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PERIOD2 GENE DELETION ABOLISHES αMELANOCYTE STIMULATING HORMONE RESPONSE TO ETHANOL

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INTRODUCTION

Propiomelanocortin (POMC)-producing neurons in the arcuate nucleus of the hypothalamus secrete αMSH (1). Studies have shown that αMSH plays an important role in the regulation of several biological functions including a role in physiological responses to drug of abuse (2). There is accumulating evidence that the neuropeptide αMSH modulates neurobiological responses to ethanol. First, αMSH is expressed in brain regions implicated in ethanol’s effects (3, 4). Second, chronic exposure to ethanol significantly reduced, while abstinence following chronic ethanol exposure increased, αMSH mRNA immunoreactivity in specific brain regions (3, 4). Our lab has shown that in adults and fetal rats, alcohol exposure alters POMC gene expression and the POMC-derived peptide release from the hypothalamus (5, 6). Since, both αMSH and αMSH peptides are produced from POMC precursor gene, the possibility arise that the level of αMSH in the hypothalamus is similarly altered by alcohol exposure. Thus, in this study we used the monoallelic mice model to determine whether acute or chronic alcohol exposures alter levels of αMSH and POMC mRNA in the hypothalamus.

Recently, an interaction between αMSH and a clock gene Period 2 (Per2) has been demonstrated in the hypothalamus. Yang et al. (2009) demonstrated that Per2 suppresses feeding during the inactive period by regulating the circadian rhythm of αMSH in the hypothalamus (7). In addition, our lab provided evidence that alcohol feeding in fetuses and adults alters circadian rhythm of Period genes (Per1, 2, 3) in the hypothalamus (8). However, Per2 appears to be a targeted gene which alcohol may act on to alter circadian functions. In order to elucidate the role of Per2 gene in modulation of neurobiological responses to ethanol, we examined the effect of Per2 mutation on hypothalamic αMSH neuronal responses to acute and chronic ethanol.

MATERIALS AND METHODS

**Animals**

To study the effect of both acute and chronic ethanol administration on αMSH level of hypothalamic neurons of control and Per2 knockout mice. The Pregnant C57BL/6 and Per2 mutant mice were individually housed in 12 hour light/12 hour dark cycles. The newborn mice (C57BL/6 and Per 2 mutant) were treated with ethanol in the following way: the acute ethanol exposure animals were only given treatment on day P7, whereas the chronic ethanol exposure animals received treatment from PD2-PD7. At day of treatment two pups from each litter were fed by intubation with milk formula containing 5% alcohol (alcohol fed - AF), a solution (0.1-0.2 ml/animal; 2.5g/kg; or an isocaloric volume of maltose dextrin (pair fed - PF) as in Goodlet et al., (1998); or pups were immediately returned to the litter. One hour after the last feeding, brains were collected and the medial hypothalamic tissue was divided into halves. One half was used for gene expression assay and other was used to measure αMSH levels in the hypothalamus. Six animals per treatment were used (a total of 18 animals were used per experiment). The samples were stored accordingly. Animal care and treatment were performed in accordance with institutional guidelines, and were approved by the Rutgers Animal Care and facilities Committee and complied with National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

**Real time Reverse transcription-polymerase chain reaction**

The total RNA was isolated from the hypothalamic tissue of each treatment group (Ad libitum, pair-fed, and alcohol-fed) by using the Trizol RNeasy kit (Applied Biosystems, Foster City, CA) was used for the RT reaction. The cDNA was subjected to real-time PCR on an ABI Prism 7000 sequence detector (Applied Biosystems, Foster City, CA). The POMC primer were acquired from Applied Biosystems (Foster City, CA). The samples were normalized to total glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression. The POMC values were presented as fold change over ad libitum controls.

**RESULTS**

In this study we showed that C57BL/6 mice acutely exposed to ethanol exhibited an increase in αMSH levels in the hypothalamus, however no response to ethanol was seen in Per2 mutant mice. Chronic exposure to ethanol reduced αMSH levels in hypothalamic αMSH mice but not in Per2 mutant mice.

The αMSH gene expression in both C57BL/6 and Per2 mutant mice were not altered upon acute ethanol treatment. Under chronic ethanol exposure, C57BL/6 mice showed a significant reduction in αMSH levels whereas Per2 mice did not show any response to ethanol.

**CONCLUSION**

In light of the above observations, the data support the involvement of Per2 gene in mediating the αMSH neuronal responses to ethanol.

**REFERENCES**


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