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Behavioral, biological, and chemical perspectives on targeting CRF_1 receptor antagonists to treat alcoholism

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Abstract

Background—Alcohol use disorders are chronic disabling conditions for which existing pharmacotherapies have only modest efficacy. In the present review, derived from the 2012 Behavior, Biology and Chemistry "Translational Research in Addiction" symposium, we summarize the anti-relapse potential of corticotropin-releasing factor type 1 (CRF_1) receptor antagonists to reduce negative emotional symptoms of acute and protracted alcohol withdrawal and stress-induced relapse to alcohol seeking.

Methods—We review the biology of CRF_1 systems, the activity of CRF_1 receptor antagonists in animal models of anxiolytic and antidepressant activity, and experimental findings in alcohol addiction models. We also update the clinical trial status of CRF_1 receptor antagonists, including pexacerfont (BMS-562086), emicerfont (GW876008), verucerfont (GSK561679), CP316311, SSR125543A, R121919/NBI30775, R317573/19567470/CRA5626, and ONO-2333Ms. Finally, we discuss the potential heterogeneity and pharmacogenomics of CRF_1 receptor pharmacotherapy for alcohol dependence.

Results—The evidence suggests that brain penetrant-CRF_1 receptor antagonists have therapeutic potential for alcohol dependence. Lead compounds with clinically desirable pharmacokinetic properties now exist, and longer receptor residence rates (i.e., slow dissociation) may predict greater CRF_1 receptor antagonist efficacy. Functional variants in genes that encode CRF system molecules, including polymorphisms in *Crhr1* (rs110402, rs1876831, rs242938) and *Crhbp* genes

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(rs10055255, rs3811939) may promote alcohol seeking and consumption by altering basal or stress-induced CRF system activation.

Conclusions—Ongoing clinical trials with pexacerfont and verucerfont in moderately to highly severe dependent anxious alcoholics may yield insight as to the role of CRF_1 receptor antagonists in a personalized medicine approach to treat drug or alcohol dependence.

Keywords

corticotropin-releasing factor or hormone receptor antagonist; CRF or CRH; anxiety disorder; major depression; alcohol or ethanol; drug addiction or alcoholism or alcohol dependence or alcohol use disorder or binge drinking; acute or protracted withdrawal or abstinence; treatment or clinical trial; stress-induced relapse or reinstatement or craving

1. INTRODUCTION

Each year, almost half of all American adults (47%) suffer from an addictive disorder (Sussman et al., 2011). Alcohol misuse alone has an annual prevalence of 10% (Sussman et al., 2011) and accounts for 10% of total disability in developed countries (Rehm et al., 2009). Most of the disability and cost to society results from alcohol dependence, or alcoholism, which has lifetime prevalence in the United States of more than 12% (Hasin et al., 2007). Available pharmacotherapies for alcoholism have only modest long-term efficacy and are underutilized (Heilig et al., 2011). Novel treatment options are needed. Here, we review the current state of therapeutically targeting corticotropin-releasing factor (CRF) systems to prevent alcohol dependence and relapse.

Brain and pituitary CRF_1 receptors mediate many endocrine, behavioral, and autonomic responses to stress (Heinrichs and Koob, 2004). Accordingly, the pharmaceutical industry has sought to develop blood-brain barrier-penetrating CRF_1 receptor antagonists for stress-related psychiatric conditions, including anxiety disorders and major depression (Holsboer and Ising, 2008; Koob and Zorrilla, 2012; Zorrilla and Koob, 2004, 2010). Indeed, the search for CRF receptor antagonists began from the time that Vale and colleagues isolated the stress-secreted CRF peptide in 1981 (Vale et al., 1981). In addition to CRF, the CRF/urocortin (Ucn) system includes genes encoding three CRF paralogs (Ucn 1, Ucn 2, and Ucn 3) and two G-protein coupled receptors (CRF_1, CRF_2), with which the CRF/Ucn peptides interact (Fekete and Zorrilla, 2007). Extensive, previously reviewed preclinical data support the therapeutic potential of blood-brain barrier penetrating CRF_1 receptor antagonists for different facets of alcohol dependence (Breese et al., 2011; Ciccocioppo et al., 2009; Heilig et al., 2010a, 2011; Heilig and Koob, 2007; Heilig et al., 2010b; Koob and Zorrilla, 2010, 2012; Le and Shaham, 2002; Logrip et al., 2011; Martin-Fardon et al., 2010; Shalev et al., 2010; Zorrilla and Koob, 2010). Here, we will focus on (i) recent developments in the medicinal chemistry of CRF_1 receptor antagonists, (ii) the progress of specific small molecule CRF_1 receptor antagonists in clinical trials, and (iii) issues related to the potential heterogeneity and pharmacogenomics of CRF_1 receptor pharmacotherapy for alcoholism. The review is derived from our 2012 Behavior, Biology and Chemistry “Translational Research in Addiction” symposium in San Antonio, Texas.

2. BIOLOGY OF CRF/UROCORTIN RECEPTOR SYSTEMS

2.1 CRF/Urocortin system molecules

CRF_1 and CRF_2 receptors are class B1 (“secretin-like”) G-protein coupled receptors that share ~70% sequence identity with one another. The CRF_1 receptor exists in multiple isoforms (e.g., CRF_{1a}-CRF_{1h}), with the $CRF_{1(a)}$ subtype being the major functional isoform. In humans, the CRF_2 receptor has three known membrane-associated functional subtypes --

$CRF_{2(a)}$, $CRF_{2(b)}$, and $CRF_{2(c)}$. CRF peptide has preferential agonist activity for CRF_1 *vs.* CRF_2 receptors. Ucn 1 is a high-affinity agonist at both receptors, but the type 2 urocortins (Ucn 2 and Ucn 3) are much more selective agonists for CRF_2 receptors. A CRF-binding protein (CRF-BP) putatively sequesters CRF and Ucn 1 with equal or greater affinity than do CRF receptors. Most CRF receptor antagonists do not bind the CRF-BP, because of the different structural requirements for binding to CRF receptors vs. the CRF-BP (Fekete and Zorrilla, 2007; Zorrilla and Koob, 2004).

2.2 Hypothalamic vs. extrahypothalamic CRF systems

CRF initiates the hypothalamic-pituitary-adrenal (HPA) axis neuroendocrine stress response by binding CRF_1 receptors in the anterior pituitary after release into portal blood. In addition, CRF_1 receptors are widely distributed in stress-responsive brain regions, including the neocortex, central extended amygdala, medial septum, hippocampus, thalamus, cerebellum, and autonomic midbrain and hindbrain nuclei (Grigoriadis et al., 1996; Primus et al., 1997; Sanchez et al., 1999; Van Pett et al., 2000). The CRF_1 receptor distribution resembles the distribution of its natural ligands CRF and Ucn 1 and accounts for the dissociable role of extrahypothalamic CRF_1 systems (i.e., outside the HPA-axis) to mediate behavioral and autonomic stress responses (Fekete and Zorrilla, 2007; Kozicz et al., 1998; Swanson et al., 1983; Zorrilla and Koob, 2004).

3. CRF_1 RECEPTOR ANTAGONISTS IN ANIMAL MODELS OF ANXIETY AND DEPRESSION

3.1 CRF_1 receptor antagonists in anxiety models

CRF_1 receptor antagonists produce anxiolytic-like effects in animal models of anxiety. In rodents, CRF_1 receptor antagonists reduced acoustic startle responding (Chen et al., 1997; Schulz et al., 1996), conditioned fear (Hikichi et al., 2000; Kikusui et al., 2000), shock-induced freezing (Weninger et al., 1999), and defensive burying behavior (Heinrichs et al., 2002; Richardson et al., 2008; Zhao et al., 2007a; Zorrilla et al., 2003). CRF_1 receptor antagonists also produced anxiolytic-like effects in exploration models of anxiety, but only under high anxiety baseline conditions (Gilligan et al., 2000; Griebel et al., 2002; Lelas et al., 2004; Okuyama et al., 1999; Zorrilla et al., 2002b). CRF_1 receptor antagonists blunted the anxiogenic-like effects of social stressors; they reduced ultrasonic vocalization responses of rat pups to neonatal isolation (Griebel et al., 2002; Kehne et al., 2000) and reduced anxiogenic-like responses of rodents (Millan et al., 2001; Overstreet and Griebel, 2004) and non-human primates (Habib et al., 2000) to unfamiliar conspecifics. A CRF_1 receptor antagonist (R317573/JNJ19567470/CRA5626) also produced anxiolytic-like effects in a rodent panic model (Shekhar et al., 2011).

3.2 Tolerance and side effect profile of CRF_1 receptor antagonists

Little tolerance is seen to the anxiolytic-like actions of CRF_1 receptor antagonists (Zorrilla and Koob, 2004). Unlike benzodiazepines, CRF_1 receptor antagonists did not have sedative or ataxic effects or impair attention or spatial learning (Hogan et al., 2005; Zorrilla and Koob, 2004; Zorrilla et al., 2002a). CRF_1 receptor antagonists may have less addiction liability than benzodiazepines; they did not promote formation of a conditioned place preference (Sahuque et al., 2006; Stinus et al., 2005) or support intravenous self-administration (Broadbear et al., 2002).

3.3 CRF_1 receptor antagonists in models of antidepressant activity

The efficacy of CRF_1 receptor antagonists in animal models of antidepressant activity is less consistent. Antalarmin reduced forced swim immobility in CRF_2 receptor null mutant mice

(Bale and Vale, 2003), an antidepressant-like effect, as did antalarmin, SSR125543A, LWH234, and CRA1000 in outbred rats (Griebel et al., 2002; Harro et al., 2001; Jutkiewicz et al., 2005). Subchronic treatment with R121919 and DMP696 also reduced forced swim immobility in mice (Nielsen et al., 2004) as did chronic SSR125543 treatment in Flinder Sensitive Line rats (Overstreet and Griebel, 2004; Overstreet et al., 2004a). R278995 reduced hyperemotionality (Chaki et al., 2004) in the olfactory bulbectomy model of depression (Song and Leonard, 2005). Finally, chronic treatment with antalarmin or SSR125543A reversed negative effects of chronic mild stress on coat appearance and hippocampal neurogenesis (Alonso et al., 2004; Ducottet et al., 2003; Griebel et al., 2002).

However, negative findings also are common. R121919, CP-154,526, and R278995 did not reduce forced-swim immobility in rats (Chaki et al., 2004; Jutkiewicz et al., 2005); antalarmin, CP-154,526, DMP904, R121919, and DMP696 did not reduce forced-swim immobility in mice (Nielsen et al., 2004; Oshima et al., 2003; Yamano et al., 2000). Antalarmin, CP-154,526, DMP904, R121919, DMP696, and R278995 did not produce antidepressant-like effects in the mouse tail-suspension test (Chaki et al., 2004; Liu et al., 2003; Nielsen et al., 2004). Although CP-154,526 Although CRF_1 antagonist treatment was initially reported to reverse “learned helplessness” (Mansbach et al., 1997), subsequent studies with CP-154,526, DMP904, DMP696, R2789995, and CRA1000 failed to replicate this finding (Chaki et al., 2004; Li et al., 2005; Takamori et al., 2001). Finally, R278995 did not produce antidepressant-like effects in the rat differential-reinforcement-of-low-rate 72-s model (Chaki et al., 2004).

3.3 Conditions under which CRF_1 receptor antagonists are effective

A possible reconciliation of these contradictory findings is that CRF_1 receptor antagonists may differentially show antidepressant-like activity in dysfunction models that involve genetic (e.g., CRF_2 null mutant mice) or environmental factors (e.g., chronic mild stress) that amplify or disinhibit CRF_1 receptor signaling. Alternatively, perhaps CRF_1 receptor antagonists are more effective against dynamic, active responses to acute stressors as compared to chronic, sustained stressors (Koob and Zorrilla, 2012). CRF_1 receptor antagonists consistently reduce anxiety-like behavior in genetic, environmental and pharmacologically-induced models of high anxiety, but not low anxiety, conditions (Zorrilla and Koob, 2004).

4. RATIONALE FOR TARGETING CRF_1 RECEPTORS TO TREAT ALCOHOL DEPENDENCE AND PREVENT RELAPSE

4.1 CRF_1 receptor antagonists in withdrawal/negative affect models of alcohol dependence

The reviewed preclinical data suggest that extrahypothalamic CRF_1 systems subserve some negative emotional states. Activation of CRF systems may therefore contribute to the *withdrawal/negative affect* stage of the addiction cycle. Individuals with high levels of innate anxiety or depression may be more likely to consume alcohol for its anxiolytic or dysphoria-relieving effects (Pohorecky, 1991). By reducing dysphoria, CRF_1 receptor antagonists may help treat individuals who “self-medicate” antecedent anxiety or depression with alcohol. Consistent with this idea, small molecule CRF_1 receptor antagonists reduce alcohol drinking in rodent models with high innate anxiety (Ciccocioppo et al., 2006; Hansson et al., 2007, 2006; Heilig and Koob, 2007; Lodge and Lawrence, 2003; Sommer et al., 2008), at doses that do not alter intake of normal, outbred rodents.

Chronic alcohol use itself, even if initiated for its rewarding effects, can lead to negative emotional symptoms and negatively reinforced alcohol use. An extension of the opponent process theory of affective regulation (Solomon and Corbit, 1974), this hypothesis of

addiction proposes that alcohol initially engages brain structures that subserve positive emotional states (e.g., pleasure, contentment). To restore emotional homeostasis, however, a counterregulatory, opponent-process then decrements mood and increases vigilance/tension via downregulation of brain reward circuitry and recruitment of brain stress circuitry (Breese et al., 2011; Heilig et al., 2010a, 2011; Heilig and Koob, 2007; Heilig et al., 2010b; Koob and Zorrilla, 2010, 2012; Logrip et al., 2011). With repeated cycles of intoxication/withdrawal, the opponent-process allostatically predominates over the rewarding primary process. Consequently, progressively more alcohol is required to maintain euthymia. In the absence of alcohol, negative affective signs emerge (i.e., acute withdrawal: anxiety, dysphoria, irritability). With a sufficient alcohol use history, dysphoria can episodically and spontaneously resurge and heightened responses to otherwise innocuous stressors can be seen despite sustained abstinence (i.e., protracted withdrawal). Accordingly, fMRI activation responses to negative affective pictures are sensitized in detoxified alcoholics (Gilman and Hommer, 2008). Under this conceptualization, alcohol use escalates and relapse occurs because alcohol prevents and relieves the intrinsically-determined negative emotional symptoms of acute and protracted withdrawal (Heilig and Koob, 2007; Koob and Zorrilla, 2010).

The opponent process putatively involves activation of otherwise quiescent brain CRF_1 receptor stress systems. Accordingly, cerebrospinal CRF levels are elevated in recently withdrawn alcoholics (Adinoff et al., 1996). In animal models, acute alcohol withdrawal activates CRF systems in the central nucleus of the amygdala (Funk et al., 2006; Merlo Pich et al., 1995; Roberto et al., 2010; Zorrilla et al., 2001) and bed nucleus of the stria terminalis (Olive et al., 2002), components of the central extended amygdala. Alcohol exposure also activates the HPA-axis via neuroendocrine CRF-dependent release of ACTH and glucocorticoids (Allen et al., 2011; Ogilvie et al., 1998; Rivier, 1996; Rivier and Plotsky, 1986),

Supporting a functional role for central extended amygdala CRF_1 receptor activation in the *negative affect/withdrawal* stage, site-specific injections of CRF receptor antagonists into the central amygdala reduce anxiety-like behavior, motivational deficits for other reinforcers, and excessive self-administration of addictive substances during acute withdrawal (Heilig and Koob, 2007; Heilig et al., 2010b; Koob and Zorrilla, 2010; Logrip et al., 2011; Parylak et al., 2011). CRF_1-dependent activation of the HPA-axis is also implicated because glucocorticoid receptor antagonists reduce the development and expression of excessive alcohol self-administration that results from repeated, intermittent intoxication (Vendruscolo et al., 2012). Consistent with both sets of findings, systemic injections of small molecule CRF_1 receptor antagonists reduce the heightened anxiety-like behavior (Breese et al., 2005a, 2005b; Gehlert et al., 2007; Knapp et al., 2004; Overstreet et al., 2004b; Sommer et al., 2008) and escalated alcohol self-administration of dependent rodents acutely withdrawn from alcohol (Chu et al., 2007; Funk et al., 2007; Gehlert et al., 2007; Gilpin et al., 2008; Richardson et al., 2008; Sabino et al., 2006) at doses that do not alter intake of non-dependent animals.

Neuroadaptations in amygdala CRF_1 systems still can be observed several weeks following detoxification from repeated cycles of intoxication/withdrawal in animal models (Sommer et al., 2008; Zorrilla et al., 2001). Accordingly, CRF_1 receptor antagonists reduce the potentiated anxiogenic-like and ethanol intake behavior responses to otherwise ineffectual stressors seen during protracted withdrawal (Rimondini et al., 2002; Sommer et al., 2008; Valdez et al., 2002, 2003). CRF_1 antagonists also attenuate the increased spontaneous anxiety-like behavior (Breese et al., 2005a, 2005b; Overstreet et al., 2002; Sommer et al., 2008; Valdez et al., 2002; Zhao et al., 2007b) and alcohol intake that can be seen in post-dependent rats even under low exteroceptive stress conditions (Rimondini et al., 2002;

Sommer et al., 2008; Valdez et al., 2002). Both sets of findings are critical, because the resurgence of negative emotional states during protracted withdrawal, whether in the presence or absence of external stressors, is a major predictor of relapse in alcoholics (Mossberg et al., 1985; Pickens et al., 1985).

4.2 CRF_1 receptor antagonists in relapse models of alcohol-seeking

CRF_1 receptors also have been implicated in stress-induced reinstatement of alcohol seeking (Le and Shaham, 2002; Shalev et al., 2010). In the reinstatement model of relapse, animals are trained to self-administer drugs and are then subjected to extinction training during which lever presses are not reinforced. Reinstatement of extinguished lever responding (the operational measure of drug seeking) is determined after exposure to drug priming injections, drug-associated cues, or different stressors (Shaham et al., 2003; Stewart and de Wit, 1987), such as intermittent footshock (Le et al., 2000; Liu and Weiss, 2002) or the pharmacological stressor yohimbine (Le et al., 2012; Marinelli et al., 2007). Systemic injection of yohimbine, an alpha-2 adrenoceptor antagonist, induces stress- and anxiety-like responses in humans and laboratory animals (Bremner et al., 1996a, b). Furthermore, yohimbine reinstates drug seeking in rats (Feltenstein et al., 2012; Shepard et al., 2004) and monkeys (Lee et al., 2004) and palatable food seeking in rats via a CRF_1 receptor-mediated mechanism (Ghitza et al., 2006). Yohimbine injections also induce alcohol and heroin craving in human drug addicts (Stine et al., 2002; Umhau et al., 2011).

In an initial study, Le et al. (2000) demonstrated that systemic injections of CP154,526 attenuate footshock-induced reinstatement of alcohol seeking in non-dependent rats. Subsequently, Hansson et al (2006) and Gehlert et al. (2007) reported that antalarmin and MTIP also attenuate footshock-induced reinstatement of alcohol seeking and additionally found that the CRF_1 receptor antagonists were more effective in alcohol dependent rats or in genetically selected, Marchigian Sardinian alcohol-preferring rats. In the latter rat line, the *Crhr1* transcript is innately upregulated in several brain areas, including the amygdala (Hansson et al., 2006). Finally, Marinelli et al. (2007) showed that antalarmin attenuates yohimbine-induced reinstatement of alcohol seeking.

The ability of CRF_1 receptor antagonists to reduce stress-induced reinstatement is mediated by extrahypothalamic sites. Intermittent fooshock-induced reinstatement was not affected by adrenalectomy (Le et al., 2000), and antalarmin had no effect on yohimbine-induced corticosterone secretion (Marinelli et al., 2007). Furthermore, blockade of CRF receptors in the median raphe nucleus (Le et al., 2012, 2002) was sufficient to attenuate footshock- and yohimbine-induced reinstatement of alcohol seeking. The results agree with reports that stress-induced reinstatement of cocaine and heroin seeking is mediated by stress-induced activation of extrahypothalamic CRF sites (Blacktop et al., 2011; Erb et al., 1998; Shaham et al., 1997; Wang et al., 2005, 2006).

Taken together, results from the studies described above have established a critical role of CRF_1 receptors in three main alcohol addiction-related behaviors in animal models: escalation of alcohol intake by the induction of alcohol dependence, acute and protracted aversive psychological withdrawal symptoms that can occur even in the absence of exteroceptive stress, and stress-induced relapse to alcohol seeking.

5. NONPEPTIDE CRF_1-SELECTIVE RECEPTOR ANTAGONISTS

5.1 Pharmacophore and selectivity of nonpeptide CRF_1 receptor antagonists

Almost all disclosed nonpeptide CRF_1 receptor antagonists conform to a single pharmacophore. Prototypical compounds (Figure 1) share one or two aliphatic top units that occupy a hydrophobic pocket of the receptor, a central mono-, bi-, or tricyclic ring core, and

an orthogonal, conformation-stabilizing, di- or -tri-substituted aromatic bottom group. Each ring core contains a putative proton-accepting ring nitrogen separated from the pendant aromatic by a one- or, more commonly, two-atom spacer. The core ring is typically methylated on the opposite position adjacent to the bonding nitrogen. Small molecules of this pharmacophore are potent and selective CRF_1 receptor antagonists in relation to their interactions with regions of the third (His-199) and fifth (Met-276) transmembrane CRF_1 receptor domains, residues not shared by the CRF_2 receptor (Zorrilla and Koob, 2004, 2010).

Rare exceptions to this pharmacophore include oxo-7*H*-benzo[*e*]pyrimidine-4-carboxylic acid derivatives (subtype nonselective CRF receptor antagonists discovered by Alanex), CC 2064460 (a moderately potent arylamidrazone CRF_1 receptor antagonist that lacks a central ring core with the customary hydrogen-bond accepting nitrogen), and stereospecific *N*-phenylphenylglycines (which also lack a ring core but were identified through computational screening based on a classic pharmacophore training set; Zorrilla and Koob, 2004, 2010).

5.2 Identification of "drug-like" CRF_1 receptor antagonists

Early CRF_1 receptor antagonist leads did not have drug-like physiochemical and pharmacokinetic properties. They violated Lipinski s "Rule of 5" heuristic for clinical drug candidates (e.g., excessively lipophilic and water insoluble with Log*P*>5) and correspondingly showed poor oral bioavailability ($F\% < 20\%$) and undesirably high volumes of distribution at steady state ($V_D > 10$ L/kg) and/or plasma clearance ($Cl_{plasma} > 45$ ml/min/kg; Chen, 2006; Zorrilla and Koob, 2004, 2010). As shown in Table 1, however, concerted medicinal chemistry efforts have procured compounds with more favorable properties. Each of these compounds is active *in vivo* at minimum effective systemic doses of 2–10 mg/kg in preclinical behavioral or endocrine animal models that are sensitive to CRF_1 receptor signaling. Notably, for MTIP and MPZP, these *in vivo* activities include published positive findings in animal models of alcohol dependence and relapse (Table 1).

Selected CRF_1 receptor antagonists that are currently in clinical trials are discussed below (see also Table 1 and Figure 1). GlaxoSmithKline developed moderately potent (IC_{50} = 32–100 nM) substituted tetrahydrotetraazaacenaphthylenes and diydropyrrolo[2,3-*d*]pyrimidines with excellent oral bioavailability (52–86%), distribution/clearance balance, and central accumulation (B/P = 2.3–3.7). These include verucerfont (NBI-77860/GSK561679; (Tellew et al., 2010) and emicerfont (GW876008; (Di Fabio et al., 2008). In addition, pexacerfont (Gilligan et al., 2009a), the potent (IC_{50} = 6.1 nM) lead candidate from Bristol Myers Squibb, is a substituted pyrazolo[1,5-*a*]-1,3,5-triazine that showed good pharmacokinetics in rat, dog, and nonhuman primate models, no evidence of gastrointestinal or respiratory toxicity, and mild renal effects at doses ~1 order greater than those needed to substantially occupy brain CRF receptors (Gilligan et al., 2009a; Zhou et al., 2012). Supporting its therapeutic potential, pexacerfont was more potent than MTIP in reversing acute alcohol withdrawal-induced anxiety-like behavior of rats in the elevated plus-maze. (Thorsell, Heilig, unpublished results).

5.3 Clinical trials for CRF_1 receptor antagonists

5.3.1 Completed trials for psychiatric indications—Clinical anticipation for CRF_1 receptor antagonists for stress-related psychiatric disorders has been high since an early, open-label Phase IIa trial found that increasing doses of R121919 normalized sleep EEG and reduced depressive and anxious symptoms in depressed patients with few side effects (Held et al., 2004; Kunzel et al., 2005, 2003; Zobel et al., 2000). Unfortunately, to date, no CRF_1 receptor antagonist has successfully completed a Phase III trial for any stress-related

psychiatric illness. Development programs for R121919 (Neurocrine Press Release April 5, 2000; Zobel et al., 2000) and PF-00572778 (NCT00580190) were discontinued due to liver enzyme elevations. ONO-2333Ms (NCT00514865; (Ono Pharmaceutical Co Ltd, 2008), verucerfont (GSK561679; GlaxoSmithKline Results Summary for CRS106139, 2010) and CP-316,311 (Binneman et al., 2008) all lacked efficacy in controlled trials for major depression. Pexacerfont (BMS-562086) did not relieve generalized anxiety disorder symptoms (Coric et al., 2010), suicidal ideation in anxious patients (Coric et al., 2009), or diarrhea-predominant irritable bowel syndrome (Sweetser et al., 2009). Emicerfont (GW876008) also lacked efficacy in a Phase II trial for irritable bowel syndrome (GlaxoSmithKline Results Summary for CRI105626, 2008). Trials of verucerfont and emicerfont for social anxiety disorder were completed with undisclosed results (NCT00555139), as were trials of pexacerfont (NCT00135421) and SSR125543 (NCT01034995) for major depression. Finally, verucerfont is still being evaluated for efficacy to reduce post-traumatic stress disorder in women (NCT01059227).

5.3.2 Receptor residence rate and clinical efficacy—Recent pharmacological findings may provide an explanation for the discrepancy between the early positive findings obtained with R121919 and the negative findings more recently obtained with ONO-2333Ms, CP-316,311, and pexacerfont. Specifically, the nominal binding affinities shown in Table 1 that were attributed to each small molecule during compound screening and development were based on room temperature, standard competition assays. Such non-equilibrium single-point measurements do not capture the actual kinetics by which the small molecule binds to and, most importantly, subsequently resides on the human CRF_1 receptor *in vivo* (Fleck et al., 2012; Ramsey et al., 2011). Greater receptor antagonist efficacy is putatively associated with greater residence time on the receptor (Brinkerhoff et al., 2008; Fleck et al., 2012; Tummino and Copeland, 2008; Vauquelin et al., 2006; Vauquelin and Van Liefde, 2006), as exemplified in the greater efficacy and duration of action of candesartan, a slowly dissociating angiotensin II AT1 receptor antagonist (Lacourciere and Asmar, 1999; Verheijen et al., 2004), versus the more rapidly dissociating antagonist losartan (Hansson, 2001). Kinetic analysis of receptor association and dissociation rates at physiological temperatures demonstrated that R121919, with which positive preliminary clinical findings were obtained, exhibits a "kinetic" K_i of ~0.36 nM, a potency underestimated by binding constants for it of 3–4 nM in standard competition assays (Fleck et al., 2012). In contrast, ONO-2333Ms, CP-316,311 and pexacerfont, for which negative clinical findings have been obtained, exhibit "kinetic" K_is of 1–2 orders poorer potency than R121919 (15, 12 and 19 nM, respectively), indicating that their nominal affinities (~2, 2 and 8 nM) may have overestimated their actual kinetic potencies (Fleck et al., 2012). Another way to conceptualize the kinetics of the antagonists is with respect to their residence time on the receptor, as reflected in the receptor dissociation rate. R121919 exhibits a slow off-rate (dissociation $t_{1/2}$) of 130 min, an order longer than those for ONO-2333Ms, CP316,311 and pexacerfont (17, 4 and 14 min, respectively). SSR125543A, for which clinical results have not yet been reported, exhibits very favorable receptor residence kinetics (dissociation $t_{1/2}$ = 430 min, kinetic K_i = 0.049 nM); results with SSR125543A may therefore provide discriminative information as to the importance of CRF_1 receptor kinetics for clinical efficacy (Fleck et al., 2012).

5.3.3 Recent positive experimental results with CRF_1 receptor antagonists in humans—Against the disappointing clinical outcomes to date, a few recent positive results may be instructive. As compared to placebo, oral emicerfont administration reduced blood oxygen level-dependent (BOLD) fMRI signal reductions in the hypothalamus, amygdala, hippocampus, insula, anterior cingulate, and orbitomedial prefrontal cortices during anticipation of pain in patients with irritable bowel syndrome (Hubbard et al., 2011). The

inhibitory effects of emicerfont on hypothalamic activation were specific to patients who were experiencing (anticipatory) state anxiety. Furthermore, R317573/JNJ19567470/CRA5626 decreased regional glucose utilization in the amygdala of 12 healthy adults (Schmidt et al., 2010) and also decreased anxiety responses to 7.5% acute CO_2 inhalation in a double-blind, placebo-controlled trial with healthy men (Bailey et al., 2011). The collective clinical findings are compatible with the revisionist hypothesis that CRF_1 receptor antagonists may be differentially effective for conditions that involve dynamic, active responses to acute stressors, as opposed to low stress or chronic, sustained negative emotional states (Koob and Zorrilla, 2012).

5.3.4 Ongoing clinical trials of CRF_1 receptor antagonists in addiction—In that context, and relevant to the reviewed stress-induced reinstatement preclinical literature, GlaxoSmithKline and NIH are collaboratively evaluating verucerfont for its ability to reduce stress-induced alcohol craving in anxious, stress-reactive alcoholic women (NCT01187511). Similarly, a comprehensive collaboration of Bristol Myers Squibb and NIH is currently testing oral daily pexacerfont for its efficacy to prevent: 1) stress-induced craving for palatable food in dieters (NCT01656577), 2) stress-induced craving for tobacco in smokers attempting to quit (NCT01557556), and 3) stress- or alcohol cue-induced craving in anxious, alcoholic women (NCT01227980). Even in the absence of a history of dependence or withdrawal state, CRF_1 antagonism is effective to suppress alcohol self-administration in rats that show high innate levels of anxiety-like behavior (Hansson et al., 2006). Selecting for alcohol-dependent patients with high trait anxiety therefore may offer a strategy to enrich for subjects sensitive to CRF_1 antagonism. This is critical for the ability of early human studies to detect a drug effect signal. Unselected populations of alcoholics diagnosed according to DSM-IV are heterogeneous, with many patients falling in an externalizing – impulsive cluster. Such patients are not necessarily expected to be sensitive to an anti-stress medication, and may therefore dilute a drug signal. Clinical assessments that distinguish trait from state anxiety are well validated and have successfully been used in prior studies that applied this type of enrichment strategy (George et al., 2008).

The inpatient study in anxious alcoholics involves a randomized, double-blind, placebo-controlled, parallel group design. Alcohol detoxification and associated withdrawal treatment is completed prior to inclusion in the experimental protocol. Participants are then randomized to 3 weeks of treatment (pexacerfont vs. placebo) in a 1:1 ratio. The oral dose in the pexacerfont trials involves a daily 300 mg/kg loading dose for 7 days, which results in ≥90% of subjects attaining a target circulating concentration of 0.5 μM within 5 days, followed by a 100 mg/day maintenance dose for 2 weeks. Co-primary outcomes include cravings induced by personalized, auditory scripts of stressful or alcohol-related guided imagery as compared to neutral imagery, as well as fMRI blood oxygenation level dependent (BOLD) responses to negative emotional stimuli. The stressful and alcohol-cue imagery used in the trial both induce alcohol craving, but only the stressful imagery also elevates state anxiety. This allows for testing the hypothesis that CRF_1 antagonists have the potential to selectively target pathways to alcohol relapse that are associated with stressful triggers.

By using surrogate efficacy markers such as craving and fMRI BOLD responses, this experimental medicine based approach offers a high level of safety, and may accelerate development because it can go forward in the absence of drug – alcohol interaction data. If positive, it will provide a compelling rationale for such interaction data to be obtained and for full scale, outpatient relapse prevention studies to be carried out. While the predictive validity of animal models is frequently debated, less attention has been devoted to whether surrogate outcomes in human experimental models, such as stress-induced craving or brain responses to aversive stimuli, can predict clinical efficacy. This important issue is by no

means settled, but some early observations give reason for measured optimism. Thus, both in alcohol and cocaine addiction, cravings in response to an experimental stressor in the laboratory have been shown to predict relapse during outpatient follow-up over three months (Sinha et al., 2011, 2006).

6. HETEROGENEITY OF ALCOHOLISM: A NEED FOR PERSONALIZED MEDICINE?

6.1 Heterogeneity of alcohol use disorders between and within individuals

The effectiveness of medications for alcohol use disorders may vary both across individuals and also within individuals at different times (Heilig et al., 2011, 2010b; Koob and Zorrilla, 2010; Logrip et al., 2011, 2012). For example, treatments may be differentially effective for different diagnoses because of the different underlying biological and psychological mechanisms, and, for similar reasons, during different stages of the addiction cycle. Based on the evidence described, CRF_1 receptor antagonists may be more effective for alcoholism treatment after use has transitioned to primarily negatively-reinforced drinking (*withdrawal/negative affect*) or to protect against stress-induced relapse (*stress-related craving*). They may be less effective in preventing alcohol craving that is not related to negative emotional states or stressor precipitants, such as alcohol cue-induced relapse (Liu and Weiss, 2002). CRF_1 receptor antagonists also may less effectively reduce reward-motivated, recreational binge drinking earlier in the addiction process (Heilig and Koob, 2007).

6.2 CRF system genetic variants and alcohol drinking phenotypes

6.2.1. Polymorphisms in animal models—The effectiveness of CRF_1 receptor antagonists, as for other medicines, also may depend on genetic factors (Heilig et al., 2011; Sinha et al., 2011). Alcohol dependence has an estimated heritability of 50 – 60% (Goldman et al., 2005), with many susceptibility loci contributing individually to a small degree (Treutlein et al., 2009). Animal models support the hypothesis that variants in the genes that encode CRF system molecules, by altering CRF system activation under basal conditions or in response to stressful life events, may promote negatively-reinforced alcohol intake. For example, msP alcohol-preferring rats show two G-to-A polymorphisms in allelic identity with one another in the distal promoter of the *Crhr1* gene, mutations that are not seen in other alcohol-preferring lines or outbred rats (Hansson et al., 2006); Logrip, Ciccocioppo, Walker, Koob and Zorrilla, unpublished observations). Perhaps as a result, the msP line exhibits increased CRF_1 receptor expression in several stress-related brain regions, increased anxiety-like behavior and alcohol preference ratios, and increased sensitivity to the ability of CRF_1 receptor antagonists to reduce alcohol self-administration and stress-induced reinstatement of alcohol seeking (Ciccocioppo et al., 2006; Gehlert et al., 2007; Hansson et al., 2006); Logrip, Ciccocioppo, Walker, Koob and Zorrilla, unpublished observations). Similarly, rhesus monkeys that carry a C-to-T single nucleotide polymorphism in the promoter of the *Crh* gene exhibit CRF peptide expression that is unrestrained by glucocorticoid feed-back inhibition. While without effect on voluntary alcohol intake in individuals with a normal life history, this is associated with a doubling in consumption in monkeys exposed to early life stress (Barr et al., 2009).

6.2.2 Polymorphisms in humans—Supporting the translational relevance of the genetic results in rats and monkeys, polymorphisms in human CRF system molecules also have been associated with alcohol use phenotypes. For example, variant *Crhr1* haplotypes in adolescents predict binge drinking and lifetime prevalence of intoxication and alcohol dependence (Treutlein et al., 2006). *Crhr1* single nucleotide polymorphisms (SNPs) also predicted greater alcohol consumption in already dependent individuals (Treutlein et al., 2006) and were associated with reduced P300 amplitude, an endophenotype seen in

alcoholics (Chen et al., 2010). Adolescents homozygous for the C allele of the rs1876831 SNP of the *Crhr1* gene exhibited greater future drinking (Blomeyer et al., 2008; Schmid et al., 2010) and an earlier onset of drinking in an interactive relation to stress history (Schmid et al., 2010). Conversely, adolescents homozygous for the H2 haplotype that contains the minor allele of rs1876831 are protected against early child abuse-associated increases in alcohol consumption and dependence (Nelson et al., 2010). Adolescent carriers of the A allele of the rs242938 *Crhr1* SNP also reported greater alcohol drinking when exposed to stress in some (Schmid et al., 2010), but not other (Blomeyer et al., 2008), studies.

Genetic associations of CRF system polymorphisms to human alcohol phenotypes also have been seen for the CRF-binding protein (CRF-BP), which moderates the ability of CRF to interact with its receptors. For example, certain *Crhbp* gene SNPs are associated with decreased EEG alpha wave power, an endophenotype of alcoholism, and are more prevalent in alcohol use disorders (Enoch et al., 2008), including in alcoholics with comorbid anxiety disorders (Enoch et al., 1999). A *Crhbp* polymorphism (rs10055255) also has been associated with severity of stress imagery-induced alcohol craving and dysphoria (Ray, 2011). Recently, *Crhbp* (rs3811939) and *Crhr1* SNPs (the widely-studied rs110402 polymorphism) were found to jointly predict comorbid alcohol use disorder in patients with schizophrenia (Ribbe et al., 2011). Elevated levels of CRF_1 receptor mRNA relative to CRF-BP mRNA were seen in mononuclear blood cells from individuals carrying the dual polymorphism (Ribbe et al., 2011), supporting the hypothesis that that CRF-CRF_1 receptor interactions predominate over CRF-CRF-BP interactions in those at risk for alcohol use disorders. Altogether, the human genetic findings further support the hypothesis that CRF_1 receptor activation may contribute to the pathophysiology of alcohol use disorders. Furthermore, these findings suggest that pharmacogenomic profiling could identify genetically-vulnerable patients for whom CRF_1 receptor antagonist pharmacotherapy may be especially useful to prevent alcohol relapse (Sinha et al., 2011).

7. CONCLUDING REMARKS

We briefly reviewed basic pharmacological and behavioral properties of small-molecule CRF_1 receptor antagonists as well as their clinical trial status for psychiatric disorders. We discussed their potent effect in three main alcohol addiction-related behaviors in animal models: dependence-induced escalation of alcohol intake, negative emotional symptoms of acute and protracted withdrawal, and stress-induced relapse to alcohol seeking. These data provide a strong rationale for — translating these basic research findings to assess the efficacy of CRF_1 receptor antagonists for the treatment of alcohol dependence and relapse. The future also holds great promise for discovering ways to individualize medications to suit the genetic profile of the individual. Genetic factors are likely to influence both the individual's form of alcoholism and the pharmacokinetic and pharmacodynamic response to medications. In particular, CRF-related genes play an important role in alcohol dependence and relapse in humans. The outcome of clinical trials may depend on targeting the CRF_1 receptor antagonists to a selected set of alcoholics for whom, due to experiential or genetic reasons, stress and negative reinforcement play a major role in the disease process. Personalized treatment, while holding promise for the long-term, poses a challenge for clinical development, because it requires studies to predict, identify and enrich for subjects likely to be responsive.

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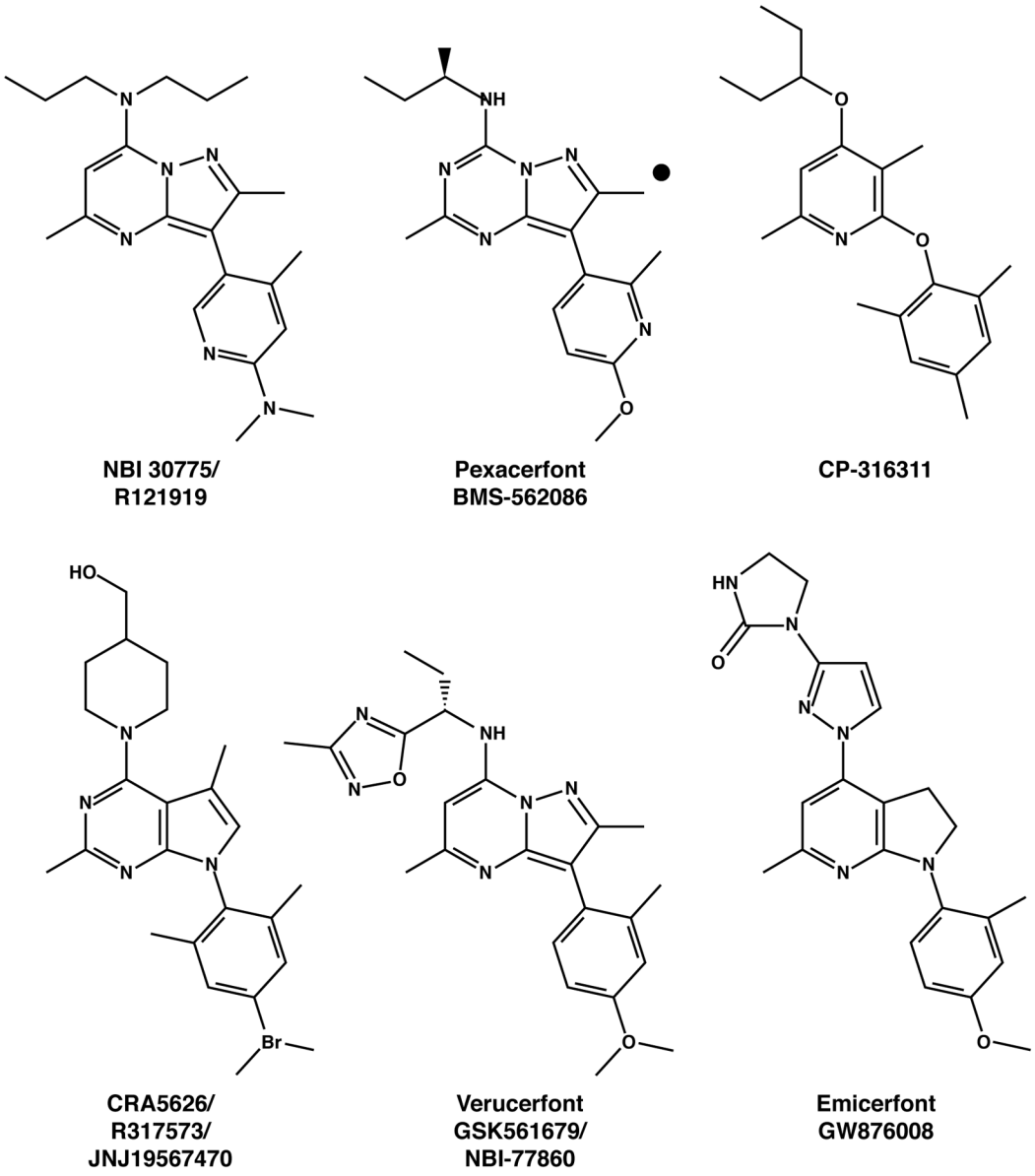

Figure 1.
Prototypical CRF_1 receptor antagonists that have been tested in humans.

Table 1

Selected nonpeptide CRF_1receptor antagonists with “drug-like” properties

Familiar name	CAS registry	Nominal CRF_1 affinity (nM)	cLog *P*	Oral bioavailability (F%)	C_l plasma (ml/min·kg)	V_d (L/kg)	$t_{1/2}$ (h)	Notes	Refs
Emicerfont (GW876008)	786701-83-5	IC_{50} = 66	1.5	66%	19	2.4	1.6	B/P = 3.7; reduced i.c.v. CRF-induced gerbil forepaw treading (10 mg/kg), marmoset defensive postures (10 mg/kg), and rat pup ultrasonic vocalization (30 mg/kg)	(Di Fabio et al., 2008)
Verucerfont (NBI-77860/GSK561679A)	885220-61-1	pK_i=8.2 (~16 nM)	4.3	66%	14	7.5		B/P=1.6	(Tellew et al., 2010)
MTIP	910551-43-8	K_d = 0.22	3.6	91%	4.9	1.7	3.9	Reduced conflict behavior; reduced hangover’ anxiety-like behavior in elevated plus-maze (10 mg/kg); reduced alcohol self-administration (10 mg/kg); reduced ethanol-intake in alcohol post-dependent or alcohol-preferring rats; decreased stress-induced reinstatement	(Gehlert et al., 2007; Sommer et al., 2008)
MPZP	202579-76-8	K_d = 4.9	2.9	—	—	—	—	Reduced defensive burying behavior; reduced ethanol, nicotine and cocaine self-administration in dependence models	(George et al., 2007; Richardson et al., 2008; Specio et al., 2008)
Pexacerfont (BMS-562,086)	459856-18-9	K_d = 6.1	2.9	40%	17.9	14.9	13.5	Anxiolytic-like activity in elevated plus-maze and defensive withdrawal models (10 mg/kg, p.o.); solubility of 16 μg/ml in water (pH 7.4) and 16.3 mg/ml in 0.01 N HCl (pH 2.5); good pharmacokinetics in nonhuman primates	(Gilligan et al., 2009a; Zhou et al., 2012)
BMS-561,388	202578-88-9 (free base)	K_d = 4.7	2.2	51%	20	14.6	9.7	Anxiolytic-like activity in elevated plus-maze and defensive withdrawal models (10 mg/kg, p.o.); observed log*P* = 4.76; solubility < 1 μg/ml in water (pH 7.4) and 2.5 mg/ml in 0.01 N HCl (pH 2.5); inferior oral pharmacokinetics in chimpanzee compared with pexacerfont	(Gilligan et al., 2009b)

Note: Intravenous pharmacokinetic parameters are C_l (clearance from plasma), V_d (volume of distribution at steady state) and $t_{1/2}$ (plasma half-life).

Abbreviations: ACTH, adrenocorticotropic hormone; B/P, brain/plasma ratio; *c*Log*P*, calculated Log*P*; CRF, corticotropin-releasing factor. cLog*P* was calculated with Advanced Chemistry Development (ACD/Labs) Software v. 8.19 for Solaris.