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

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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

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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 periculomotor urocortin-containing population of neurons (pIU, 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 pIU 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-Phe¹²,Nle^{21,38},CαMeLeu³⁷]-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-methylphenyl)-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)-7H-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-methoxyethyl)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 Ucn1-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 α hCRF [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.

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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	Periocular motor 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	(<i>N,N</i> -bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5- <i>a</i>]pyrimidin-7-amine
MTIP	3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethyl-imidazo[1,2- <i>b</i>]pyridazine
LWH-63	(4-ethyl-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-7 <i>H</i> -pyrrolo[2,3- <i>d</i>]pyrimidin-4-yl]amino-1-butanol
R121919	2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1,5- <i>a</i>]pyrimidine
MJL-1-109-2	[8-(4-bromo-2-chlorophenyl)-2,7-dimethyl-pyrazolo[1,5- <i>a</i>][1,3,5]triazin-4-yl]- <i>bis</i> -(2-methoxyethyl)amine
CP-154,526	butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7 <i>H</i> pyrrolo[2,3- <i>d</i>]pyrimidin-4-yl]amine
CRA1000	(2-(<i>N</i> -(2-methylthio-4-isopropylphenyl)- <i>N</i> -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

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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]
ahCRF	CRF1 = CRF2 (K_i = 35 vs. 11 nM) [106]	i.c.v.	-- cue-induced reinstatement	[70]
		i.c.v.	-- ethanol preference of high consuming animals	[39]
		i.c.v.	↑ ethanol preference of low consuming animals transiently	[39]
Antalarmin	CRF1 > CRF2 (K_i = 1.0 vs. >10000 nM) [107]	i.c.v.	↓ binge-like ethanol consumption	[47]
		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
CP-154,526	CRF1 > CRF2 (K_i = 0.44 vs. >10000 nM) [41]	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]
		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]
MPZP	CRF1 > CRF2 (K_i = 4.9 nM at CRF1R) [40]	i.p.	↓ stress-induced reinstatement	[69]
		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]
LWH-63	CRF1 > CRF2 (K_i = 0.68-0.7 nM at the CRF1R) [42, 108]		↓ ethanol self-administration in dependent animals	40,58]
		i.p.	-- ethanol self-administration in non-dependent animals	[42]
MJL-1-109-2	CRF1 > CRF2 (K_i = 1.9 nM at CRF1R) [60]	i.p.	↓ ethanol self-administration in dependent animals	[42]
		i.p.	-- ethanol self-administration in non-dependent animals	[60]
R121919	CRF1 > CRF2 (K_i = 0.24 vs. >1000 nM) [41]	i.p.	↓ ethanol self-administration in dependent animals	[60]
			-- ethanol self-administration in non-dependent animals	[60]
MTIP	CRF1 > CRF2 (K_i = 0.22 vs > 1000 nM [41])		-- stress-induced ethanol consumption	[57]
			↓ ethanol self-administration in dependent animals	[60]
		i.p.	-- ethanol self-administration in non-dependent animals	[41,60]
CRA1000	CRF1 > CRF2 (K_i = 16-21 vs. >10000 nM) [107]	i.p.	↓ ethanol self-administration in non-dependent msP rats	[41]
			↓ ethanol self-administration in dependent animals	[41,60]
			↓ 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)