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BRAIN RESEARCH

Research Report

Prenatal choline supplementation in rats increases the expression of IGF2 and its receptor IGF2R and enhances IGF2-induced acetylcholine release in hippocampus and frontal cortex

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ABSTRACT

Choline is an essential nutrient whose availability during the second half of gestation produces long-lasting cognitive effects. Rats that obtain supplemental choline during embryonic day (E) 11–17 have enhanced depolarization-evoked acetylcholine (ACh) release from hippocampal slices, whereas choline deficiency during this time reduces this release. Previously we reported that rats whose mothers consumed a choline-supplemented diet during E11–17 have higher levels of insulin-like growth factor II (IGF2) mRNA and protein in the frontal cortex compared to control and prenatally choline-deficient animals. Since IGF2 has been shown to stimulate endogenous ACh release, we measured the release of ACh from hippocampal and frontal cortical slices from rats on postnatal day (P) 18, P24, P34 and P80 in response to a depolarizing concentration of potassium (45 mM or 25 mM) or to IGF2 treatment in the absence or presence of a depolarizing concentration of potassium (25 mM). On P18, IGF2/depolarization-evoked ACh release from hippocampal slices was enhanced by prenatal choline supplementation. In the frontal cortex on P80, prenatal choline supplementation dramatically potentiated ACh release induced by depolarization, IGF2 or the combination of the two. On P18 and P90 and in both brain regions, IGF2 mRNA and protein levels, as well as protein levels of the IGF2 receptor (IGF2R), were higher in prenatally choline-supplemented rats. Choline supplementation also increased IGF2R mRNA levels in the septum. In summary, prenatal choline supplementation produced alterations in IGF2 signaling, via increased levels of IGF2 and IGF2R, which may enhance cholinergic neurotransmission and confer neuroprotection against insult.

1. Introduction

Normal development and function of the brain requires the supply of fundamental nutrients, such as choline, during the period of embryonic and fetal growth. Choline is an essential nutrient that is required for several cellular functions, including growth and maintenance of structural integrity of phospholipid membranes, and it is necessary to establish a pool of acetylcholine (ACh), the neurotransmitter of cholinergic neurons. Maternal dietary choline availability during

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pregnancy plays a critical role in the organization of the brain and influences the cognitive functions. When rat maternal diets were supplemented with choline during the second half of gestation (embryonic day (E) 11–17), the offspring had improved spatial and temporal memory as well as improved attention compared to control rats at both a young age and in adulthood (Meck et al., 1989; Meck et al., 1988; Meck and Williams, 1997a,b,c; Meck and Williams, 1999; Meck and Williams, 1997a,b,c; Meck and Williams, 1997a,b,c). In contrast, rats whose mothers consumed a diet that was deficient in choline during the E11–17 period had deficits in certain memory tasks (Meck and Williams, 1997a,b,c). The neurochemical mechanisms by which choline supplementation *in utero* leads to the improvement in memory are not known. However, it has been shown that prenatal choline manipulation modulates ACh synthesis and release in basal forebrain cholinergic neuron (Cermak et al., 1998), which are known to participate in memory processes (Fibiger, 1991). Hippocampi of prenatally choline-supplemented rats had enhanced depolarization-evoked ACh release, whereas ACh synthesis from choline transported by high-affinity choline transporter (CHT) was reduced. In contrast, prenatally choline-deficient rats were characterized as having increased synthesis of ACh following choline uptake by CHT but reduced ACh content relative to the control and prenatally choline-supplemented rats. Prenatally choline-deficient animals were also unable to sustain depolarization-evoked ACh release relative to the choline-supplemented animals (Cermak et al., 1998). Recently, we found that prenatally choline-deficient rats have a higher amount of CHT mRNA in the septum and CHT protein in the hippocampus (Mellott et al., 2007a,b). The augmentation of CHT levels supports the observed increase in ACh synthesis from choline transported by CHT in hippocampal slices from prenatally choline-deficient rats. This pattern of changes suggests that the hippocampus of the prenatally choline-deficient animals is characterized by fast ACh recycling and efficient choline reutilization for ACh synthesis, presumably to maintain adequate ACh release despite the decrease of the ACh pool, whereas ACh turnover and choline recycling is slower while the evoked release of ACh is high in the prenatally choline-supplemented animals.

Recently, we analyzed the effects of prenatal choline availability on gene expression by oligonucleotide microarrays. Insulin-like growth factor 2 (IGF2) was among the genes that were identified as being one whose expression was modified by prenatal choline availability. Its mRNA and protein expression in the frontal cortex was significantly increased by prenatal choline supplementation (Mellott et al., 2007a,b). The insulin-like growth factors (IGFs), their receptors, and binding proteins constitute a family of cellular modulators that play essential roles in the regulation of growth, differentiation and survival, as well as metabolic processes. This family is comprised of three structurally-related peptides: insulin, IGF1, and IGF2. IGF1 stimulates the proliferation of neuron progenitors, induces the differentiation of oligodendrocytes, and increases the survival of neurons and oligodendrocytes *in vitro* (Barres et al., 1992; D'Mello et al., 1993; Drago et al., 1991; McMorris and Dubois-Dalcq, 1988; Mozell and McMorris, 1991; Pons and Torres-Aleman, 1992; Torres-Aleman et al., 1990; Werther et al., 1993). Although less is known about the role of IGF2 in the brain, *in vitro* studies have shown that IGF2 stimulates proliferation of neuronal and glial cells (Konishi et al., 1994; Lim et al., 1985), promotes survival of several neuronal cell types (Haselbacher et al., 1989; Knusel and Hefti, 1991), regulates the development and turnover of neuromuscular synapses (Ishii, 1989), and potentiates ACh release from hippocampal slices (Kar et al., 1997). IGF2 expression is also reportedly upregulated in response to a penetrating brain injury (Walter et al., 1999). The brains of IGF2 knockout mice ($Igf2^{-/-}$) did not display any significant differences in morphological structures or in myelination levels compared to control mice ($Igf2^{+/+}$), suggesting that IGF2 may not be critical for brain development under normal conditions; however, IGF1, IGF2 and insulin receptor binding sites, as well as their response to neurotoxic insult, were altered by the loss of IGF2 (Dikkes et al., 2007).

IGF1 and IGF2 are selectively localized in distinct cell types and specific regions of the brain (Kar et al., 1993; Lesniak et al., 1988; Werther et al., 1990) and their physiological responses are presumed to be mediated by specific interactions with cell surface receptors. IGFs bind the tyrosine kinase IGF type 1 receptor (IGF1R), as well as the IGF type 2/mannose-6-phosphate receptor (IGF2R or M6PR) that binds IGF2 with higher affinity than IGF1 (Kar et al., 1997). IGF2R is widely distributed in various tissues including the brain. It is a multifunctional single pass transmembrane glycoprotein that mediates the trafficking of lysosomal enzymes and also participates in the degradation of non-glycosylated IGF2 (Hawkes and Kar, 2004). Recently, it was shown that the IGF2R is involved in the regulation of ACh release in response to stimulation by IGF2 in the rat hippocampus and the response is mediated through the activation of protein kinase Cα (Hawkes et al., 2006).

Since IGF2 and IGF2R are mediators of endogenous acetylcholine release (Hawkes et al., 2006) and prenatally choline-supplemented rats have increase expression of IGF2 (Mellott et al., 2007a,b), this study was designed to examine the effects of prenatal choline availability on the expression of IGF2 and its receptors, as well as to determine if IGF2-evoked acetylcholine release from frontal cortical and hippocampal slices of these rats is altered. We found that prenatal choline intake altered the mRNA and protein expression of IGF2 and IGF2R in young and adult rats. Moreover, prenatal choline availability affected IGF2-evoked ACh release in hippocampal and frontal cortical slices. The results of this study suggest that the IGF system may help to mediate the differences in cholinergic transmission observed in animals that were exposed to various levels of choline *in utero*.

2. Results

2.1. *Hippocampus*

Previously, we have shown that prenatal choline availability modulates IGF2 gene expression in the rat frontal cortex (Mellott et al., 2007a,b). Here, we examined the effects of prenatal choline availability on IGF2 and IGF2R mRNA, as well as protein, expression in the hippocampus during early postnatal development (P18) and adulthood (P90). On P18,