur in the absence of other physical evidence of alcohol-related birth defects. Along with cognitive deficits, ARND has been associated with an increased risk of psychiatric disorders such as Major Depressive Disorder (MDD). Major Depressive Disorder (MDD) has been linked to polymorphisms in the Brian Derived Neurotrophic Factor (BDNF) gene and patients with MDD have low serum BDNF levels (Levinson 2005, Chen et al 2001). Also post-mortem depressed patients who were on anti-depressant drugs have shown increased levels of BDNF (Chen et al 2001). BDNF given to stressed animals can even produce an antidepressant-like effect through the antagonism of a model of depression called learned helplessness behavior (Karege 2002). Efforts to better understand how prenatal ethanol increases susceptibility to MDD and the mechanism(s) by which ethanol produces these defects will rely on the study of these effects in animal models of prenatal ethanol exposure. Recently, our laboratory developed a mouse Fetal Alcohol Exposure (FAE) model which displays neurochemical and learning deficits. This model was used to explore the relationship of FAE-induced learned helplessness and changes in BDNF in the

adult brain. Prenatal alcohol exposure was shown to produce an increase in learned helplessness and this was associated with a decrease in BDNF levels in the brain. These findings support the hypothesis that ARND is associated with an increase in depressive behavior and that lower levels of BDNF following prenatal exposure to alcohol may be involved.

Introduction

Prenatal ethanol exposure can produce a myriad of teratogenic defects in offspring. Learning disabilities are among the more subtle, yet most prevalent and pervasive, fetal alcohol-related defects in children (Jones and Smith, 1973). These learning deficits, which may not become apparent until a child is school-aged, can occur in the absence of other physical evidence of alcohol-related birth defects (Abel, 1995). The seriousness of this problem is reflected in a recent Institute of Medicine report recommending an expansion of the diagnostic classification of fetal alcohol-related defects to include a new category called Alcohol-Related Neurodevelopmental Disorders (ARND) (Streissguth et al., 1994, Stratton et al., 1995). Efforts to better understand the basis of prenatal ethanol-induced learning disabilities and the mechanism(s) by which ethanol produces these defects will rely on the study of these effects in animal models of prenatal ethanol exposure. In previous work both mouse and rat offspring whose mothers consumed moderate quantities of ethanol throughout gestation, demonstrate electrophysiological, behavioral and neurochemical deficits in hippocampal neurotransmission and synaptic plasticity. Recently, we developed a mouse fetal alcohol exposure (FAE) model which displays neurochemical and learning deficits seen in ARND. Using this model, these studies explored the relationship of FAE and depression using a learned helplessness task and associated these data with changes in brain BDNF levels.

Neurotrophins, like Brain Derived Neurotropic Factor (BDNF), have a variety of functions in the central nervous system. They are made as precursors, or proneurotrophins which are cleaved into mature proteins. They function then as signaling molecules that activate Trk receptor tyrosine kinases to promote neuronal survival and enhance synaptic plasticity. Mature BDNF facilitates early phase and late phase long term potentiation in the hippocampus, the region of the brain which has been shown to have the highest neuroanatomical expression of BDNF and its TrkB receptor in the mammalian brain (Lu 2005, Tyler 2002). Also, antibodies to BDNF have been shown to reduce the survival of cortical neurons, further validating the importance of BDNF in neuronal plasticity (Ghosh 1994).

Patients that have fetal alcohol syndrome or fetal alcohol exposure effects have been shown to have a very high instance of drug or alcohol dependence and major depression has been shown to be the second most common psychiatric disorder among this group (Famy 1998). Childhood depression has also been linked to prenatal alcohol exposure, resulting in children with more negative affect and mothers that are less emotionally connected to their children (O'connor 2005).

A recent etiological hypothesis about depression suggests that depressive symptoms may be mediated by neurotoxic effects which kill or damage hippocampal cells. These factors may involve excessive corticotropin activity and/or inflammatory cytokines, and may cause an inordinate amount of damage because of a lack of neuroprotective responses to stress, such as

neuroprotective peptides. BDNF is one of these neuroprotective proteins and major depressive disorder has been linked to polymorphisms in the BDNF gene (Levinson 2005). Patients with major depressive disorder have low serum BDNF levels, and there is a correlation between decreased BDNF levels and the severity of depression. While BDNF levels in postmortem human brains of untreated depressed patients have not been studied, postmortem depressed patients who were on anti-depressant drugs showed increased levels of BDNF (Chen et al 2001). Also, stress reduces expression of BDNF, while long term anti-depressant treatment and electroconvulsive therapy can increase BDNF expression. BDNF given to stressed animals can even produce an antidepressant-like effect through the antagonism of learned helplessness behavior (Karege 2002).

Recently, our laboratory developed a mouse FAE model which displays neurochemical and learning deficits. This model will be used to explore the relationship of FAE-induced learned helplessness and changes in brain derived growth factor in the adult brain. The goal was to develop a prenatal alcohol exposure model that would (1) produce a voluntary and stable drinking of moderate concentrations of ethanol throughout pregnancy, (2) not significantly affect maternal care, and (3) replicate the behavioral effects found previously in the rat moderate alcohol drinking model. This paradigm facilitated the study of the impact of FAE without negatively influencing diet or natural consumption routes or requiring surrogate maternal care, all of which are thought to contribute to maternal stress (Allan 2003). This model has allowed us to investigate BDNF

levels in prenatal alcohol exposed mice that underwent the learned helpless task. Thus, we postulated that prenatal alcohol exposure should produce an increase in learned helplessness and this should be associated with a decrease in BDNF levels in the brain.

Methods

Animals — All of the procedures involving animals were approved by the University of New Mexico Laboratory Animal Care and Use Committee.

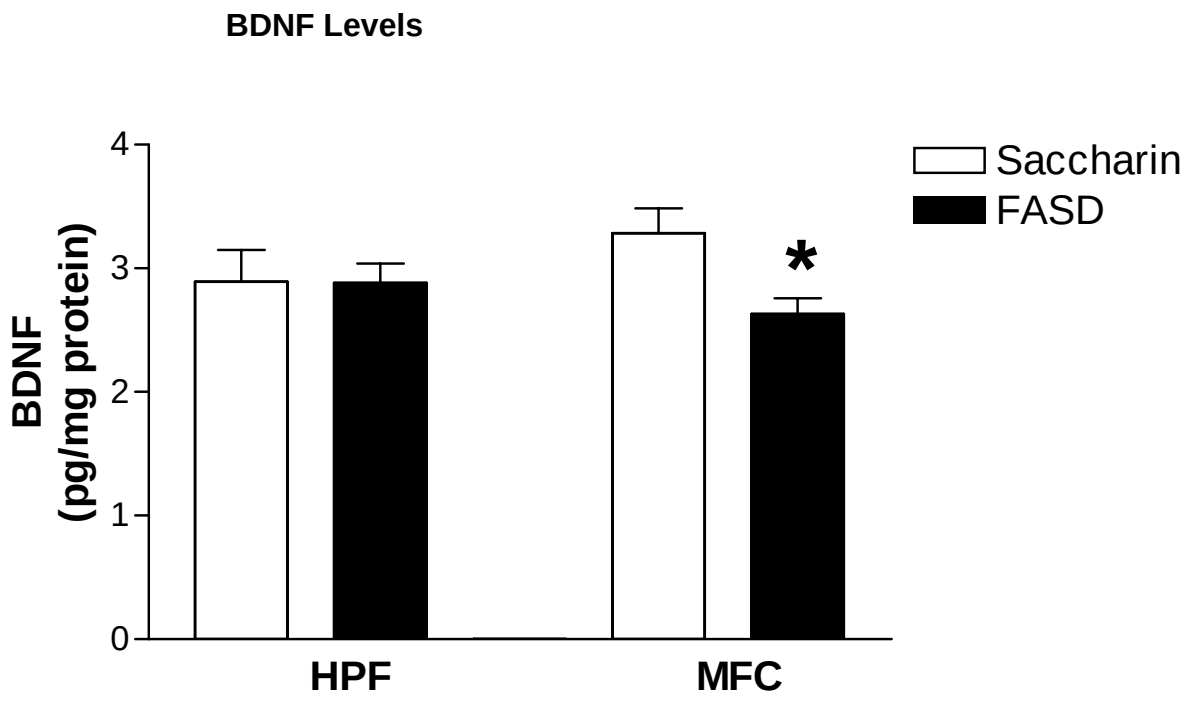
Prenatal ethanol exposure (FASD) — Prenatal exposure of mice to ethanol was performed using a saccharine sweetened 10% (w/v) ethanol solution as described (Allan et al., 2003). C57BL/6J female mice were offered 22 hr free access to either 0.066% saccharin or water for 2 weeks. Following this introduction, ethanol was introduced to the saccharin tube for the experimental groups, while the control group continued to have access to saccharin alone. For mice in the 10% ethanol group, ethanol concentration was increased from 0% to 2% then 5% then finally to 10% every 3 days. In the group drinking 10% ethanol, average blood levels of 90-110 mg% were routinely achieved and maintained throughout gestation. After two weeks of drinking, females were placed on breeder chow and a male was introduced into the female's cage. Once the female was determined to be pregnant, the male was removed. Females continue to drink stably throughout pregnancy. Within one day of birth, the alcohol and the saccharin concentrations were reduced by ½ every two days until

the mice were consuming only water. Saccharin consuming mothers were weaned off of the sweetened water in a similar step down fashion. Pups were weaned at 23 days and maintained in same –sex litter-mate housed cages with free access to water and chow until age 60-90 days when they were then used in the experiment. Pups underwent learned helplessness training and escape testing in order to assess depressive behavior between the 2 groups. After training and testing, brains were removed and the hippocampi and medial frontal cortices were dissected out and prepared for use in the BDNF ELISA assay. Following the testing mice were euthanized and the brains rapidly dissected on ice. The hippocampi were removed and prepared for use in the BDNF ELISA assay. All dissected samples were first weighed individually to get their wet weights. Each sample was then transferred to an ice-cold lysis buffer containing NaCl (137 mM), Tris–HCL (20 mM), NP40 detergent (1%), glycerol (10%), sodium vanadate (0.5 mM), and the following protease inhibitors (Sigma): phenylmethanesulfonylfluoride (PMSF, 1 mM), aprotinin (10 Ag/ml), leupeptin (1 Ag/ml). Then, each sample was sonicated in 250 Al of icecold lysis buffer using Sonic Dismembrator for 10 s. The lysate from each sample was centrifuged at 14,000 x g for 15 min at 4-C and the supernatant solutions were collected. The supernatant from each sample was diluted 5 times with Dulbecco's PBS and acidified to pH 2.6. After 15 min of incubation at room temperature, the diluted supernatants were neutralized to pH 7.6, aliquoted and frozen for subsequent measurement of BDNF using the instructions provided by the manufacturer of the ELISA kit. Levels of BDNF were calculated by

generating a standard curve. The chromogen reaction was stopped by adding 1 N hydrochloric acid. 96 well plates were then read at a wavelength of 450 nm on a microplate reader (Molecular Devices).

Results

Mice were tested for learned helplessness behavior in an earlier study by a different student so those data are not presented here. However, it is important to point out that the FASD mice displayed significantly higher levels of learned helpless behavior than the saccharin control mice. The observed increase in depressive behaviors in the FASD mice could be due to the presence of lower levels of brain BDNF protein (Ridder et al., 2005). To test this hypothesis we measured BDNF levels in the hippocampus and medial frontal cortex taken from FASD and control mice using ELISA techniques. Figure 1 shows that BDNF levels were significantly decreased in the medial frontal cortex of FASD mice compared to saccharin control mice. No differences were seen in the hippocampus.



t(13)=2.7, p<0.02

Figure 1 *Homogenates of the medial frontal cortex (MFC) and hippocampus (HPF) were prepared from adult saccharin (n= 7) and FASD (n=7) mice. BDNF levels were quantified by ELISA. Data were expressed relative to the wet weight of the tissue sample. Compared to the saccharin MFC, BDNF levels were significantly reduced in the MFC of the FASD mice.*

Conclusion

The regulation of BDNF signaling is an established target of both alcohol's actions in the adult and its actions on the developing nervous system. Light et al. (2001) reported that perinatal exposure to alcohol reduced BDNF mRNA levels in the rat cerebellum. Heaton et al. (2003) showed that treatment of postnatal day 7 rats with ethanol either increased or decreased BDNF levels in the cortex, depending on the time between administration of the drug and sacrifice of the animals. In this study we were able to show that BDNF levels were reduced in the frontal cortex, but not in the hippocampal formation of adult mice that had been exposed to moderate levels of alcohol throughout pregnancy compared to tissue isolated from saccharin control offspring. In the interest of future research, at least 3 mechanisms may be considered to account for this observed decrease in BDNF in the medial frontal cortex: 1) the reduction in BDNF is secondary to a reduction in the level of its precursor form, proBDNF; 2) the reduction in BDNF protein is the result of decreased activity of the proteolytic enzymes (e.g., plasmin) that cleave proBDNF, or; 3) the degradation of BDNF is elevated. In order to study these possible causes and further elucidate the regulatory mechanisms that account for decreased BDNF levels, several studies will follow. These may include the analysis of pro-BDNF levels in the brain as well as differences in histone methylation and acetylation which may underlie the reductions in BDNF mRNA. These results would help to identify candidate targets for molecular and pharmacologic intervention as well as the development of pharmacologic studies to test hypotheses that predict the therapeutic efficacy

of different antidepressants based on their known effects on BDNF and histone modifications. The information gained from these studies could produce a great advance in our understanding of prenatal alcohol and its known detrimental long-term effects in humans.

Prenatal ethanol exposure can produce subtle learning disabilities in children, which may not become apparent until school-age and can occur in the absence of other physical evidence of alcohol-related birth defects. Major depressive disorder has been linked to polymorphisms in the BDNF gene and patients with Major depressive disorder have been shown to have low serum BDNF levels (Levinson 2005, Chen et al 2001). However, post-mortem depressed patients who were on antidepressant drugs have shown increased levels of BDNF (Chen et al 2001). Also, BDNF given to stressed animals can even produce an anti-depressant-like effect through the antagonism of learned helplessness behavior (Karege 2002). In our study, by demonstrating that BDNF levels are lower in the brains of FAE mice compared to saccharin controls, we support the hypothesis that even moderate amounts of alcohol can have a significant effect on fetal neurological and behavioral development. BDNF appears to be a vital link between fetal alcohol exposure, depression, and behavioral deficits such as learned helplessness.

References

Abel EL (1995). An update on incidence of FAS: FAS is not an equal opportunity birth defect. Neurotoxicology and Teratology. 17(4):437-443.

Allan, AM, Chynoweth, J, Tyler, LA and Caldwell, KK (2003). A mouse model of prenatal ethanol exposure using a voluntary drinking paradigm. Alcoholism: Clinical & Experimental Research. 27(12):2009-16.

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with anti-depressant medication. Biological Psychiatry. 50:260–265.

Famy C, Streissguth AP, Unis AS (1998). Mental Illness in Adults With Fetal Alcohol Syndrome or Fetal Alcohol Effects. American Journal of Psychiatry. 155(4):552-554.

Ghosh A, Carnahan J, Greenberg ME (1994). Requirement for BDNF in activity-dependent survival of cortical neurons. Science. 263(5153):1618-23.

Heaton et al. (2003). Ethanol effects on neonatal rat cortex: comparative analyses of neurotrophic factors, apoptosis-related proteins, and oxidative

processes during vulnerable and resistant periods. Dev. Brain Res. 145: 249-262.

Jones KL, Smith DW (1973). Recognition of the fetal alcohol syndrome in early infancy. The Lancet. 2:999-1001.

Karege F, et al. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Research. 109:143–148.

Levinson DF (2005). The Genetics of Depression: A Review. Biological Psychiatry. *Internet publication ahead of print.*

Light KE, Ge Y, Belcher SM (2001). Esrly postnatal ethanol exposure selectively decreases BDNF and truncated TrKB-T2 receptor mRNA expression in the rat cerebellum. Mol. Brain Res. 93:46-55.

Lu B, Pang PT, Woo NH (2005). The yin and yang of neurotrophin action. Nature Reviews Neuroscience. 6(8):603-614.

O'Connor MJ, Paley B (2005). The Relationship of Prenatal Alcohol Exposure and the Postnatal Environment to Child Depressive Symptoms. Journal of Pediatric Psychology. 30(1):50-64.

Ridder S, et al (2005). Mice with genetically altered glucocorticoid expression show altered sensitivity to stress-induced depressive reactions. J. Neuroscience. 25(26): 6243-50.

Sampson PD, Streissguth AP, Booksteiin FL, Little RE, Clarren SK, Dehane P, Hanson JW, Graham JM (1997). Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. Teratology 56:317-326.

Stratton K, Howe C, Battaglia FC (1995). Fetal Alcohol Syndrome: Diagnosis, epidemiology, prevention and treatment. Washington, DC: National Academy Press.

Streissguth AP, Sampson PD, Olson HC, Bookstein FL, Barr HM, Scott M, Feldman J, Mirsky AF (1994). Maternal drinking during pregnancy: attention and short-term memory in 14-year-old offspring-a longitudinal prospective study. Alcohol: Clinical and Experimental Research. 18:202-218.

Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD (2002). From acquisition to consolidation: On the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learning and Memory. 9:224-237.