Ethanol exposure interferes with the IGF-1R pathway and induces progenitor apoptosis, reduces neural progenitor proliferation, and causes abnormal differentiation. Specifically, we believe that ethanol distorts the differentiation. The aims of the experiments are to study the molecular effects of ethanol on IGF-1R signaling during embryogenesis. This study focuses on three major roles of the IGF-1R system during brain growth: regulating various cellular processes and maintaining precise proliferation and differentiation in the CNS determine how ethanol-dependent changes in IGF-1R signaling alter neural progenitor differentiation. We immunolabeled neural progenitors with markers for neurons, astrocytes, and oligodendrocytes and, after treatment with EtOH to assess differentiation and proliferation, and we used an MTS assay to determine cell viability following EtOH exposure. MAPK, NOS1, NR2B, and Nestin were used as markers for neurons, astrocytes, and oligodendrocytes, respectively. This analysis is significant in clarifying possible molecular mechanisms of IGF-1R signaling using a neural progenitor model and its relation to the mental abnormalities associated with FAS and FASD.

The U.S. spends nearly $7 billion per year to study and care for the physiologic, behavioral, and mental defects in children suffering from FASD. Evidence has shown that ethanol disturbs 68% of the IGF-1R signaling pathway that is responsible for many aspects of brain growth including neural progenitor proliferation and differentiation. Neural progenitors are contained within the neural tube during embryogenesis. These neural progenitors specialize into neurons, astrocytes, or oligodendrocytes, or undergo programmed cell death as maturation occurs in the embryonic CNS. These three differentiated cell types form the mature CNS and are critical to correct function. Neural progenitors must mature sequentially through differentiation into neurons, astrocytes, or oligodendrocytes to transmit neurotransmitters to other neurons, muscle cells or gland cells, while astrocytes and oligodendrocytes work to support neuronal function and survival. A disruption in the IGF-1R system results in improper ratios of these cell types, seen in FAS and FASD. Individuals with IGF-1R mutations suffer severe physiologic development and mental retardation. Additionally, genetic manipulations of the IGF system in mouse models have led to major developmental irregularities and death shortly after birth. Therefore, proper regulation of the IGF-1R system is critical for CNS development especially during embryogenesis.

Methods

1. Isolate neural progenitors from embryonic mice and culture in 100 mm dishes
2. MTS Assay of neural progenitors following acute EtOH exposure (24hrs) measuring cell viability. Cells are cultured in 1% FBS and stimulated with EtOH and control; 3 conditions within each: 1 plate w/ EtOH; 1 plate w/ control; 1 plate w/ EtOH & control.

3. BrdU Assay of neural progenitors following acute EtOH exposure (24hrs) measuring proliferation. Cells are cultured in 1% FBS, stimulated with EtOH or control and BrdU for 24hrs. Neurons present in the CNS. Cell nuclei are stained. The overall trend is toward hyper-active signaling through the IGF-1R receptor system after acute alcohol exposure.

Results

1. MTS assay: *EtOH treated dishes in 37°C 50 mM EtOH incubator; Control dishes in 37°C incubator

2. BrdU Assay: *EtOH treated dishes in 37°C 50 mM EtOH incubator; Control dishes in 37°C incubator

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