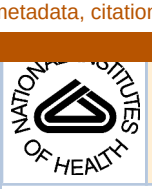

Published in final edited form as:

CNS Neurol Disord Drug Targets. 2010 March 1; 9(1): 77–86.

Pre-Clinical Evidence that Corticotropin-Releasing Factor (CRF) Receptor Antagonists are Promising Targets for Pharmacological Treatment of Alcoholism

Emily G. Lowery[1] and **Todd E. Thiele**[1,2,*]

[1]Department of Psychology University of North Carolina, Chapel Hill, NC 27599-3270

[2]Bowles Center for Alcohol Studies University of North Carolina, Chapel Hill, NC 27599-3270

Abstract

Alcoholism is a chronic disorder characterized by cycling periods of excessive ethanol consumption, withdrawal, abstinence and relapse, which is associated with progressive changes in central corticotropin-releasing factor (CRF) receptor signaling. CRF and urocortin (Ucn) peptides act by binding to the CRF type 1 (CRF1R) or the CRF type 2 (CRF2R) receptors, both of which have been implicated in the regulation of neurobiological responses to ethanol. The current review provides a comprehensive overview of preclinical evidence from studies involving rodents that when viewed together, suggest a promising role for CRF receptor (CRFR) antagonists in the treatment of alcohol abuse disorders. CRFR antagonists have been shown to protect against excessive ethanol intake resulting from ethanol dependence without influencing ethanol intake in non-dependent animals. Similarly, CRFR antagonists block excessive binge-like ethanol drinking in non-dependent mice but do not alter ethanol intake in mice drinking moderate amounts of ethanol. CRFR antagonists protect against increased ethanol intake and relapse-like behaviors precipitated by exposure to a stressful event. Additionally, CRFR antagonists attenuate the negative emotional responses associated with ethanol withdrawal. The protective effects of CRFR antagonists are modulated by the CRF1R. Finally, recent evidence has emerged suggesting that CRF2R agonists may also be useful for treating alcohol abuse disorders.

Keywords

Corticotropin-releasing factor; urocortin; CRF receptor; alcoholism; dependence; withdrawal; relapse; ethanol

Corticotropin-releasing factor (CRF) is a 41-amino acid poly-peptide that is widely expressed throughout the central nervous system (CNS) and modulates a range of neurobiological responses through activation of the G_s-protein coupled CRF type 1 (CRF1R) and type 2 (CRF2R) receptors [1-4]. While CRF binds to both receptors, it has greater affinity to the CRF1R [1,5,6]. CRFRs are also stimulated by the 40-amino acid urocortin (Ucn) family of peptides, with Urocortin I (Ucn1) displaying equal affinity for both CRF1R and CRF2R, and Urocortin II (Ucn2) and Urocortin III (Ucn3) displaying affinity primarily for the CRF2R [1, 6,7]. In rodents, expression of the CRF1R is ubiquitous throughout the brain, with high density found in hypothalamic, cortical, and limbic regions, while CRF2R expression is limited to specific regions, including the raphe nuclei, lateral septum, and subregions of the amygdala

*Address correspondence to: Department of Psychology, CB#3270 University of North Carolina Chapel Hill, NC 27599-3270, USA Phone: 919-966-1519; Fax: 919-962-2537; thiele@unc.edu.

and hypothalamus [1]. Agonist binding of these receptors induces distinct outcomes with respect to cellular signaling pathways, downstream mechanisms, and behavior [1,8,9]. CRF and Ucn signaling through CRF1Rs and CRF2Rs have been implicated in a number of biobehavioral processes, including regulation of the hypothalamic-pituitary-adrenal (HPA) axis stress response, anxiety, depression, feeding, and excessive alcohol consumption [1,3,6, 10-14].

Alcoholism is a chronic and progressive disorder characterized by cyclic patterns of excessive ethanol self-administration intermixed with periods of withdrawal and abstinence, followed by relapse [12,15]. As such, alcoholism can be conceptualized in terms of shifts in allostatic load, wherein repeated exposure and withdrawal from alcohol promote gradual neurobiological alterations within the brain which translate into psychological and behavioral changes leading to excessive uncontrolled ethanol consumption [12]. A growing literature suggests that the central CRFR signaling system exhibits plastic changes as ethanol dependence emerges [3, 12,16,17]. In this review, we provide a comprehensive overview of preclinical evidence from studies involving rodents that when viewed together, suggests a promising role for CRF receptor (CRFR) antagonists (and possibly CRF2R agonists) in the treatment of alcohol abuse disorders, including excessive ethanol intake resulting from ethanol dependence and exposure to stressful stimuli, relapse of ethanol-seeking behavior precipitated by stress, and negative emotional responses (such as anxiety) stemming from ethanol withdrawal. Interestingly, more recent evidence has emerged suggesting that CRFR antagonists may be effective in treating binge drinking prior to the development of ethanol dependence. The converging evidence within the preclinical literature, and the continued development of improved CRFR antagonists, make compounds aimed at CRFRs attractive targets for potential treatment of alcohol abuse disorders and alcoholism.

The Effects of Ethanol on the CRF/Ucn System

Ethanol produces immediate effects on CRF and Ucn signaling. Acute ethanol administration is accompanied by increases in levels of CRF [18] and CRF-like immunoreactivity (CRF-IR) [19], as well as increased levels of CRF heteronuclear RNA (hnRNA) and messenger RNA (mRNA) [18,20,21] in the hypothalamus. Acute ethanol administration also induces activation of Ucn-positive cells in the perioculomotor urocortin-containing population of neurons (pIIIu, also known as the Edinger-Westphal nucleus) [22]. With respect to receptors, acute ethanol exposure is correlated with increased CRF1R mRNA expression in the hypothalamus [23]. No changes in CRF2R mRNA expression or binding have been noted following acute ethanol exposure in any brain region assessed to date [23,24]. Together, these findings show that the CRF/Ucn system is modified in the hypothalamus and plllu during the early stages of ethanol exposure.

With repeated administration and withdrawal, ethanol induces further alterations in the CRF/ Ucn system. For example, upregulation of CRF markers, including extracellular CRF, pre-pro CRF mRNA, and CRF mRNA have been reported in the amygdala [25], and more specifically, within the central nucleus of the amygdala (CeA) [26-28] in dependent, ethanol-withdrawn rats relative to non-dependent controls. Likewise, increased levels of extracellular CRF have been observed in the bed nucleus of the stria terminalis (BNST) [29] and enhanced CRF mRNA expression has been noted in the paraventricular nucleus of the hypothalamus (PVN) after chronic ethanol exposure [30,31]. Additionally, increased CRF1R expression has been observed in the basolateral amygdala (BLA) and the medial nucleus of the amygdala (MeA) [26], as well as the hypothalamus [32] in dependent, ethanol-withdrawn rats. Marked alterations in CRF-induced excitability in the BNST have been observed following prolonged exposure to ethanol [for review, see 33]. Additionally, decreases in Ucn1 fibers were noted in the lateral septum and dorsal raphe of mice with a history of ethanol exposure [24]. Decreases

in CRF2R expression were observed in the BLA of ethanol dependent rats [26], while increases have been observed in the dorsal raphe of mice [24], and the hypothalamus of rats [32] with a history of ethanol exposure. Long-term investigations show that some of these neurobiological changes in CRFR signaling persist months into abstinence, which may contribute to the enhanced anxiety-like behaviors and stress responsiveness that are observed long after ethanol administration has ceased [15,34-37]. Interestingly, follow-up investigations show that some of these changes can be normalized through reinstatement of ethanol self-administration [29]. Thus, the literature suggests that chronic ethanol exposure and withdrawal promote alterations in CRF/Ucn signaling in regions of the amygdala, the lateral septum, the dorsal raphe, and the hypothalamus. These observations are consistent with the hypothesis that a dysregulation of CRFR signaling emerges over the course of ethanol dependence, and that this dysregulation may contribute to the excessive and uncontrolled ethanol intake associated with ethanol dependence. The effects of ethanol on CRFR activity lead to the predictions that a) CRFR antagonists may protect against excessive ethanol drinking in non-dependent animals (since initial ethanol exposure augments CRFR signaling) and b), CRFR antagonists may protect against dependence-induced increases of ethanol intake as well as the negative emotional responses associated with ethanol dependence (since CRFR signaling is upregulated in dependent animals). In general, the studies that are reviewed below are consistent with these predictions.

Pharmacological Evidence for a Role of CRFR Signaling in Ethanol Consumption

The Effects of CRFR Compounds on the Early Stages of Ethanol Consumption

A growing body of preclinical literature is consistent with the idea that CRFR antagonists (and possibly CRF2R agonists) are promising targets for preventing excessive ethanol intake (see Table 1). With respect to moderate ethanol intake in the early stages of ethanol drinking, results from numerous investigations indicate that the involvement of CRFR signaling is limited. For example, central administration of non-selective CRFR antagonists, such as [$_{D}$-Phe^{12},Nle^{21,38},CαMeLeu^{37}]-rCRF$_{(12-41)}$ ($_{D}$-Phe-CRF) and α-helical CRF$_{(9-41)}$ (ahCRF), does not significantly alter ethanol consumption or self-administration in non-dependent rats or mice with a history of ethanol exposure akin to social drinking in humans [27,38,39]. Similar results have been obtained using peripheral administration of antagonists selective for the CRF1R, including (*N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylpheenyl)-2,5-dimethyl-pyrazolo [1,5-*a*]pyrimidin-7-amine (MPZP) [40], 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine (MTIP) [41], (4-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amino-1-butanol (LWH-63) [42], 2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo [1,5-*a*]pyrimidine (R121919, also called NBI 30775) [42], and [8-(4-bromo-2-chlorophenyl)-2,7-dimethyl-pyrazolo[1,5-*a*][1,3,5]triazin-4-yl]-*bis*-(2-methyoxyethyl)amine (MJL-1-109-2) [42]. These observations indicate that CRFR signaling does not modulate moderate levels of ethanol consumption in non-dependent animals.

Interestingly, recent work suggests that CRFR signaling modulates ethanol intake in non-dependent rodents when the level of ethanol intake is high. “Drinking in the Dark” procedures were recently described to cause significant amounts of ethanol intake in a limited period of time by C57BL/6J mice, akin to an ethanol “binge” in humans [43-46]. DID procedures involve giving C57BL/6J mice access to a 20% ethanol solution for 2 to 4-hours starting 3-hours into their dark cycle. With these procedures, mice will drink enough ethanol to achieve blood ethanol levels (BELs) of 80 mg/dL or greater and exhibit signs of behavioral intoxication after a binge-like drinking episode [43,44]. Pretreatment with the CRF1R antagonist butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7Hpyrrolo[2,3-d]pyrimidin-4-yl]amine

(CP-154,526) significantly attenuated binge-like drinking by C57BL/6J mice (which achieved BELs of greater than 80 mg/dL under control conditions). On the other hand, the CRF1R antagonist was ineffective in altering ethanol consumption in mice drinking moderate amounts of ethanol and which achieved BELs of less than 40 mg/dL [45]. These observations suggest that CRF1R signaling is recruited during excessive, but not moderate, ethanol drinking. More recently, the protective effects of CP-154,526 against binge-like drinking were found to be independent of HPA axis signaling, and central administration of the CRFR antagonist ahCRF or the CRF2R agonist Ucn3 blunted binge-like ethanol drinking in C57BL/6J mice [47]. Additionally, microinjection of Ucn1 into the lateral septum significantly reduced binge-like ethanol drinking in C57BL/6J mice [48]. Since Ucn1 stimulates CRF2Rs, which are abundant in the lateral septum [1], and because activation of CRF2R with Ucn3 reduced ethanol intake [49] and binge-like ethanol drinking [47], it is possible that Unc1-induced reduction of binge-like ethanol drinking was modulated by the CRF2R in the lateral septum. Together, these findings show that CRFR signaling can modulate ethanol consumption in non-dependent animals, particularly when the levels of ethanol intake are high (and which are associated with significant BELs as in the case of binge-like drinking mice). An interesting possibility is that CRF1R antagonists and CRF2R agonists may be useful for treating problematic binge drinking in humans. On the other hand, pretreatment with the CRF1R antagonist MPZP failed to alter binge-like consumption in rats [50]. Inconsistencies between studies may be accounted for by differences in the species used, or the use of sweetened ethanol in the rat study.

The effects of exogenous CRF on ethanol intake has also been examined, though with less conclusive results. Central administration of CRF either had no effect on ethanol intake in mice [39] or significantly reduced ethanol consumption by rats [51,52] that were not ethanol-dependent. The counterintuitive results of such experiments may be explained by CRF's dual role in ethanol consumption and stress, as central CRF administration triggers a stress response which can disrupt behavioral activity [53] therefore making it difficult to disseminate the effects of stress from those of CRF on ethanol consumption in these experiments.

Finally, the effects of CRFR antagonists on stress-induced ethanol intake have also been assessed in non-ethanol dependent rodents. Pretreatment with the CRF1R antagonist CP-154,526 or antalarmin before exposure to stress-inducing stimuli significantly attenuated stress-induced increases in ethanol consumption by mice [54] and stress-induced increases of ethanol self-administration in rats [55], respectively. Additionally, antalarmin attenuated increased ethanol drinking stemming from early life stress exposure in rats with inherently high levels of anxiety [56]. On the other hand, pretreatment with either of two CRF1R antagonists (R121919 or antalarmin) failed to prevent stress-induced increases of ethanol consumption in mice [57]. While more work is necessary, these initial observations suggest that CRF1R antagonist may be useful for treating excessive ethanol drinking triggered by stressful life events in humans.

The Effects of CRFR Compounds on Dependence-Induced Ethanol Intake

Perhaps the most convincing evidence of a role for CRFR signaling in ethanol consumption is revealed by investigations of animals in which ethanol dependence has been induced by repeated exposure to, and withdrawal from, ethanol vapor or an ethanol-containing diet. A converging body of literature indicates a pivotal role for CRF1R signaling in dependence-induced ethanol consumption, and recent studies have suggested a role for the CRF2R. Central administration of the non-selective CRF antagonist $_{D}$-Phe-CRF into the ventricles attenuated dependence-induced increases in ethanol consumption in rats [34], as did peripheral administration of selective CRF1R antagonists, including antalarmin [58], MPZP [40,59], LWH-63 [42], MJL-1-109-2 [60], R121919 [60], and MTIP [60]. Importantly, as noted above manipulation of CRFR signaling with these antagonists did not alter ethanol drinking in non-

dependent animals that drank moderate amounts of ethanol. Further evidence indicates that the role of CRF1R signaling in dependence-induced increases in ethanol consumption is brain region-specific, as microinjections of $_{D}$-Phe-CRF into the CeA, but not the BNST, attenuated increased levels of ethanol consumption in ethanol-dependent rats to the levels of non-dependent controls [27,38]. Likewise, activation of the CRF2R by ventricular [61], or site-directed infusion into the CeA [62] of Ucn3 also reduced ethanol consumption by ethanol-dependent rats. Together, these observations show the CRFR antagonists (and specifically those aimed at the CRF1R) and CRF2R agonists protect against dependence-induced increases in ethanol drinking. Furthermore, the CeA is a key brain region in which CRF1R blockade and CRF2R stimulation modulates dependence-induced ethanol intake.

Relative to low ethanol drinking Wistar rats, high ethanol drinking msP rats, which were selectively bred for high ethanol intake, exhibit evidence of an inherent upregulation of CRFR signaling in a pattern that resembles ethanol-dependent animals [63]. Thus, these animals provide a model in which the effects of pharmacological agents can be verified in genetically predisposed populations. Recent investigations revealed that the CRF1R antagonists MTIP [41] and antalarmin [64] attenuated ethanol self-administration in non-dependent msP rats, without effects in non-dependent outbred rats. Thus, alterations of normal CRFR signaling can be achieved by ethanol dependence or genetic selection, and high levels of ethanol intake associated with either genetic predisposition or a history of ethanol dependence can be significantly attenuated by treating animals with CRF1R antagonists. An exciting possibility based on these results is that CRF1R antagonists will be effective in curbing excessive ethanol intake in genetically predisposed individuals, as well as those who are ethanol-dependent.

The Effects of CRFR Compounds on Relapse-Like Behaviors

Pharmacological evidence demonstrates a role for CRFR signaling in ethanol relapse-like behaviors in rodents with models of reinstatement and alcohol deprivation. Reinstatement experiments typically involve operant self-administration procedures in which rodents learn to perform a specific behavior (e.g., press a lever) to gain access to ethanol reinforcement. Following the establishment of stable ethanol-reinforced lever pressing, the operant behavior is extinguished by withholding the ethanol reinforcer. Over the course of extinction, the rate of lever pressing declines. Reinstatement of ethanol-seeking behavior is assessed by exposing the subject to specific stimuli during extinction responding. Stimuli that can promote reinstatement of ethanol-seeking behavior include stressful stimuli such as foot-shock or ethanol-associated cues [65-68]. Reinstatement of ethanol-seeking behavior (e.g., increased pressing of the lever that was previously reinforced with ethanol) is thought to model relapse of ethanol seeking in abstinent humans, which can be triggered by stressful events or by exposure to stimuli associated with ethanol. CRFR signaling has been shown to modulate reinstatement of ethanol-seeking behavior, particularly reinstatement associated with exposure to stressful stimuli. For example, central administration of the non-selective CRF receptor antagonist $_{D}$-Phe-CRF attenuated stress-induced reinstatement of ethanol-seeking behavior in rats with a history of prolonged ethanol exposure [69] and in ethanol-dependent rats [70]. Furthermore, reinstatement of ethanol-seeking behavior is modulated by CRFR signaling within the medial raphe nucleus (MRN), as a microinjection of $_{D}$-Phe-CRF into this brain region attenuated, while microinjection of CRF exacerbated, stress-induced reinstatement of ethanol-seeking behavior in rats [71]. Similar results have been obtained using peripheral administration of the CRF1R antagonists antalarmin [55] and CP-154,526 [69]. The role of CRFR signaling is specific to stress-induced reinstatement, as $_{D}$-Phe-CRF did not interfere with reinstatement of ethanol-seeking behavior elicited by ethanol-associated cues in rats [70]. Taken together, these observations show the CRFR signaling modulates reinstatement of ethanol-seeking behavior triggered by exposure to stressful stimuli, but is not involved in reinstatement induced by exposure to ethanol-associated cues. As CRF1R antagonists protect

against stress-induced reinstatement, such compounds may have therapeutic value for preventing relapse in ethanol dependent individuals vulnerable to stress-related disorders or in individuals that are confronted with stressful environmental stimuli.

Forced abstinence from ethanol following a history of ethanol consumption is associated with a transient increase in the amount of ethanol consumed upon re-exposure. This phenomenon has been labeled the alcohol deprivation effect (ADE), and is thought to model the robust increase in ethanol consumption that is characteristic of human alcoholics during the initial phases of relapse [65,66,72-74]. Recent evidence suggests the involvement of CRF1R signaling in the modulation of the ADE, as peripheral pretreatment with CP-154,526 attenuated deprivation-induced increases in ethanol self-administration by mice with a history of ethanol exposure without influencing self-administration of a sucrose solution [75]. Furthermore, exacerbation of the ADE by stress exposure in rats was attenuated by pretreatment with CP-154,526 and the CRF1R antagonist (2-(*N*-(2-methylthio-4-isopropylphenyl)-*N*-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine) (CRA1000) [76].These studies suggest that CRF1R antagonists may be useful for curbing the amount of ethanol that is consumed following relapse.

Pharmacological Evidence for a Role of CRFR Signaling in Withdrawal-Induced Emotional Responses

Relapse is hypothesized to be precipitated, in part, by heightened levels of anxiety experienced by individuals during periods of abstinence [12,15]. As such, alleviation of withdrawal-induced anxiety and other negative emotional responses may be one strategy to reduce the risk of relapse and thus has been the focus of many preclinical investigations (see Table 2). Dysregulated CRF signaling contributes to increased anxiety-like behaviors experienced during acute ethanol withdrawal [27,77] as well as during protracted abstinence [78], and a converging body of evidence suggests that both CRF1R and CRF2R signaling modulate these effects. For example, central administration of CRF potentiated withdrawal-induced anxiety-like behaviors in rats, while central pretreatment with the non-selective CRFR antagonists ahCRF [79] or $_{D}$-Phe-CRF [37,80] alleviated withdrawal-induced anxiety-like behaviors. The amygdala appears to modulate this behavior, since reductions in withdrawal-induced anxiety-like behaviors were observed following microinjections of $_{D}$-Phe-CRF into the CeA [80]. Likewise, peripheral administration of the CRF1R antagonists MTIP [41], CP-154,526 [77,81,82], or CRA-1000 [76,77,83-85] significantly attenuated withdrawal-induced anxiety-like behavior in ethanol-dependent rats, results which strongly suggest that CRF1R signaling is up-regulated during periods of ethanol withdrawal. In contrast to CRF1Rs, activation of central CRF2R appears to attenuate withdrawal-induced anxiety-like behavior, as pretreatment with the CRF2R-selective ligand Ucn3 effectively reduced anxiety-like behavior in ethanol-dependent rats withdrawn from ethanol [61]. Furthermore, pretreatment with the CRF2R antagonist antisauvagine-30 had no effect on withdrawal-related anxiety-like behavior [77]. Together, these observations suggest that CRF1R antagonists, and CRF2R agonists, may be useful treatments in the prevention of anxiety experienced by abstinent alcoholics, which may further reducing the risk of relapse.

Summary and Translational Perspectives

The current preclinical literature indicates a broad role for CRFR signaling in modulating a spectrum of neurobiological responses to ethanol. Consistently, CRF1R antagonists protect against 1) excessive binge-like ethanol consumption and increases of ethanol consumption resulting from exposure to stressful stimuli, 2) excessive ethanol intake resulting from ethanol dependence, 3) heightened anxiety-like behavior stemming from ethanol withdrawal, and 4) stress-induced reinstatement ethanol-seeking behavior as well as excessive ethanol intake

following periods of ethanol abstinence. These observations suggest that CRF1R antagonists are attractive targets for the development of pharmacological compounds aimed at treating ethanol abuse disorders, ethanol dependence, and relapse in abstinent alcoholics. A preclinical literature is also emerging suggesting a potential therapeutic role for CRF2R agonists, though this literature is limited and the role of the CRF2R requires additional characterization.

The increase of ethanol consumption in ethanol-dependent animals has been hypothesized to be modulated, in part, by the ability of ethanol to alleviate the negative emotional responses that result from ethanol dependence [12,16,17,86,87]. The negative emotional state associated with ethanol dependence is thought to be modulated by increases of CRF1R signaling, and thus the ability of CRF1R antagonists to protect against dependence-induced drinking (and relapse in ethanol-withdrawn rodents) is hypothesized to stem from the ability of CR1R antagonists to blunt negative affect [3]. Consistently, as noted above, CRF1R antagonists blunt dependence-induced drinking but do not alter drinking in non-dependent animals (which exhibit normal CRF activity).

More recently, evidence has emerged suggesting that CRF1R antagonist may also protect against excessive binge-like drinking in non-dependent mice without altering ethanol drinking in mice consuming moderate amounts of ethanol [45]. These observations expand the literature by showing that CRF1R signaling is recruited during the early phases of ethanol ingestion, and expand the potential therapeutic role for CRF1R antagonists. Frequent binge drinking during young adulthood is associated with an increased risk for developing alcoholism later in life [88-90] and an interesting possibility is that repeated ethanol binges lead to the development of ethanol dependence by inducing significant allostatic neuroadaptations in CRFR signaling. Viewed this way, repeated activation of the CRF system during binge drinking episodes leads to a progressive and chronic upregulation of CRFR signaling which culminates in ethanol dependence. Thus, treating binge drinking with CRF1R antagonists (or CRF2R agonists as noted above) may be an effective strategy for preventing ethanol dependence.

While considering the potential for CRFR antagonists in the treatment of alcohol abuse disorders and alcoholism, it is important to note potential caveats. First, CRFR signaling has been implicated in the modulation of multiple neurobiological systems, including those that regulate feeding, anxiety and depression, HPA axis signaling, and ethanol consumption [1,3, 91-97]. As such, careful attention must be given to potential unwanted side-effects when assessing the therapeutic role of CRFR antagonists in treating alcoholism in clinical populations. Second, the etiology of alcoholism is complex and multifaceted. Therefore, the effectiveness of CRFR antagonists may be limited to specific sub-populations of clinically diagnosed alcoholics. Similarly, CRFR antagonists are likely to be useful in treating specific components of alcoholism. In preclinical work, CRFR antagonists protected against stress-induced reinstatement in rats, but were ineffective in blocking reinstatement induced by stimuli associated with ethanol [70]. Thus, CRFR antagonists may be useful for reducing the risk of relapse trigger by stressful events, but not relapse stemming from ethanol-associated stimuli. Third, the viability of CRFR compounds as pharmacological treatments has been historically limited, as obstacles to clinical use include the solubility and oral bioavailability of the CRFR compounds [41,98]. Though these issues have limited clinical testing, several new compounds with improved bioavailability and receptor selectivity are currently being evaluated [40,41, 99,100]. Thus, the converging evidence within the preclinical literature, and the continued development of better CRFR antagonists, make compounds aimed at CRFRs attractive targets for treating alcohol abuse disorders and alcoholism.

Acknowledgments

This work was supported by the NIH grants AA017803, AA013573, and AA015148, and the Department of Defense grants W81XWH-06-1-0158 and W81XWH-09-1-0293.

ABBREVIATIONS

PEPTIDE

CRF	Corticotropin-releasing factor
Ucn	Urocortin
Ucn1	Urocortin I
Ucn2	Urocortin II
Ucn3	Urocortin III

RECEPTOR

CRF1R	Corticotropin-releasing factor type 1 receptor
CRF2R	Corticotropin-releasing factor type 2 receptor
CRFR	Corticotropin-releasing factor receptor

QUANTITATIVE MEASURE

CRF-IR	Corticotropin-releasing factor-like immunoreactivity
hnRNA	Heteronuclear ribonucleic acid
mRNA	Messenger ribonucleic acid
BEL	Blood ethanol levels
K_i	Dissociation constant
IC_{50}	Median inhibitory concentration
ADE	Alcohol deprivation effect

BRAIN REGION

HPA	Hypothalamic-pituitary-adrenal
pIIIu	Perioculomotor urocortin-containing population of neurons
CeA	Central nucleus of the amygdala
BNST	Bed nucleus of the stria terminalis
PVN	Paraventricular nucleus of the hypothalamus
BLA	Basolateral amygdala
MeA	Medial nucleus of the amygdala
NAcSh	Nucleus accumbens shell
MRN	Medial raphe nucleus
LS	Lateral septum
DRN	Dorsal raphe nucleus

DRUG NAME	
$_{D}$-Phe-CRF	[$_{D}$-Phe^{12},Nle^{21,38},CαMeLeu^{37}]-rCRF(12-41)
ahCRF	α-helical CRF$_{(9-41)}$
MPZP	(*N*,*N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylpheenyl)-2,5-dimethyl-pyrazolo[1,5-*a*]pyrimidin-7-amine
MTIP	3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine
LWH-63	(4-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*] pyrimidin-4- yl]amino-1-butanol
R121919	2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1,5- *a*]pyrimidine
MJL-1-109-2	[8-(4-bromo-2-chlorophenyl)-2,7-dimethyl-pyrazolo[1,5-*a*][1,3,5] triazin-4-yl]-*bis*-(2-methyoxyethyl)amine
CP-154,526	butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7Hpyrrolo[2,3-d] pyrimidin-4-yl]amine
CRA1000	(2-(*N*-(2-methylthio-4-isopropylphenyl)-*N*-ethylamino-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl)-6-methylpyrimidine)

ROUTE OF ADMINISTRATION	
i.c.v.	Intracerebroventricular
i.p.	Intraperitoneal
s.c.	Subcutaneous

References

1. Hauger RL, Risbrough V, Brauns O, Dautzenberg FM. Corticotropin releasing factor (CRF) receptor signaling in the central nervous system: new molecular targets. CNS & neurological disorders drug targets 2006;5:453–79. [PubMed: 16918397]
2. Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. The Journal of comparative neurology 2000;428:191–212. [PubMed: 11064361]
3. Heilig M, Koob GF. A key role for corticotropin-releasing factor in alcohol dependence. Trends in neurosciences 2007;30:399–406. [PubMed: 17629579]
4. Gulyas J, Rivier C, Perrin M, Koerber SC, Sutton S, Corrigan A, Lahrichi SL, Craig AG, Vale W, Rivier J. Potent, structurally constrained agonists and competitive antagonists of corticotropin-releasing factor. Proceedings of the National Academy of Sciences of the United States of America 1995;92:10575–9. [PubMed: 7479843]
5. Pioszak AA, Parker NR, Suino-Powell K, Xu HE. Molecular recognition of corticotropin-releasing factor by its G-protein-coupled receptor CRFR1. The Journal of biological chemistry 2008;283:32900–12. [PubMed: 18801728]
6. Ryabinin AE, Bachtell RK, Heinrichs SC, Lee S, Rivier C, Olive MF, Mehmert KK, Camarini R, Kim JA, Koenig HN, Nannini MA, Hodge CW, Roberts AJ, Koob GF. The corticotropin-releasing factor/urocortin system and alcohol. Alcohol Clin Exp Res 2002;26:714–22. [PubMed: 12045481]
7. Venihaki M, Sakihara S, Subramanian S, Dikkes P, Weninger SC, Liapakis G, Graf T, Majzoub JA. Urocortin III, a brain neuropeptide of the corticotropin-releasing hormone family: modulation by stress and attenuation of some anxiety-like behaviours. Journal of neuroendocrinology 2004;16:411–22. [PubMed: 15117334]

8. Fu Y, Neugebauer V. Differential mechanisms of CRF1 and CRF2 receptor functions in the amygdala in pain-related synaptic facilitation and behavior. J Neurosci 2008;28:3861–76. [PubMed: 18400885]
9. Zhao Y, Valdez GR, Fekete EM, Rivier JE, Vale WW, Rice KC, Weiss F, Zorrilla EP. Subtype-selective corticotropin-releasing factor receptor agonists exert contrasting, but not opposite, effects on anxiety-related behavior in rats. The Journal of pharmacology and experimental therapeutics 2007;323:846–54. [PubMed: 17855476]
10. Kozicz T. CRF and CRF-related peptides in stress adaptation: from invertebrates to man. General and comparative endocrinology 2007;153:198–9. [PubMed: 17666206]
11. Kozicz T. On the role of urocortin 1 in the non-preganglionic Edinger-Westphal nucleus in stress adaptation. General and comparative endocrinology 2007;153:235–40. [PubMed: 17517410]
12. Koob GF. Alcoholism: allostasis and beyond. Alcohol Clin Exp Res 2003;27:232–43. [PubMed: 12605072]
13. Clark MS, Kaiyala KJ. Role of corticotropin-releasing factor family peptides and receptors in stress-related psychiatric disorders. Seminars in clinical neuropsychiatry 2003;8:119–36. [PubMed: 12728411]
14. Latchman DS. Urocortin. The international journal of biochemistry & cell biology 2002;34:907–10.
15. Breese GR, Chu K, Dayas CV, Funk D, Knapp DJ, Koob GF, Le AD, O'Dell L, Overstreet DH, Roberts AJ, Sinha R, Valdez GR, Weiss F. Stress enhancement of craving during sobriety: A risk for relapse. Alcohol Clin Exp Res 2005;29:185–95. [PubMed: 15714042]
16. Koob GF. A role for brain stress systems in addiction. Neuron 2008;59:11–34. [PubMed: 18614026]
17. Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. The American journal of psychiatry 2007;164:1149–59. [PubMed: 17671276]
18. Li Z, Kang SS, Lee S, Rivier C. Effect of ethanol on the regulation of corticotropin-releasing factor (CRF) gene expression. Molecular and cellular neurosciences 2005;29:345–54. [PubMed: 15914027]
19. Redei E, Branch BJ, Gholami S, Lin EY, Taylor AN. Effects of ethanol on CRF release in vitro. Endocrinology 1988;123:2736–43. [PubMed: 2848685]
20. Lee S, Selvage D, Hansen K, Rivier C. Site of action of acute alcohol administration in stimulating the rat hypothalamic-pituitary-adrenal axis: comparison between the effect of systemic and intracerebroventricular injection of this drug on pituitary and hypothalamic responses. Endocrinology 2004;145:4470–9. [PubMed: 15205375]
21. Rivier C, Lee S. Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. Brain Res 1996;726:1–10. [PubMed: 8836539]
22. Li W, Challis JR. Corticotropin-releasing hormone and urocortin induce secretion of matrix metalloproteinase-9 (MMP-9) without change in tissue inhibitors of MMP-1 by cultured cells from human placenta and fetal membranes. The Journal of clinical endocrinology and metabolism 2005;90:6569–74. [PubMed: 16174714]
23. Lee S, Rivier C. Alcohol increases the expression of type 1, but not type 2 alpha corticotropin-releasing factor (CRF) receptor messenger ribonucleic acid in the rat hypothalamus. Brain Res Mol Brain Res 1997;52:78–89. [PubMed: 9450680]
24. Weitemier AZ, Ryabinin AE. Brain region-specific regulation of urocortin 1 innervation and corticotropin-releasing factor receptor type 2 binding by ethanol exposure. Alcohol Clin Exp Res 2005;29:1610–20. [PubMed: 16205361]
25. Pich EM, Lorang M, Yeganeh M, Rodriguez d. F. Raber J, Koob GF, Weiss F. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. Journal of Neuroscience 1995;15:5439–47. [PubMed: 7643193]
26. Sommer WH, Rimondini R, Hansson AC, Hipskind PA, Gehlert DR, Barr CS, Heilig M. Upregulation of voluntary alcohol intake, behavioral sensitivity to stress, and amygdala *Crhr1* expression following a history of dependence. Biol Psychiatry. 2008
27. Funk CK, O'Dell LE, Crawford EF, Koob GF. Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. J Neurosci 2006;26:11324–32. [PubMed: 17079660]

28. Lack AK, Floyd DW, McCool BA. Chronic ethanol ingestion modulates proanxiety factors expressed in rat central amygdala. Alcohol 2005;36:83–90. [PubMed: 16396741]
29. Olive MF, Koenig HN, Nannini MA, Hodge CW. Elevated extracellular CRF levels in the bed nucleus of the stria terminalis during ethanol withdrawal and reduction by subsequent ethanol intake. Pharmacol Biochem Behav 2002;72:213–20. [PubMed: 11900791]
30. Oliva JM, Manzanares J. Gene transcription alterations associated with decrease of ethanol intake induced by naltrexone in the brain of wistar rats. Neruopysychopharmacology 2007;32:1358–69.
31. Rivier C, Imaki T, Vale W. Prolonged exposure to alcohol: effect on CRF mRNA levels, and CRF- and stress-induced ACTH secretion in the rat. Brain research 1990;520:1–5. [PubMed: 2169950]
32. Pickering C, Avesson L, Liljequist S, Lindblom J, Schioth HB. The role of hypothalamic peptide gene expression in alcohol self-administration behavior. Peptides 2007;28:2361–71. [PubMed: 17976860]
33. Francesconi W, Berton F, Repunte-Canonigo V, Hagihara K, Thurbon D, Lekic D, Specio SE, Greenwell TN, Chen SA, Rice KC, Richardson HN, O'Dell LE, Zorrilla EP, Morales M, Koob GF, Sanna PP. Protracted withdrawal from alcohol and drugs of abuse impairs long-term potentiation of intrinsic excitability in the juxtacapsular bed nucleus of the stria terminalis. J Neurosci 2009;29:5389–401. [PubMed: 19403807]
34. Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, Koob GF. Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. Alcohol Clin Exp Res 2002;26:1494–501. [PubMed: 12394282]
35. Falco AM, Bergstrom HC, Bachus SE, Smith RF. Persisting changes in basolateral amygdala mRNAs after chronic ethanol consumption. Physiol Behav 2009;96:169–73. [PubMed: 18938187]
36. Zhang Z, Morse AC, Koob GF, Schulteis G. Dose- and time-dependent expression of anxiety-like behavior in the elevated plus-maze during withdrawal from acute and repeated intermittent ethanol intoxication in rats. Alcohol Clin Exp Res 2007;31:1811–9. [PubMed: 17877783]
37. Valdez GR, Zorrilla EP, Roberts AJ, Koob GF. Antagonism of corticotropin-releasing factor attenuates the enhanced responsiveness to stress observed during protracted ethanol abstinence. Alcohol 2003;29:55–60. [PubMed: 12782246]
38. Finn DA, Snelling C, Fretwell AM, Tanchuck MA, Underwood L, Cole M, Crabbe JC, Roberts AJ. Increased Drinking During Withdrawal From Intermittent Ethanol Exposure Is Blocked by the CRF Receptor Antagonist d-Phe-CRF(12-41). Alcohol Clin Exp Res 2007;31:939–49. [PubMed: 17403068]
39. O'Callaghan MJ, Croft AP, Jacquot C, Little HJ. The hypothalamopituitary-adrenal axis and alcohol preference. Brain research bulletin 2005;68:171–8. [PubMed: 16325017]
40. Richardson HN, Zhao Y, Fekete EM, Funk CK, Wirsching P, Janda KD, Zorrilla EP, Koob GF. MPZP: a novel small molecule corticotropin-releasing factor type 1 receptor (CRF1) antagonist. Pharmacol Biochem Behav 2008;88:497–510. [PubMed: 18031798]
41. Gehlert DR, Cippitelli A, Thorsell A, Le AD, Hipskind PA, Hamdouchi C, Lu J, Hembre EJ, Cramer J, Song M, McKinzie D, Morin M, Ciccocioppo R, Heilig M. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. J Neurosci 2007;27:2718–26. [PubMed: 17344409]
42. Sabino V, Cottone P, Koob GF, Steardo L, Lee MJ, Rice KC, Zorrilla EP. Dissociation between opioid and CRF1 antagonist sensitive drinking in Sardinian alcohol-preferring rats. Psychopharmacology (Berl) 2006;189:175–86. [PubMed: 17047935]
43. Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol Behav 2005;84:53–63. [PubMed: 15642607]
44. Rhodes JS, Ford MM, Yu CH, Brown LL, Finn DA, Garland T Jr. Crabbe JC. Mouse inbred strain differences in ethanol drinking to intoxication. Genes Brain Behav 2007;6:1–18. [PubMed: 17233637]
45. Sparta DR, Sparrow AM, Lowery EG, Fee JR, Knapp DJ, Thiele TE. Blockade of the corticotropin releasing factor type 1 receptor attenuates elevated ethanol drinking associated with drinking in the dark procedures. Alcohol Clin Exp Res 2008;32:259–65. [PubMed: 18162072]

46. Lyons AM, Lowery EG, Sparta DR, Thiele TE. Effects of food availability and administration of orexigenic and anorectic agents on elevated ethanol drinking associated with drinking in the dark procedures. Alcoholism, clinical and experimental research 2008;32:1962–8.
47. Lowery EG, Spanos M, Navarro M, Lyons AM, Hodge CW, Thiele TE. CRF-1 antagonist and CRF-2 agonist decrease binge-like ethanol drinking in C57BL/6J mice independent of the HPA axis. Neuropsychopharmacology. in press.
48. Ryabinin AE, Yoneyama N, Tanchuck MA, Mark GP, Finn DA. Urocortin 1 microinjection into the mouse lateral septum regulates the acquisition and expression of alcohol consumption. Neuroscience 2008;151:780–90. [PubMed: 18164138]
49. Sharpe AL, Phillips TJ. Central urocortin 3 administration decreases limited-access ethanol intake in nondependent mice. Behavioural pharmacology 2009;20:346–51. [PubMed: 19581799]
50. Ji D, Gilpin NW, Richardson HN, Rivier CL, Koob GF. Effects of naltrexone, duloxetine, and a corticotropin-releasing factor type 1 receptor antagonist on binge-like alcohol drinking in rats. Behavioural pharmacology 2008;19:1–12. [PubMed: 18195589]
51. Thorsell A, Slawecki CJ, Ehlers CL. Effects of neuropeptide Y and corticotropin-releasing factor on ethanol intake in Wistar rats: interaction with chronic ethanol exposure. Behavioural brain research 2005;161:133–40. [PubMed: 15904720]
52. Bell SM, Reynolds JG, Thiele TE, Gan J, Figlewicz DP, Woods SC. Effects of third intracerebroventricular injections of corticotropin-releasing factor (CRF) on ethanol drinking and food intake. Psychopharmacology 1998;139:128–35. [PubMed: 9768550]
53. Campbell BM, Morrison JL, Walker EL, Merchant KM. Differential regulation of behavioral, genomic, and neuroendocrine responses by CRF infusions in rats. Pharmacol Biochem Behav 2004;77:447–55. [PubMed: 15006454]
54. Lowery EG, Sparrow AM, Breese GR, Knapp DJ, Thiele TE. The CRF-1 receptor antagonist, CP-154,526, attenuates stress-induced increases in ethanol consumption by BALB/cJ mice. Alcohol Clin Exp Res 2008;32:240–48. [PubMed: 18162074]
55. Marinelli PW, Funk D, Juzytsch W, Harding S, Rice KC, Shaham Y, Le AD. The CRF(1) receptor antagonist antalarmin attenuates yohimbine-induced increases in operant alcohol self-administration and reinstatement of alcohol seeking in rats. Psychopharmacology (Berl) 2007;195:345–55. [PubMed: 17705061]
56. Lodge DJ, Lawrence AJ. The CRF1 receptor antagonist antalarmin reduces volitional ethanol consumption in isolation-reared fawn-hooded rats. Neuroscience 2003;117:243–7. [PubMed: 12614667]
57. Yang X, Wang S, Rice KC, Munro CA, Wand GS. Restraint stress and ethanol consumption in two mouse strains. Alcohol Clin Exp Res 2008;32:840–52. [PubMed: 18336636]
58. Chu K, Koob GF, Cole M, Zorrilla EP, Roberts AJ. Dependence-induced increases in ethanol self-administration in mice are blocked by the CRF1 receptor antagonist antalarmin and by CRF1 receptor knockout. Pharmacol Biochem Behav 2007;86:813–21. [PubMed: 17482248]
59. Gilpin NW, Richardson HN, Koob GF. Effects of CRF1-receptor and opioid-receptor antagonists on dependence-induced increases in alcohol drinking by alcohol-preferring (P) rats. Alcohol Clin Exp Res 2008;32:1535–42. [PubMed: 18631323]
60. Funk CK, Zorrilla EP, Lee MJ, Rice KC, Koob GF. Corticotropin-releasing factor 1 antagonists selectively reduce ethanol self-administration in ethanol-dependent rats. Biol Psychiatry 2007;61:78–86. [PubMed: 16876134]
61. Valdez GR, Sabino V, Koob GF. Increased anxiety-like behavior and ethanol self-administration in dependent rats: reversal via corticotropin-releasing factor-2 receptor activation. Alcohol Clin Exp Res 2004;28:865–72. [PubMed: 15201629]
62. Funk CK, Koob GF. A CRF(2) agonist administered into the central nucleus of the amygdala decreases ethanol self-administration in ethanol-dependent rats. Brain research 2007;1155:172–8. [PubMed: 17512918]
63. Ciccocioppo R, Economidou D, Cippitelli A, Cucculelli M, Ubaldi M, Soverchia L, Lourdusamy A, Massi M. Genetically selected Marchigian Sardinian alcohol-preferring (msP) rats: an animal model to study the neurobiology of alcoholism. Addiction biology 2006;11:339–55. [PubMed: 16961763]

64. Hansson AC, Cippitelli A, Sommer WH, Fedeli A, Bjork K, Soverchia L, Terasmaa A, Massi M, Heilig M, Ciccocioppo R. Variation at the rat Crhr1 locus and sensitivity to relapse into alcohol seeking induced by environmental stress. Proceedings of the National Academy of Sciences of the United States of America 2006;103:15236–41. [PubMed: 17015825]

65. Koob GF. Animal models of craving for ethanol. Addiction 2000;95(Suppl 2):S73–S81. [PubMed: 11002904]

66. Le A, Shaham Y. Neurobiology of relapse to alcohol in rats. Pharmacology & therapeutics 2002;94:137–56. [PubMed: 12191599]

67. Le AD, Kiianmaa K, Cunningham CL, Engel JA, Ericson M, Soderpalm B, Koob GF, Roberts AJ, Weiss F, Hyytia P, Janhunen S, Mikkola J, Backstrom P, Ponomarev I, Crabbe JC. Neurobiological processes in alcohol addiction. Alcohol Clin Exp Res 2001;25:144S–51S. [PubMed: 11391064]

68. Weiss F, Ciccocioppo R, Parsons LH, Katner S, Liu X, Zorrilla EP, Valdez GR, Ben-Shahar O, Angeletti S, Richter RR. Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. Annals of the New York Academy of Sciences 2001;937:1–26. [PubMed: 11458532]

69. Le AD, Harding S, Juzytsch W, Watchus J, Shalev U, Shaham Y. The role of corticotropin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. Psychopharmacology 2000;150:317–24. [PubMed: 10923760]

70. Liu X, Weiss F. Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. J Neurosci 2002;22:7856–61. [PubMed: 12223538]

71. Le AD, Harding S, Juzytsch W, Fletcher PJ, Shaham Y. The role of corticotropin-releasing factor in the median raphe nucleus in relapse to alcohol. J Neurosci 2002;22:7844–9. [PubMed: 12223536]

72. Colombo G, Lobina C, Carai MA, Gessa GL. Phenotypic characterization of genetically selected Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats. Addiction biology 2006;11:324–38. [PubMed: 16961762]

73. Bell RL, Rodd ZA, Lumeng L, Murphy JM, McBride WJ. The alcohol-preferring P rat and animal models of excessive alcohol drinking. Addiction biology 2006;11:270–88. [PubMed: 16961759]

74. Rodd ZA, Bell RL, Sable HJ, Murphy JM, McBride WJ. Recent advances in animal models of alcohol craving and relapse. Pharmacology, biochemistry, and behavior 2004;79:439–50.

75. Sparta DR, Ferraro FM 3rd, Fee JR, Knapp DJ, Breese GR, Thiele TE. The alcohol deprivation effect in C57BL/6J mice is observed using operant self-administration procedures and is modulated by CRF-1 receptor signaling. Alcohol Clin Exp Res 2009;33:31–42. [PubMed: 18945225]

76. Overstreet DH, Knapp DJ, Breese GR. Drug challenges reveal differences in mediation of stress facilitation of voluntary alcohol drinking and withdrawal-induced anxiety in alcohol-preferring P rats. Alcohol Clin Exp Res 2007;31:1473–81. [PubMed: 17624999]

77. Overstreet DH, Knapp DJ, Breese GR. Modulation of multiple ethanol withdrawal-induced anxiety-like behavior by CRF and CRF1 receptors. Pharmacol Biochem Behav 2004;77:405–13. [PubMed: 14751471]

78. Zorrilla EP, Valdez GR, Weiss F. Changes in levels of regional CRF-like-immunoreactivity and plasma corticosterone during protracted drug withdrawal in dependent rats. Psychopharmacology (Berl) 2001;158:374–81. [PubMed: 11797058]

79. Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton TK. CRF antagonist reverses the “anxiogenic” response to ethanol withdrawal in the rat. Psychopharmacology 1991;103:227–32. [PubMed: 2027923]

80. Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. Brain research 1993;605:25–32. [PubMed: 8467387]

81. Wills TA, Knapp DJ, Overstreet DH, Breese GR. Sensitization, duration, and pharmacological blockade of anxiety-like behavior following repeated ethanol withdrawal in adolescent and adult rats. Alcohol Clin Exp Res 2009;33:455–63. [PubMed: 19120055]

82. Overstreet DH, Knapp DJ, Breese GR. Pharmacological modulation of repeated ethanol withdrawal-induced anxiety-like behavior differs in alcohol-preferring P and Sprague-Dawley rats. Pharmacol Biochem Behav 2005;81:122–30. [PubMed: 15894069]

83. Breese GR, Knapp DJ, Overstreet DH. Stress sensitization of ethanol withdrawal-induced reduction in social interaction: inhibition by CRF-1 and benzodiazepine receptor antagonists and a 5-HT1A-receptor agonist. Neuropsychopharmacology 2004;29:470–82. [PubMed: 12955093]
84. Breese GR, Overstreet DH, Knapp DJ, Navarro M. Prior multiple ethanol withdrawals enhance stress-induced anxiety-like behavior: inhibition by CRF1- and benzodiazepine-receptor antagonists and a 5-HT1a-receptor agonist. Neuropsychopharmacology 2005;30:1662–9. [PubMed: 15726114]
85. Knapp DJ, Overstreet DH, Moy SS, Breese GR. SB242084, flumazenil, and CRA1000 block ethanol withdrawal-induced anxiety in rats. Alcohol 2004;32:101–11. [PubMed: 15163561]
86. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 2001;24:97–129. [PubMed: 11120394]
87. Koob GF, Le Moal M. Review. Neurobiological mechanisms for opponent motivational processes in addiction. Philosophical transactions of the Royal Society of London 2008;363:3113–23. [PubMed: 18653439]
88. Treutlein J, Kissling C, Frank J, Wiemann S, Dong L, Depner M, Saam C, Lascorz J, Soyka M, Preuss UW, Rujescu D, Skowronek MH, Rietschel M, Spanagel R, Heinz A, Laucht M, Mann K, Schumann G. Genetic association of the human corticotropin releasing hormone receptor 1 (CRHR1) with binge drinking and alcohol intake patterns in two independent samples. Mol Psychiatry 2006;11:594–602. [PubMed: 16550213]
89. Bonomo YA, Bowes G, Coffey C, Carlin JB, Patton GC. Teenage drinking and the onset of alcohol dependence: a cohort study over seven years. Addiction 2004;99:1520–8. [PubMed: 15585043]
90. Jennison KM. The short-term effects and unintended long-term consequences of binge drinking in college: a 10-year follow-up study. The American journal of drug and alcohol abuse 2004;30:659–84. [PubMed: 15540499]
91. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. Journal of Endocrinology 1999;160:1–12. [PubMed: 9854171]
92. Heinrichs SC, Richard D. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. Neuropeptides 1999;33:350–9. [PubMed: 10657512]
93. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues in clinical neuroscience 2006;8:383–95. [PubMed: 17290797]
94. Menzaghi F, Rassnick S, Heinrichs S, Baldwin H, Pich EM, Weiss F, Koob GF. The role of corticotropin-releasing factor in the anxiogenic effects of ethanol withdrawal. Annals of the New York Academy of Sciences 1994;739:176–84. [PubMed: 7832471]
95. Kehne JH. The CRF1 receptor, a novel target for the treatment of depression, anxiety, and stress-related disorders. CNS Neurol Disord Drug Targets 2007;6:163–82. [PubMed: 17511614]
96. Kuperman Y, Chen A. Urocortins: emerging metabolic and energy homeostasis perspectives. Trends in endocrinology and metabolism: TEM 2008;19:122–9. [PubMed: 18337115]
97. Ryabinin AE, Weitemier AZ. The urocortin 1 neurocircuit: ethanol-sensitivity and potential involvement in alcohol consumption. Brain research reviews 2006;52:368–80. [PubMed: 16766036]
98. Sanghvi R, Mogalian E, Machatha SG, Narazaki R, Karlage KL, Jain P, Tabibi SE, Glaze E, Myrdal PB, Yalkowsky SH. Preformulation and pharmacokinetic studies on antalarmin: a novel stress inhibitor. Journal of pharmaceutical sciences 2009;98:205–14. [PubMed: 18428980]
99. Gilligan PJ, He L, Clarke T, Tivitmahaisoon P, Lelas S, Li YW, Heman K, Fitzgerald L, Miller K, Zhang G, Marshall A, Krause C, McElroy J, Ward K, Shen H, Wong H, Grossman S, Nemeth G, Zaczek R, Arneric SP, Hartig P, Robertson DW, Trainor G. 8-(4-Methoxyphenyl)pyrazolo[1,5-a]-1,3,5-triazines: selective and centrally active corticotropin-releasing factor receptor-1 (CRF1) antagonists. Journal of medicinal chemistry 2009;52:3073–83. [PubMed: 19361210]
100. Gilligan PJ, Clarke T, He L, Lelas S, Li YW, Heman K, Fitzgerald L, Miller K, Zhang G, Marshall A, Krause C, McElroy JF, Ward K, Zeller K, Wong H, Bai S, Saye J, Grossman S, Zaczek R, Arneric SP, Hartig P, Robertson D, Trainor G. Synthesis and structure-activity relationships of 8-(pyrid-3-yl)pyrazolo[1,5-a]-1,3,5-triazines: potent, orally bioavailable corticotropin releasing factor receptor-1 (CRF1) antagonists. Journal of medicinal chemistry 2009;52:3084–92. [PubMed: 19361209]

101. Perrin MH, Sutton SW, Cervini LA, Rivier JE, Vale WW. Comparison of an agonist, urocortin, and an antagonist, astressin, as radioligands for characterization of corticotropin-releasing factor receptors. J Pharmacol Exp Ther 1999;288:729–34. [PubMed: 9918582]

102. Perrin MH, Vale WW. Corticotropin releasing factor receptors and their ligand family. Annals of the New York Academy of Sciences 1999;885:312–28. [PubMed: 10816663]

103. Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, Vaughan J, Reyes TM, Gulyas J, Fischer W, Bilezikjian L, Rivier J, Sawchenko PE, Vale WW. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. Proceedings of the National Academy of Sciences of the United States of America 2001;98:7570–5. [PubMed: 11416224]

104. Weitemier AZ, Ryabinin AE. Urocortin 1 in the dorsal raphe regulates food and fluid consumption, but not ethanol preference in C57BL/6J mice. Neuroscience 2006;137:1439–45. [PubMed: 16338088]

105. Zorrilla EP, Schulteis G, Ormsby A, Klaassen A, Ling N, McCarthy JR, Koob GF, De Souza EB. Urocortin shares the memory modulating effects of corticotropin-releasing factor (CRF): mediation by CRF1 receptors. Brain research 2002;952:200–10. [PubMed: 12376180]

106. Steckler T, Dautzenberg FM. Corticotropin-releasing factor receptor antagonists in affective disorders and drug dependence-- an update. CNS Neurol Disord Drug Targets 2006;5:147–65. [PubMed: 16611089]

107. Valdez GR. Development of CRF1 receptor antagonists as antidepressants and anxiolytics: progress to date. CNS drugs 2006;20:887–96. [PubMed: 17044726]

108. Hsin LW, Tian X, Webster EL, Coop A, Caldwell TM, Jacobson AE, Chrousos GP, Gold PW, Habib KE, Ayala A, Eckelman WC, Contoreggi C, Rice KC. CRHR1 Receptor binding and lipophilicity of pyrrolopyrimidines, potential nonpeptide corticotropin-releasing hormone type 1 receptor antagonists. Bioorganic & medicinal chemistry 2002;10:175–83. [PubMed: 11738619]

109. Rivier J, Gulyas J, Kirby D, Low W, Perrin MH, Kunitake K, DiGruccio M, Vaughan J, Reubi JC, Waser B, Koerber SC, Martinez V, Wang L, Tache Y, Vale W. Potent and long-acting corticotropin releasing factor (CRF) receptor 2 selective peptide competitive antagonists. Journal of medicinal chemistry 2002;45:4737–47. [PubMed: 12361401]

Table 1

The Effects of CRF, Ucn1, Ucn3, and CRFR Antagonists on Ethanol Consumption

Compound	Receptor specificity	Mode of administration	Effect on ethanol consumption	Study
CRF	CRF1 = CRF2 (K_i = 11 vs. 44 nM) [101,102]	i.c.v.	-- ethanol preference	[39]
		i.c.v.	↓ limited access ethanol consumption in non-dependent and dependent animals	[48,51, 52]
		Microinjection into the MRN	↑ reinstatement to ethanol-seeking	[71]
Ucn 1	CRF1 = CRF2 (K_i = 0.32 vs. 2.2 nM) [103]	Microinjection into the LS	↓ acquisition and expression of limited access ethanol consumption	[48]
		Microinjection into DRN	-- continuous access ethanol consumption	[104]
Ucn 3	CRF1 > CRF2 (K_i = >100 vs. 5.0 nM) [103]	i.c.v.	-- ethanol self-administration in non-dependent animals	[61]
		i.c.v.	↓ limited access ethanol consumption	[49]
		i.c.v.	↓ binge-like ethanol consumption	[47]
		Microinjection into the CeA	↑ ethanol self-administration in non-dependent animals	[62]
		i.c.v.	↓ ethanol self-administration in dependent animals	[61]
		Microinjection into the CeA	↓ ethanol self-administration in dependent animals	[62]
D-Phe-CRF	CRF1 = CRF2 (K_i = 20 vs. 50 nM) [105]	i.c.v.	-- ethanol self-administration in non-dependent animals	[34]
		i.c.v.	↓ ethanol self-administration in dependent animals	[34]
		Microinjection into CeA	-- ethanol self-administration in non-dependent animals	[27,38]
		Microinjection into CeA	↓ ethanol self-administration in dependent animals	[27,38]
		Microinjection into lateral BNST	↓ ethanol self-administration in non-dependent and dependent animals	[27]
		Microinjection into NAcSh	↓ ethanol self-administration in non-dependent and dependent animals	[27]
		i.c.v.	↓ stress-induced reinstatement	[69]
		Microinjection into MRN	↓ stress-induced reinstatement	[71]
		i.c.v.	↓ stress-induced reinstatement	[70]
		i.c.v.	-- cue-induced reinstatement	[70]
ahCRF	CRF1 = CRF2 (K_i = 35 vs. 11nM) [106]	i.c.v.	-- ethanol preference of high consuming animals	[39]
		i.c.v.	↑ ethanol preference of low consuming animals transiently	[39]
		i.c.v.	↓ binge-like ethanol consumption	[47]
Antalarmin	CRF1 > CRF2 (K_i = 1.0 vs. >10000 nM) [107]	i.p.	-- ethanol self-administration in non-dependent animals	[58,64]
		i.p.	↓ ethanol self-administration in non-dependent msP rats	[64]
		i.p.	↓ continuous ethanol consumption in anxious animals	[56]

Compound	Receptor specificity	Mode of administration	Effect on ethanol consumption	Study
		i.p.	↓ ethanol self-administration in dependent animals	[58]
		i.p.	↓ stress-induced ethanol self-administration	[55]
			-- stress-induced ethanol consumption	[57]
		i.p.	↓ stress-induced reinstatement	[55,64]
CP-154,526	CRF1 > CRF2 (K_i = 0.44 vs. >10000 nM) [41]	i.p.	-- limited access ethanol consumption	[45]
		i.p.	↓ binge-like ethanol consumption	[45,47]
		i.p.	↓ binge-like ethanol consumption in ADX animals	[47]
		i.p.	↓ stress-induced ethanol consumption	[54]
		i.p.	↓ deprivation-induced ethanol self-administration	[75]
		i.p.	↓ stress and deprivation-induced ethanol consumption in P rats	[76]
		i.p.	↓ stress-induced reinstatement	[69]
MPZP	CRF1 > CRF2 (K_i = 4.9 nM at CRF1R) [40]	i.p.	-- ethanol self-administration in non-dependent animals	[40,59]
		s.c.	-- binge-like consumption of sweetened ethanol	[50]
		s.c.	-- binge-like self-administration of sweetened ethanol	[50]
			↓ ethanol self-administration in dependent animals	40,58]
LWH-63	CRF1 > CRF2 (*K*i= 0.68-0.7 nM at the CRF1R) [42, 108]	i.p.	-- ethanol self-administration in non-dependent animals	[42]
		i.p.	↓ ethanol self-administration in dependent animals	[42]
MJL-1-109-2	CRF1 > CRF2 (*K*1= 1.9 nM at CRF1R) [60]	i.p.	-- ethanol self-administration in non-dependent animals	[60]
		i.p.	↓ ethanol self-administration in dependent animals	[60]
R121919	CRF1 > CRF2 (K_i= 0.24 vs. >1000 nM) [41]	i.p.	-- ethanol self-administration in non-dependent animals	[60]
			-- stress-induced ethanol consumption	[57]
			↓ ethanol self-administration in dependent animals	[60]
MTIP	CRF1 > CRF2 (K_i = 0.22 vs > 1000 nM [41]	i.p.	-- ethanol self-administration in non-dependent animals	[41,60]
		i.p.	↓ ethanol self-administration in non-dependent msP rats	[41]
			↓ ethanol self-administration in dependent animals	[41,60]
CRA1000	CRF1 > CRF2 (K_i= 16-21 vs. >10000 nM) [107]	i.p.	↓ stress and deprivation-induced ethanol consumption in P rats	[76]

(--, no change; ↓, decrease or attenuation; ↑, increase; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala, DRN, dorsal raphe nucleus; LS, lateral septum; MRN, median raphe nucleus; NAcSh, nucleus accumbens shell)

Table 2

The Effects of CRF, Ucn3 and CRFR Antagonists on Withdrawal-Induced Anxiety-Like Behavior

Compound	Receptor specificity	Mode of administration	Effect on ethanol-related behavior	Study
CRF	CRF1 = CRF2 (K_i = 11 vs. 44 nM) [101,102]	i.c.v.	↑ withdrawal-induced anxiety in dependent animals	[77]
Ucn 3	CRF1 < CRF2 (K_i = >100 vs. 5.0 nM) [103]	i.c.v.	↓ withdrawal-induced anxiety in dependent animals	[61]
ahCRF	CRF1 = CRF2 (K_i = 35 vs. 11nM) [106]	i.c.v.	↓ withdrawal-induced anxiety in dependent animals	[79]
		i.c.v.	-- withdrawal-induced anxiety in dependent animals	[80]
		Microinjection into the CeA	↓ withdrawal-induced anxiety in dependent animals	[80]
$_D$-Phe-CRF	CRF1 = CRF2 (K_i = 20 vs. 50 nM) [105]	i.c.v.	↓ enhanced withdrawal-induced anxiety following stress exposure in dependent animals	[37]
MTIP	CRF1 > CRF2 (K_i = 0.22 vs > 1000 nM [41]	i.p.	↓ withdrawal-induced anxiety	[41]
CP-154,526	CRF1 > CRF2 (K_i = 0.44 vs. >10000 nM) [41]	i.p.	↓ withdrawal-induced anxiety in dependent adolescent animals	[81]
		i.p.	↓ withdrawal-induced anxiety in dependent P rats	[82]
		i.p.	↓ enhanced withdrawal-induced anxiety following stress exposure in dependent P rats	[82]
		i.p.	↓ withdrawal-induced anxiety following stress exposure in dependent animals	[77]
CRA-1000	CRF1 > CRF2 (K_i= 16-21 vs. >10000 nM) [107]	i.p.	↓ enhanced withdrawal-induced anxiety following stress exposure in dependent P rats	[83]
		i.p.	↓ enhanced withdrawal-induced anxiety following stress exposure in dependent animals	[84]
		i.p.	↓ withdrawal-induced anxiety in dependent animals	[77,85]
		i.p.	↓ enhanced withdrawal-induced anxiety in dependent P rats	[76]
Antisauvagine-30	CRF1 < CRF2 (IC_{50} = 400 vs 1.1 nM) [109]	i.c.v.	-- withdrawal-induced anxiety in dependent animals	[77]

(--, no change; ↓, decrease or attenuation; ↑, increase; CeA, central nucleus of the amygdala)