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Naltrexone and alcohol drinking in mice lacking β-endorphin by site-directed mutagenesis

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Abstract

Alcohol-induced activation of the opioid system may contribute to the reinforcing properties of alcohol. This study investigated whether elimination of β-endorphin (BE) synthesis via site-directed mutagenesis in embryonic stem cells would alter alcohol intake in mice. Both BE-deficient and wildtype (WT) mice generated from the targeted stem cells were backcrossed for nine generations onto a C57BL/6 background, and were maintained with ad libitum food and water. Mice had access to alcohol (10% v/v) under the following conditions: 24 h, scheduled access for 2 h/day, following acute (1 or 2 days) or chronic (5 weeks) alcohol deprivation, and scheduled access following six doses of naltrexone (0.125–16.0 mg/kg BW, ip) or saline treatment. Alcohol intake was similar in BE-deficient and WT mice given chronic access to alcohol, but greater in BE-deficient compared with WT mice during the first 10 days of scheduled access to alcohol, but not after more extensive experience with scheduled access. BE-deficient, but not WT mice, increased alcohol intake following 2 days, but not 1 day or 5 weeks, of deprivation. Naltrexone reduced alcohol drinking both in BE-deficient and WT mice, suggesting that drinking is mediated, in part, by activation of opioid receptors in both genotypes. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Alcohol deprivation effect; β-Endorphin-deficient mice; Alcohol preference

1. Introduction

Endorphin and enkephalin peptides, as well as opioid analogs, have rewarding effects, as evidenced by peripheral and central self-administration [5–7,19], by opioid peptide-induced alterations in pattern of responding for electrical brain stimulation [8,39], and by preference for environmental stimuli associated with opioid peptide administration [1,31,32,37]. Blocking the action of opioid peptides with opioid receptor antagonists attenuates or blocks the rewarding effects of opioid peptides in a variety of paradigms [5,24,39].

Three lines of evidence suggest that activation of endogenous opioids, such as β-endorphin (BE) in response to alcohol ingestion, may mediate some of the reinforcing properties of alcohol. First, alcohol administration alters BE and enkephalin (ENK) gene expression [2,18,22,24,36] and opioid peptide release [9,14,15]. Second, pretreatment with opioid receptor antagonists decreases alcohol consumption in both rodents [3,10–12,21–23] and humans [26,29,40]. Third, genetic differences in sensitivity of the opioid system to alcohol have been found in animal models of alcoholism [9,10,24,25] and in individuals that differ in family history of alcoholism [16].

While this indirect evidence suggests that alcohol-induced BE release may contribute to alcohol drinking, several methodological problems hinder direct study of the influence of this peptide on alcohol-drinking behavior. There are three families of endogenous opioid peptides: dynorphins, endorphins, and enkephalins. There are also three major opioid receptor subtypes: μ, δ, and κ [25,28,34]. Although dynorphins have been shown to bind selectively to the κ receptor subtype, BE and the enkephalins have been shown to bind to both μ and δ receptor subtypes with high affinity in cloned receptor populations [34]. While specific antagonists can be used to block several ligands, including BE, from binding to a particular

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opioid receptor subtype, there is currently no pharmacological method for blocking a specific endogenous ligand from binding to all of its cognate receptors. In addition, because BE is synthesized in the brain, as well as in the anterior pituitary, isolating the influence of different anatomical sources of BE is difficult.

Rubinstein et al. [35] have developed a mutant strain of mice (B6.129S2-*Pomc*^{tm1Low}) that cannot synthesize BE. The *Pomc* gene was mutated by homologous recombination in embryonic stem cells derived from the 129S2/SvPas inbred mouse strain to encode a premature translational stop codon. These altered stem cells were then injected into blastocysts from B6 mice to produce germ-line-penetrant chimeras. After nine generations of backcrossing the mutant allele onto a B6 background, heterozygous mice were bred together to generate congenic homozygous BE-deficient and wildtype (WT) mice. The mutant *Pomc* allele was expressed at normal levels and translated to a carboxy-terminal truncated proopiomelanocortin (POMC) prohormone. The absence of BE was confirmed in the hypothalamus and pituitary of BE-deficient mice using RIA of HPLC-fractionated tissue extracts and imunohistochemistry [35]. However, the mice expressed normal levels of other POMC peptides, α-MSH and ACTH, from the truncated prohormone. Furthermore, these mice lacked certain types of endogenous opioid-dependent analgesia, confirming the functional significance of the loss of BE [35].

Because these mutant mice lack BE, and, hence, are deficient in alcohol-induced BE release, one might predict that alcohol intake would be lower in BE-deficient mice than in WT mice. Contrary to expectations, however, a previous study [18] reported that intake of a 10% alcohol solution was similar in BE-deficient and WT mice, while intake of a 7% alcohol solution was higher in BE-deficient mice, under chronic free-choice conditions. It is not known whether alcohol intake differs between genotypes in other access conditions. In mice heterozygous for the BE-deficient allele, intake of alcohol was higher than in WT mice at both alcohol concentrations, which may represent an attempt to elevate BE in the face of compromised function. Another study [17] indicated that operant intravenous self-administration was higher in BE-deficient mice than WT mice, under limited-access conditions. However, it is unclear whether this finding was due to the fact that ethanol was self-administered intravenously rather than orally, or was due to the fact that ethanol was available for only 2 h daily. Therefore, the present study examined alcohol intake in BE-deficient mice when alcohol was concurrently available with water for 24 h a day, and when alcohol access was scheduled for 2 h daily at the beginning of the dark cycle with food and water freely available (scheduled access). We also examined alcohol intake following various alcohol-deprivation conditions. Based on pharmacological and genetic findings, we hypothesize that alcohol intake will be lower in BE deficient mice, in keeping with the postulated role of BE in mediating alcohol drinking. Nevertheless, compensatory mechanisms may develop in mice lacking BE, providing other substrates for promoting alcohol-drinking behavior. In order to address whether compensation within the opioid system has occurred in BE-deficient mice, alcohol drinking in BE-deficient mice was assessed following treatment with naltrexone, a nonselective μ- and δ-opioid antagonist, which might be expected to decrease alcohol intake if endogenous opioid ligands other than BE are involved in mediating alcohol-drinking behavior via binding at the μ- and δ-opioid receptor subtypes.

2. Methods

2.1. Subjects

The male mice used in this study were the offspring of breeders derived from the transgenic animal colony at Oregon Health Sciences University, and were bred at the AAALAC-approved colony at Indiana University School of Medicine in a single generation. The original F1 hybrid mice were a cross of 129S2/SvPas and C57BL/6N, and these mice were subsequently backcrossed for five generations to C57BL/6N mice (Simonsen, Gilroy, CA). Four additional backcrossings were performed onto C57BL/6J mice (The Jackson Laboratories, Bar Harbor, ME). All backcrossings were done from heterozygous mice to WT C57BL/6 mice at each backcross generation and then at the final N9 generations, Het $\times$ Het crosses generated both WT and BE-deficient mice that formed the parents of the mice used in this study. Mice were genotyped at OHSU by a standard PCR reaction. Male mice, which were all born within 1 week of each other, were individually housed in standard polycarbonate shoebox cages with wire tops and Cell-Sorb bedding. Lights were on a reverse cycle (on from 19:30–07:30 hours daily) to facilitate drinking behavior during limited-access drinking sessions. Temperature was maintained at $21 \pm 1°C$. Mice were given ad libitum access to standard laboratory chow, and were 14 weeks old at the beginning of the experiment. There were 29 WT and 31 BE-deficient mice at the beginning of the experiment.

2.2. Procedures

All mice were given ad libitum access to food and water and access to alcohol (10% v/v) under different access conditions. During all conditions, mice received concurrent access to both alcohol (10% v/v) and water presented in narrow polycarbonate graduated cylinders with sipper tubes. During chronic access, 25-ml cylinders were used, readable to ± 0.25 ml, and during limited access, 10-ml cylinders, readable to the nearest to ± 0.05 ml, were used. Ethanol and water intake were measured throughout all phases of the experiment.

2.2.1. Phase 1: 24-h access to alcohol for 28 days

Fluid intakes were examined every 2 days, and after each reading, the position of the ethanol bottle was alternated with that of water. Mice were weighed once every 8 days.

2.2.2. Phase 2: scheduled access to alcohol for 2 h/day (08:00–10:00 hours) for 51 days

During the first 3 weeks, mice were run 7 days/week, except as noted below, during Phase 3. Subsequently, mice were run for 5 days/week. Mice were weighed once per week immediately prior to onset of alcohol access. The position of the bottle containing alcohol was alternated daily.

2.2.3. Phase 3: scheduled daily access to alcohol for 2 h/day following 1 and 2 days of alcohol deprivation or no deprivation

Mice were counterbalanced on the basis of daily 2-h alcohol intake over days 5–8 of scheduled access and were assigned, in matched pairs, to the deprivation or nondeprivation condition. The 1-day deprivation manipulation was conducted first, occurring on the 11th consecutive day of scheduled access to alcohol (i.e., day 41). Six days of scheduled access to alcohol for 2 h/day separated the two acute alcohol-deprivation conditions. During these short deprivation manipulations, alcohol-deprived mice were treated identically to mice with alcohol access, except that they received access to two tubes, both of which contained water.

2.2.4. Phase 4: scheduled access to alcohol for 2 h/day following 5 weeks of chronic alcohol deprivation

During chronic deprivation, mice were left undisturbed in the home cage. Chronic deprivation was preceded by 5 weeks of scheduled access to alcohol for 2 h/day for 5 days/week, Monday through Friday.

2.2.5. Phase 5: scheduled access to alcohol for 2 h/day, 5 days/week, following pretreatment with naltrexone (0.125–16 mg/10 ml/kg ip) or an equal volume of saline administered 30 min prior to onset of the scheduled alcohol access period

Mice were matched for alcohol intake based on the previous week's alcohol consumption, and assigned to groups in matched pairs. One member of the pair received saline and one received naltrexone. Over repeated injections, mice were assigned to saline or naltrexone injections randomly, with the stipulation that no more than four of the injections were of one type for each mouse. Doses were administered in the following order: 0.5, 8.0, 2.0, 0.125, 16.0, and 4.0 mg/kg. Each dose was given for 2 consecutive days, with no less than 1 week separating administration of each dose. Injections were given on Tuesdays and Wednesdays (to avoid potential effects of the weekend deprivation on the response to naltrexone or saline). In total, 9 weeks elapsed between administration of the first and last doses of naltrexone. The data from the first injection of each dose is presented, rather than the mean of the 2 days, because alcohol consumption in both saline-treated and naltrexone-treated animals was decreased on the second day of treatment, which may reflect a short-term sensitization to the disruptive effects of handling and injection.

3. Results

3.1. Phase 1

Mean alcohol intake, cumulated over 4-day blocks, did not differ between BE-deficient and WT mice given 24-h access to alcohol with food and water freely available (Fig. 1). Intake was analyzed in 4-day blocks with a Genotype (WT or BE-deficient) × Blocks mixed factorial ANOVA. This indicated a main effect of 4-day blocks, $F(6, 348) = 12.11$, $P < .0001$. However, there was neither a main effect of genotype $F(1, 58) = 1.4$, $P > .24$, nor a Genotype × Blocks interaction, $F(6, 348) = 1.00$, $P > .40$, indicating that there were no differences between WT and BE-deficient mice in alcohol intake during this period. Results for preference were similar, with a main effect of 4-day blocks, $F(6, 348) = 15.06$, $P < .0001$, but no effect of genotype nor a Genotype × Days interaction, P's > .17. Percent of fluid intake that was comprised of 10% alcohol was higher initially and tended to decrease over time. Percent of intake during the first 4-day block was 59.1 ± 3.3% for WT and 60.0 ± 3.5% for BE deficient, and by the last 4-day block preference was 38.2 ± 4.4% for WT and 44.3 ± 3.7% for BE-deficient. Notably, water intake was the same for both lines over the 28-day period,

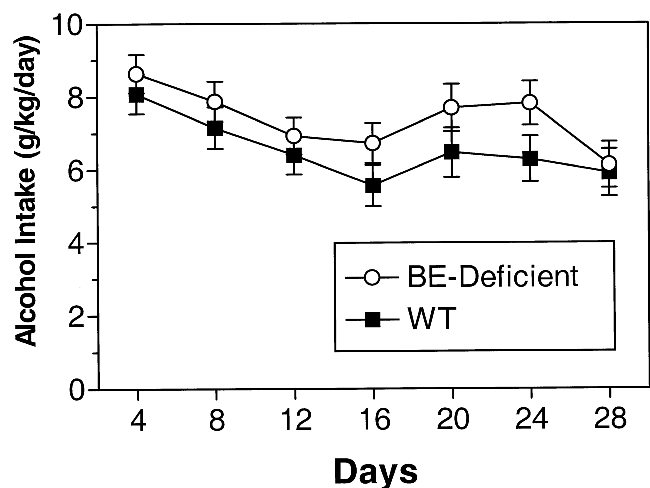

Fig. 1. Ethanol intake during 24-h, two-bottle choice access to 10% (v/v) ethanol and water, with food freely available. Intakes did not differ between BE-deficient ($n = 31$) and WT ($n = 29$) mice. Each point represents the mean of 4 days of drinking, with the day number indicating the last day of each block. Bars indicate standard errors.

averaging 2.41 ± 0.16 ml/day for WT, and 2.31 ± 0.17 ml/day for BE-deficient, P>.50.

3.2. Phase 2

Alcohol intake during scheduled access to alcohol for 2 h/day is shown in Fig. 2. Days 40–49 were omitted from the figure, as they include days in which groups of animals within each genotype either were being deprived, or had just been deprived, and, hence, were treated differently within genotypes. To simplify the data, analysis was conducted as a 2 (Genotype) × 7 (Blocks of days) ANOVA, with repeated measures on blocks. All blocks of days were the mean of 5 consecutive days, with the exception of block 3, which was the mean of 4 days (50–53). This block began 2 days following the conclusion of the 2-day deprivation study, when intakes had returned to baseline. The ANOVA indicated an interaction of Genotype × Blocks, $F(6, 348) = 2.31$, $P < .05$, as well as a main effect of blocks, $F(6, 348) = 13.03$, $P < .001$. The effect of genotype did not reach significance, P>.10. The interaction indicates that differences between genotypes depended upon blocks.

Inspection of the figure reveals that the difference between genotypes was most prominent during the first two 5-day blocks. Therefore, follow-up analyses were conduced on daily intakes during this period. During the first 10 days of scheduled access to alcohol for 2 h/day, daily alcohol intake was higher in BE-deficient compared with WT mice. Alcohol intake was analyzed using a Genotype (WT or BE-deficient) × Days ANOVA. This indicated a main effect of genotype, $F(1, 54) = 4.51$, P=.05, as well as a main effect of days, $F(9, 486) = 21.62$, $P < .001$, but no interaction, P>.4. The main effect of genotype and the lack of an interaction with days indicated that throughout this 10-day period, BE-deficient mice drank more alcohol than WT mice. Average daily fluid intake during the limited-access drinking sessions did not differ between genotypes over this period (0.55 ± 0.026 ml for WT mice and 0.601 ± 0.024 ml for BE-deficient mice, P>.10). Weights did not differ between genotypes; WT mice weighed 28.39 ± 0.50 g, and BE-deficient mice weighed 29.53 ± 0.48 g, P>.10.

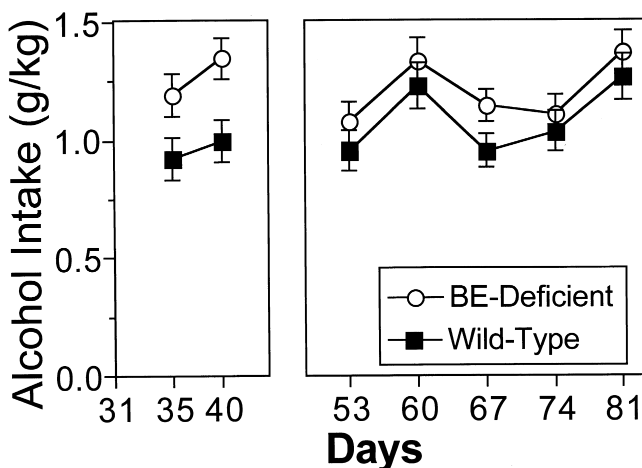

Fig. 2. Ethanol intake during days 31–81, during which mice received scheduled access to alcohol for 2 h/day. Each point represents the mean of 4–5 consecutive days of drinking, the day number indicating the last day of the block. Mice were run daily for the first two blocks of days, and 5 days/week during subsequent blocks. Days 41–49 are not shown, as animals were subjected to deprivation manipulations during this period (see Fig. 3). BE-deficient mice drank more than WT mice only during the first two blocks of days.

Alcohol preference during the 10 days of limited-access alcohol drinking was higher in BE-deficient mice than in WT mice. Preference at the beginning of the limited-access period (days 31–32) was 56.7% ± 4.6% in WT and 68.0% ± 4% in BE-deficient mice). At the end of the first 10 days (days 38–39), preference was 62.5% ± 3.8% in WT and 75.0% ± 3.3% in BE-deficient mice. Preference data, analyzed in a manner similar to that used for the first 10 days of limited-access intake data, also indicated a main effect of genotype, $F(1, 54) = 7.94$, $P < .01$, as well as a genotype × days interaction $F(9, 486) = 2.18$, $P < .05$, and a main effect of days, $F(9, 486) = 7.00$, $P < .001$. These data indicate that the higher ethanol intake in BE-deficient mice is not the result of higher fluid intake per se.

3.3. Phase 3

One BE-deficient and two WT mice died prior to the onset of deprivation studies. One day of alcohol deprivation did not alter alcohol intake in either BE-deficient or WT mice (data not shown, P's>.20), but 2 days of deprivation resulted in a difference in alcohol intake between alcohol-deprived and non-deprived animals in the BE-deficient mice but not in WT mice (see Fig. 3). To assess whether 2 days of deprivation affected intake, a 2 (Genotype) × 2 (Deprivation condition) × 2 (Days: baseline or post-deprivation) ANOVA with repeated measures on the last factor was conducted. Baseline was defined as intake averaged over days 44–46, the 3 days preceding the deprivation assessment. The ANOVA showed a main effect of days, $F(1, 56) = 14.19$, $P < .001$, and a Days × Deprivation interaction, $F(1, 56) = 7.06$, $p = 0.01$, consistent with intake increasing only in deprived animals. There were no main effects of, nor interactions with genotype. Nevertheless, follow-up pairwise comparisons indicated that intake increased from baseline only in BE-deficient mice, $t(14) = 3.49$, $P < .01$.

3.4. Phase 4

When the alcohol-deprivation period was extended to 5 weeks, a drop in alcohol intake was noted in both BE-

deficient and WT mice when scheduled access to alcohol was reintroduced. A paired *t* test within each genotype indicated that alcohol intake was lower on the first day of alcohol access following alcohol deprivation when compared with intake on the last day of scheduled access prior to deprivation in both genotypes, t's ≥ 3.03, P's $\leq .005$ (data not shown).

3.5. Phase 5

Naltrexone dose-dependently reduced alcohol intake in both genotypes (Fig. 4). Two additional BE-deficient mice died prior to onset of this phase of the study, and one WT mouse died during this phase. To assess naltrexone's effect on alcohol consumption within each genotype, the drinking score (in g/kg) of each naltrexone-treated mouse was subtracted from its paired saline-injected control to derive a difference score that was positive when naltrexone treatment reduced drinking. In cases in which an odd number of mice were present within a particular genotype during baseline, the lowest-drinking mouse was excluded from the study. Additionally, mice were excluded from analysis if bottle spills were found on the injection day. The resulting number of pairs of mice per genotype for each dose group were as follows: in WT mice, 10, 14, 12, 12, 13, and 11; and in KO mice, 13, 14, 14, 14, 14, and 14 for doses 0.125, 0.5, 2.0, 4.0, 8.0, and 16 mg/kg, respectively. Notably, no difference between the genotypes was seen during the entire baseline for the naltrexone phase, $P > .15$. Difference scores from injection days were then subjected to a Genotype × Naltrexone dose between subjects factorial ANOVA. This indicated a main effect of naltrexone dose, $F(5, 143) = 2.53$, $P < .04$, but no effect of genotype and no Genotype × Naltrexone interaction, P's ≥ 0.18, indicating

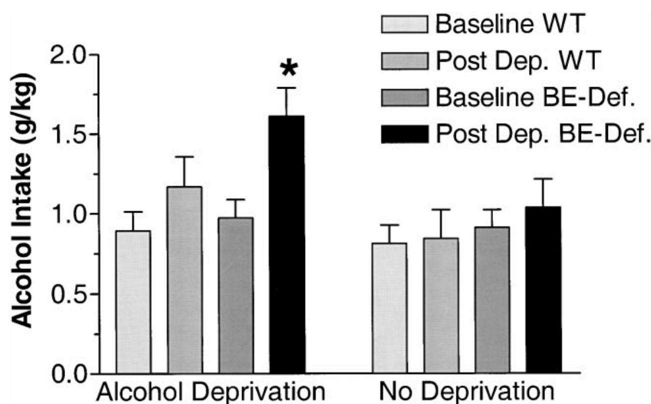

Fig. 3. BE-deficient, but not WT, mice showed an increase in alcohol consumption after 2 days of alcohol deprivation during limited-access drinking sessions. Baseline was the mean of days 44–46 of the experiment, and day 49 was the alcohol reinstatement day for the deprived animals. * Denotes a significant ($P = .01$) difference from baseline as a result of deprivation.

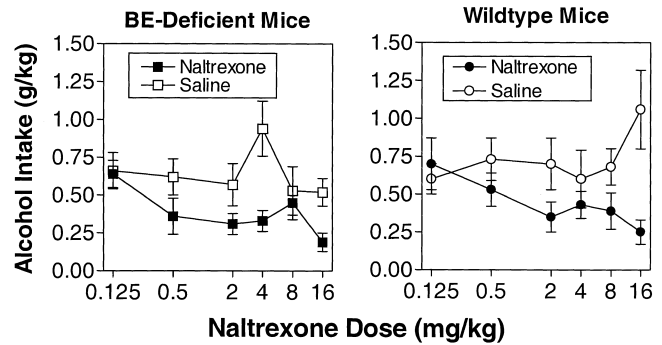

Fig. 4. Alcohol intake in both genotypes was suppressed in a dose-dependent manner by naltrexone during limited-access (2 h/day) drinking sessions when 10% alcohol and water were concurrently available.

that naltrexone had a similar, dose-dependent effect on alcohol drinking in both genotypes. Importantly, there was no dose-effect of naltrexone on the rate (ml/day) of water intake, $F(5, 143) = 1.70$, $P > .14$.

4. Discussion

Receptor antagonists that evidence selectivity for the three major opioid receptor subtypes, μ, δ, and κ, have been used extensively to determine the relative contribution of opioid receptors to alcohol-drinking behavior. However, very few studies have selectively eliminated opioid ligands from the entire physiological system in order to determine whether the presence or absence of a specific ligand alters alcohol-drinking behavior. The present study used transgenic mice lacking BE to isolate the contribution of BE to alcohol drinking in a variety of experimental conditions. Two differences in alcohol drinking were found in transgenic mice lacking BE: increased alcohol drinking in a limited-access paradigm, and increased alcohol drinking after a 2-day alcohol-deprivation period. No differences were seen between genotypes in chronic drinking of 10% ethanol, replicating a prior report by Grisel et al. [18]. The greater alcohol intake and preference exhibited by BE-deficient mice during limited-access drinking was reliable, but dissipated after 9 days of experience with the limited-access condition. Finally, it appeared that for both WT and transgenic mice, consumption of 10% alcohol during limited-access drinking sessions was opioid dependent, because naltrexone reduced drinking in both genotypes.

These results are consistent with a prior report of greater alcohol self-administration in BE-deficient mice than in WT mice. Grahame et al. [17] found that BE-deficient mice acquired operant intravenous (iv) self-administration of ethanol while WT mice did not. Although the route of administration in that study differed from the present study, the parameters of alcohol access are similar in the two

studies in that mice had access to alcohol only during 2-h daily sessions. Additionally, the genetic background of mice in that study differed from the present study only in that backcrossing had been carried out for seven, instead of nine generations onto C57BL/6 as in the present study. Because no other intravenous self-administration conditions were tested in the study by Grahame et al. [17], it is unclear whether the BE-deficient and WT mice would have differed in alcohol intake under deprivation conditions, or would have been similar in a chronic access condition. The concordance of results during limited access between the intravenous study and in this drinking study, that is, the greater intake of alcohol in the BE-deficient mice during limited access, indicates that the present findings are not likely due to genotypic differences in sensitivity to the preingestive effects of ethanol [4]. Interpretation of the present results should be tempered by the relatively modest levels of alcohol intake seen during some phases of the present study. Typically, drinking in both genotypes was about 1 g/kg BW. However, following alcohol deprivation, and early in the limited-access drinking phase, alcohol consumption in BE-deficient mice was closer to 1.5 g/kg. Interestingly, this is similar to the amount of alcohol self-administered intravenously by BE-deficient mice once they acquired operant responding for alcohol during 2-h daily sessions [17].

It is unclear why the genotypes differ in alcohol intake during limited, but not chronic access conditions, and why this difference does not persist indefinitely. The increased intake seen in BE-deficient mice during limited access may be viewed as a form of alcohol deprivation when compared to a chronic access condition [38]. Alcohol intake following deprivation in BE-deficient mice was about 50% higher after 2 days of deprivation than during daily limited access, and was about 50% higher in BE-deficient mice during the first week or so on limited access when compared both to WT mice at the same time, and BE-deficient mice following more experience with the limited-access drinking situation. These findings may suggest a role for BE in regularizing alcohol intake following an alcohol-deprivation period.

This interpretation of the present findings is supported by a recent report by Holter and Spanagel [20]. They found that chronic endogenous opioid receptor blockade induced by delivering naloxone via osmotic mini-pumps increased the alcohol-deprivation effect in rats following extensive drinking experience. In contrast, intermittent blockade of opioid receptors using daily subcutaneous injections of naltrexone attenuated the alcohol-deprivation effect. Differences in alcohol intake produced by chronic vs. intermittent naloxone treatment have also been reported by Phillips et al. [33]. They found that intermittent naloxone injections decreased alcohol intake during limited-access drinking sessions in C57BL/6J mice, but mice receiving naloxone pellets (chronic naloxone) drank more alcohol during 24-h alcohol access conditions than did mice receiving placebo pellets. The apparent concordance of alcohol-drinking behavior under alcohol-deprivation conditions in the Holter and Spanagel experiment, and BE-deficient mice in the present study, suggests that BE can regularize alcohol intake in the intact animal under deprivation conditions.

Because the BE-deficient mice in the present study lack BE throughout the lifespan, they may be expected to have compensated physiologically for the absence of the peptide. In order to determine whether potential compensatory changes are opioid in nature, naltrexone pretreatment was used to determine whether there were differences between the genotypes in the extent to which alcohol drinking depended on the endogenous opioid system. Perhaps surprisingly, both genotypes showed fairly orderly, dose-dependent suppression of alcohol intake by naltrexone. One way of interpreting this finding is that in the BE-deficient mice, which still have other endogenous opioids such as enkephalins present, the remaining endogenous opioids play a role akin to BE in mediating alcohol drinking. The enkephalins bind to μ- and δ-opioid receptors [34], and alcohol-induced enkephalin release may mediate the reinforcing actions of alcohol. Prior work has demonstrated that blocking either μ- or δ-opioid receptors can attenuate alcohol drinking in rodents [10,12,21–23]. It is possible, in other words, that BE-deficient mice have compensated for the absence of the peptide by developing a disproportionate reliance on endogenous opioids such as the enkephalins. This view is supported by the absence of a difference between genotypes in sensitivity to naltrexone, which may suggest that the endogenous opioidergic basis of drinking in BE-deficient and WT mice is similar. Another more conservative, but not incompatible way to interpret the present findings is that BE is not necessary for acquisition and maintenance of alcohol drinking or intravenous self-administration of alcohol [17].

It is important to bear in mind that phenotypic differences between mutant and WT mice in studies such as the present one may not be attributed solely to the targeted gene allele. Differences apparently due to the mutation can in fact be due to "hitchhiking donor alleles" from the 129-strain stem cells, or can arise from epistatic interactions with those alleles [13,30]. Nevertheless, an advantage of the present study is that the mice used were backcrossed for nine generations onto the C57BL/6 background, and, hence, only a small proportion (less than 0.5%) of the 129S2/SvPas gene alleles could remain.

The results of the present study suggest that BE activity may normalize alcohol intake in situations in which alcohol drinking is intermittent, which is characteristic of alcohol-drinking patterns in humans. These findings, along with those of other research groups, suggest that opioid receptor blockade is more effective in attenuating alcohol intake when blockade is produced intermittently rather than chronically. Hence, pharmacotherapeutic regimens using opioid antagonists for the treatment of alcohol dependence should

allow blood levels of drugs, such as naltrexone, to fluctuate, and should avoid maintaining steady, high levels of opioid antagonists for optimal efficacy.

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