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Don't stress about CRF: Assessing the translational failures of CRF_1 antagonists

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Abstract

BACKGROUND—Dr. Athina Markou sought treatments for a common neural substrate shared by depression and drug dependence. Antagonists of corticotropin-releasing factor (CRF) receptors, a target of interest to her, have not reached the clinic despite strong preclinical rationale and sustained translational efforts.

METHODS—We explore potential causes for the failure of CRF_1 antagonists and review recent findings concerning CRF-CRF_1 systems in psychopathology.

RESULTS—Potential causes for negative outcomes include: 1) poor safety and efficacy of initial drug candidates due to bad pharmacokinetic and physicochemical properties 2) specificity problems with preclinical screens, 3) the acute nature of screens vs late-presenting patients, 4.) positive preclinical results were limited to certain models and conditions with dynamic CRF-CRF_1 activation not homologous to tested patients, 5) repeated CRF_1 activation-induced plasticity that reduces the importance of ongoing CRF_1 agonist stimulation, 6) therapeutic silencing may need to address CRF_2 receptor or CRF-BP molecules, constitutive CRF_1 activity, or molecules that influence agonist-independent activity or to target structural regions other than the allosteric site bound by all drug candidates We describe potential markers of activation towards individualized treatment, human genetic and functional data that still implicate CRF_1 systems in emotional disturbance, sex differences, and suggestive clinical findings for CRF_1 antagonists in food craving and CRF-driven HPA-axis overactivation.

CONCLUSION—The therapeutic scope of selective CRF_1 antagonists now appears narrower than had been hoped. Yet, much remains to be learned about CRF's role in the neurobiology of dysphoria and addiction and the potential for novel anti-CRF therapies therein.

Keywords

CRF or CRH or corticotropin-releasing hormone or corticotropin-releasing factor or CRF1 receptor antagonist or CRH1 receptor antagonist; drug addiction or alcoholism or alcohol dependence or alcohol use disorder or binge drinking or binge eating or food addiction; major depression; generalized anxiety disorder or panic disorder or post-traumatic stress disorder; antidepressant or anxiolytic; irritable bowel syndrome; translation or clinical trial or treatment

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In an early, influential contribution, Dr. Athina Markou, with Drs. Kosten and Koob, cited comorbidity data, preclinical findings on the neurobiological consequences of substances of abuse, and similar neurotransmitter alterations to propose that drug dependence and major depression share a common neurobiological substrate. In this conceptual model, drug use is motivated by negative reinforcement mechanisms to relieve depression-like symptoms – the so-called "self-medication" hypothesis (Markou et al 1998; Paterson et al 2007). From that time on, a thrust of research in their and other laboratories has been to identify novel compounds with antidepressant and anxiolytic activity (Markou and Cryan 2012) in order to reduce the suffering of emotional disorders and of the abstinent state in drug-dependent individuals. Relatedly, novel compounds (e.g., mGluR5 antagonists; Markou 2007; Stoker et al 2012) and recognized antidepressants, such as fluoxetine, bupropion and desipramine (Lin et al 1999; Harrison and Markou 2001; Harrison et al 2001; Cryan et al 2003a; Bruijnzeel and Markou 2003; Takamatsu et al 2006, 2011; Paterson et al 2008a, b; Paterson et al 2007) have been used as pharmacological tools to understand better the neurobiology of drug dependence.

At the same time that the "shared neurobiology and self-medication hypothesis" was published, there was mounting interest in the therapeutic potential of corticotropin-releasing factor (CRF) receptor antagonists to treat stress-related psychiatric disorders. Preclinical studies during the previous 15 years had strongly supported the hypothesis that CRF was a key physiological mediator of not only neuroendocrine, but also behavioral, responses to psychosocial stress, and stress was a known etiologic factor in depression, anxiety disorders and addiction. The cloning of a second CRF receptor subtype in 1995 (CRF_2) raised uncertainty as to the roles of each subtype in mediating the actions of CRF (Lovenberg et al 1995), including vis-à-vis the "depressed" neural substrate hypothesized to be common to major depression and drug dependence (Macey et al 2000).

In this context, there was much interest to determine the role of each CRF receptor subtype (CRF_1, CRF_2) in mediating dysphoria and, by inference, the anti-dysphoria therapeutic potential of subtype-selective CRF receptor antagonists (Zorrilla et al 2002; Henry et al 2006; Cryan et al 2003b). In one project, Dr. Markou organized a collaboration between colleagues at The Scripps Research Institute and a pharmaceutical partner at Novartis-Basel to determine whether antalarmin, a recently identified, first-generation, small molecule CRF_1 antagonist (see Figure 1), had anxiolytic-like activity in the rat. The findings were among the first to show that selective CRF_1 antagonists reduced naturally-occurring anxiety-like behavior (Zorrilla et al 2002), joining reports that a structurally-related CRF_1 antagonist, CP-154,526, had antidepressant-and anxiolytic-like activity in rodent models (Mansbach et al 1997; Kehne et al 2000).

In the intervening 15 years, an enormity of medicinal chemistry, preclinical testing, and clinical trials concerning CRF_1 antagonists has been performed. Unfortunately, since initial promising results of an open-label Phase IIa trial of the CRF_1 antagonist R121919 for the treatment of major depression reported in 2000 (Zobel et al 2000; see Figure 1), a series of disappointing clinical failures have followed. Table 1 summarizes the many drug-like, small molecule CRF_1 antagonists that have failed to successfully complete double-blind, placebo-controlled trials for a wide range of stress-related psychiatric disorders. We and others have

reviewed details of these trials previously (Zorrilla et al 2013; Shaham and De Wit 2016; Sanders and Nemeroff 2016). In light of these setbacks, a recent commentary noted that CRF_1 receptor antagonists, which were considered by many to have some of the strongest preclinical evidence of recent therapeutic candidates for psychiatric disorders, appeared to have been lost in translation from the laboratory to the bedside (Shaham and De Wit 2016). Here, we analyze, and in some cases revisit (Zorrilla and Koob 2010; Koob and Zorrilla 2012; Zorrilla et al 2013; Shaham and De Wit 2016), possible explanations for the negative outcomes, in order to assess constructively the most current state of the field. In the spirit of avoiding translational obstacles, we also review recent (2014 to present) findings in humans and non-human primates, many of which continue to implicate a role for CRF_1 receptors in psychiatric conditions.

Performance in animal models

Given the perception that CRF_1 antagonists performed well in preclinical models but have performed poorly in the clinic, one might prematurely conclude that existing preclinical models are invalid predictors of clinical efficacy in psychiatric disorders (see also Hyman 2012) This alarmist interpretation is not well-supported. In the alcohol research literature, for example, Shaham and De Wit (2016) noted that other drugs that were effective in animal models of stress-induced reinstatement (e.g., alpha-2 adrenoceptor agonists such as clonidine and lofexidine) translated to showing efficacy against stress-induced drug craving in human laboratory studies (Mantsch et al 2016; Sinha et al 2011). Similarly, acamprosate and the opioid receptor antagonists naltrexone and nalmefene reduce operant oral ethanol self-administration in rats under a variety of conditions (Rassnick et al 1992; Heyser et al 1998; Heyser et al 2003; Sabino et al 2006; Ji et al 2008; Gilpin et al 2008; Walker and Koob 2008) and, analogously, show some efficacy to mitigate alcohol use disorders (see Stevenson et al 2015; Keating 2013; Rösner et al 2010; Plosker 2015; but see Palpacuer et al 2015). Gabapentin reduced both the anxiogenic-like behavior and the increased ethanol self-administration observed in withdrawn, ethanol dependent rats, but not non-dependent rats (Roberto et al 2008; Besheer et al 2016; Watson et al 1997) and was found to improve emotional function and reduce insomnia and alcohol use in abstinent alcoholics (Bonnet et al 2007; Malcolm et al 2007; Brower et al 2008; Myrick et al 2009; Mason et al 2014). Most recently, the glucocorticoid receptor antagonist mifepristone, which like CRF_1 antagonists more efficaciously reduces ethanol intake in dependent rodents during abstinence than in non-dependent rodents (Yang et al 2008; Simms et al 2012; Vendruscolo et al 2012; Vendruscolo et al 2015), was found to reduce alcohol-cued craving in the laboratory as well as naturalistic measures of alcohol use in a double-blind, placebo-controlled study of 56 alcohol-dependent human subjects (NCT01548417; Vendruscolo et al 2015). Thus, the preclinical models do show predictive *sensitivity* to detect effective treatments.

On the other hand, Haller et al., have pointed out that, like CRF_1 antagonists, 40% of compounds that showed activity in so-called “classical” or “popular” animal models of anxiety-like behavior (which are used in ~90% of anxiety studies), ultimately failed to show therapeutic activity in humans (Haller et al 2013). Accordingly, many neurokinin, cholecystokinin, and 5-hydroxytryptamine type 3 receptor antagonists that showed activity in these preclinical models and were developed contemporaneously with CRF_1 receptor

antagonists also were then found to be ineffective to treat anxiety disorders. Thus, even when preclinical models show predictive sensitivity to detect therapeutic compounds (i.e., ~60% of compounds that advanced to human trials based on promising results in anxiety models did ultimately show efficacy), they may have suboptimal specificity. Identical issues hamper preclinical models of antidepressant activity. Solutions to improve not only the sensitivity, but also specificity, of preclinical anxiety and depression models have been proposed and apply similarly to all psychiatric domains in which CRF_1 antagonists have yet to show clinical efficacy (Haller et al 2013; Griebel et al 2013; Stewart et al 2015; Belzung 2014).

The concept of predictive, validity, in the literature currently is often used to refer, to whether an effective treatment is detected by a model In reality, however, predictive validity refers to whether a model distinguishes effective vs, ineffective, treatments, which jointly reflects the identification of true positives and true negatives in a summary measure of accuracy. Analogous to how positive and negative predictive value jointly determine the accuracy of diagnostic tests in receiver-operating-characteristics (ROC) analyses, both sensitivity and specificity must be considered to determine the predictive validity of animal models. From a screening perspective, the joint use of an animal model with high predictive sensitivity with another having high predictive specificity may yield better outcomes than current screening approaches that focus more on predictive sensitivity (see Abruzzo et al 2015 for analogous approaches with diagnostic tests). The suboptimal specificiiy of commonly used models of depression and anxiety disorders may reflect an incomplete implementation of the pathognomonic constructs and pathophysiological bases of these disorders, in contrast with more recently developed models for alcohol use disorder.

Another consideration is the reality that CRF_1 antagonists did not show activity in some models or conditions under which some clinically efficacious treatments do. For example, CRF_1 antagonists did not reduce substance- or cue-induced reinstatement of substance-seeking in animal models, so it is not surprising that they did not reduce alcohol cue-induced craving in human laboratory studies (Schwandt et al 2016; Kwako et al 2015). Similarly, CRF_1 antagonists did not reduce and even exacerbated fear-potentiated acoustic startle responses in rat models (Walker et al 2009). Accordingly, the CRF_1 antagonist GSK561679 ultimately increased fear-potentiated acoustic startle reactivity in 31 healthy women (Grillon et al 2015). Thus, for a few endpoints, the “negative” clinical results may actually translate from the preclinical findings.

Along the same lines, whereas several clinically effective treatments like naltrexone, nalmefene, and acamprosate reduce alcohol self-administration in rat models of non-dependent alcohol self-administration, including in rats genetically selected for high alcohol preference or in outbred rats receiving alcohol under intermittent schedules of alcohol access (Rassnick et al 1992; Heyser et al 1998; Heyser et al 2003; Sabino et al 2006; Ji et al 2008; Gilpin et al 2008; Walker and Koob 2008), CRF_1 antagonists frequently did not (Sabino et al 2006; Gilpin et al 2008; Sabino et al 2013). Rather, they differentially or more strongly showed effects in rats that had been made dependent on alcohol due to chronic intermittent exposure (but, see also some positive findings in non-dependent rat (Simms et al 2014; Cippitelli et al 2012) and mouse models (Lowery et al 2010; Sparta et al 2009; Lowery et al

2008; Sparta et al 2008)). Similarly, unlike benzodiazepines, CRF_1 antagonists did not typically show activity under baseline conditions in most models of anxiety-like behavior, including the elevated plus-maze (see Zorrilla and Koob 2004). Likewise, unlike tricyclic antidepressants and noradrenergic- or serotonergic-reuptake inhibitors, CRF_1 antagonists did not consistently show activity under baseline conditions in rodent forced swim tests and several other models that have been used to screen for antidepressant-like compounds Rather, they required environmental, pharmacological, or genetic manipulation to induce a stress-like phenotype during testing (or, for some antidepressant-predictive models, did not show activity even under those conditions; see Zorrilla and Koob 2010). One skeptical interpretation of these results might have been that, even though the forced swim test is subject to false positive results (specificity issue, low PPV+), perhaps the predictive value of a negative result in the model is high (sensitivity issue, high NPV+) CRF_1 antagonists might thereby have not been expected to show antidepressant-like activity. Instead, the collective findings were regarded as being conceptually appealing and heralded as evidence that pathological substance use and dysphoria are associated with recruitment of otherwise quiescent CRF-CRF_1 synaptic transmission, a hypothesis also supported by molecular and electrophysiological studies in preclinical models.

Revisionist hypotheses and their implications

In light of the negative findings to date in clinical studies, revised hypotheses concerning the manner of "CRF-CRF_1 recruitment" needed for therapeutic activity have been offered For example, CRF_1 antagonists were proposed to be effective in "specific psychiatric disorders in which stress was a dynamic rather than chronic condition" (to include, for example, PTSD, panic and addiction disorders and exclude major depression and generalized anxiety disorder; Koob and Zorrilla 2012) or, in which central CRF overactivation was explicitly present. Neither of these hypotheses has been fully evaluated yet, but they raise several testable predictions.

One interpretation of the "dynamic" revision is not only that only certain types of stress-related disorders may be treatable by CRF_1 antagonists, but also that a given patient may be more sensitive to CRF_1 antagonist treatment earlier in the course of their disorder (before CRF activation has become chronic). The "dynamic" revision also suggests that sensitivity to CRF_1 antagonists might decrease with greater chronicity in preclinical models and that repeated CRF_1 activation (as in chronic stress) may lead to plasticity within or downstream of CRF_1 receptor signaling that comes to perpetuate the maladaptive behavior comparatively less dependent on subsequent acute CRF_1 agonist stimulation. Possible mechanisms for such plasticity have been described, including kindling, priming, heterologous sensitization, altered G-protein coupling, altered splicing, and long-term potentiation (Lee et al 2008; Ray et al 2011; Sajdyk et al 1999; Rainnie et al 2004; Narla et al 2016; Zmijewski and Slominski 2010; Dunn et al 2016; Magalhaes et al 2010; Bunson et al 1998; Rajbhandari et al 2015; Huang et al 2010; Krishnan et al 2010). It may be that patients present for treatment later in their disease course, after more such plasticity has occurred, as compared to preclinical models, which are designed for expeditious testing. Finally, the "dynamic" revision suggests that patients may be more responsive to CRF_1 antagonists during particular circumstances or stages of their disorder during which stress responses play a greater role in driving

symptoms. In support of the final proposition, oral CRF_1 antagonist emicerfont administration (and not placebo) selectively reduced BOLD fMRI signal in the hypothalamus, amygdala, hippocampus, insula, anterior cingulate, and orbitomedial prefrontal cortices in patients with irritable bowel disease who were actively experiencing anxiety in anticipation of visceral pain (Hubbard et al 2011). Similarly, the CRF_1 antagonist R317573/JNJ19567470/CRA5626 decreased regional glucose utilization in the amygdala (Schmidt et al., 2010) and anxiety responses to 7.5% acute CO_2 inhalation challenge in a double-blind, placebo-controlled trial with healthy men (Bailey et al 2011).

Relatedly, the "CRF overactivation" revision suggests that CRF_1 antagonists may be effective in patients who show high activity in CRF-CRF_1 systems (on either a trait or state basis). This could be probed via biochemical (e.g., high CSF CRF), neuroimaging (e.g., altered CRF_1 receptor availability), endophenotypic (e.g., increased REM sleep/pressure signs of high CRF drive; see also. Heilig et al 2016, and Heilig and Leggio 2016), or genetic means (e.g., functional single nucleotide polymorphisms [SNPs] in CRF system molecules; Holsboer and Ising 2010; Zorrilla et al 2013; Sanders and Nemeroff 2016, Treutlein et al 2006, Barr et al 2008, Nelson, et al 2010, Heilig et al 2011) Significant limitations of the often cited initial clinical study that obtained promising results with R121919 in. patients with major depression (Zobel et al 2000) include that it was small, not double-blind, and did not show a significant cross-sectional difference between subjects treated with the high- versus low-escalating dose schedule. With regard to potential markers of treatment response, however, a reanalysis of the study found that patients with increased rapid eye movement (REM) sleep density during the first half of the baseline night showed a greater reduction of Hamilton-Depression scores with high-dose R121919 treatment than those that did not. Low-dose R121919 treatment was ineffective in all groups (Held et al 2004). On the other hand, several SNPs for CRF_1 and CRF-binding protein (CRF-BP) did not predict treatment response to pexacerfont in patients with generalized anxiety disorder (Coric et al 2010). Thus, validating therapeutically prognostic markers of CRF-CRF_1 activation in double-blind, placebo-controlled studies may be key.

Targeting and validating drug action in humans

Clinical trial failures alternatively might reflect the inadequacy of prioritized drug candidates to quiet central CRF_1 signaling in humans. Indeed, early CRF_1 antagonists had unacceptably high lipophilicities and other physiochemical properties not characteristic of successful CNS drugs, leading to high toxicity potential and poor bioavailability (see Zorrilla and Koob, 2010). Some compounds were suggested to yield negative results because they had lower CRF_1 affinity than R121919 (e.g., CP 316,311; Holsboer and Ising 2010). More recently, it was proposed that a long duration of receptor residency may be key for efficacy (Fleck et al 2012; Zorrilla et al 2013), because R121919 had slower receptor dissociation rates than compounds that had failed clinically. In support of the importance of this property for antagonist action *in vivo*, verucerfont (NBI-77860; see Figure 1), a high-affinity, drug-like (Zorrilla and Koob, 2010), CRF_1 antagonist with long receptor residence, was found, like R121919, and unlike the faster-dissociating compounds pexacerfont and CP316,311, to reduce circulating adrencocorticotropic hormone (ACTH) in adrenalectomized rats. In anxious individuals with alcohol use disorder, verucerfont also reduced ACTH and cortisol

responses to dexamethasone/CRF challenge and blunted right amygdala fMRI activation responses to fearful faces, activities that had not been seen for the faster-dissociating pexacerfont (Schwandt et al 2016; Kwako et al 2015). These findings validate the drug's pharmacological action Also, as expected, verucerfont did not reduce alcohol cue-induced craving or anxiety, negative findings consistent with the lack of action of CRF_1 antagonists in animal models of cue-induced craving Unexpectedly, however, verucerfont still did not reduce alcphol craving or anxiety induced by public speaking or by stress-related guided imagery; rather, it worsened anxiety associated with the New Trier social stress test of public speaking (Schwandt et al 2016). Thus, although the verucerfont trial was negative, the confirmation that receptor residence, a previously underappreciated property, was key for pharmacological action, supports the possibility that not all mechanisms critical for achieving desired CRF_1 silencing in humans have been identified.

To the degree that the biology of human vs rodent CRF_1 receptors differ (or that of several, molecular partners that influence CRF_1 signaling; Dunn et al 2016; Bonfiglio et al 2013; Bangasser et al 2010 Walther et al 2015), cryptic species differences also may be impeding therapeutic silencing of CRF_1 receptors in humans. Furthermore, selective CRF_1 antagonists that have been tested to date have no activity at CRF_2 receptors or the CRE-binding protein (CRF-BP). The CRF_2 subtype in rodents has often been regarded as having a net null or perhaps even anxiolytic-like action, but as Dr. Markou and others showed, stimulation of CRE_2 receptors in the lateral septum is anxiogenic in rodents (Bakshi et al 2007, Henry et al 2006, Anthony et al 2014). Furthermore, in contrast to rodents, which mainly express only CRF_1 receptors in the central nucleus of the amygdala (CeA) (Van Pett et al 2000), primates also express substantial numbers of CRF_2 receptors of unknown behavioral significance in the CeA (Sanchez et al 1999). Third, humans, but not rodents, possess a unique CRF_2 gamma subtype (Kostich et al 1998).Finally,CRF_2receptors interact with the CRF-B.P to produce actions independent from CRF_1 (Wang et al 2007, Ungless et al 2003, Slater et al 2016a, Slater et al 2016b, Milan-Lobo et al 2009).

In this context, the CRF-BP initially had been regarded as serving an inhibitory role in the CRF system; but, it has increasingly been recognized to have other modulatory roles in the brain (Westphal and Seasholtz 2006). Indeed, the CRF-BP has recently received attention as a potential target for its role in alcohol use disorder (Haass-Koffler et al 2016, Ketchesin et al 2016); and, particularly, its role in the escalation of alcohol drinking may involve interaction with CRF_2 receptors (Albrechet-Souza et al 2015, Quadros et al 2016). Receptor activity modifying proteins (RAMPs) are other molecules that interact directly with the CRF system, as RAMP2 binds CRF_1 and increases its surface expression and signaling sensitivity (Wootten et al 2013). But CRF_1 antagonists to date likewise have not explicitly considered CRF_1-RAMP2 complexes.

Thus, perhaps small molecules that: 1) act as non-selective antagonists at both CRF subtypes, 2) exhibit inverse agonist activity, or 3) also modulate activities of other CRF system molecules, such as CRF-BP or receptor activity modifying protein-2 (RAMP2) (Wooten et al 2013; Weston et al 2016; Gingell et al 2016) would have greater therapeutic activity than the many selective CRF_1 neutral antagonists tested to date. Similarly, the structural manner of binding the receptor may be important; perhaps small molecules that

bind differentially to certain residues of the atypical allosteric binding site (e.g., compare MTIP vs. CP-376395; Xu et al 2015) may slow the antagonist's escape kinetics (Bai et al 2014) or direct its anti-signaling pathway bias (Suen et al 2014; Zhang et al 2016). Alternatively, a small molecule that binds to the orthosteric (agonist)-binding site, rather than to the atypical, deep allosteric binding site that is bound by all clinically-evaluated CRF_1 antagonists to date (see Zorrilla and Koob, 2010; Hollenstein et al 2013; Hausch 2013) may yield different pharmacological effects. Finally, there are different degrees of "inactivity" in terms of receptor confirmation, and a recent study showed that a cooperative, "double antagonist" approach (one at orthosteric, one at allosteric site) led to the most inactive state for the CC chemokine receptor 2, another G-protein coupled receptor (Zheng et al 2016; Miao and McCammon 2016).

The unavailability of a CRF_1 radiotracer for PET/SPECT imaging to confirm adequate receptor occupancy in humans, despite continuing efforts to obtain one that exhibits specific binding *in vivo* (Stehouwer et al 2015a; Stehouwer et al 2015b; Lodge et al 2014) as well as the uncertain density of CRF_1 receptors in human brain vs. other species (see discussion in Lodge et al 2014) also may be leading to suboptimal prioritization of drug candidates and dosing. A surrogate, non-PET biomarker approach (Schwandt et al 2016) used to predict central receptor occupancy found that even with high estimated (~90%) occupancy, verucefont still did not produce therapeutic action The development of CRF_1 PET/SPECT radioligands could further validate and refine such surrogate approaches for estimating receptor occupancy.

Recent genetic and molecular findings in humans

In addition to results already cited, several genetic and molecular findings since 2014 in humans and non-human primates continue to implicate CRF-CRF_1 systems in emotional dysfunction, addiction, or stress-related phenotypes (see also Zorrilla et al 2013, for review of earlier studies of CRF system SNPs in addiction).

As prelude, major limitations of the genetic variant studies to date are that many of them have not been replicated and, for most, their molecular effect, if any, on the CRF_1 system (as opposed to a putative surrogate marker of CRF_1 system activity) is unknown. Additionally, in general, individual genetic variants are associated with a low percentage of psychiatric disease prevalence. Furthermore, with only a few recent exceptions (see Clarke et al 2014, Crist et al 2016, Crist et al 2013 Heinzerling et al 2013) genetic variants have not yet reliably and reproducibly predicted treatment response in psychiatric diseases (Jones and Comer 2015 Berrettini 2016 Qedegaard et al 2016) A molecular, and not only phenotype-based, understanding, of a variant's functional effect (if any) may ultimately be needed to understand its prognostic relation to CRF_1 antagonist treatment response.

Major Depression

With respect to major depression, *CRHR1* SNPs of rs7209436, rs110402, and rs242924 previously had been associated with peak cortisol responses to the Trier Social Stress Test in healthy adults (Mahon et al 2013). Recently it was reported that a TATGA haplotype combination that includes the above 3 polymorphic loci (rs17689966, rs173365, rs7209436,

rs110402, and rs242924) increased the risk for major depression by 68% in a community-based study in South Spain (Ching-Lopez et al 2015). Furthermore, the T allele at *CRHR1* rs242941, which forms part of a haplotype that previously had been linked to major depression and antidepressant response (Liu et al 2006; Licinio et al 2004; Liu et al 2007) was linked to family history of mental illness (Tan et al 2015). Smoller recently reviewed genome-wide association studies that implicate *CRHR1* genotype X early childhood maltreatment environmental interactions for major depression risk (Smoller 2016). Finally, an A-Deletion-A *CRHR1* haplotype (rs77032924, rs3832590, rs6159) was associated with a trend for poorer response to treatment with mirtazapine or escitalopram in patients with major depression not experiencing stressful life events, perhaps suggesting a role for this haplotype in driving CRF-mediated intrinsic dysphoria that is resistant to non-CRFergic intervention (Chang et al 2015).

Suicidality

CRF-CRF_1 systems also were linked to suicidality, with ~2-fold increased CRF mRNA in the anterior cingulate of depressed patients who committed suicide as opposed to those who died from natural causes (Zhao et al 2015). Allele C at the *Crhr1* rs878886 locus was overrepresented in suicide attempters from both Russian and Tatar ethnicity samples (Khalilova et al 2014). In addition, *CRHR1* loci that mitigated (rs2664008) or tended to potentiate suicide risk (rs1724425, rs1526123, rs6503447, rs11655764) were identified in a case-control study of individuals with bipolar disorder, the former especially buffering the effects of early childhood abuse (Breen et al 2015)

Anxiety disorders

With respect to anxiety disorders, a recent case-control study found that the minor (A) allele of *CRHR1* rs17689918 and, relatedly, a CGTGA haplotype (rs7209436, rs4458044, rs12936181, rs3785877, rs17689918) increased risk for panic disorder selectively in women. Unexpectedly, post-mortem studies showed that this risk allele was associated with *decreased* CRF_1 mRNA in human forebrains and amygdala. Neuroimaging studies found that allele carriers showed a pattern of altered fMRI signal in the prefrontal cortex and amygdala that was interpreted to reflect overgeneralization of fear conditioning and underprocessing of safety signals; allele carriers also showed less “flight” and more “anxious apprehension” behaviors in response to fear provoking-stimuli. While the results implicate CRF_1 in anxious processing in panic disorder, they do so in an unexpected direction and also indicate that pharmacogenetic selection of patients in which CRF_1 receptors play a role may need to be considered in a sex-specific manner (Weber et al 2016). An additional concern here as well is if a patient has fewer CRF_1 receptors, then they have less available drug target.

In apparent opposition to the findings in people with panic disorder, Kalin, Oler and colleagues observed that viral vector mediated overexpression of CRF in the CeA of young rhesus monkeys led to increased anxious temperament (freezing, cooing and cortisol reactivity in response to a human intruder). The increase in anxious temperament correlated directly with increased fluorodeoxyglucose metabolism (by PET) and fMRI functional

connectivity within a circuit that included the dorsal amygdala, orbital proisocortex/anterior insula and hippocampus (Kalin et al 2016).

Addiction

With respect to addiction, the *CRHBP* rs1875999 locus was associated with risk for both cocaine and heroin addiction in African Americans in a study of heroin addicts ($n = 314$), cocaine addicts ($n = 281$), and healthy controls ($n = 208$) (Levran et al 2014). SNPs in the *CRHBP* (10kD) fragment, *rs10055255, rs10062367*, and *rs7728378* were each shown to be associated with increased risk of alcohol drinking and/or anxiety in patients with alcohol use disorder (Haass-Koffler et al 2016).

Irritable bowel syndrome

Several recent human studies further implicated CRF-CRF_1 activity in irritable bowel syndrome (IBS), a heterogeneous diagnosis that involves abdominal pain, altered bowels habits, gastrointestinal (GI) symptom-specific anxiety, and altered, stress-sensitive brain-gut interactions, often in association with comorbid anxiety or mood disorders. First, men with IBS showed increased sensitivity to intravenous CRF infusion, with greater right amygdala activation (by $H_2{}^{15}O$-PET) and peripheral noradrenaline secretion than in healthy controls (Tanaka et al 2016). Second, the major alleles of *CRHR1* rs110402, rs242924 and rs720943 (all C) were associated with increased risk for IBS and, within IBS patients, increased GI-symptom-related anxiety. Unexpectedly, IBS patients with the same risk alleles showed reduced acoustic startle responses vs. healthy controls, whereas those with the minor alleles did not, indicating a complex influence of the *CRHR1* SNPs on different symptoms of anxiety per diagnostic group (Orand et al 2016). Finally, a separate study of young Japanese individuals observed that the *CRHR1* rs10474485 locus was associated with increased psychometric scores for depression, perceived stress or state/trait anxiety in IBS patients with diarrhea or mixed symptoms, with the *CRHR1* variant more predictive of differences in emotional scales in women than in men (Sasaki et al 2016). Two. *CRHR2.* variants, *rs4722999* and *rs3779250*, have also been associated with genotype frequency of IBS and the distribution of the major allele was significantly different in IBS patients compared to controls (Komuro et al 2016).

Other emotion-related phenotypes

A recent neuroimaging study of school-age children was performed involving a genetic profile score that predicts HPA-axis reactivity, wherein 5 of the 10 SNPs used to calculate the genetic profile score involved *CRHR1* loci (rs4792887, rs110402, rs242941, rs242939, rs1876828). Higher genetic profile scores were found to predict greater amygdala and hippocampal fMRI activational responses to facial stimuli in pubertal, but not non-pubertal, children. In pubertal children, differential activation to fearful faces was seen in girls and to neutral faces in boys (Pagliaccio et al 2015). The results again are consistent with the possibility of sex differences in the functional significance of CRF_1 genetic variants in a manner that depends upon developmental milestones associated with puberty. Indeed, gonadal hormones influence the regulation of CRF system molecules in both male and female rats in a puberty-relevant manner (Bangasser and Valentino 2012; Gomez et al 2004). Furthermore, Valentino and colleagues have described sex differences in the signaling

pathway bias of CRF_1 receptors in rodents (Valentino et al 2013a; Valentino et al 2013b), a mechanistic difference that may be relevant to some of the reviewed sex differences in the functional impact of *CRHR1* genetic variants.

A separate imaging study at the Duke Neurogenetics Center examined the widely studied *CRHR1* locus rs110402, which, as reviewed above, has been associated with increased HPA-axis reactivity, in interaction with a locus relevant to a gene encoding fatty acid amide hydrolase, an enzyme that degrades the endocannabinoid anandamide. Individuals with a genetic background of increased CRF_1 signaling (A homozygotes) in combination with increased anandamide inhibitory tone (*FAAH* 385A carriers) showed decreased habituation of the BOLD fMRI response of the basolateral amygdala during emotional facial processing. The blunted amygdala habituation, in turn, was associated with increased risk for an anxiety disorder (Demers et al 2016). Interestingly, the rs110402 locus also was associated recently with premature decline in working memory, but not other measures of neuropsychological function, across the lifespan. The finding has been interpreted to reflect a chronic deleterious influence of stress reactivity (Grimm et al 2015).

Finally, the GGA haplotype at polymorphic loci of the *CRHR1* gene (rs4458044, rs242924, and rs1768996) was associated with aggressive behavior towards others as determined in a Han Chinese sample of violent criminals (Chen et al 2014).

Summary

Variants of the *CRHR1* and *CRHBP* genes continue to be associated with the diagnoses, phenomenology and/or non-CRFergic treatment response of major depression, suicidality, panic disorder, and irritable bowel syndrome. Findings also implicate other stress-related (endo)phenotypes, including not only HPA-axis reactivity and startle reactivity, but also more novel findings, such as altered amygdalar habituation or activation during facial processing, premature impairment of working memory, and physical aggression. A new development includes the finding that there may be sex differences or developmental (pubertal) moderation of the predictive relation of some genetic variants, and that for some phenotypes (e.g., panic disorder, startle reactivity between IBS patients and controls). Particular CRF_1 variants also appeared to have effects opposite to those anticipated from a simple model of greater CRF_1 activation always having anxiogenic action. The latter result may reflect that CRF_1 activation in some brain regions may have anxiolytic-like effects, including via circuit action to inhibit anxiogenic-like effects of CRF_1 activation elsewhere (Sztainberg et al 2011; Walker et al 2009). These results may also relate to why CRF_1 antagonists even exacerbated anxious or fearful symptomatology in two human studies (Grillon et al 2015; Schwandt et al 2016).

Variants that have been replicated across studies and which, at the least, associate with an endophenotype of CRF_1 activation, such as rs7209436, rs110402, or rs242924, are hypothesized to be more likely to predict better treatment response to CRF_1 antagonists. However, as alluded to previously, important criticisms of these genetic variant studies include that their associated odds ratios, even when significant, have been modest (Levran et al 2014), and many genetic variants have unknown molecular effects, if any, on the. CRF_1 system. Additionally, many genetic variant studies failed to replicate (Buttenschøn et al

2016, Ventura-Juncá et al 2014) or have not yet been replicated. Finally, the finding that variants of *CRHBP* and *CRHR2* have been associated with psychiatric and stress-related disease lends support to the hypothesis that other components of the CRF system may be involved and warrants further exploration of polymorphs of *CRHBP*, *CRHR2*, and/or *RAMP2.*

Recent promising CRF_1 antagonist trials in humans

Two clinical trials with CRF_1 antagonists reported potentially promising results during 2016. First, in a randomized, double-blind, placebo-controlled study that was stopped by the NIH. IRB for reasons unrelated to adverse drug effects or efficacy (reinterpretation of the Common Rule for human snbject protection under HHS, 45 CFR 46A), pexacerfont was found to produce effect sizes consistent with reduction of food craving and laboratory stress-induced eating in a small sample of healthy individuals with restrained eating. Although statistical significance was not seen, the study was stopped prematurely and thereby only had 30% power to detect the stated effect size of.interest; thus, it would be inappropriate to interpret it as a negative result and observed effect sizes may inform whether future appropriately-powered studies are warranted. The effect size for pexacerfont's reduction of laboratory stress-induced eating was $r = 0.30$ (counternull $r = 0.55$; Rosenthal and Rubin 1994) and for its reduction of craving for sweet foods (brownies and Swedish fish) ranged from $r = 0.28$ to 0.49 (counternull $rs = 0.52–0.79$). Furthermore, in bogus taste tests designed to mask the true dependent measure of interest (intake), pexacerfont reduced eating of palatable foods independent of which imagery script was presented before food access (neutral, food cue, stress) with an effect size of $r = 0.34$ (counternull $r = 0.61$). Finally, nightly Yale Food Addiction Scores were consistently lower in subjects receiving pexacerfont (vs. placebo) beginning the evening after the first loading dose of pexacerfont with an effect size of $r = 0.39$ (counternull $r = 0.68$). Because the study was stopped prematurely ($n = 11–13$/group for laboratory studies and $n = 13–17$/group for YFAS ratings), only the YFAS result was significant at the $p < 0.05$ level. However, Bayes factor analysis, a ratio that relates to the relative probability of an effect actually being present vs. the null effect (Goodman 1999), and counternull analysis, which describes the effect size as likely to be true as the null, indicate a strong positive potential of CRF_1 antagonists to reduce palatable food craving and eating in restrained eaters (Epstein et al 2016) and justify a well-powered clinical trial in this domain. A concern with these results is that the YFAS scores had changed as early as 24-hours post-treatment, and the degree of CNS exposure obtained at that time is uncertain, leading one to question the CRF_1 antagonist mechanism of action Still, the preliminary results concord with preclinical studies showing that systemic administration of CRF_1 antagonists reduce overeating of a palatable, high sucrose diet in rats receiving intermittent access to the diet (Cottone et al 2009) and also reduce stress-induced reinstatement of palatable food-seeking (Ghitza et al 2006). Why the clinical results differ from those obtained for alcohol craving with pexacerfont in anxious alcoholics (Kwako et al 2015) is unclear.

The second promising clinical result involved a phase Ib, single-blind, placebo-controlled, fixed-sequence, single-dose trial of verucerfont, (NBI-77860; see Figure 1) for 21-hydroxylase deficiency, the most common cause of congenital adrenal hyperplasia. In 21-

hydroxylase deficiency, the cortisol synthetic pathway is impaired, leading to loss of glucocorticoid negative feedback over the HPA-axis (similar to adrenalectomy) and consequent hypothalamic CRF-driven hypersecretion of ACTH, with accumulation of upstream precursors of cortisol, including 17α-hydroxyprogesterone (17OHP). Because 17OHP cannot be processed to cortisol, it instead is converted along the androgen pathway, leading to clinical manifestations of congenital adrenal hyperplasia. Following CRF_1 antagonist treatment, dose-dependent reductions of ACTH and/or 17OHP were observed in six of eight subjects, with overall mean reductions of 41–43% for ACTH and, at the higher antagonist dose, 27% for 17OHP. Thus, the results validate the reviewed clinical finding with verucerfont (Schwandt et al 2016) that CRF_1 antagonists with long receptor residence can reduce CRF-driven chronic overactivation of the HPA-axis and indicate one possible therapeutic indication for this action (Turcu et al 2016). This positive finding also is consistent with the revisionist hypotheses that robust pathophysiological overactivation of CRF signaling is a key for therapeutic potential.

Conclusion

We reviewed a range of issues that may explain why CRF_1 antagonists could be said to have been lost in translation from the bench to the bedside. These include not only potential specificity limitations of the preclinical models themselves, but also the reality that, for some predictive endpoints, CRF_1 antagonists produced therapeutic-like results only under certain circumstances (e.g., high stress, withdrawal), unlike some clinically effective compounds that act more generally. In some models, CRF_1 antagonists had null or even exacerbating actions, the latter consistent with analogous findings in gene variant and clinical studies. Recognition of this leads to the revised view that the efficacy of CRF_1 antagonists may be correspondingly circumscribed to particular psychiatric disorders or symptoms, patient subgroups, or circumstances in which the activation of pro-stress-like CRF-CRF_1 circuits is dynamically heightened. We described genetic and non-genetic markers that could be evaluated as markers of such activation towards individualized treatment and obstacles that remain for such approaches (e.g.., CRF_1 *in vivo* radiotracer; molecularly validated, functional SNPs). We also discussed both solved and unresolved issues concerning whether small molecules that have been advanced to the clinic adequately engaged human CRF system molecules in the manner needed to attain therapeutic silencing. We also noted mechanisms via which CRF_1 stimulation-induced plasticity within and downstream of CRF_1 receptors may reduce the need for high ongoing CRF_1 agonist stimulation to perpetuate the maladaptive behavior. Finally we review promising, recent human trials which suggest that CRF_1 antagonists may have potential to reduce craving for and stress-induced eating of palatable food as well as CRF-driven overactivation of the HPA-axis. One must be cognizant of the significant opportunity cost in continuing to pursue selective CRF_1 antagonists for therapeutic use, but given the increasing understanding in the field, several therapeutie avenues with these or, especially, novel anti-CRF compounds remain underexplored Thus, while it appears that the therapeutic scope of selective CRF_1 antagonists is narrower than had been hoped when Dr. Markou organized those early studies of antalarmin, much remains to be learned about the shared molecular roles of CRF receptors in the neurobiology of

stress, dysphoria and addictive behavior in humans and the potential individualized role of novel anti-CRF approaches therein.

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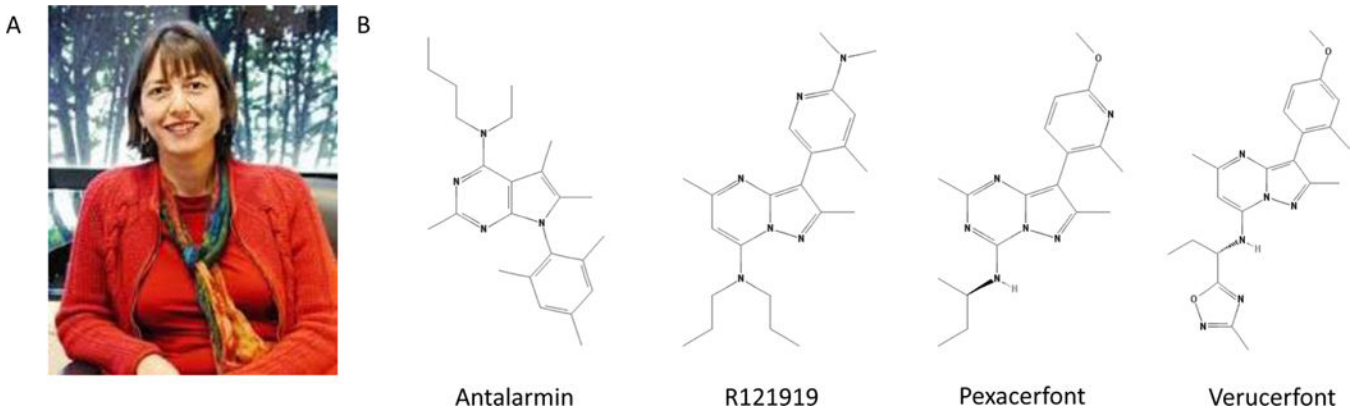

Figure 1.

A. Dr. Athina Markou, one of the pioneers in the preclinical study of selective CRF_1 antagonists for potential therapeutic use in emotional disorders B. The chemical structure of antalarmin (CAS: 157284-96-3), an early CRF_1 antagonist shown to have anxiolytic-like activity by Dr. Markou and colleagues, and similar structures of CRF_1 antagonists that have been evaluated in clinical trials, including compounds R121919 (CAS: 195055-03-9), pexacerfont (CAS: 459856-18-9), and verucerfont (CAS: 885220-61-1)

Table 1

Clinical trial outcomes of small-molecule CRF_1 antagonists in selected samples with stress-like psychiatric symptoms

Trial for	Drug	Notes	Refs
Major depression	R121919	Reduced anxiety and depression; normalized sleep EEG But, withdrawn due to liver enzyme elevations	Held et al 2004; Kunzel et al 2005; Kunzel et al 2003; Zobel et al 2000; Neurocrine Press Release April 5, 2000
	PF-00572778	Withdrawn due to liver enzyme elevations	NCT00580190
	ONO-2333Ms	Lacked efficacy	NCT00514865; Ono Pharmaceutical Co Ltd, 2008
	CP-316,311	Lacked efficacy	Binneman et al 2008
	SSR125543	Lacked efficacy	NCT01034995; Sanofi Report DFI5687, September 2011
	Verucerfont (NBI-77860/GSK561679A)	Lacked efficacy	Tellew et al 2010; GlaxoSmithKline Results Summary for CRS106139, 2010
	Pexacerfont (BMS-562,086)	Study completed October, 2007; no results reported	NCT00135421
Generalized	Pexacerfont	Lacked efficacy	Coric et al 2010
Anxiety Disorder	(BMS-562,086)		
Post-traumatic stress disorder	Verucerfont (NBI-77860/GSK561679A)	Lacked efficacy	NCT01018992
Suicidal ideation in anxious patients	Pexacerfont (BMS-562,086)	Lacked efficacy	Coric et al 2010
Social anxiety disorder	Emicerfont (GW876008)	Study completed January, 2008; no results reported	NCT00555139
	Verucerfont (NBI-77860/GSK561679A)	Study completed January, 2008; no results reported	NCT00555139
Alcohol dependence	Pexacerfont (BMS-562,086)	Lacked efficacy to reduce alcohol craving, emotional responses to alcohol- or stress-related imagery, or anxiety	Kwako et al 2015
	Verucerfont (NBI-77860/GSK561679A)	Lacked efficacy to reduce alcohol craving, emotional responses to alcohol- or stress-related imagery, or anxiety in anxious alcoholic women.	Schwandt et al 2016
Irritable bowel syndrome	Emicerfont (GW876008)	Lacked efficacy	GlaxoSmithKline Results Summary for CRI105626, 2008
	Pexacerfont (BMS-562,086)	Lacked efficacy	Sweetser et al 2009