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**ETHANOL AND
INTRACELLULAR SIGNALING:
FROM MOLECULES TO
BEHAVIOR**

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Chapter 15

Effects of β -Endorphin Levels on Ethanol Self-Administration in Mice

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β -Endorphin, a member of the opioid peptide family, is an endogenous ligand for the metabotropic μ opioid receptor. This receptor, like other opioid receptors, is negatively coupled to adenyl cyclase and thus upon activation results in decreased cyclic adenosine monophosphate (cAMP) production. The peptide β -endorphin derives from the proopiomelanocortin (POMC) gene along with adrenocorticotrophic hormone (ACTH), β -lipotropin, α -melanocyte-stimulating hormone, and other related peptides. The primary sites of POMC biosynthesis are the anterior and intermediate lobes of the pituitary gland, the arcuate nucleus of the hypothalamus, and a small group of neurons in the nucleus tractus solitarii (Eipper and Mains

1980). The primary endorphinergic projections in the central nervous system are from the arcuate nucleus to brain regions involved in both reward (e.g., septum and nucleus accumbens [Wise and Bozarth 1982]) and stress (periaqueductal gray and amygdala [Khachaturian et al. 1985]). In fact, mice lacking β -endorphin are deficient in opioid-mediated stress-induced analgesia (Rubinstein et al. 1996). This chapter summarizes the rationale and findings for an investigation of β -endorphin's role in the reinforcing effects of ethanol.

OPIOIDS AND ALCOHOL

Endogenous opioids have been hypothesized to be an important component of

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the neural substrate for ethanol actions, and the relationship between ethanol and these peptides has been investigated for a number of years (see Herz 1997 for a review). To date, research on the specific relationship between ethanol and β -endorphin has taken one of two general strategies.

The first strategy has involved studying the β -endorphin response to ethanol, and much is currently known about these effects. For instance, acute ethanol administration increases β -endorphin synthesis and release, whereas chronic exposure to ethanol appears to have the opposite effects (Schulz et al. 1980; Keith et al. 1986; Gianoulakis 1990; Scanlon et al. 1992; De Waele and Gianoulakis 1993; Przewlocka et al. 1994). These changes are postulated to underlie some of the important behavioral consequences of ethanol, such as those resulting from dopamine release in the mesolimbic pathway (Widdowson and Holman 1992). Alcohol consumption, craving, and relapse have thus been linked to the endogenous opioid system and, by implication, to β -endorphin in particular (e.g., Gianoulakis et al. 1996; O'Brien et al. 1996; Davidson et al. 1996).

A genetic risk for alcoholism has been well established (e.g., Goodwin et al. 1973, Cloninger et al. 1981). Clearly polygenic, in recent years specific candidate genes have been postulated to contribute to this complex trait. Thus, the second general line of inquiry looks for differences in β -endorphin between populations of animals (including humans) that differ in their sensitivity to ethanol. Many studies have suggested that heritable differences in the opioid system may be related to alcoholism (e.g., Topel

1988; Gianoulakis et al. 1989; Aguirre et al. 1995, del Arbol et al. 1995; Herz 1997). One such hypothesis specifies a role for β -endorphin.

Several strains of rodents that differ in their preference for ethanol have been shown also to differ with respect to β -endorphin. For example, differences in the brain content of β -endorphin have been reported between AA rats (selectively bred to be alcohol preferring) relative to ANA rats (alcohol avoiding) as well as between inbred mouse strains that differ in voluntary consumption of ethanol (De Waele and Gianoulakis 1994; De Waele et al. 1994). Furthermore, the ethanol-induced rise in hypothalamic β -endorphin is more robust for ethanol-preferring C57BL/6 mice than ethanol-avoiding DBA/2 mice (De Waele et al. 1992; De Waele and Gianoulakis 1994).

The differences in β -endorphin observed in rodents that are correlated with ethanol consumption appear to hold for humans as well. Relative to nonalcoholics, lower plasma β -endorphin has been found in alcoholics (Vescovi et al. 1992; Aguirre et al. 1995; del Arbol et al. 1995) as well as those at high risk for the development of alcoholism (Gianoulakis et al. 1989). Moreover, Gianoulakis and colleagues (1995, 1996) found that nonalcoholic subjects from families with at least a two-generation history of alcoholism showed an enhanced dose-dependent increase in β -endorphin plasma levels following oral consumption of ethanol relative to those from families without a history of alcoholism. Froehlich and colleagues

(2000) confirmed that the β-endorphin rise in response to ethanol is a heritable trait.

Pharmacotherapeutic support for the contention that opioids influence ethanol self-administration comes from the large body of evidence indicating that ethanol drinking decreases following administration of opiate antagonists. This was first demonstrated in rodents (e.g., Altshuler et al. 1980; Froehlich et al. 1990) and subsequently confirmed in clinical trials (O'Malley et al. 1992; Volpicelli et al. 1992). The supposition here is that by blocking the effects of the ethanol-induced opioid release, naltrexone attenuates the pleasure associated with drinking. In a recent study by Gonzales and Weiss (1998), naltrexone blocked the ethanol-mediated dopamine increase in the nucleus accumbens. This finding supports a model in which endorphinergic neurons disinhibit dopamine activity in the mesolimbic "reward" pathway.

These studies, either examining β-endorphin changes in response to ethanol, or correlating β-endorphin activity with ethanol consumption, provide ample rationale for investigating the way in which β-endorphin might alter ethanol sensitivity. Fortunately, the mechanism for such investigations has recently been made available.

β-ENDORPHIN MUTANTS AND ETHANOL CONSUMPTION

In order to study the physiological roles of β-endorphin, our laboratory (Rubinstein et al. 1996) used the

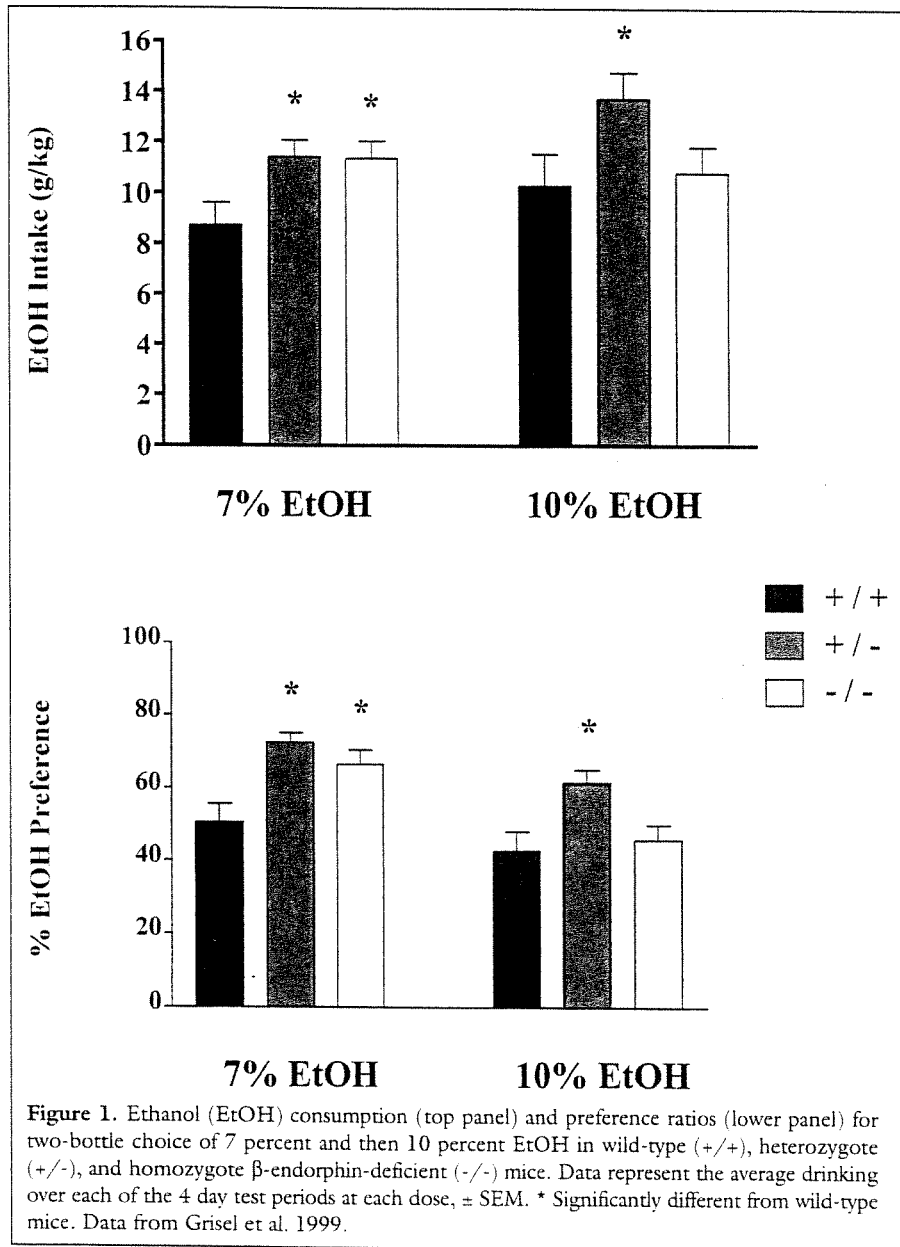
genetic approach of homologous recombination in embryonic stem cells to produce mutant mice that are unable to synthesize β-endorphin. Because β-endorphin is posttranslationally processed from the carboxyl-terminal amino acid region of a multifunctional precursor peptide (POMC), the introduction of a premature stop codon (point mutation) into the POMC gene results in normal processing of all POMC products in the mutant mice with the exception of β-endorphin. These knockout mice appear normal in many respects, such as fertility, birth weight, activity, and systemic morphine analgesia (Rubinstein et al. 1996).

The purpose of this investigation was to begin asking the question: how do varying levels of endogenous β-endorphin affect ethanol-dependent responses? We tested genetically homogenous mice that express either 100 percent, 50 percent, or 0 percent of normal β-endorphin levels for their preference and consumption of ethanol using a two-bottle, free choice paradigm. Our findings support the notion that availability of the endogenous peptide is one of the factors that contributes to oral self-administration of ethanol.

Experimental animals were born and reared at the animal care facilities of Oregon Health Sciences University and then transferred to the Portland VA Medical Center animal research facility after weaning. Mating pairs consisted of heterozygote (+/-) males crossed with (+/-) females. The offspring segregated into the three genotypes (+/+, +/-, and -/-) in a Mendelian ratio of 1:2:1 as predicted

for a recessive allele. These derived from original F₁ hybrid mice (129/Sv, C57BL/6N) that had been backcrossed to C57BL/6J for seven gen-

erations and were thus nearly congenic on the C57BL background. Genotyping was performed on genomic DNA samples obtained from



mouse tails by polymerase chain reaction under standard conditions.

Male and female mice of each genotype were individually housed in a temperature- (21 ± 2 °C) and light-controlled (12L:12D) colony room. There were 117 test subjects, approximately equally divided by sex and genotype. Two fluid-filled 25-mL graduated cylinders were placed on each cage, food hoppers were filled with rodent block chow, and the tube volumes were recorded. Following 4 days of water drinking, one of the tubes (counterbalanced for side across cages) was filled with 7 percent ethanol for 8 days, and then 10 percent ethanol for 8 more days. Ethanol consumption was expressed as grams per kilogram per day (dividing the 48-hour consumption scores by 2), and preference ratios were calculated as the percent of total fluid intake consumed from the ethanol tube over that same period. Tube positions were changed every other day to dissuade the development of side preferences.

The results of this study are summarized in figure 1. Although the dose of self-administered ethanol did not significantly differ between genotypes ($F_{[2,107]} = 2.5$, $p < 0.1$ [top panel of the figure]), there was a main effect of dose ($F_{[1,107]} = 9.6$, $p < 0.01$) and, importantly, an interaction between genotype and dose ($F_{[2,107]} = 3.1$, $p < 0.05$). The three genotypes did differ significantly with respect to their overall preference for the ethanol solution ($F_{[2,114]} = 6.9$, $p < 0.01$ [lower panel of the figure]). The concentration of ethanol affected preferences ($F_{[1,114]} = 60.1$, $p < 0.001$), and there was an

interaction between concentration and genotype, indicating that genotypes were not equally affected by the change in concentration ($F_{[2,114]} = 5.6$, $p < 0.01$). Taken together, these data reflect the fact that heterozygote mice consistently drank more ethanol than wild-type mice, while drinking in the knockouts was dependent on the concentration of ethanol.

DISCUSSION

The results of these studies support the hypothesis that β-endorphin modulates the reinforcing effects of ethanol. Mice that express low levels of β-endorphin (50 percent of normal) drink more ethanol than wild-type mice. One possible explanation for this is that those mice with deficient basal levels of β-endorphin find the ethanol-induced release particularly reinforcing. Because ethanol cannot produce such a surge in genetically null mice, drinking might be particularly dependent on preabsorptive factors, and therefore more variable as a function of concentration, as seen in the homozygous knockouts

The enhanced administration of ethanol in mice with lower β-endorphin levels is concordant with previous findings from the clinical literature, as well as current thought underlying the use of antiopioid pharmacotherapies for alcoholism treatment (Herz 1997). The relationship between ethanol's effects and endogenous opioid peptides can be uniquely studied using mutant mice engineered for different levels of β-endorphin, and future directions are likely to

include further neuropharmacological and behavioral analyses of the differential sensitivity between these strains to ethanol. It is hoped that these studies will contribute to our understanding of the relationship between opioids and ethanol and thereby expedite the successful treatment and prevention of alcoholism.

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