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Research report

Effects of prenatal alcohol exposure on brain-derived neurotrophic factor and its receptor tyrosine kinase B in offspring

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Abstract

Prenatal alcohol exposure produces many developmental defects in the central nervous system. The underlying molecular mechanism, however, has not been fully understood. The present study was undertaken to examine the effects of prenatal alcohol exposure on brain-derived neurotrophic factor (BDNF) and its receptor tyrosine kinase B (TrkB) in offspring. The pregnant Sprague–Dawley rats received 1 or 3 g/kg of alcohol or an isocaloric solution by intragastric intubation once a day from gestational day (GD) 5 to GD 20. On postnatal day 7–8, pups were killed and the hippocampus, striatum, cortex, and cerebellum dissected out. Levels of BDNF mRNA and proteins, total TrkB proteins and receptor phosphorylation were measured. The results showed that prenatal alcohol exposure at the dose of 1 g/kg/day did not significantly affect BDNF protein levels in any region examined. However, administration of alcohol at the dose of 3 g/kg/day markedly reduced levels of BDNF protein and mRNA in the cortex and hippocampus of offspring. Western blotting showed that prenatal alcohol exposure at the dose of 3 g/kg/day also inhibited TrkB phosphorylation in the hippocampus although no changes in total TrkB protein levels were observed in any region examined. Our data suggest that prenatal alcohol exposure alters both presynaptic and postsynaptic BDNF function in certain brain areas of offspring. These alterations in BDNF function may contribute to the development of alcohol-related birth defects.
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1. Introduction

Alcohol consumption during pregnancy is a major public health problem. Fetal alcohol syndrome (FAS) in the US is believed to occur with an incidence in the range of 0.5 to 1 per thousand live births in the general population [13]. Children who do not meet all the criteria for a diagnosis of FAS may still show physical and/or mental deficits consistent with a partial phenotype, termed alcohol-related neurodevelopmental disorders (ARNDs). It has been reported that the incidence of ARNDs is higher than that of FAS [13].

Animal studies show that numerous mechanisms such as increased oxidative stress, mitochondrial damage, and impaired development and function of chemical messenger systems involved in neuronal communication likely contribute to the damaging effects of prenatal alcohol exposure on the developing central nervous system [14]. Recent evidence suggests that brain-derived neurotrophic factor (BDNF), in addition to its well-known roles in neuronal development and survival, is an important molecular mediator of synaptic and morphological plasticity [27]. It has been reported that chronic alcohol consumption alters BDNF activities in adult animals [2,23,32]. Since alcohol

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can readily cross the placenta and reach concentrations in the fetus that are similar to those in maternal blood [26], it is likely that prenatal alcohol exposure may affect the offspring's BDNF system and that altered BDNF function may act simultaneously or consecutively with other mechanisms to contribute to alcohol-induced brain malformations. However, there are few reports in the literature concerning effects of prenatal alcohol exposure on BDNF function in offspring.

In the present study, BDNF protein and mRNA, as indexes of presynaptic BDNF function, were measured and compared between pups prenatally exposed to alcohol or isocaloric control solutions. Moreover, in order to gain a more complete picture of the BDNF system, the present study also sought to determine whether prenatal alcohol exposure affected BDNF receptor tyrosine kinase B (TrkB) or whether alcohol-induced alterations in presynaptic BDNF activities caused any compensatory change in postsynaptic receptors. To this end, total protein levels of TrkB were measured. Since phosphorylation at several intracellular tyrosine residues is essential for TrkB activation and for TrkB-mediated intracellular signal transduction, TrkB phosphorylation is also an important index for TrkB receptor activity [18]. For this reason, TrkB phosphorylation was assessed and compared as well in offspring prenatally exposed to alcohol or control solutions. In this study, the hippocampus, cortex, cerebellum, and striatum were chosen because BDNF and TrkB receptors were highly expressed in these brain regions [27] and because these brain structures were particularly vulnerable to prenatal alcohol treatments [4,9,25].

2. Materials and methods

2.1. Animals and treatments

Timed-pregnant Sprague–Dawley rats were obtained from Harlan Sprague–Dawley Inc (Indianapolis, IN). After arrival, they were individually housed under 12 h light/12 h dark conditions with ad libitum access to food and water and maintained at 21 ± 3 °C, 40–60% relative humidity. All animal care and experimentation in this study were carried out in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of the University of Illinois College of Medicine at Peoria.

On gestational day (GD) 5, the animals were divided into two groups. Dams assigned to the alcohol group received 1 or 3 g/kg of alcohol by intragastric intubation once a day from GD 5 through GD 20 and had ad libitum access to laboratory chow and tap water. Previous reports showed that intragastric intubation of alcohol at a dose of 6 g/kg caused offspring body weights, whole brain weights, and the granule number of olfactory bulbs to be decreased significantly [24,25]. This dose was tested in our preliminary studies and found to produce evident deleterious effects on both dams and pups. As a result, we decided to use lower doses. The previous studies showed that intragastric administration of alcohol at the dose less than 4.5 g/kg/day did not cause offspring body weights, whole brain weights, and weights of forebrains, cerebellums, and brainstems to be decreased significantly [25]. Thus, the doses of 1 and 3 g/kg/day were chosen in this study. Dams assigned to the control (paired) group received an isocaloric dextrose solution (rather than alcohol) by intragastric intubation once a day from GD 5 to GD 20. In addition, food access was limited in each individual control (paired) dam to the amount consumed by an individual dam in the alcohol group with which it was weight-matched on GD 5. The intubation time was 16:00–16:30. On the day of birth, litters from both groups were culled to 8 pups/litter.

On postnatal day (PD) 7–8, pups were killed by decapitation. 2–3 pups (either male or female) with each one being obtained from a different litter were used for each determination. Each experimental group consisted of 5–9 determinations. The hippocampus, striatum, cortex, and cerebellum were dissected out under a surgical magnifier at 0–4 °C.

2.2. BDNF protein measurement

The tissues of interest were sonicated in lysis buffer (137 mM NaCl, 20 mM Tris [pH 8.0], 1% NP-40, 10% glycerol, 1 mM phenylmethylsulfonylfluoride [PMSF], 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate). Lysates were clarified by centrifugation at 25,000 rpm for 20 min at 4 °C before acidification with HCl and neutralization to pH 7.5–8.0. BDNF proteins were measured with the enzyme-linked immunosorbent assay (ELISA) procedure using BDNF E_{max} ImmunoAssay System kits (Promega, Madison, WI) according to the protocol of the manufacturer. In brief, 96-well microplates were treated with a monoclonal anti-BDNF antibody in carbonate buffer (pH 9.7) and incubated overnight at 4 °C. The plates were emptied, washed with Tris-buffered saline containing Tween-20 (TBST), and blocked for 1 h at room temperature. A standard curve was prepared with authentic BDNF, and diluted (in blocking buffer) tissue extracts (100 μl/well) were added to other wells. The plates were incubated with shaking for 2 h at room temperature and washed 5 times with TBST. A polyclonal anti-human BDNF was then added to the wells followed by shaking for 2 h at room temperature. After 5 washes with TBST, a horseradish peroxidase-conjugated anti-IgY antibody was added to the wells and the plates shaken for 1 h at room temperature. After 5 more washes with TBST, a peroxidase substrate was added to the wells, and the plates shaken for 15 min at room temperature. The reaction was stopped by addition of 1 N HCl, and absorbance read in a microplate reader at 450 nm. Total protein contents in the

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