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Prenatal protein malnutrition reduces β_2, β_3 and γ_{2L} $GABA_A$ receptor subunit mRNAs in the adult septum

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Abstract

Rats exposed to prenatal protein malnutrition are less sensitive to the amnestic effects of chlordiazepoxide when administered directly into the medial septum. Here we report that prenatal malnutrition selectively decreases γ-aminobutyric acid A ($GABA_A$) receptor γ_{2L} mRNA levels in the medial septum, consistent with malnutrition-induced decreases in the amnestic effects of chlordiazepoxide infusion. In the lateral septum, β_2 and β_3 mRNA levels are also decreased, suggesting that prenatal malnutrition alters $GABA_A$ receptor gene expression in the septal complex. © 2002 Elsevier Science B.V. All rights reserved.

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Fast inhibitory synaptic transmission in the central nervous system (CNS) is largely mediated by γ-aminobutyric acid (GABA) interacting with the $GABA_A$ receptor. A defining feature of the $GABA_A$ receptor is its capacity for allosteric modulation by benzodiazepines (Rabow et al., 1995). Evidence from behavioral studies indicates that the medial septum plays an important role in mediating benzodiazepine-induced impairment of spatial memory (McNamara and Skelton, 1993). Furthermore, direct infusion of the benzodiazepine chlordiazepoxide into the medial septum has revealed behavioral differences between prenatally protein-malnourished and control rats on the Morris water maze (Tonkiss et al., 2000). The findings suggest that malnourished rats are less sensitive to 30 and 60 nmol doses of chlordiazepoxide. It has been postulated that changes in $GABA_A$ receptor function underlie this differential effect. Decreased chlordiazepoxide sensitivity in the medial septum of prenatally protein-malnourished rats may reflect either alterations in the affinity of $GABA_A$ receptors for benzodiazepines or alterations in the allosteric interactions between the benzodiazepine and GABA sites.

There are at least 20 subunits that can be found in $GABA_A$ receptors (α_{1-6}, β_{1-4}, γ_{1-3}, ρ_{1-3}, π, ε, δ and θ). Benzodiazepine binding affinity may, in part, be ascribed to α and γ subunits. Results of recombinant receptor studies indicate that receptors containing different α subunits confer high affinity (Type I) or low affinity (Type II) pharmacology (Rabow et al., 1995). Moreover, α_4- and α_6-containing receptors constitute a subfamily of $GABA_A$ receptors that are insensitive to benzodiazepine modulation (Luddens et al., 1990; Wisden et al., 1991). The γ_2 subunit is essential for the potentiating actions of benzodiazepines (Pritchett et al., 1989). Thus, selective alterations in the expression of $GABA_A$ receptor subunit genes consequent to prenatal malnutrition may contribute to the synthesis of novel $GABA_A$ receptors that are less sensitive to benzodiazepines.

To this end, we monitored the levels of $GABA_A$ receptor subunit mRNAs in the medial and lateral septum of well-nourished and prenatally protein-malnourished rats. In this animal model of human malnutrition (Tonkiss et al., 2000), rat dams are fed a low protein diet (6% caesin) before mating and during pregnancy, and the offspring are cross-fostered to well-nourished females. After weaning, the pups are maintained on an adequate protein diet (25% caesin). At postnatal day 70, the medial and lateral septum was isolated and RNA was extracted from tissue pooled from 10 rats. Ribonuclease protection assays were performed using $GABA_A$ receptor subunit-specific riboprobes and a cyclophilin internal standard riboprobe. Since the quantity of medial septum RNA was limiting, we specifically targeted

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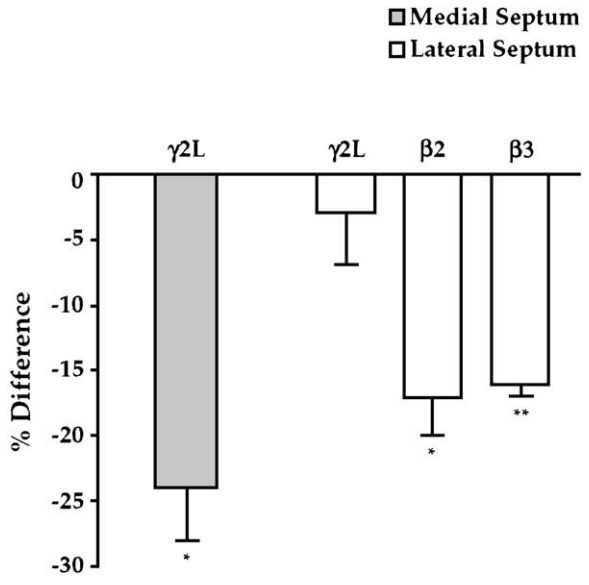

Fig. 1. Prenatal protein malnutrition alters $GABA_A$ receptor β_2, β_3 and γ_{2L} subunit mRNA levels in the adult septum. Total RNA was extracted from the medial and lateral septum of prenatally malnourished and control rats. RNase protection was performed using subunit-specific cRNA probes and a cyclophilin internal standard cRNA probe. $GABA_A$ receptor γ_{2L} subunit mRNA levels were reduced in the medial septum (filled bars), but not in the lateral septum (open bars). Additionally, β_2 and β_3 subunit mRNAs were reduced in the lateral septum. Data are presented as a percentage of expression levels in controls. All values shown are the summary (mean ± S.E.M.) of three independent determinations. $**P \leq 0.01$; $*P \leq 0.05$.

seven $GABA_A$ receptor subunit mRNAs (α_{1-5}, γ_{2S} and γ_{2L}). However, lateral septum RNA was not limiting and we were able to assess the expression of 10 $GABA_A$ receptor subunit mRNAs (α_{1-5}, β_{1-3}, γ_{2S} and γ_{2L}). Data from three independent breedings were analyzed using the 95% confidence interval.

Differential changes in $GABA_A$ receptor γ subunit mRNAs were observed in the medial and lateral septum (Fig. 1). The levels of γ_{2L} mRNAs were downregulated in the medial septum, but not in the lateral septum. Moreover, β_2 and β_3 mRNAs were reduced in the lateral septum (Fig. 1). The levels of α_1, α_2 and γ_{2S} mRNAs may be downregulated in the medial septum, while α_3 and α_4 may be downregulated in the lateral septum, as these differences approached statistical significance (data not shown). The true magnitude of these changes in gene expression is difficult to assess given the fact that the small size of septum precludes analysis of individual specimens. Yet it is tempting to speculate that prenatal protein malnutrition-induced changes in $GABA_A$ subunit gene expression may underlie the resultant changes in behavior.

Based on our data, the effects of prenatal protein malnutrition may include regulation of β- and γ-containing $GABA_A$ receptors in the septum. The observed changes in β_2 and β_3 subunit mRNA levels consequent to prenatal protein malnutrition may reflect alterations in channel properties (Bureau and Olsen, 1990) and receptor assembly at the cell surface (Connolly et al., 1996). In contrast, reduced levels of γ_{2L} mRNAs in the medial septum may support changes in benzodiazepine responsiveness. The fact that γ_2 knockout mice lack benzodiazepine sensitivity (Gunther et al., 1995) highlights the importance of γ_2-containing receptors to $GABA_A$ receptor function in the CNS. Thus, decreased levels of γ_{2L} transcripts consequent to prenatal protein malnutrition may underlie the observed decrease in sensitivity to medial septal infusions of chlordiazepoxide (Tonkiss et al., 2000). Now that target genes have been identified, future studies using single-cell analysis in the medial septum will be performed to correlate individual differences in behavioral performance with alterations of $GABA_A$ receptor expression profiles and GABAergic function.

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