Oral Self-Administration Of Ethanol In Transgenic Mice Lacking β-Endorphin

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Ethanol (EtOH) modifies the production and/or release of endogenous opioid peptides, including β-endorphin (Gianoulakis, 2004; Przewlocka et al., 1994; Schulz et al., 1980). Opioids subsequently influence the reinforcing properties of EtOH and the development of alcoholism (Terenius, 1996; Van Ree, 1996). In this study, β-endorphin deficient mutant mice were used to examine the effects of a specific opioid peptide on EtOH consumption. A two-bottle free choice EtOH oral self-administration paradigm was administered to homozygous mutant mice (void of all β-endorphin), heterozygous mice (50% β-endorphin expression), and sibling wildtype mice (C57BL/6J; B6). Subjects received increasing concentrations of EtOH (0%, 3%, 6%, 12%, and 15%) each given over an eight day span, and were evaluated for preference and consumption each day. Overall, females drank more than males. Homozygous mutant mice (KO) showed decreased preference for EtOH at all concentrations, and self-administered significantly less than heterozygous mice (HT) and wildtype mice (B6). The HTs had a tendency to drink the most followed by the B6s, and the KOs drank the least. These data support the hypothesis that β-endorphin influences the reinforcing effects of EtOH.

Keywords: Ethanol, β-endorphin, self-administration, transgenic mice

Introduction

Biological contributions to excessive ethanol (EtOH) drinking that can lead to alcoholism have been investigated for decades (Nestler and Aghajanian, 1997; Cowen et al., 2004). Animal models evaluating the chemical and genetic substrates for self-administration of alcohol (EtOH) have been used in an attempt to identify mechanisms that promote the development of alcoholism in humans.

The relationship between EtOH and endogenous opioid peptides is of particular interest. For instance, EtOH modifies the synthesis and release of β-endorphin (Scanlon et al., 1992; Froehlich, 1995), and these changes in turn affect dopamine release in the mesolimbic pathway, a critical neural substrate for reward (Widdowson and Holman, 1992). Numerous studies in animals (Altshuler et al., 1980; Froehlich et al., 1990) and in humans (O’Malley et al., 1992; Volpicelli et al., 1992) have shown that administration of an opiate antagonist can decrease EtOH consumption. The EtOH-mediated release of dopamine in the nucleus accumbens can also be blocked by opiate antagonists (Gonzalez and Weiss, 1998). Thus, β-endorphin is thought to play an important role in the reinforcing effects of EtOH, mediating consumption (Grisel et al., 1999; Roberts et al., 2000), craving (Van Ree, 1996; Marinelli et al., 2000), and relapse (Terenius, 1996).

Some genotypes of rodents that prefer EtOH have lower endogenous β-endorphin when compared to non-preferring lines (Aguirre et al., 1995; del Arbol et al., 1995). Moreover, humans with a positive family history for alcoholism also tend to have lower basal levels of β-endorphin, as
well as an exaggerated EtOH-mediated release of this peptide, than those without a genetic liability for alcoholism (Gianoulakis et al., 1989). From data such as these it has been argued that those prone to alcoholism are “self-medicating” an opioid deficiency and/or especially benefiting from an opioid surge, following administration of EtOH (Oswald and Wand, 2004; Reid et al., 1991).

In an earlier study, Grisel et al. (1999) found evidence to support the hypothesis that EtOH-mediated reward depends upon β-endorphin. We investigated EtOH consumption in a two-bottle, free-choice paradigm in transgenic mice with varying levels of endogenous β-endorphin. Although strain differences were small, there was a tendency for mice with low levels of β-endorphin to consume the most EtOH, supporting the idea that consumption would be especially reinforcing for subjects deficient in this peptide. Unfortunately, in this study, we only evaluated two concentrations of EtOH: 7 and 10%, and so were unable to fully elucidate the dose-response relationship as it varied with respect to β-endorphin levels. In the present experiment we expanded the range of concentrations tested and also included sufficient numbers of male and female subjects to test for sex-dependent effects. Furthermore, we evaluated sucrose preference to test for differences in sweet responsivity. We predicted that mice lacking all β-endorphin (knockout; KO) would consume the least, because they would not be able to benefit from any β-endorphin release. Mice with half the normal amounts of β-endorphin (heterozygotes; HT) were predicted to drink the most EtOH and wildtype mice (B6) were expected to drink intermediate levels.

Our data support our hypothesis that β-endorphin does influence the reinforcing properties of EtOH. HT mice with 50% β-endorphin expression prefer and consume the most EtOH, KO the least, and B6 in between. With this data we can further examine the neurological substrates that modulate EtOH consumption and addiction.

**Methods**

Progenitor mice used to obtain subjects for this study were derived from those made by Rubinstein et al. (1996) and obtained from The Jackson Laboratories, Bar Harbor, ME. The original lines were constructed by inserting a point mutation in exon 3 of the POMC gene, causing a shortened proopiomelanocortin (POMC) prohormone. The gene has since been fully backcrossed onto the C57BL/6J inbred line.

All experimental animals were born and reared at Furman University in the animal care facilities. Mating pairs were arranged and offspring produced by either homozygous (B6, KO) or heterozygous mating pairs. Mice were group housed by sex and genotype following weaning at 20-21 days, and maintained on a reverse 12:12 light: dark cycle at 21°C ± 2°C with *ad libitum* access to food and water. All experimental procedures were in accordance with the Furman University Institutional Animal Care and Use Committee and the principles of laboratory animal care from the National Institutes of Health guidelines. On Day 1, adult (50-103 day old, experimentally naïve) subjects were taken from the colony room to a procedural room where they were weighed and single housed in corn-cob bedding lined Plexiglas cages with wire lids. Two 25 mL graduated cylinders containing tap water were placed on each cage, food hoppers were filled with rodent block chow, and the tube volumes were recorded. Cage locations were counterbalanced so that genotype and sex were equally distributed on the rack. A sentinel cage was added to each side of the rack in order to obtain control volumes of leakage/evaporation. Approximately 24 h later, tube volumes were recorded again, and refilled as necessary. For the following 48 days, tube readings occurred every day, and after every 48 h tube positions were switched to control for development of side preferences. Following 8 days of water drinking, one of the tubes (counterbalanced
for side across cages) was filled with 3% EtOH for 8 days. Remaining concentrations of EtOH and a test for sweet preference (6%, 12%, 15% EtOH, and 8% sucrose) were also tested for 8 days each. EtOH consumption was expressed as g/kg/day, and preference ratios were calculated. The experiment was conducted in three separate runs, which were procedurally identical, but separated by 12 days. A total of 77 test subjects were used in the study. Twenty-four of these subjects were wildtype (B6), 28 heterozygous (HT), and 25 knockout (KO). There were 37 females and 40 males, approximately equally divided across genotype and test run.

Data were analyzed by two-way (genotype and sex) repeated measure ANOVA on average g/kg of EtOH administered and preference at each dose. Because of significant effects of sex, males and females were subsequently evaluated separately by single factor ANOVAs at each concentration. Post-hoc analysis of significant differences was evaluated using the Scheffe test. Criterion for significance was set at $p \leq .05$.

Results

There was no difference in any measure of EtOH consumption across runs, so data from all three runs were combined following two-way analysis with run and genotype. No differences in water or sucrose intake were observed among the runs or between strains (data not shown), nor were there any significant interactions with sucrose consumption.

Preference

$\beta$-endorphin levels influenced the preference for EtOH as evidenced by a significant effect of genotype in the two way repeated measures ANOVA on preference across the dose range: ($F_{(2,69)} = 8.361, p < .01$). As is evident in Figure 1, the KOs tended to drink the least, the HTs drank the most, and the B6s were intermediate drinkers, with this pattern becoming more evident at higher concentrations of available EtOH. Although there was no main effect of sex in this analysis ($F_{(1,69)} = .103, p > .05$), there was a significant sex x genotype interaction ($F_{(2,69)} = 3.496, p < .05$). In addition, although there was no overall effect of EtOH concentration ($F_{(3,207)} = 1.898, p > .05$), there was a significant interaction between EtOH concentration and strain ($F_{(6,207)} = 6.306, p < .01$), but no significant interactions with sex ($F_{(3,207)} = .693, p > .05$).

Post hoc analysis of EtOH preference was evaluated in males and females separately at each concentration. KO males drank significantly less than either B6 or HT males at 3 or 6 % EtOH, and less than B6 males at 12 or 15 % EtOH, and there was a strong tendency for them to drink less than HTs at these concentrations ($p = .056$ and $.091$, respectively). In addition, there was a tendency for HT males to prefer 6% EtOH more than B6 males ($p = .056$).

There were only genotypic differences in females at 6% EtOH, as KO’s consumed relatively less EtOH than either other line.

Dosage

Figure 2 shows the dosage consumed by different groups across experimental days. There was no main effect of genotype on dose ($F_{(2,64)} = 1.477, p > .05$). As expected, there was a significant effect of sex on the amount of EtOH administered ($F_{(1,64)} = 20.357, p < .01$), as females are well known to drink more than males in rodent studies (Jones and Whitfield, 1995). There was also no genotype by sex interaction ($F_{(2,64)} = 1.568, p = .216$). However, animals self-administered different amounts of EtOH depending upon the concentration available, as there was a significant effect of dose ($F_{(3,192)} = 107.904, p < .01$). Of particular interest to us was the significant interaction between the dose (g/kg) administered across concentrations and genotype, indicating that the different
lines of mice were differentially affected by the change in concentration ($F_{(6,192)} = 2.321$, $p < .05$). This interaction reflects the fact that at higher EtOH concentrations, KO's tended to drink less than B6, and especially HT mice. There was also a significant interaction between concentration and sex ($F_{(6,192)} = 5.467$, $p < .01$). There was not a significant 3-way interaction.

**Fig.1.** EtOH preference ratio for two-bottle choice of 3%, 6%, 12% and 15% EtOH in wildtype (B6), heterozygous (HT) and β-endorphin knockout mice (KO). Data represent mean ± SEM. Panel A shows the average preference ratio at all four concentrations of EtOH for each genotype. Panel B shows the female average preference ratio at all four concentrations of EtOH for each genotype. Panel C shows the male average preference ratio at all four concentrations of EtOH for each genotype. Panel D shows the female preference ratios across the 40 day testing period. Panel E shows the male preference ratios across the 40 day testing period.
Fig. 2. EtOH consumption (g/kg) in two-bottle choice of 3%, 6%, 12%, and 15%EtOH in wildtype (B6), heterozygous (HT), and Knockout (KO) mice. Data represent mean ± SEM. Panel A shows average amount of EtOH consumption by each genotype at each concentration in a 48 hour period. Panel B shows average amount of EtOH consumption by females of each genotype. Panel C shows average amount of EtOH consumption by males of each genotype. Panel D shows consumption by females across the 40 day testing period as measured every day. Panel E shows consumption by males across the 40 day testing period.
Discussion

Current theories of addiction emphasize the rewarding effects produced by the drug (Wise, 1988). One mediator of these rewarding effects is β-endorphin (Herz, 1997). Many studies show that EtOH drinking in mice is correlated with the animal’s genetic ability to release β-endorphin (Gianoulakis et al., 1989). It has also been shown that people with a family history of alcoholism have an increase in β-endorphin release after EtOH administration compared to controls with no family history of alcoholism (Gianoulakis et al., 1989).

The purpose of this study was to see how β-endorphin influenced EtOH drinking in an animal model. The results support our hypothesis that β-endorphin modulates the rewarding properties of EtOH. Mice completely lacking β-endorphin (KO) self-administered less EtOH in a free-choice paradigm.

Overall, females drank more EtOH than the males, which is a well-known result in rodents (Jones and Whitfield, 1995). Notably, the effect of β-endorphin on drinking was also sex-specific. This peptide did not influence EtOH self-administration in females, but did effect drinking in males. The male KOs, entirely lacking β-endorphin, appeared to be insensitive to the rewarding effects of EtOH as they self administered at chance levels (50% of the fluid they consumed was from the EtOH-spiked tube). On the other hand, male HTs showed a pattern of drinking consistent with enhanced EtOH reward, and tended to drink more than control mice. Both of these findings are consistent with a theory implicating β-endorphin release in EtOH reinforcement, though why this effect should be sex-specific is unclear at present. One possibility is that sex specific hormones, such as progesterone metabolites, make up for the lack of β-endorphin in females (Morrow, 2007; Morrow et al., 2006) though further research is needed to clarify the mechanisms of this sex-dependent effect.

There is a main concern that should be acknowledged when interpreting data collected from induced mutant mice. It is possible that the observable phenotypic behaviors could be the cause of a “hitchhiking” gene polymorphism and not purely a consequence of the intentional genetic mutation (Gerlai, 1996; Low et al., 1998). A more likely possibility is that some compensatory reaction to the genetic mutation could cause the different phenotypic behaviors and not the mutation alone (Mogil and Grisel, 1998). The use of inducible mutant mice in future studies will lead to a better understanding of the mechanism by which β-endorphin induces behavior, including behaviors related to alcoholism.

Further investigation should be done to evaluate the role of β-endorphin in EtOH sensitivity more generally. Currently, we are performing place conditioning tests with the β-endorphin KO and HT mice. This is also a test for EtOH reinforcement, but β-endorphin may affect other consequences of EtOH too.

The main purpose of this study was to evaluate the neurobiological substrates that modulate EtOH consumption in an animal model where β-endorphin levels vary. We demonstrated that β-endorphin,
especially in males, influences the reinforcing properties of EtOH. A more complete assessment of a possible vulnerability to alcoholism, giving us a broader understanding of the predispositions to addiction and aiding in the development of successful interventions to that would ameliorate the negative consequences of excessive alcohol use.

References


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2007


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