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Novel Roles for CRF-Binding Protein and CRF Receptor 2 in Binge Drinking

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Stress is a key environmental factor in alcohol addiction, and the stress system has been linked to alcohol use prior to dependence, after dependence, and in relapse to drinking after abstinence in both humans and rodent model systems (reviewed in Lowery and Thiele, 2010; Phillips et al., 2015, Ray, 2011). The key central nervous system regulator of the mammalian stress response is Corticotropin-Releasing Factor (CRF). This peptide mediates its primary effects through binding to G-protein coupled receptors, CRF receptor 1 (CRF-R1) and CRF receptor 2 (CRF-R2), and its activity is modulated by the CRF-binding protein (CRF-BP). Dysregulation of the CRF system has been observed in a number of rodent models of binge drinking and alcohol dependence, with most studies focusing on the roles of CRF and CRF receptors, particularly CRF-R1 (reviewed in Lowery and Thiele, 2010; Phillips et al., 2015). CRF-R1 antagonists decreased binge drinking and self-administration in alcohol-dependent animals (Lowery and Thiele, 2010), suggesting potential therapeutic uses for CRF-R1 antagonists. However, fewer studies have investigated the roles of CRF-R2 and CRF-BP in binge drinking and alcohol dependence.

In the recent study by Albrechet-Souza and colleagues (2015), a variety of drinking paradigms and pharmacological tools were utilized to examine the roles of CRF-BP and CRF-R2 in the ventral tegmental area (VTA) and central nucleus of the amygdala (CeA) in binge drinking and alcohol dependence. To model binge drinking, the authors used the drinking in the dark (DID) paradigm, in which mice were given limited access to 20% ethanol in the dark phase of their circadian cycle, resulting in drinking to intoxication and pharmacologically relevant blood ethanol concentration. Two variations of the DID paradigm were employed, both one-bottle and two-bottle choice (Rhodes et al., 2007). Alcohol dependence was modeled using a 4-week two-bottle choice, intermittent access to alcohol (IAA) paradigm. To investigate the role of CRF-BP in each of these paradigms, the CRF-BP ligand inhibitor $CRF_{6–33}$ was utilized (Sutton et al., 1995). The authors found that CRF-BP in the VTA, but not CeA, regulates one-bottle choice binge drinking, but not dependence-induced alcohol consumption. To investigate the role of CRF-R2 in the VTA in

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binge drinking, the CRF-R2 selective antagonist astressin 2B (A2B) was used. A2B injected alone into the VTA at a high dose led to a decrease in ethanol intake in two-bottle choice DID, while low dose A2B had no effect. Together, these exciting results reveal novel roles for CRF-BP and CRF-R2 in the VTA in binge drinking.

Roles for CRF-binding protein (CRF-BP)

The CRF-BP is a 37 kDa secreted glycoprotein that binds CRF and the CRF-like ligand, urocortin 1 (Ucn1), with very high affinity. It is co-localized with CRF or CRF receptors at numerous sites (i.e. amygdala and BNST), suggesting potential sites of interaction in stress-reward pathways (reviewed in Westphal and Seasholtz, 2006). In humans, it is estimated that 40–60% of CRF in the brain is bound by CRF-BP, and *CRHBP* SNPs have been associated with alcohol use disorder and stress-induced alcohol craving (Ray, 2011), suggesting a role for the CRF-BP in susceptibility to alcohol use and addiction.

The CRF-BP has been studied for over 20 years with many postulated roles. In an inhibitory role, CRF-BP reduces CRF receptor activation, likely by sequestering CRF or Ucn1 and/or targeting them for degradation. Consistent with this model, purified CRF-BP reduces CRF-mediated ACTH release from anterior pituitary cultures or AtT-20 cells (Cortright et al., 1995; Potter et al., 1991; Sutton et al., 1995). Recombinant CRF-BP also attenuates CRF-R1-mediated increases in cAMP (Boorse et al., 2006). Similarly, CRF-BP-deficient mice show increased baseline anxiety-like behavior and slowed return to homeostasis after lipopolysaccharide stress, consistent with elevated free levels of CRF in the absence of an inhibitory CRF-BP (Karolyi et al., 1999). In contrast, the study by Albrechet-Souza and colleagues (2015) suggests an enhancing or facilitatory role. In this role, CRF-BP may bind CRF and increase CRF signaling, likely by delivering CRF to the receptor, extending the half-life of CRF, or modulating its interaction with the receptor. Consistent with this hypothesis, VTA CRF-BP may normally facilitate alcohol consumption, as administration of $CRF_{6–33}$ into VTA reduced binge drinking in mice (Albrechet-Souza et al., 2015). Similarly, intra-VTA administration of $CRF_{6–33}$ attenuated CRF-induced relapse to cocaine seeking in rats (Wang et al., 2007). Altogether, these results suggest that CRF-BP may have different roles depending on the specific cell type or context in which it is expressed, consistent with ultrastructural localization studies (Peto et al., 1999). Finally, the CRF-BP may exert ligand and/or receptor independent actions. Administration (i.c.v.) of $CRF_{6–33}$ elicits c-fos activation not only in a subset of CRF receptor-expressing cells, but also in CRF-BP-expressing cells that do not express ligand or CRF receptors, suggesting additional actions of $CRF_{6–33}$ and/or CRF-BP (Chan et al., 2000). Hence, further studies are needed to elucidate the multiple roles of CRF-BP *in vivo*, as the actions of CRF-BP are likely brain region and cell type-dependent.

CRF Receptors in Binge Drinking and Dependence

The CRF receptors are highly homologous G-protein coupled receptors with similar, yet distinct ligand binding profiles. CRF binds with very high affinity to CRF-R1, but binds to CRF-R2 with about 30 fold lower affinity. Both CRF receptors couple predominantly to $G\alpha_s$, activating adenylyl cyclase and increasing cAMP levels and protein kinase A activity.

However, they have also been shown to act through other G protein subtypes, including $G\alpha_{q/11}$, $G\alpha_o$, $G\alpha_i$, in certain cellular contexts (reviewed in Hillhouse and Grammatopoulos, 2006).

There is a large body of literature implicating the role of CRF-R1 in excessive alcohol drinking (reviewed in Lowery and Thiele, 2010; Phillips et al., 2015). Altered CRF-R1 expression has been observed in rats with a history of alcohol dependence, and peripheral administration of CRF-R1 antagonists reduced both binge drinking and dependence-induced alcohol consumption (Lowery and Thiele, 2010). Furthermore, CRF receptor regulation of alcohol consumption may be brain region-specific, with CeA and VTA as major sites of action. Intra-CeA (but not basolateral amygdala) administration of a CRF-R1 antagonist decreased binge drinking (Lowery-Gionta et al., 2012). Similarly, intra-VTA CRF-R1 antagonist administration decreased binge drinking in a DID paradigm (Sparta et al., 2013) and decreased excessive drinking during the two-bottle choice IAA paradigm (Hwa et al., 2013). Although less studied, CRF-R2 has also been implicated in excessive alcohol drinking, but often in an inverse fashion to CRF-R1. For example, i.c.v. administration of the CRF-R2-selective agonist Urocortin 3 dose-dependently decreased binge drinking, and intra-CeA administration of Urocortin 3 resulted in decreased alcohol self-administration in alcohol dependent rats (reviewed in Lowery and Thiele, 2010; Phillips et al., 2015). Hence, CRF-R1 antagonists and CRF-R2 agonists often elicit similar responses. In contrast, in the recent study by Albrechet-Souza et al. (2015), intra-VTA CRF-R2 antagonist (astressin 2B) administration decreased alcohol drinking in the two-bottle DID paradigm, suggesting that VTA CRF-R2 is facilitating drinking. Additional studies that directly compare the effects of CRF-R1 and CRF-R2, via genetic approaches and/or receptor-selective agonists and antagonists, in the VTA, CeA and other brain regions will be required to fully elucidate the roles of these two receptors in alcohol binge drinking and dependence.

Potential CRF-BP/CRF Receptor Interactions and Future Directions

The CRF system is unique in that very few neuropeptides have a high affinity binding protein that is totally distinct from its receptors. CRF and Ucn1 bind to CRF-BP with very high affinity (K_d <1nM), equal to or greater than the affinity of these ligands for CRF-R1 and/or CRF-R2. Interestingly, the CRF-BP is widely expressed in brain, localizing to sites of CRF or Ucn1 expression or release, sites of CRF-R1 or CRF-R2 expression, and sites where neither the receptors nor ligands are detected (Chan et al., 2000; Potter et al., 1992). CRF-BP synthesis and release are also highly responsive to environmental cues, as stress, CRF, glucocorticoids and IL-6 all regulate CRF-BP expression (Westphal and Seasholtz, 2006). Yet we still know little about the molecular mechanisms of its actions. Purified CRF-BP attenuates CRF or Ucn1 activity at endogenous CRF receptors in cultured cells (Westphal and Seasholtz, 2006). In contrast, *in vivo* or slice studies suggest a facilitatory role for CRF-BP in VTA, with $CRF_{6–33}$ administration into the VTA decreasing ethanol consumption, CRF-induced relapse to cocaine, and CRF-mediated potentiation of NMDA EPSCs on dopamine neurons (Albrechet-Souza et al., 2015; Ungless et al., 2003; Wang et al., 2007). Strikingly, many of the facilitatory effects of CRF-BP in VTA appear to be associated with CRF-R2 (Ungless et al. 2003; Wang et al. 2007). In fact, Ungless et al. (2003) has proposed that CRF-BP is required for CRF activation of CRF-R2 and the downstream phospholipase

C/protein kinase C, but not PKA, signaling pathway in VTA dopamine neurons. The study by Albrechet-Souza et al. (2015) begins to address this VTA CRF-BP/CRF-R2 interaction in the two-bottle choice DID paradigm, but does not test $CRF_{6–33}$ alone in this protocol, making it difficult to ascertain whether CRF-BP and CRF-R2 are interacting.

Hence, while this interesting study by Albrechet-Souza et al. (2015) reveals new roles for the CRF system, especially CRF-BP and CRF-R2, in alcohol binge drinking, many questions remain. We propose that CRF-BP plays multiple roles in stress and alcohol addiction, likely in a brain-region and cell-type specific manner, with CRF receptor subtype and signaling pathway as potential determinants. Clearly, additional *in vitro* and *in vivo* studies are needed to define the mechanisms of CRF-BP/CRF/CRF receptor interactions. Future studies should also include viral and genetic approaches allowing cell-type specific overexpression or knockdown of CRF-BP, and/or CRF receptors, in various brain regions within the stress-reward pathway, as the transition from pre-dependent to dependent states in alcohol addiction involves different sites and circuits. Together, these multidisciplinary approaches will allow us to better understand the multiple roles of the CRF-BP in inhibiting and facilitating CRF and/or Ucn1 interactions with CRF-R1 and CRF-R2 and the functional outcomes of these interactions in stress and addiction.

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