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Short communication

Gender-selective effects of ethanol dependence on NMDA receptor subunit expression in cerebral cortex, hippocampus and hypothalamus

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Abstract

Previous investigations have shown subunit-selective alterations in NMDA receptors in ethanol dependent male rats. In the present study, we found pronounced gender differences in the effects of ethanol dependence on NMDA receptor subunit expression in all brain regions investigated. Ethanol dependent female rats exhibited increased NR1 subunit levels in cerebral cortex and hypothalamus, whereas males displayed increased NR1 levels only in hippocampus. NR2A subunit levels were significantly increased only in hippocampus from ethanol dependent male rats, whereas NR2B subunit levels significantly increased in cerebral cortex of both female and male rats. These findings suggest that gender influences neuroadaptations elicited by ethanol dependence at the level of NMDA receptor subunit expression.

Keywords: Ethanol dependence; Gender; NMDA receptor

1. Introduction

Chronic ethanol intake leads to the development of ethanol tolerance and dependence in both humans and lab animals. Adaptations in NMDA and $GABA_A$ receptor systems in brain appear to play an important role in ethanol tolerance and dependence (see Crews et al., 1996 for review). There is increased responsiveness of NMDA receptors (Iorio et al., 1992; Sanna et al., 1993). Concurrently, decreased activation of $GABA_A$ receptors is observed (Morrow et al., 1988; Sanna et al., 1993; Devaud et al., 1996). One mechanism mediating these changes in responsiveness may involve alterations in receptor subunit assembly. Selective alterations in NMDA (Trevisan et al., 1994; Follesa and Ticku, 1995; Snell et al., 1996; Kalluri et al., 1998) and $GABA_A$ (Montpied et al., 1991; Mhatre et al., 1993; Devaud et al., 1995b, 1997) receptor subunit gene expression have been observed following chronic ethanol exposure. This suggests that neuroadaptations associated with ethanol dependence may result, at least in part, from the expression of NMDA and/or $GABA_A$ receptors with different subunit assemblies than those expressed in the native state. These alterations are likely to be associated with ethanol dependence as subunit expression levels return to normal following ethanol withdrawal (Devaud et al., 1996; Kalluri et al., 1998). The pharmacological properties of these receptors are conferred by subunit composition (Levitan et al., 1988; Hollmann et al., 1989; Boulter et al., 1990; Sieghart, 1995). Therefore, the expression of receptors with altered subunit assembly may provide a basis for the observed alterations in function of NMDA and $GABA_A$ receptors noted during ethanol dependence.

To date, these studies have all been conducted in male rodents or cell culture systems. However, we found gender selective effects of ethanol dependence on $GABA_A$ receptor subunit gene expression in cerebral cortex of ethanol dependent rats (Devaud et al., 1998). Therefore, the present study investigated whether there were gender selective effects of ethanol dependence on NMDA receptor subunit

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gene expression in several brain regions implicated in the effects of ethanol.

2. Methods

Intact male and female Sprague–Dawley rats (200–240 g) were utilized in all experiments. Rats were administered 6% ethanol in a nutritionally complete liquid diet for 14 days as previously reported (Devaud et al., 1996). Control animals were pair fed the same diet with dextrose substituted isocalorically for the ethanol. Female rats were monitored daily for stage of estrus by histological examination of vaginal smears. Termination of experiments was scheduled to coincide with the majority of female rats in the estrus stage of their cycle.

Immunoblotting was performed on rat cerebral cortical (20 μg protein/well), hypothalamic (20 μg protein/well) or hippocampal (16 μg protein/well) membrane homogenates as previously described (Devaud et al., 1997). Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 8–16% Tris–glycine gels using a mini blot apparatus and then transferred to polyvinylidene fluoride (PVDF) membranes as previously described. Blots were incubated with selective antibodies for NMDA receptor NR1, NR2A or NR2B subunits (Chemicon, Temecula, CA) followed by incubation with anti-rabbit horseradish peroxidase conjugated secondary antibodies. Blots were exposed to chemiluminescent substrate (Super Signal, Pierce, Rockford, IL), apposed to X-ray film and quantified by densitometric measurements. Blots were subsequently exposed to an antibody directed against β-actin to verify equivalent protein loading. β-Actin peptide levels did not change following chronic ethanol administration. Data were normalized to β-actin levels when necessary. Data were analyzed by paired *t*-test using non-transformed optical density values.

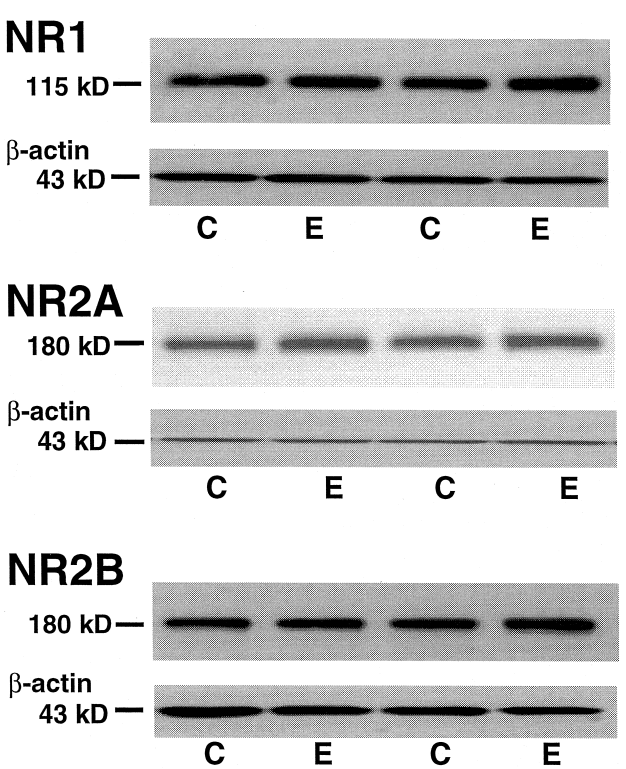

Fig. 1. Representative Western Blots of NR1, NR2A and NR2B subunits with corresponding β-actin from hypothalamus of female rats. C = pair-fed control, E = ethanol-dependent. This figure is computer generated from capturing of the X-ray film images and is included to demonstrate the selectivity of the antibodies.

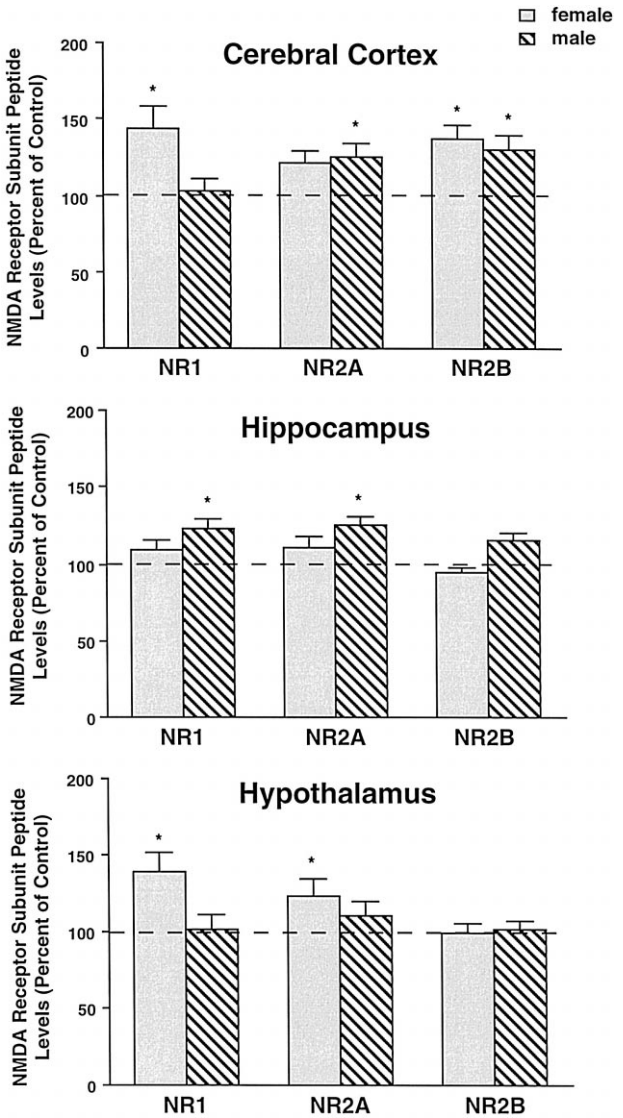

Fig. 2. Ethanol dependence elicits gender and region selective effects on NMDA receptor subunit peptide expression. Ethanol dependent values are presented as percent of pair-fed control levels and were collected by densitometric imaging of the X-ray film. Values are a summary of duplicate determinations of 8–10 pairs of animals (16–20 per group) from three separate chronic ethanol experiments and are presented as the mean ± S.E. transformed from optical density values to percent of control. $^{*}P < 0.05$.

3. Results

Chronic ethanol exposure produced selective alterations in gene expression of NMDA receptor subunits that differed between male and female rats across several brain regions. Fig. 1 is a representation of the film images collected for densitometric measurements. There was no obvious difference in subunit peptide levels between female and male rats for any of the brain areas studied.

As shown in Fig. 2, NR1 subunit protein levels in cerebral cortex increased $144 \pm 12\%$ compared to control levels in ethanol dependent female, but not male, rats. Ethanol dependent female rats also showed a significant increase ($145 \pm 11\%$) in NR1 subunit peptide expression in hypothalamus. However, a significant increase in the NR1 subunit peptide level ($123 \pm 6\%$) was observed in hippocampus from ethanol dependent male, but not female, rats. NMDA NR2A subunit peptide levels were significantly increased to $126 \pm 5\%$ in hippocampus only for male rats. NR2A levels were increased in female hypothalamus ($123 \pm 10\%$) and male hippocampus ($126 \pm 5\%$). Both males and females showed a trend toward an increase in cerebral cortex. The NR2B subunit peptide was significantly increased (130 ± 10 and $137 \pm 9\%$) only in cortex for ethanol dependent male and female rats, respectively. Our findings in ethanol dependent male rats are consistent with previous reports investigating the effects of prolonged ethanol exposure on NMDA receptor subunit gene expression (Snell et al., 1996; Chen et al., 1997; Kalluri et al., 1998).

4. Discussion

Chronic ethanol exposure elicits neuroadaptations by NMDA receptors that are suggested to be involved in the behavioral and functional changes observed with ethanol dependence. The present investigation adds to the increasing evidence that ethanol dependence is associated with alterations in gene expression for NMDA receptors. If chronic ethanol-induced adaptations result in the expression of receptors containing a differing assembly than in the native state, this could be a mechanism for the alterations in function of NMDA receptors associated with ethanol dependence.

The present investigation also observed regional differences in the effects of ethanol dependence on NMDA receptor gene expression. Interestingly, there were gender differences in NR1 subunit expression for all three areas studied. As the NR1 subunit is a required constituent of NMDA receptors, this has implications for significant gender influences on NMDA receptor activity. Furthermore, these regional variations suggest there may be gender differences in the sensitivity/responsiveness of particular brain areas to chronic ethanol exposure.

The effects of chronic ethanol exposure overlay a hormonal milieu that differs between females and males due to the presence of gender-specific steroid hormones. Some of these steroid hormone derivatives, termed neuroactive steroids exert effects on NMDA and $GABA_A$ receptors rather than via classical genomic mechanisms (Baulieu and Robel, 1990; Paul and Purdy, 1992; Irwin et al., 1992; Farb and Gibbs, 1996). Therefore, the effects of chronic ethanol administration may be influenced by endogenous regulation at the level of NMDA and $GABA_A$ receptors. We have found that ethanol withdrawn female rats show greater sensitization to the anticonvulsant effects of neuroactive steroids than ethanol withdrawn male rats (Devaud et al., 1995a, 1998). The observed gender differences in the effects of ethanol dependence on NMDA receptor gene expression may involve influences of the endogenous environment.

Taken together with our previous observations of the gender-selective effects of ethanol dependence on $GABA_A$ receptor gene expression, the present findings suggest there is plasticity in the type of neuroadaptations elicited by chronic ethanol exposure. The functional ramifications of these differential adaptations are yet to be determined but could prove to have significant impact on treatment of alcoholism and alcohol withdrawal in men as well as women.

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