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ETHANOL MODULATES THE EXPRESSION OF $GABA_B$ RECEPTOR mRNAs IN THE PRENATAL RAT BRAIN IN AN AGE AND AREA DEPENDENT MANNER

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Abstract—Prenatal ethanol exposure has various deleterious effects on neuronal development. As $GABA_B$ receptor is known to play an important role during the development of the CNS, we now focused on its mRNA expression pattern in the rat brain during the late gestational days (GD) from 15.5 to GD 21.5. Ethanol's effect was also observed from GD 11.5 to GD 21.5. $GABA_{B1}$ receptor mRNA showed a high expression level in GD 15.5 and 19.5, while $GABA_{B2}$ receptor mRNA did in GD 15.5 and 21.5. The mRNAs levels depended on age and area during development. Ethanol exposure decreased $GABA_{B1}$ receptor from GD 11.5 to GD 19.5 with slight increases in GD 21.5. The decreasing effects were area dependent, with the highest effects in the forebrain including cortex, whereas slight effects were observed in the midbrain and hindbrain. The present results suggest an important role of $GABA_B$ receptor in the effects of ethanol on prenatal brain developmental processes.

Key words: development, ethanol, $GABA_B$ receptors, mRNA, brain area, cortex.

Exposure to ethanol during prenatal development causes a wide range of structural and functional abnormalities, resulting in fetal alcohol syndrome (Clarren et al., 1978). One of the most striking effects of in utero ethanol exposure is damage to the developing CNS (Streissguth et al., 1994; Guerri, 1998). Previous studies demonstrate that ethanol exposure during brain development induces a wide array of adverse effects, including defects in neuronal migration, cell loss in several brain regions, and deficits in the number of neurons and synaptic connections (Miller, 1997; Guerri, 1998). Although the detailed mechanisms of these alterations are far from clear, increasing evidences suggest that ethanol interferes with the action of some neurotransmitter, which might underlie some of the deficits observed in neuronal development (Costa et al., 2000). Indeed, ethanol could modulate the synthesis, release, receptor binding, and signaling of a variety of neurotransmitters and neuromodulators (Diamond and Gordon, 1997).

Specific neurotransmitter receptors are present on progenitor cells of the developing CNS and may regulate, during neural development, such developmental events as proliferation, growth, migration, differentiation, and survival of neural precursors cells. In the developing brain, the majority of early transmitter signaling is mediated by the GABA system (Owens and Kriegstein, 2002). GABA appears to be important for the migration and the maturation of neuronal precursors, since it enhances rat embryonic spinal and cortical neuronal cell motility (Behar et al., 2000). Also, prenatal alcohol exposure increases GABA levels from gestational days (GD) 1 to GD 20 (Maier et al., 1996). Most researches have focused on the importance of ligand-gated ion channels in mediating the effects of ethanol and find that moderate ethanol consumption during gestation can produce long-lasting alterations in the neuromodulatory influence of $GABA_A$ receptor on inhibitory neurotransmission in adult offspring (Allan et al., 1998). However, little is known about $GABA_B$ receptor, the metabolic GABA receptor that mediates the slow onset and prolonged effects of GABA and its role in ethanol's effects. In the present study, to further explore the potential effects of $GABA_B$ receptor on ethanol exposure, we investigated the effects of ethanol on $GABA_B$ receptor mRNA expression in the fetal rat brain from GD 15.5 to GD 21.5, when neuronal differentiation and the rapid growth of the brain parenchyma is most prominent. Our results demonstrated that both $GABA_{B1}$ and $GABA_{B2}$ receptor mRNAs had different expression patterns in various brain areas during normal neuronal development. Ethanol caused a great decrease of $GABA_{B1}$ expression in the whole brain area from GD 11.5 to GD 13.5 and in the forebrain areas, as compared with the midbrain and hindbrain from GD 15.5 to GD 21.5.

EXPERIMENTAL PROCEDURES

Animals and tissue preparation

Female (n=120) Sprague–Dawley rats (250 g, Gyeongsang National University, Neurobiology Laboratory, Chinju, South

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Abbreviations: B, basal ganglia; C, cerebral cortex; DEPC, diethyl pyrocarbonate; GD, gestational days; K, hippocampus; L, cerebellum; M, midbrain; RPA, RNase protection assay; SSC, sodium chloride–sodium citrate; T, thalamus.

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Korea) were housed in a temperature-controlled environment with lights from 06:00–20:00 h with food *ad libitum*. Pregnant rats were divided into two groups. Rats in experimental groups were treated with 10% ethanol orally and control rats were treated with water. Timed pregnant [the day of insemination equals to GD 0.5] Sprague–Dawley rats from control group and ethanol groups were killed by decapitation, after an i.v. injection of pentobarbital sodium (3 mg/100 g b.w.). Fetuses were removed and freed from the ammiotic membranes at various GD. The whole fetus was used for GD 11.5 and the brain and spinal cord were used on GD 12.5 and GD 13.5. The brains in later developmental stages (GD 15.5, 17.5, 19.5, and 21.5) were divided into forebrain, midbrain, and hindbrain. Samples were allocated for *in situ* hybridization or for RNase protection assay (RPA) preparation. For *in situ* hybridization, the fetuses were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate (NBP) for 48 h at 4 °C and cryoprotected by immersion into 20% sucrose phosphate buffer for 24 h at 4 °C. Tissues were frozen at Optimal Cutting Temperature compound (Tissue Tek, USA) and after freezing 15-μm sections were made in the sagittal (mid and lateral) planes (Leica cryostat CM 3050C, Germany). Sections were thaw-mounted on the probe-on plus charged slides (Fisher) and stored at −70 °C until use. For RPA, fresh whole head, spinal cords, brains, forebrains, midbrains, and hindbrains were removed aseptically, and frozen in liquid nitrogen. All solutions used in this experiment were 0.1% diethyl pyrocarbonate (DEPC) treated.

Synthesis of cRNA probes

The full length cDNA clones for $GABA_{B1}$ and $GABA_{B2}$ receptors were obtained from Dr. Rønneklive (OHSU, OR, USA). A 438 bp partial $GABA_{B1}$ receptor and a 550 bp partial $GABA_{B2}$ receptor fragment containing sequences encoding C-terminus were cloned into the RNA synthesizing vector pAMP 1 (Promega, Madison, WI, USA). These vectors contain a polylinker and the promoters for T7 and SP6 polymerase. These clones were analyzed by sequencing (Sequenase 2.0; USB).

$GABA_{B1}$ and $GABA_{B2}$ receptor cRNA probes were synthesized from the pAMP1 recombinant subclones. Antisense $GABA_{B1}$ and $GABA_{B2}$ receptor cRNA probes were transcribed with Sp6 RNA polymerase from the $GABA_{B1}$ and $GABA_{B2}$ construct linearized with EcoRI, while sense $GABA_{B1}$ and $GABA_{B2}$ receptor cRNA probes were transcribed with T7 RNA polymerase. The ^{35}S-UTP-labeled probes with specific activity of 1.010^9 cpm/μg were prepared using *in vitro* transcription kit (Promega). Antisense and sense cRNA probes were purified by Sephadex G-50 DNA grade column and eluted with SET buffer (0.1% SDS, 1 mM EDTA, 10 mM Tris, 10 mM DTT). Polyacrylamide gel analysis of purified probes revealed that >90% of the probes were of the expected length.

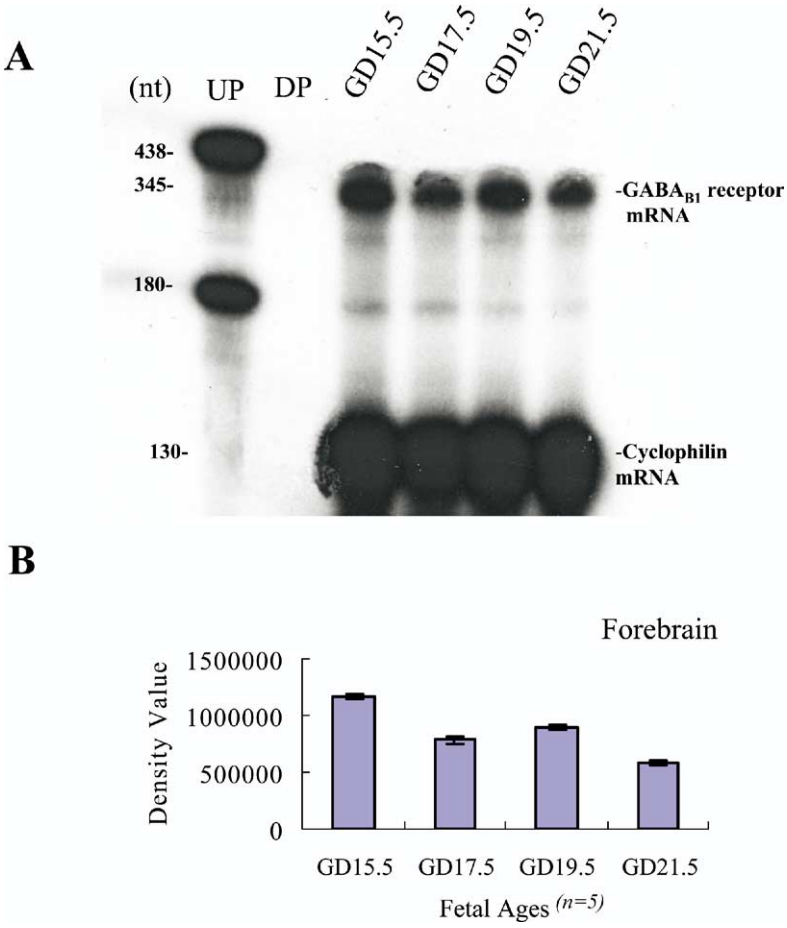

Fig. 1. (A) Representative RPA showing $GABA_{B1}$ receptor mRNA (20 μg of total RNA/lane) in the forebrain. *UP* represents the incubated undigested $GABA_{B1}$ receptor (438 nt), and cyclophilin (180 nt) cRNA probes. *DP* represents the ribonuclease-digested probes (345 nt and 130 nt). (B) Distribution and quantitative analysis of $GABA_{B1}$ receptor mRNAs. Densitometric value were normalized to cyclophilin mRNA.