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# PERIOD2 GENE DELETION ABOLISHES $\alpha$ MELANOCYTE STIMULATING HORMONE RESPONSE TO ETHANOL



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## INTRODUCTION

Proopiomelanocortin (POMC)-producing neurons in the arcuate nucleus of the hypothalamus secrete  $\alpha$ MSH (1). Studies have showed that  $\alpha$ MSH 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  $\alpha$ MSH modulates neurobiologic responses to ethanol. First,  $\alpha$ MSH 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, endogenous  $\alpha$ MSH immunoreactivity in specific brain regions of Sprague-Dawley rats (4, 5). Our lab has showed that in adults and fetal rats, alcohol exposure alters POMC gene expression and the  $\beta$ -endorphin peptide release from the hypothalamus (6,7). Since, both  $\beta$ -endorphin and  $\alpha$ MSH peptides are produced from POMC precursor gene, the possibility arose that the level of  $\alpha$ MSH in the hypothalamus is similarly altered by alcohol exposure. Thus, in this study we used the neonatal mice model to determine whether acute or chronic alcohol exposures alter levels of  $\alpha$ MSH and POMC mRNA in the hypothalamus.

Recently, an interaction between  $\alpha$ MSH and a clock gene *Period 2* (*Per2*) has been demonstrated in the hypothalamus. Yang *et al.* (2009) demonstrated that *mPer2* suppresses feeding during the inactive period by regulating the circadian rhythm of  $\alpha$ MSH in the hypothalamus (8). In addition, our lab provided evidence that alcohol feeding in fetuses and adults alters circadian rhythms of *Period* genes (*Per1*, 2, 3) in the hypothalamus (6,7). Clinical studies revealed that alcoholics with a specific set of polymorphisms in the *Per2* gene consume less alcohol than alcoholics without the polymorphisms (9). Hence, *Per2* appears to be a targeted gene where 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  $\alpha$ MSH neuronal responses to acute and chronic ethanol.

## MATERIALS AND METHODS

### Animals Methods

To study the effect of both acute and chronic ethanol administration on  $\alpha$ MSH 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 *Per2* mutant mice) were treated with ethanol in the following way: the acute ethanol exposure animals were only given treatment on day P07, whereas the chronic ethanol exposure animals received treatment from P02-P07. At day of treatment two pups from each litter were fed by intubation with milk formula containing 11.34% (vol/vol) alcohol (alcohol fed - AF), a solution (0.1-2 ml/animal; during a period of 1 minute) containing ethanol and milk formula, yielding a total daily ethanol dose of 2.5g/kg; or an isocaloric volume of maltose dextrin (pair fed - PF) as in Goodlett *et al.*, (1998); or leave alone (ad libitum - AD). The feeding was conducted at 1000 and 1200 h daily. After feeding, the pups were immediately returned to the litter. One hour after the last feeding, brains were collected and the medial basal hypothalamic tissue was divided into halves. One half was used for gene expression analyses and the other was used to measure  $\alpha$ MSH 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 con-



### $\alpha$ MSH immune assay

The  $\alpha$  melanocyte stimulating hormone ( $\alpha$ MSH) assay (EK-043-01) was purchased from Phoenix Pharmaceuticals, Inc. (Burlingame, CA). The assay was prepared according to the manufacturer's protocol. The calculation was based according to the manufacturer's protocol. The samples were normalized to total  $\mu$ g/ml protein.

### Real time Reverse transcriptase-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 plus RNA purification system (Invitrogen, CA). Then the high-capacity cDNA reverse transcription kit from Applied Biosystems (Foster City, CA) was used for the RT reaction. The cDNA was subjected to real-time PCR on an ABI Prism 7500 sequence detector (Applied Biosystems, Foster City, CA). The POMC primer were acquired from Applied Biosystems (Foster City, CA).

### Statistics

Quantitative results are indicated as mean  $\pm$  SEM. Data obtained in the studies dealing with ethanol effects on  $\alpha$ MSH level of each strain were compared using one-way ANOVA followed by Newman Keuls post-hoc test. The ethanol effects between *Per2* mutant and wildtype mice were assessed with two-way ANOVA with post hoc analysis using the Bonferroni post-test. A value of  $p < 0.05$  was considered significant.

## Comparing the response of hypothalamic $\alpha$ melanocyte stimulating hormone ( $\alpha$ -MSH) levels in C57BL/6 and *Per2* mutant mice upon acute and chronic ethanol (ETOH) exposures

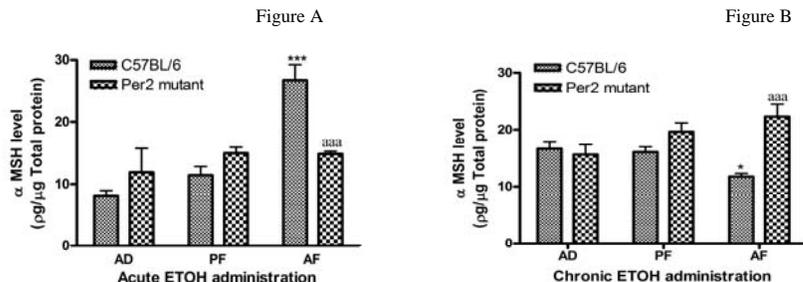


Figure 1. Effects of ethanol administration on  $\alpha$ MSH levels in mediobasal hypothalami of C57BL/6 and *Per2* mutant mice. A. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 3h. Data are  $\pm$  SEM of six independent observations. \*\*\* $P < 0.001$ , significantly different from controls of the same strain. \*\*\*\* $P < 0.0001$ , significantly different between two strains at the same dose. B. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 5 days. Data are  $\pm$  SEM of six independent observations. \* $P < 0.05$ , significantly different from controls of the same strain. \*\*\*\* $P < 0.0001$ , significantly different between two strains at the same dose.

## Comparing the response of proopiomelanocortin (POMC) mRNA levels in C57BL/6 and *Per2* mutant mice upon acute and chronic ethanol (ETOH) exposures

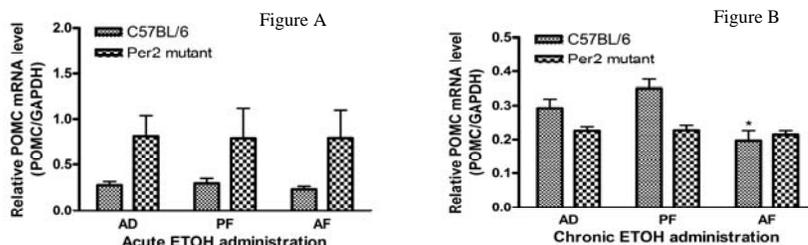


Figure 2. Effects of ethanol administration on POMC gene expression in mediobasal hypothalami of C57BL/6 and *Per2* mutant mice. A. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 3h. Data are  $\pm$  SEM of six independent observations. B. Postnatal mice were fed milk formula containing ethanol (AF) or no ethanol (PF) or left in the litter (AD) for 5 days. Data are  $\pm$  SEM of six independent observations. \* $P < 0.05$ , significantly different from controls of the same strain.

## RESULTS

- In this study we showed that C57BL/6 mice acutely exposed to ethanol exhibited an increase in  $\alpha$ MSH levels in the hypothalamus, however no response to ethanol was seen in *Per2* mutant mice.
- Chronic exposure to ethanol reduced  $\alpha$ MSH levels in hypothalami of C57BL/6 mice but not in *Per2* mutant mice.
- The POMC 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 POMC expression 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 POMC neurons response to ethanol.

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## REFERENCES

- Bloh B, Bugnon C, Fellmann D, Lenys D, Gougout A (1979) Neurons of the rat hypothalamus reactive with antisera against endorphins, ACTH, MSH, and beta-LPH. *Cell Tissue Res*. 204:1-15.
- Berolini A, Tachci R, Vergoni AV (2009) Brain effects of melanocortins. *Pharmacological Research* 59:13-47.
- Kokare DM, Singru PS, Dandekar MP, Chopde CT, Subhedar NK. (2008) Involvement of alpha - melanocyte stimulating hormone (MSH) in differential ethanol exposure and withdrawal related depression in rat: neuroanatomical-behavioral correlates. *Brain research* 1216:53-67
- Navarro M, Cubero I, Chen AS, Chen HY, Knapp DJ, Breesse GR, Marsh DJ, Thiele TE (2005) Effects of melanocortin receptor activation and blockade on ethanol intake: a possible role for the melanocortin-4-receptor. *Alcohol Clin Exp Res* 29:949-957.
- Navarro M, Cubero I, Knapp DJ, Breesse GR, Thiele ET. (2008) Decreased immunoreactivity of the melanocortin neuropeptide  $\alpha$ Melanocyte-Stimulating Hormone (MSH) after chronic ethanol exposure in Sprague-Dawley rats. *Alcohol Clin Exp Res*. 29:949-57.
- Chen CP, Kuhn P, Advis JP, Sarkar DK (2004) Chronic ethanol consumption impairs the circadian rhythm of proopiomelanocortin and period genes mRNA expression in the hypothalamus of the male rat. *J Neurochem* 88:1547-54.
- Chen CP, Kuhn P, Advis JP, Sarkar DK (2006) Prenatal ethanol exposure alters the expression of period genes governing the circadian function of  $\beta$ -endorphin. *J Neurochem* 97:1026-1033.
- Yang S, Liu A, Weidenhammer A, Cooksey RC, McClain D, Kim M K, Aguilera G, E. Abel D and Chung JH. (2008) The Role of *mPer2* clock gene in glucocorticoid and feeding rhythms. *Endocrinology* 150:2153-2160
- Spannagel R, Pandeyla G, Abarca C, Zghoul T, Sanchis-Segura C, Magnone MC, Lascorz J, Depner M, Holzberg D, Soyka M, Schreiber S, Matsuda F, Luthrop M, Schumann G, Albrecht U. (2005) The clock gene *Per2* influences the glutamatergic system and modulates alcohol consumption. *Nat Med*. 11:35-42.