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Corticotropin releasing factor acting on corticotropin releasing factor receptor type 1 is critical for binge alcohol drinking in mice

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Abstract

Background—The corticotropin releasing factor (CRF) system has been implicated in the regulation of alcohol consumption. However, previous mouse knockout (KO) studies using continuous ethanol access have failed to conclusively confirm this. Recent studies have shown that CRF receptor 1 (CRFR1) antagonists attenuate alcohol intake in the limited access “drinking in the dark” (DID) model of binge drinking. To avoid the potential non-specific effects of antagonists, in the present study we tested alcohol drinking in CRFR1, CRFR2, CRF and Ucn1 KO and corresponding wild-type (WT) littermates using the DID paradigm.

Methods—On days 1–3, the CRFR1, CRFR2, Ucn1 and CRF KO mice and their respective wildtype (WT) littermates were provided with 20% ethanol or 10% sucrose for 2 hours with water available at all other times. On day 4, access to ethanol or sucrose was increased to 4 hours. At the end of each drinking session, the volume of ethanol consumed was recorded and at the conclusion of the last session, blood was also collected for blood ethanol concentration (BEC) analysis.

Results—CRFR1 KO mice had lower alcohol intakes and BECs and higher intakes of sucrose compared to WT. In contrast, CRFR2 KO mice, while having reduced intakes initially, had similar alcohol intakes on days 2–4 and similar BECs as the WT. In order to determine the ligand responsible, Ucn1 and CRF KO and WT mice were tested next. While Ucn1 KOs had similar alcohol intakes and BECs to their WT, CRF KO mice showed reduced alcohol consumption and lower BECs compared to WT.

Conclusions—Our results confirm that CRFR1 plays a key role in binge drinking and identify CRF as the ligand critically involved in excessive alcohol consumption.

Keywords

Alcoholism; stress; CRH; ethanol drinking; limited access

The corticotropin releasing factor (CRF) system regulates many important physiological functions and includes the ligands, CRF, urocortins 1, 2 and 3 (Ucn1, Ucn2 and Ucn3) and their receptors, CRF receptor type 1 (CRFR1) and type 2 (CRFR2) (Bale and Vale, 2004; Hauger et al., 2006). CRF binds with greater affinity to CRFR1 than CRFR2 while Ucn1 binds both receptor types with equal affinity. Ucn 2 and 3 act only at CRFR2 (Hauger et al., 2006; Pioszak et al., 2008; Ryabinin et al., 2002).

The CRF system is involved in the regulation of alcohol consumption (Cowen and Lawrence, 2006; Cowen et al., 2004; Heilig and Koob, 2007). In particular, CRFR1 antagonists attenuated drinking in dependent rats but not in non-dependent controls (Funk et al., 2007; Gehlert et al., 2007; Gilpin et al., 2008; Ji et al., 2008; Sommer et al., 2008). In agreement, Chu et al. (2007) reported that a CRFR1 antagonist attenuated the increase in alcohol consumption observed after dependence and an abstinent period in C57BL/6J mice. In contrast, a CRFR2 agonist was found to decrease alcohol self-administration in dependent rats while having the opposite effect in non-dependent animals (Funk and Koob, 2007). These findings suggest that the CRF system is especially important in the transition from excessive drinking to alcohol dependence. In agreement with pharmacological studies, polymorphisms in the human gene encoding CRFR1 have been shown to be associated with excessive drinking (Treutlein et al., 2006). Similarly, a polymorphism in the gene encoding CRFR1 in rats selectively bred for alcohol preference was found to be associated with their high alcohol intake (Hansson et al., 2006).

However, studies using genetically-modified mice have provided inconclusive and contradictory evidence on the role of CRF system in alcohol drinking. For example, CRFR1-deficient mice were found to consume similar amounts of alcohol as wild-type (WT) littermates in a continuous 2-bottle choice procedure and did not increase their alcohol intake after induction of dependence. On the other hand, prolonged alcohol consumption and repeated stress resulted in higher alcohol intake in these KOs compared to WTs (Chu et al., 2007; Sillaber et al., 2002). A recent study by Pastor et al. (2011) showed that drinking in CRFR1 KO mice was sensitive to ethanol concentration and only consumption of 20% ethanol was reduced compared to the WTs, while there was no difference in the consumption of 3%, 6% and 10% ethanol. A deletion of the *CRFR2* gene only slightly increased alcohol intake in the limited access procedure while having no effect when access was continuous (Sharpe et al., 2005). On the other hand, CRF knockout (KO) mice consumed more alcohol than controls in continuous and limited access experiments, and CRF overexpressing mice consumed less alcohol than WTs (Olive et al., 2003; Palmer et al., 2004).

The discrepancies between pharmacological and KO studies could be due to developmental compensations in the genetically-manipulated mice or may preclude a role for the system in moderate alcohol consumption and instead suggest a role in binge drinking. More recent pharmacological studies have suggested exactly this, i.e., that this system is involved in excessive binge-like alcohol consumption in non-dependent animals (Lowery et al., 2008; 2010; Sparta et al., 2008).

Effects of genetic manipulations of specific components of the CRF system have never been addressed in models of binge drinking. Therefore, in the studies detailed here, we investigated the roles of several components of the CRF system in binge alcohol consumption, utilizing the “drinking-in-the-dark” (DID) paradigm (Rhodes et al., 2005; 2007; Ryabinin et al., 2003; Sharpe et al., 2005). Specifically we used CRFR1, CRFR2, CRF and *Ucn1* KO mice to avoid potential non-specific actions of pharmacological agents and to identify the specific ligand(s) regulating alcohol drinking in this procedure.

MATERIALS AND METHODS

Animals

Four lines of male and female KO and WT mice were used in our studies: namely the CRF receptor 1 (CRFR1), CRF receptor 2 (CRFR2), Urocortin 1 (*Ucn1*) and CRF KO lines. The mice had a single gene inactivated at the embryonic stem cell stage. In CRFR1 KO mice exons 4–7 of the *Crrh1* gene were deleted and the mice were generated on a 129P2/OlaHsd

× CD1 background (Timpl *et al.*, 1998). In CRFR2 KO mice exons 3–4 of the *Crhr2* gene were deleted and the mice were generated on a 129X1/SvJ × C57BL/6 (B6) background (Coste *et al.*, 2000). In *Ucn1* KO mice exon 2 of the *Ucn* gene was deleted and the mice were generated on a 129X1/SvJ × B6 background (Vetter *et al.*, 2002). In CRF KO mice exon 2 of the *Crh* gene was deleted and the mice were generated on a 129S2/SvPas × B6 background (Muglia *et al.*, 1995). Each KO line was then backcrossed onto a B6 background for at least 8 generations and the mice used in the studies here were the offspring produced by heterozygous pairs. The mice were all weaned at approximately 21 days of age and housed with same-sex siblings with a maximum of 5 animals per cage. Prior to acclimation to the shifted light cycle, the mice were transferred to individual cages and remained single-housed throughout the duration of the experiment. At testing, the mice ranged in age from 7 to 13 weeks. Food was available *ad libitum* and animals had access to water at all times unless noted, i.e., when animals had access to either 20% ethanol or 10% sucrose. All experimental procedures used were approved by the OHSU Animal Care and Use Committee (IACUC) and complied with NIH ethical guidelines for the treatment of laboratory animals.

Experimental Design

Male and female CRFR1, CRFR2, *Ucn1* and CRF KO and WT mice were tested in a drinking in the dark (DID) paradigm. In the DID procedure, mice typically consume high enough amounts of ethanol to reach behavioral signs of intoxication and show blood ethanol concentrations (BECs) upwards of 100 mg% (Rhodes *et al.*, 2005; 2007; Ryabinin *et al.*, 2003; Sharpe *et al.*, 2005). On days 1 to 3, the mice were provided access to 20% ethanol (v/v), three hours into the dark cycle, for 2 hours. On day 4, access to ethanol was increased to 4 hours. Water was available at all other times. The volume of fluid consumed was recorded at the end of each session and on day 4, at the conclusion of the drinking session, trunk blood was collected for BEC analysis. In a separate set of experiments, CRFR1 KO and WT mice were also tested for 10% sucrose (w/v) consumption in the same DID procedure used for alcohol consumption in order to determine the specificity of the effect.

Blood ethanol concentration analysis

Blood samples were centrifuged and plasma removed and frozen at −20°C until analyzed. Blood ethanol concentrations (BECs in mg/dl) were obtained using an Analox alcohol analyzer (GL5 Analyser, Analox Instruments, London, UK).

Statistical analysis

Data are presented as means + S.E.M. Alcohol intakes (g/kg) for the first three experimental days were compared using analysis of variance (ANOVA) with repeated measures and for the CRFR2 data, ANOVAs were also carried out for each day with genotype and sex as the variables. For day 4, statistical comparisons were carried out using ANOVA with genotype and sex as the variables. An effect was considered to be statistically significant when $p < 0.05$.

RESULTS

Effects of genetic deletions of CRF receptors on alcohol consumption

As pharmacological studies have implicated the CRFR1 in binge-like excessive alcohol consumption and to avoid potential non-specific effects of pharmacological agents, we used KO mice deficient in CRFR1 or CRFR2 to investigate the contribution of specific CRF receptors to this behavior.

CRFR1 KO mice showed reduced alcohol intakes (g/kg) when compared to the corresponding WT mice. Figure 1A shows alcohol intakes, and 1B shows the BEC levels observed on day 4 at the conclusion of the drinking session. On days 1 to 3, while there was a sex and a genotype difference in alcohol intakes, there was no interaction between the two variables [Repeated measures ANOVA: Sex ($F_{1,88} = 5.85$, $P < 0.05$); Genotype ($F_{1,88} = 6.13$, $P < 0.05$)]. On day 4, CRFR1 KO consumed markedly less alcohol than their WT counterparts and as before there was also a sex effect but no interaction between sex and genotype. [ANOVA: Sex ($F_{1,88} = 12.54$, $P < 0.001$); Genotype ($F_{1,88} = 11.69$, $P = 0.001$)]. The resulting blood ethanol concentrations (BECs) were lower in CRFR1 KO mice compared to the WT mice [ANOVA: Genotype ($F_{1,73} = 6.26$, $P < 0.05$)]. There were fewer mice in the BEC analysis as blood was not collected for one cohort.

In contrast, CRFR2 KO mice showed sex and genotype effects as well as an interaction between sex and genotype on days 1 to 3 but only a sex difference on day 4 [Days 1–3, Repeated measures ANOVA: Sex ($F_{1,63} = 22.53$, $P < 0.001$), Genotype ($F_{1,63} = 5.58$, $P < 0.05$), Sex $\times$ Genotype $\times$ Ethanol intake ($F_{2,126} = 4.37$, $P < 0.05$); ANOVA, Day 4: Sex ($F_{1,63} = 34.09$, $P < 0.001$)]. When ANOVAs were carried out on the individual days, there was a significant difference in alcohol consumption between male and female mice over all three days (days 1–3), but the genotype effect was seen only on day 1 (ANOVA: Genotype ($F_{1,63} = 6.03$, $P < 0.05$)) (Fig. 2A). There were no differences in the BECs between male and female mice or between KO and WT mice (Fig. 2B).

Therefore, as deletion of CRFR1 and not CRFR2 results in decreased alcohol consumption in the DID model, it is CRFR1 and not CRFR2 that plays a critical role in excessive alcohol consumption.

The effect on CRFR1 deletion on sucrose consumption

Since CRFR1-deficient mice consumed less alcohol than their WT counterparts, we needed to test whether the involvement of CRFR1 was specific to alcohol or generalized to other rewarding substances. Therefore, we provided an independent set of CRFR1 KO and WT mice access to 10% sucrose and recorded sucrose consumption using the same DID protocol that we used in alcohol consumption study.

While there was a sex effect evident on all four experimental days [Days 1–3, Repeated measures ANOVA: Sex ($F_{1,77} = 12.16$, $P < 0.001$); Day 4, ANOVA: Sex ($F_{1,77} = 21.18$, $P < 0.001$)], CRFR1 KO mice consumed significantly greater amounts of 10% sucrose than the WTs only on days 1–3 [Days 1–3, Repeated measures ANOVA: Genotype ($F_{1,77} = 16.35$, $P < 0.001$)], but not on day 4 (Fig. 3). These data indicated that CRFR1 is not critical for sucrose consumption in this procedure.

Effects of the deletion of CRF and Ucn1 on alcohol consumption

Since in the previous experiments, we determined that CRFR1 promotes DID alcohol consumption, and since CRFR1 is the target for both CRF and Ucn1, we next investigated the ligand responsible using CRF and Ucn1 KO and their respective WT counterparts.

Ucn1 KO mice consumed similar amounts of alcohol as the WT mice. There was only a sex effect evident over all 4 days [Repeated measures ANOVA, Days 1–3: Sex ($F_{1,87} = 19.16$, $P < 0.001$); ANOVA, Day 4: Sex ($F_{1,87} = 17.77$, $P < 0.001$)] (Fig. 4A). There was no statistically significant difference in the BECs between male and female mice and between Ucn1 KOs and WTs (Fig. 4B).

In contrast, CRF KO mice consumed less alcohol over all four days when compared to the WT mice and there was also a sex effect evident [Repeated measures ANOVA, Days 1–3:

Sex ($F_{1,86} = 13.70$, $P < 0.001$), Genotype ($F_{1,86} = 12.41$, $P < 0.001$); ANOVA, Day 4: Sex ($F_{1,86} = 15.06$, $P < 0.001$); Genotype ($F_{1,86} = 6.47$, $P < 0.05$) (Fig. 5A). There was a significant genotype effect but no sex effect in BECs [ANOVA, Day 4, Genotype ($F_{1,86} = 5.78$, $P < 0.05$)] (Fig. 5B).

As there was a clear reduction in alcohol intakes in CRF KO mice, which was not observed in Ucn1 KO mice, when compared to their respective WT littermates, CRF appears to be the ligand acting on CRFR1 to promote alcohol consumption in the DID procedure.

DISCUSSION

Our studies for the first time tested in parallel four lines of mice with genetic deletions of components of the CRF system in a model of binge alcohol consumption, i.e., the DID paradigm. While CRFR2 KO mice did not show any distinct differences in binge drinking or BECs compared to their WT counterparts, CRFR1 KO mice showed lower alcohol intakes on all four days and lower BECs than the WT but greater sucrose intakes on days 1 to 3, indicating that the CRF receptor type 1 is involved in alcohol binge drinking. Furthermore, Ucn1 KO mice were found to drink similar amounts of alcohol and showed similar BEC levels as the WT, whereas CRF KO mice had clearly reduced alcohol intakes and BEC levels when compared to their WT littermates, indicating that CRF acting on CRFR1 promotes binge alcohol consumption. Importantly, the genetic deletion of CRFR1 and CRF prevented these mutants from reaching BECs that are considered intoxicating. These results cannot be attributed to potential differential alcohol metabolism between genotypes, as several studies have consistently shown no difference in blood alcohol elimination rates between these genotypes (Olive et al., 2003; Pastor et al., 2008; Sharpe et al., 2005; Sillaber et al., 2002).

Interestingly, for all the genotypes tested, female mice had higher alcohol intakes than male mice, but there was no difference in the BECs between the sexes, suggesting that alcohol clearance was different between females and males. This is in agreement with previous studies showing that female rodents consume greater amounts of alcohol and appear to be more sensitive to the effects of alcohol than males (Crippens et al., 1999; Lancaster and Spiegel, 1992; Middaugh et al., 1999; Peterson et al., 1991). This difference is most likely attributed to faster alcohol metabolism rates in female than male mice and rats (Crippens et al., 1999; Peterson et al., 1991). These findings may explain the discrepancy between alcohol intake and BECs between the sexes that we observed. However, for none of the genotype effects was there a significant interaction with sex, indicating that the contribution of CRF and CRFR1 receptors to binge alcohol consumption occurs in animals of both sexes.

Our results showing that CRFR1 KO mice consume less alcohol and have lower BECs than the corresponding WT mice in a binge drinking model bear out and strengthen recent pharmacological evidence showing that administration of a CRFR1 antagonist results in decreased binge drinking (Lowery et al., 2008; 2010; Sparta et al., 2008). Antagonists of the CRF receptor type 1 have also been reported to reduce alcohol intakes in dependent rats (Funk et al., 2007; Gehlert et al., 2007; Gilpin et al., 2008; Ji et al., 2008; Sommer et al., 2008). Further evidence showing the involvement of the CRFR1 in excessive drinking comes from a study carried out by Treutlein et al. (2006) where two SNPs in the CRFR1 gene were associated with heavy drinking in adolescents with little previous exposure to alcohol and in alcohol-dependent adults. The recent finding that CRFR1 KO mice had lower intakes of 20% ethanol but not of 3%, 6% and 10% ethanol when compared to WT mice in a 24 hour access two-bottle choice paradigm (Pastor et al. 2011) also provides suggestive evidence that binge, and not modest, drinking is affected. Taken together, our current genetic studies in KO mice, genetic studies in humans and previous and recent

pharmacological studies clearly implicate the CRFR1 in excessive alcohol consumption in both non-dependent and dependent subjects.

Mice deficient in CRFR2 did not show any distinct differences in alcohol intakes or in BECs, except on day 1, which may be due to a delay in acquisition of this behavior. Pharmacological studies in dependent rats have shown that administering a CRFR2 agonist, Ucn 3, into the CeA resulted in decreased drinking (Funk and Koob, 2007). Lowery et al. (2010) recently reported that Ucn 3, administered intracerebroventricularly in C57BL/6J mice, protected against binge drinking in a DID procedure similar to ours. Interestingly, Sharpe et al. (2005) showed that CRFR2 KO mice did not behave any differently to WT mice after continuous alcohol exposure but showed increased intakes at two out of ten stages of their limited access alcohol drinking procedure. Both these findings can be considered different from our observation of no difference between CRFR2 KO and WT mice in the DID procedure. However, in contrast to Sharpe et al. (2005) our procedure involves 4 hour access to ethanol and used a higher concentration of ethanol, and it has been argued that this protocol appears to be a better measure of the binge aspect of alcohol intake (Sparta et al., 2008). While our procedure is similar to the one used by Lowery et al. (2010), the mice in their study underwent stereotaxic implantation of cannulae and central administration of the CRFR2 agonist whereas our CRFR2 KO and WT mice were allowed to drink undisturbed. Another possible explanation for the discrepancy could be potential developmental compensations occurring in the CRFR2 KO mice. Importantly, none of the studies observed a decrease in intake in the CRFR2 KO mice, which implies that CRF type 2 receptors do not play a critical role in moderate or excessive binge-like alcohol consumption.

Since results from our first experiments showed that CRFR1 is critical in binge alcohol consumption and since both CRF and Ucn1 bind to CRFR1, it was logical to test Ucn1 and CRF KO and WT mice in the same DID protocol to determine the ligand responsible. A major caveat of pharmacological studies is the inability of pharmacological agents to distinguish between multiple ligands acting at the same receptor. In contrast, this can be investigated using KO mice. Ucn1 KO mice were not different from their WT counterparts in either alcohol intakes or BECs. Even though studies showing higher levels of Ucn1 immunoreactivity in mice and rats selectively bred to prefer alcohol suggest that Ucn1 plays a role in the predisposition to alcohol consumption (Fonareva et al., 2009; Ryabinin and Weitemier, 2006; Turek et al., 2005), the *Ucn1* gene appears not to be critical in the regulation of binge alcohol intake. We interpret these results as suggesting that Ucn1 may be critical in regulating alcohol acceptance or moderate drinking, but not in excessive alcohol consumption. In support of this, Bachtell et al. (2004) showed that electrolytic lesions of the Edinger-Westphal nucleus, which is the main source of Ucn1 in the brain, resulted in reduced alcohol preference and consumption in a two-bottle choice continuous access paradigm when the mice were given access to 3, 6 or 10% ethanol, but no difference was seen in preference or consumption between lesioned and sham mice when access was to 20% ethanol.

In contrast to our results with the Ucn1 KO mice, CRF KO mice consumed lower amounts of alcohol than their WT littermates and had lower BECs. Our data contrast with findings reported by Olive et al. (2003). In their study, CRF KO mice were found to consume more alcohol than the control mice in continuous and limited access experiments. A key difference between their study and ours is their use of F2 hybrids, obtained by breeding C57BL/6J and 129S1/SvImJ mice, as control mice. It is expected that F2 offspring from crossing high drinking and low drinking mouse strains would show low alcohol intake (Carr et al., 1998), as was observed in their study. In contrast, our study used WT littermates of the CRF KO mice, which have been largely backcrossed on to the C57BL/6J background and therefore show the characteristic high C57BL/6J-like alcohol intake. In addition, the

previous study did not use the DID model, so the investigated phenotypes across these studies are different, and this could serve as another argument that CRF acting on CRFR1 receptors is critical only for binge drinking, but not for moderate alcohol consumption.

Other alcohol-related phenotypes have also been investigated in these lines of knockout mice. Pastor et al. (2008) showed that CRFR1 KO and CRFR1+R2 KO, but not CRFR2 KO, mice showed attenuated psychomotor sensitization to ethanol. In the same set of experiments, Pastor et al. (2008) also showed that Ucn1 KO mice were not different compared to WT and concluded that CRF and CRFR1 were required for sensitization to ethanol to manifest. These findings on psychomotor sensitization to ethanol compare favorably with our data on binge alcohol consumption. Pastor et al. (2008) hypothesized that the hypothalamic-pituitary-adrenal (HPA) axis may underlie sensitization to alcohol. CRF, acting at the CRFR1, activates the HPA axis, which results in adrenocorticotrophic hormone (ACTH) release by the anterior pituitary and glucocorticoid release by the adrenal glands in response to stress (for reviews see Armario, 2006; Hauger et al., 2006; Smith and Vale, 2006). Alcohol is also reported to activate the HPA axis and this effect is blocked by administering CRF antiserum or antagonists and is absent in CRFR1 knockout mice, indicating that the effects of alcohol are exerted via the CRF system (Lee et al., 2001; Rivier, 1996; Rivier et al., 1996; Rivier et al., 1984; for review see Armario, 2010). Furthermore, activation of the HPA axis is also hypothesized to be a key factor in alcohol addiction (Fahlke et al., 2000; Higley et al., 1991; Uhart et al., 2006). Conversely, activating the HPA axis has also been shown to decrease alcohol consumption (Krishnan et al., 1991) or prevent relapse (Kiefer et al., 2006). Pastor et al. (2008) observed attenuated corticosterone levels in CRFR1 and CRFR1+R2 double KOs and concluded that CRFR1-mediated corticosterone release and subsequent activation of glucocorticoid receptors was necessary for sensitization to ethanol to occur.

Therefore, one could theorize that the effects of CRF and CRFR1 deletion on binge alcohol intake observed here could also be attributed to effects of these deletions on the HPA axis. To our knowledge, no studies on alcohol drinking in mice deficient in the glucocorticoid receptors have been reported. However, pharmacological experiments suggest that in contrast to locomotor sensitization, the HPA axis is not critical for binge alcohol consumption. Specifically, while Lowery and colleagues (2010) observed that administering a CRFR1 antagonist decreased binge drinking in agreement with the KO studies described here, they also assessed the contribution of the HPA axis by administering metyrapone, a corticosterone synthesis inhibitor; mifepristone, a glucocorticoid receptor antagonist and by investigating binge drinking after administering a CRFR1 antagonist in adrenalectomized (ADX) mice. They reported that metyrapone reduced both alcohol and sucrose consumption and was not selective, that mifepristone had no effect on alcohol drinking, and that plasma corticosterone levels after a DID procedure did not correlate with binge drinking. Also in agreement was the finding that the CRFR1 antagonist was effective in reducing binge drinking in ADX mice. Therefore, the HPA axis does not appear to play a critical role in binge alcohol consumption. It is likely therefore, that the contribution of CRFR1 to excessive alcohol intake is via actions of CRF on its central targets.

One caveat that has to be kept in mind when using genetic mouse models is the potential for developmental compensations to occur. Several compensations have been documented in the mice that we utilized. For example, in CRFR1 KO mice, CRF is over-expressed in the paraventricular nucleus (PVN), amygdala, hippocampus and cerebral cortex (Smith et al., 1998; Timpl et al., 1998). CRFR2 KO mice show no significant change in CRF mRNA levels in the PVN but increased levels in the central nucleus of the amygdala and increased levels of Ucn1 in the Edinger-Westphal nucleus (now known as the centrally projecting neurons of the Edinger-Westphal, EWcp; see Kozicz et al., 2011) (Bale et al., 2000; Coste et

al., 2000). Ucn 1 mRNA is increased in the EWcp of CRF KO mice (Weninger et al., 1999; 2000). In contrast, there appears to be no change in CRF or CRFR1 in Ucn1 KO mice and a slight decrease in CRFR2 gene expression in the lateral septum (Bale and Vale, 2004; Vetter et al., 2002; Wang et al., 2002). While it is theoretically possible that the increased Ucn1 expression in the CRF KO mice and increased CRF in the CRFR1 KOs contributed to our observations, the fact that both these peptides have also been reported to be increased in CRFR2 KO mice, which show no change in alcohol consumption, contradict this idea. Furthermore, pharmacological and genetic evidence discussed above suggests that these compensations would have potentially counteracted rather than contributed to a decrease in drinking. Therefore, our genetic data suggests that developmental compensations are insufficient to prevent the critical role of CRF, via actions on CRFR1, in binge alcohol consumption.

In conclusion, the studies detailed here and previous pharmacological studies show effects in the same direction and clearly implicate CRFR1 in binge drinking. Therefore, CRFR1 is a promising target for the pharmacotherapy of alcohol use disorders. Moreover, our studies with KO mice with their advantage over pharmacological studies, were able to differentiate between multiple ligands acting at the same receptor type, and identified CRF as the ligand responsible for the involvement of CRFR1 in promoting excessive alcohol drinking.

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FIG. 1.

Decreased alcohol intakes (g/kg) over all four days (A) and blood ethanol concentrations (BECs) on day 4 in female (F) and male (M) CRFR1 knockout (KO) mice compared to wild-type (WT) mice assessed in a drinking in the dark (DID) paradigm. In this experiment and the following alcohol consumption experiments, the mice had access to 20% ethanol for 2 hours on days 1–3 and for 4 hours on day 4. BECs were determined at the end of the last drinking session. All values are means + SEM. (N= 13–30 for the alcohol intake study and 6–27 for the BEC analysis). For ethanol intake for days 1–3, * denotes $p < 0.05$ comparing genotypes and # $p < 0.05$ comparing sex using a repeated measures ANOVA. For ethanol intake on day 4, *** $p = 0.001$ comparing genotypes and ### $p < 0.001$ comparing sexes using ANOVAs. In 1B, for BECs on day 4, * $p < 0.05$ comparing genotypes using an ANOVA.

FIG. 2.

Decreased alcohol intakes (g/kg) (A) in CRFR2 female (F) and male (M) knockout (KO) mice compared to wild-type mice (WT) over day 1 but there was only a sex effect on days 2–4. Blood ethanol concentrations (BECs) were not different between CRFR2 KO and WT mice. All values are means + SEM. (N=12–22). For ethanol intake for days 1–3, * denotes $p < 0.05$ comparing genotypes and ### $p < 0.001$ comparing sexes, and 'a' denotes an interaction between sex, genotype and ethanol intake $p < 0.05$ using a repeated measures ANOVA. For ethanol intake on day 4, ### $p < 0.001$ comparing sexes using ANOVAs. Ethanol intake data was also analyzed with ANOVAs for each day. For ethanol intake on day 1, * $p < 0.05$ comparing genotypes and ## $p < 0.01$ comparing sexes; for ethanol intake on day 2, # $p < 0.05$ comparing sexes and for ethanol intake on day 3, ### $p < 0.001$ comparing sexes using ANOVAs.

FIG. 3.

Increased sucrose intakes (ml/kg) CRFR1 sucrose over days 1–3 in female (F) and male (M) CRFR1 knockout (KO) mice compared to wild-type (WT) mice assessed in a drinking in the dark (DID) paradigm. The mice had access to 10% sucrose for 2 hours on days 1–3 and for 4 hours on day 4. All values are means + SEM. (N=12–27). For sucrose intake on days 1–3, analyzed using a repeated measures ANOVA, *** $p < 0.001$ comparing genotypes and ### $p < 0.001$ comparing sexes. There was only a sex effect on day 4; sucrose intake on day 4, ### $p < 0.001$ using an ANOVA.

FIG. 4.

No change was observed in alcohol intakes (g/kg) over all four days (A) and in blood ethanol concentrations (BECs) on day 4 in female (F) and male (M) Ucn1 knockout (KO) mice compared to wild-type (WT) mice assessed in a drinking in the dark (DID) paradigm. The mice had access to 20% ethanol for 2 hours on days 1–3 and for 4 hours on day 4. BECs were determined at the end of the last drinking session. All values are means + SEM. (N= 19–29). For ethanol intake for days 1–3, ### $p < 0.001$ comparing sexes using a repeated measures ANOVA. For ethanol intake on day 4, sex effect, ### $p < 0.001$ using an ANOVA.

FIG. 5. Decreased alcohol intakes (g/kg) over all four days (A) and blood ethanol concentrations (BECs) on day 4 in female (F) and male (M) CRF knockout (KO) mice compared to wild-type (WT) mice assessed in a drinking in the dark (DID) paradigm. All values are means + SEM. (N= 12–36). For ethanol intake for days 1–3, *** denotes $p < 0.001$ comparing genotypes and ### $p < 0.001$ comparing sexes using a repeated measures ANOVA. For ethanol intake on day 4, * $p = 0.05$ comparing genotypes and ### $p < 0.001$ comparing sexes using ANOVAs. In 1B, for BECs on day 4, * $p < 0.05$ comparing genotypes using an ANOVA.