MINIREVIEW—SPECIAL ISSUE IN MEMORY OF AVRAM GOLDSTEIN

Sex-Biased Stress Signaling: The Corticotropin-Releasing Factor Receptor as a Model

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Received November 8, 2012; accepted December 13, 2012

ABSTRACT

Sex differences in the prevalence or severity of many diseases and in the response to pharmacological agents are well recognized. Elucidating the biologic bases for these differences can advance our understanding of the pathophysiology of disease and facilitate the development of treatments. Despite the importance to medicine, this has been an area of limited research. Here, we review physiologic, cellular, and molecular findings supporting the idea that there are sex differences in receptor signaling and trafficking that can be determinants of pathology. The focus is on the receptor for corticotropin-releasing factor (CRF), the orchestrator of the stress response, which has been implicated in diverse stress-related diseases that show a female prevalence. Data are reviewed that show sex differences in the association of the CRF receptor (CRF1) with the Gs protein and β-arrestin 2 that would render females more responsive to acute stress and less able to adapt to chronic stress as a result of compromised CRF1 internalization. Because β-arrestin 2 serves to link CRF1 to Gs-independent signaling pathways, this sex-biased signaling is proposed to result in distinct cellular responses to stress that are translated to different physiologic and behavioral coping mechanisms and that can have different pathologic consequences. Because stress has been implicated in diverse medical and psychiatric diseases, these sex differences in CRF1 signaling could explain sex differences in a multitude of disorders. The possibility that analogous sex differences may occur with other G-protein-coupled receptors underscores the impact of this effect and is discussed.

Introduction

Many diseases exhibit sex differences, such that their prevalence and/or severity are greater in one sex. Identifying the biologic bases for sex differences in specific diseases can advance our understanding of their etiology and lead to the development of more effective individualized therapies. Sex differences are particularly prominent in neuropsychiatric diseases (Table 1). For example, autism, substance abuse, and attention deficit disorder are more prevalent in males (Table 1) (Gaub and Carlson, 1997; Ramtekkar et al., 2010). In contrast, affective disorders and many anxiety disorders are nearly twice as prevalent in females, compared with males (Table 1) (Kessler et al., 2003). Similarly, the incidence of posttraumatic stress disorder (PTSD) is greater in females despite males being exposed to more traumatic events (Breslau et al., 2001, 2002). These mood and anxiety disorders are debilitating mental illnesses and are a leading cause of disability in the United States for younger adults (Alonso et al., 2011; Bromet et al., 2011). A common underlying feature of the psychiatric disorders that are more prevalent in females is an association with stress. Stressor exposure is associated with the onset and severity of depression and several anxiety disorders (Kessler et al., 1995). For PTSD, stress is the precipitating event. Of importance, the sex bias in PTSD remains after adjusting for the type of trauma, preexisting psychiatric disorders, and sex differences in reporting (Breslau et al., 1999; Tolin and Foa, 2006; Breslau, 2009). Together, these data support the notion that the increased incidence of stress-related psychiatric diseases in females is biologically determined.

In addition to an association with stress, anxiety disorders, PTSD, and depression share a core symptom of hyperarousal, which is a defining feature of the diseases and is characterized by sleep disturbances, inability to concentrate, restlessness, and increased vigilance (Southwick et al., 1999; Gold and Chrousos, 2002). To elucidate the biologic bases for the female prevalence of these diseases, we examined a major point of intersection between stress and arousal systems. This was the interaction between the orchestrator of the stress response, corticotropin-releasing factor (CRF) and a major arousal...
system, the locus coeruleus (LC)—norepinephrine system (Valentino and Van Bockstaele, 2008). Here, we describe the role of these systems in stress and how their interaction is an important cognitive limb of the stress response but can become dysfunctional and result in stress-related pathology. We then describe sex differences in functional end points of CRF-LC interactions and parallel differences in CRF receptor signaling and trafficking as identified in cortical tissue. The global implications of the discovery that sex can determine the direction of receptor signaling are discussed with respect to clinical and therapeutic applications.

**Corticotropin-Releasing Factor and Stress-Related Psychiatric Disorders**

The hypothalamic neuropeptide that orchestrates the stress response is CRF, which functions as a neurohormone that is released from paraventricular hypothalamic neurons into the hypophysial portal system to elicit secretion of adrenocorticotropin (ACTH) from anterior pituitary corticotrophs (Vale et al., 1981). This is the initial step in a cascade that results in adrenal corticosteroid release, which is considered to be a hallmark of stress. Synchronically released CRF in extrahypophysal neural circuits that innervate the limbic forebrain, midbrain and pontine monoamine nuclei, and autonomic-related brainstem nuclei facilitates the coordination of autonomic, behavioral, and cognitive responses to stress with the endocrine limb (de Kloet et al., 2005). Although coordinated CRF release in these circuits would be well designed for coping in a dynamic environment with potential life-threatening challenges, the initiation of these responses would be maladaptive in the absence of stressors or if the responses persisted after stress termination. In this regard, inappropriate or excessive CRF is thought to be a pathophysiological factor in the stress-related psychiatric disorders that are more prevalent in females, including PTSD and depression. For example, CRF levels are elevated in the cerebrospinal fluid of patients with PTSD or depression (Nemeroff et al., 1984; Brenner et al., 1997; Arborelius et al., 1999; Baker et al., 1999; Sautter et al., 2003). CRF protein and mRNA are elevated in numerous brain regions of depressed suicide victims (Leake et al., 1990; Austin et al., 2003; Bissette et al., 2003). Finally, many of the symptoms in a subset of individuals with severe depression resemble over-activity of the stress axis, including increased basal cortisol secretion, blunted rhythm, and resistance to dexamethasone suppression (Gold et al., 1988a,b; Gold and Chrousos, 2002).

CRF effects are mediated through two CRF receptor subtypes, CRF1 and CRF2, in which genes have been cloned (Perrin et al., 1993; Lovenberg et al., 1995; Chen et al., 2005). These receptors have been well characterized with respect to their distribution and pharmacological specificity (Chalmers et al., 1996; Hauger et al., 2003, 2006; Bale and Vale, 2004). In addition, many details related to signaling and trafficking of the receptors have been identified (Hauger et al., 2006, 2009; Hillhouse and Grammatopoulos, 2006). The present review focuses on CRF1, because this is the subtype through which CRF acts to elicit ACTH release and to initiate many of the central effects associated with the stress response, including arousal and anxiogenic effects. As such, novel therapeutics for stress-related disorders are being developed to target CRF1 (Holsboer, 1999; Grammatopoulos and Chrousos, 2002). In addition, this is the CRF receptor subtype for which sex differences in signaling have been described (Bangasser et al., 2010). In contrast, the role of CRF2 in stress is less well defined, with some studies suggesting stress-protective effects and others providing evidence for pro-depressive effects mediated by this receptor (Bale et al., 2000; Hammack et al., 2003; Valentino and Commons, 2005). Reviews of CRF2 structure, signaling, and function can be found elsewhere (Hauger et al., 2006; Hillhouse and Grammatopoulos, 2006).

**Corticotropin-Releasing Factor and the Locus Coeruleus-Norepinephrine System**

The norepinephrine nucleus LC in the pons is a primary target of CRF during stress (Valentino and Van Bockstaele, 2008). The LC is the major source of norepinephrine in forebrain and has long been implicated in arousal and directing the mode of attention (Aston-Jones et al., 1995; Waterhouse et al., 1998; Berridge and Waterhouse, 2003). CRF axon terminals synapse with LC dendrites and also are apposed to afferent axon terminals in the LC, suggesting that CRF has both direct and indirect actions on LC neurons (Van Bockstaele et al., 1996). Consistent with this, CRF increases the spontaneous discharge rates of LC neurons in vivo when directly applied onto LC neurons and in vitro in the presence of tetrodotoxin (Curtis et al., 1997; Jedema and Grace, 2004). This action involves cyclic AMP-mediated inhibition of potassium conductance (Jedema and Grace, 2004). In addition, CRF decreases the signal-to-noise ratio of LC responses to sensory stimuli (Valentino and Foote, 1987; Valentino and Foote, 1988). Related to this, it is noteworthy that at least one-third of CRF axon terminals in the LC colocalize glutamate, the neurotransmitter that mediates LC activation by many stimuli (Valentino et al., 2001).

The net effect of CRF on LC neurons is to shift discharge toward a high tonic state that favors increased tonic and decreased phasic, stimulus-evoked activity (Valentino and Foote, 1987; Valentino and Foote, 1988). This mode of discharge has been associated with a shift from focused attention to a scanning mode that would promote increased arousal and behavioral flexibility (Aston-Jones et al., 1999; Aston-Jones and Cohen, 2005). Supporting this, CRF microinjection into the LC desynchronizes cortical electroencephalographic activity, a sign

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**TABLE 1**

Lifetime Prevalence of Psychiatric Disorders by Sex (>9000 Subjects)


<table>
<thead>
<tr>
<th>Disorder</th>
<th>Prevalence Females</th>
<th>Prevalence Males</th>
<th>Female:Male Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panic</td>
<td>6.2</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Generalized anxiety</td>
<td>7.1</td>
<td>4.2</td>
<td>1.7</td>
</tr>
<tr>
<td>PTSD</td>
<td>36.4</td>
<td>25.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Major depression</td>
<td>9.7</td>
<td>3.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Any affective disorder</td>
<td>20.2</td>
<td>13.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Bipolar</td>
<td>24.4</td>
<td>17.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Conduct disorder</td>
<td>4.5</td>
<td>4.3</td>
<td>1.0</td>
</tr>
<tr>
<td>ADHD</td>
<td>7.1</td>
<td>12.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>6.4</td>
<td>9.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Drug abuse</td>
<td>7.5</td>
<td>19.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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of elevated arousal, and promotes behavioral flexibility in an attentional set-shifting task (Curtis et al., 1997; Snyder et al., 2012). Stressors mimic the electrophysiological effects on LC neurons, and this can be prevented by microinfusion of CRF antagonists into the LC providing evidence for CRF neurotransmission in the LC during stress (Valentino et al., 1991; Curtis et al., 2001, 2012).

In addition to its electrophysiological effects, CRF has enduring effects on LC dendritic morphology, as seen in cultured slices from neonatal rats or in CATH.a cells, immortalized noradrenergic cells that resemble LC neurons (Cibelli et al., 2001; Swinny and Valentino, 2006). In the cultured explants, CRF increases dendritic growth, and in CATH.a cells, it promotes neurite outgrowth; these effects are CRF1 mediated. Both actions require cyclic AMP-dependent protein kinase and mitogen-activated protein kinase (MAPK) pathways. The morphologic effects of CRF on LC dendrites in cultured slices were also shown to require activation of the small GTPase Rac, which promotes and stabilizes dendritic branches (Swinny and Valentino, 2006). This was the first evidence linking CRF receptor actions to actin cytoskeletal regulation through Rho GTPases and suggested molecular mechanisms for stress-induced remodeling of the actin cytoskeleton.

The LC system is designed in a way that would allow CRF effects on LC dendritic growth to have a profound impact on how the LC-norepinephrine system is regulated. LC dendrites extend for hundreds of microns outside the LC core (Shipley et al., 1996), and afferents to these peri-coerulear (peri-LC) regions are functionally distinct from afferents to the nuclear core. The majority of CRF afferents to the nuclear core of the LC are related to autonomic function. These include Barrington’s nucleus, which regulates the parasympathetic neurons that control pelvic visceral function, and the nucleus paragigantocellularis, which regulates sympathetic preganglionic neurons involved in cardiovascular function (Valentino et al., 1992). In contrast, CRF afferents terminating outside of the nuclear core, in peri-LC regions into which LC dendrites extend, derive from limbic regions, such as the central amygdalar nucleus (Van Bockstaele et al., 1998, 1999, 2001). These afferents convey emotion-related information, and their links to the LC provide an anatomic substrate for emotional arousal. The further LC dendrites extend into the peri-LC space, the higher the probability that they will contact limbic afferents relaying emotion-related information to the LC arousal system and the more likely the system will be regulated by emotion-related stimuli. By promoting LC dendritic growth, CRF shapes a structural basis for emotional arousal. One speculation from these data is that early life stressors create a foundation for higher emotional arousal through the process of CRF release in the LC. Potential sex differences in this effect are discussed below.

In addition to these morphologic effects, CRF acting at CRF1 potentiates the ability of tropomyosin receptor kinase B (TrkB) agonists, brain-derived neurotrophic factor (BDNF), and neurotrophin-4, to induce the norepinephrine phenotype of LC neurons in culture (Traver et al., 2006). In this preparation, BDNF increases the number of tyrosine hydroxylase-expressing neurons, and although CRF has no effect on its own, it enhances this effect of BDNF. These CRF1 actions have been associated with activation of adenyl cyclase, induction of cyclic AMP, and activation of Epac (see below).

Despite the electrophysiological and immunohistochemical evidence for CRF1 in LC neurons, many in situ hybridization studies have failed to detect CRF1 mRNA in LC neurons of rodents, although LC neurons of humans and nonhuman primates express CRF1 mRNA (Sanchez et al., 1999; Van Pett et al., 2000; Hiroi et al., 2001). Although difficult to reconcile, this could be attributed to distribution of the mRNA in the extensive LC dendritic system, which could dilute the signal, making it difficult to detect. Of note, a relatively recent in situ hybridization study provided evidence for CRF1 mRNA in rat LC (Zeng et al., 2003). In addition, CRF1 mRNA (but not CRF2 mRNA) was detected in LC neurons cultured from embryonic rats (day 14) in the aforementioned study (Traver et al., 2006).

Sex Differences in Corticotropin-Releasing Factor Regulation of the Locus Coeruleus

The first evidence suggesting sex differences in CRF receptor-mediated effects came from electrophysiological comparisons of LC neuronal activity in male and female rats (Curtis et al., 2006). The electrophysiological characteristics of LC neurons are mostly comparable between sexes. For example, LC firing rate and the magnitude of sensory-evoked responses that are mediated by glutamatergic inputs are similar in male and female rats. However, the magnitude of LC activation by hypotensive challenge, which activates LC neurons through CRF release in the LC, was greater in female than in male rats. Of note, this effect was unrelated to adult hormonal status of either males or females. These sex differences in the magnitude of LC activation by hypotensive stress could be attributed to differences in postsynaptic sensitivity to CRF. Thus, the CRF dose-response curve for LC activation was shifted to the left in females, compared with males, and doses of CRF that were below threshold for increasing LC discharge rate in male rats were effective in females. In addition to sex differences in the acute effect of CRF, sex differences were apparent in the manner in which a history of stress regulated LC sensitivity to CRF (Curtis et al., 2006). For example, in male rats, a history of shock or swim stress shifts the CRF dose-response curve for LC activation to the left, with a decrease in the maximal response, such that neurons are more sensitive to low doses of CRF and less sensitive to higher doses. In contrast, in female rats, the CRF dose-response curve for LC activation is not altered by a history of stress.

With respect to the morphologic effects of CRF on LC neurons, it is noteworthy that LC dendrites of female rats are longer and more complex, having more branch points and extending further into the peri-LC than LC dendrites of male rats (Bangasser et al., 2011). This is reminiscent of the effects of CRF on LC dendrites and would result in a greater magnitude of arousal in response to emotion-related stimuli by favoring more contacts with limbic terminals that convey emotion-related information (Fig. 1). Nonetheless, the role of CRF in these sex differences has yet to be established.

CRF1 Signaling and Trafficking

The aforementioned sex differences suggest distinctions in CRF1 signaling. CRF1 is of the Class B family of seven transmembrane G protein–coupled receptors (GPCR). In brain, the primary mode of signaling is through Gsα, which binds to
Sex Differences in Corticotropin-Releasing Factor Signaling

The sex differences observed in LC neuronal responses to CRF could be attributed to differences in CRF1 signaling. Consistent with this, in females, LC activation by CRF was almost completely prevented by the PKA antagonist Rp-cAMP-S, whereas only a fraction (~50%) of the CRF-elicited LC activation was prevented by the PKA antagonist in males (Bangasser et al., 2010). To examine the possibility of sex differences in CRF1 signaling, CRF1 was immunoprecipitated from rat cortex (Bangasser et al., 2010), a tissue of high CRF1 expression and lacking CRF2 (Van Pett et al., 2000). Although the cortex exhibits one of the highest densities of CRF1 expression in brain, relatively few studies have examined the effects of activating these receptors on behavior, presumably because the region’s greater area makes it less amenable to microinjection experiments, compared with more discrete regions, such as the amygdala. Nonetheless, several animal studies provide evidence for a role of cortical CRF1 in stress-related behaviors and pathology (Jaferi and Bhatnagar, 2007; Magalhaes et al., 2010; Bijlsma et al., 2011). In addition, chronic stress increases in CRF1 mRNA in cortex in rodents, and CRF1 mRNA is decreased in the cortex of depressed suicide victims, possibly in response to increased CRF peptide (Merali et al., 2004; Anisman et al., 2007).

Immunoprecipitated CRF1 from the cortex of unstrained female rats was associated with approximately three times more Gs, compared with unstrained male rats (Bangasser et al., 2010). In contrast, there were no sex differences in Go or Gq/11 association with CRF1. Similar to the sex differences in LC responses to CRF, adult hormonal status was not a determinant of the sex differences in CRF1-Gs coupling, because similar results were obtained with intact and ovariectomized females. Of importance, in rats with a history of swim stress, CRF1-Gs association increased in males to a magnitude that matched that of unstrained females, but the same stress history had no effect in females. Although it is not feasible to perform similar receptor immunoprecipitation studies with LC tissue, these sex differences in CRF1-Gs coupling determined in cortical tissue mirrored sex differences in LC sensitivity to CRF and provide potential molecular mechanisms for these physiologic differences.
Of note, these were the first data, to our knowledge, to link sex differences in physiology to sex differences in the coupling of G proteins to receptors.

**Sex Differences in CRF₁ Receptor Trafficking**

In contrast to its ability to induce CRF₁ internalization in male LC neurons, swim stress does not result in CRF₁ internalization in LC neurons of female rats (Bangasser et al., 2010). Indeed, the cellular localization of CRF₁ is opposite in female and male LC neurons. In the un unstressed male rats, ∼50% CRF₁ immunogold labeling is on the plasma membrane of LC neurons, and 50% is cytoplasmic. Swim stress shifts the distribution of CRF₁ immunolabeling, such that ∼70% is cytoplasmic, indicative of internalization. This is consistent with the decreased maximal activation of LC discharge produced by CRF. In contrast, in female LC neurons in the un unstressed condition, CRF₁ is predominantly cytoplasmic and swim stress shifts the distribution to the plasma membrane. In this case, it is not clear whether swim stress actually recruits CRF₁ to the plasma membrane or whether there is a decrease in the synthesis of CRF₁ that would render relatively less in the cytoplasm, compared with the plasma membrane. The inability of CRF₁ to be internalized in female LC neurons by 24 hours after swim stress is consistent with the lack of change in the maximum effect of CRF.

The compromised ability to internalize CRF₁ in female LC neurons is likely the result of an inability of β-arrestin 2 to associate with CRF₁, a critical molecular step in the process of receptor internalization. In the unstressed condition, CRF₁ immunoprecipitated from cortical tissue of male and female rats pulled down an equivalent amount of β-arrestin 2 (Bangasser et al., 2010). After swim stress, the association of β-arrestin 2 with CRF₁ greatly increased in males, consistent with stress-induced CRF₁ internalization. However, in females, stress did not alter CRF₁-β-arrestin 2 association. The deficit in β-arrestin 2 association with CRF₁ in females relative to males can account for the compromised ability to internalize CRF₁ after stress, and this molecular mechanism may account for the inability of stress to decrease the maximal CRF response of female LC neurons. As for CRF₁-Gₛ coupling, the differences in β-arrestin 2 association were unrelated to adult hormonal status. Of note, the decreased ability of CRF₁ receptors in females to associate with β-arrestin 2 could also account for an enhanced association of CRF₁ with Gₛ, because β-arrestin 2 sterically hinders binding of Gₛ to GPCRs (Kohout and Lefkowitz, 2003).

Sex differences in receptor association with G proteins and β-arrestins are unique and suggest structural differences in CRF₁, perhaps as a result of post-translational modifications. CRF₁ is glycosylated at many points; however, differences in glycosylation would be predicted to alter molecular weight, and there was no indication of sex differences in the molecular weight of the receptor (Grigoriadis and De Souza, 1989). Sex differences in GRK phosphorylation at one or more of several sites at which CRF₁ is phosphorylated after agonist binding could account for differences in Gₛ or β-arrestin 2 association.

**Sex Differences in the Consequences of CRF Overexpression**

The aforementioned sex differences in CRF₁ signaling would render female neurons more sensitive to CRF and less able to adapt to excessive CRF. However, sex differences in CRF₁ signaling and trafficking have little consequence in the absence of CRF and, thus, are unlikely to be manifest in the absence of stress. Instead, the presentation of a stressor that releases CRF is necessary to reveal the consequences of these molecular differences. Sex differences in CRF signaling and trafficking would have the greatest impact in conditions of excessive CRF, as has been proposed to occur in the same stress-related psychiatric disorders that are more prevalent in females, PTSD and depression. Different strains of CRF overexpressing mice (CRF-OE) have been used to model the pathologic condition of excessive CRF (Stenzel-Poore et al., 1994; Dirks et al., 2002; Groenink et al., 2003; Lu et al., 2008). The best characterized of these is a transgenic line in which CRF expression is under control of the metallothionein promoter (Stenzel-Poore et al., 1994). These mice have elevated CRF expression in brain neurons in most regions that normally express CRF. They exhibit evidence of hypothalamic-pituitary-adrenal axis overactivity, including adrenal hypertrophy and elevated plasma adrenocorticotropic and corticosterone. In addition, these mice show anxiogenic effects in many animal models. Conditional CRF-OE mice have been generated in which CRF can be overexpressed throughout the entire brain (Lu et al., 2008). A disadvantage of the conditional model is that CRF is expressed ubiquitously in both neurons and glia in brain, and unlike the CRF transgenic mice, which exhibit CRF overexpression primarily in brain regions where CRF is typically expressed, the pattern of CRF expression in the conditional line is substantially different, with the highest expression being in the olfactory bulb, cortex, and hippocampus.

Sex differences were apparent in CRF-OE transgenic mice (metallothionein promoter) with respect to LC activity recorded from slice preparations in vitro and CRF₁ cellular localization (Bangasser et al., 2012). The characteristics of LC neuronal activity were comparable in male and female wild-type mice. Despite a greater CRF innervation of LC neurons of male CRF-OE mice, compared with wild-type mice, LC discharge rates were similar. In contrast, LC neurons of female CRF-OE mice had discharge rates that were nearly three times greater than those of wild-type mice or male CRF-OE mice, suggesting that neurons of male mice have mechanisms to adapt to excessive CRF that are not present in females. CRF₁ trafficking could account for the sex differences in LC activity of CRF-OE mice. The cellular distribution of CRF₁ in LC neurons of male wild-type and CRF-OE mice were analogous to CRF₁ distribution in unstressed and stressed male rats, respectively. In wild-type mice CRF₁ was equally distributed in the cytoplasm and on the plasma membrane, and in CRF-OE mice, 80% of CRF receptor labeling was in the cytoplasm, consistent with internalization and accounting for the lack of effect of excess CRF on male LC neurons. The situation was opposite for female wild-type and CRF-OE mice, such that CRF₁ was predominantly cytoplasmic in wild-type mice and on the plasma membrane in CRF-OE mice, where it would be available to be activated by excessive levels of CRF. The inability of neurons in female CRF-OE mice to internalize CRF₁, an effect that may be in part attributed to diminished CRF₁-β-arrestin 2 association, results in an overactivated LC-norepinephrine system.

Because overactivation of the LC-NE system translates to the hyperarousal symptoms that define stress-related psychiatric disorders, it is not surprising that these disorders are
more prevalent in females. Although the molecular sex differences described here may bias toward stress-related pathology, they may also be programmed for evolutionary advantages in species in which the female must remain vigilant and exhibit behavioral flexibility in performing multiple tasks, including foraging for food while protecting from predators.

Although sex differences in the behavioral phenotype of CRF-OE mice have not been systematically explored, some differences have been noted. Female CRF-OE mice gain more weight and have larger adrenals, compared with their wild-type counterparts, whereas these differences are not apparent in males (Bangasser et al., 2012). Female CRF-OE mice also have enhanced pelvic visceral responses to stressors and show more behavioral inhibition in novel environments, compared with males (Million et al., 2007). Finally, female CRF-OE mice exhibit impaired social interaction with males, whereas male CRF-OE mice do not show this reaction (Heinrichs et al., 1997).

**Sex-Biased CRF Signaling**

Although sex differences in CRF1 trafficking and signaling have important implications for stress-related disorders, new perspectives about β-arrestin function suggest even broader implications of the findings. In addition to its role in receptor internalization, β-arrestin 2 can engage G protein–independent signaling cascades by scaffolding receptors to signaling molecules, including mitogen-activated protein kinase (e.g., ERK2, JNK, p38), tyrosine kinases (e.g., c-SRC), and AKT, PI3 kinase, and RhoA (Lefkowitz and Shenoy, 2005; Violin and Lefkowitz, 2007). A compromised ability of female CRF1 receptors to associate with β-arrestin 2 would shunt CRF1 signaling down Gs-related pathways. In contrast, in male neurons β-arrestin 2, G-protein–independent pathways would be favored, at least relative to CRF1 signaling in females. By engaging sex-specific signaling pathways, CRF released during stress could have sex-specific cellular consequences that translate to distinct physiologic and/or behavioral responses and distinct pathology (Fig. 2).

The consequences of CRF1 sex-biased signaling would be magnified when CRF is in excess, as has been proposed to occur in stress-related psychiatric disorders that are more prevalent in females. In these conditions, sex differences in signaling cascades engaged by CRF could underlie sex differences in the pathologic presentation of stress. Because Gs-protein and β-arrestin 2 signaling regulate phosphorylation dynamics in cells, the excessive CRF would be predicted to result in sex-specific phosphoprotein profiles. Keys to understanding sex differences in stress-related psychiatric disorders may lie in the differences between these profiles. This is currently being examined by performing a deep phosphoproteomic analysis of cortex of male and female CRF-OE mice with use of stable isotope labeling of whole mouse and high-resolution mass spectrometry (Valentino et al., 2012). The initial results confirm the model of sex-biased signaling, because ~15% of the phosphopeptides that could be quantified in both groups were significantly enriched in either the female (10%) or male (5%), based on a false discovery rate of 1%, which translated to a 1.52-fold difference. Ingenuity pathway analysis supported the concept of sex-biased CRF1 signaling and indicated that kinases (including PKA) were prominent in the top five canonical pathways in which phosphopeptides that were enriched in the female CRF-OE phosphoproteome were overrepresented. In contrast, phosphopeptides that were enriched in male CRF-OE cortex were overrepresented in pathways related to Rho signaling, which has been linked to β-arrestin. Of interest, phosphopeptides enriched in the female CRF-OE were overrepresented in an amyloid processing pathway and included enzymes that process amyloid precursor protein to neuropathological amyloid β and kinases that phosphorylate tau, which leads to the formation of fibrillary tangles. Because Alzheimer’s disease is more prevalent in females and has been linked to stress (Ruitenbergen et al., 2001; Csernansky et al., 2006; Wilson et al., 2006; Figueira and Ouaknine, 2010), these findings suggest a mechanism by which sex-biased CRF1 signaling favors pathways that increase vulnerability to this disease in females.

Sex-biased CRF1 receptor signaling has important therapeutic implications for novel compounds that can shift the bias of CRF1 receptor signaling. By shifting CRF signaling toward a β-arrestin 2 pathway, such compounds could potentially make females more resilient to the pathophysiological consequences of stress, particularly hyperarousal. Biased agonists are currently being designed to direct receptor-mediated cellular events toward specific pathways to promote efficacy for desired effects and diminish adverse effects (Bohn and McDonald, 2010; Rajagopal et al., 2011; Whalen et al., 2011). One example of this is the angiotensin II receptor, which may be targeted to take advantage of the anti-apoptotic effects mediated through β-arrestin and to avoid G-protein–mediated hypertensive effects (Rajagopal et al., 2011). Pharmaceuticals aimed at shifting CRF1 signaling from G-protein mediated to β-arrestin mediated may reduce the hyperarousal symptoms associated with stress-related psychiatric diseases.

**Overview and Implications**

This review integrates convergent findings supporting the novel concept of sex differences in receptor signaling and trafficking, using CRF1 as a model. A caveat is that much of the evidence for sex-biased CRF1 signaling derives from studies using cortical tissue, and there may be region-specific effects of CRF1 signaling. Even post-translational modifications, such as receptor glycosylation, can be region specific (Grigoriadis and De Souza, 1989). Nonetheless, the finding that sex differences in CRF1 signaling identified in cortex were consistent with sex differences in CRF1 trafficking and physiologic sensitivity in LC neurons supports the notion that these differences generalize to other regions expressing CRF1. Sex differences in Gs coupling would confer differences in agonist sensitivity and, in the case of CRF, differences in acute responses to stressors. Of note, small-molecule nonpeptide CRF1 antagonists bind to transmembrane domains of the receptor that are in proximity to the Gs-protein coupling site in the third intracellular loop. Potential sex-specific phosphorylation of sites in this loop could confer sex differences in the ability of these antagonists to bind (Hoare et al., 2004; Hauger et al., 2006). Differences in receptor association with β-arrestin influence receptor trafficking and the ability to adapt to the excessive CRF that is predicted to be present in diseases related to severe or chronic stress. For females, this would translate to an enhanced sensitivity to acute stress and decreased ability to adapt to chronic or repeated stress. The broader implication of this model, given evidence for
β-arrestin 2/Gs-protein–independent signaling, is that sex can be a determinant of the coping responses and pathology elicited by stress (Fig. 2). Identifying the consequences of sex-biased CRF1 signaling will help us to understand why females are more vulnerable to certain stress-related diseases.

Sex differences in CRF function have important implications for drug development. Currently, CRF antagonists are being designed to treat mood and anxiety disorders. Because the data reviewed here suggest that CRF hypersecretion has a greater impact in females than in males, CRF antagonists may be more effective in women than in men. Alternatively, sex differences in the CRF1 receptor could affect the ability of these drugs to prevent CRF1 signaling. Pharmacotherapies designed with sex differences in mind may prove to be necessary in treating psychiatric consequences of stress.

Although this review focused on CRF1 signaling, given many of the shared characteristics of different GPCRs, it is unlikely that sex-biased signaling is limited to CRF1. Recently, sex differences were reported in phosphorylation of cannabinoid receptors that could lead to differences in trafficking (Xing et al., 2011). Sex differences have also been reported in ERK and AKT pathways and their regulation in a model of schizophrenia, the neonatal ventral hippocampal lesion model, suggesting mechanisms by which altered dopamine signaling may underlie sex differences in vulnerability to schizophrenia (Bychkov et al., 2011). This unexplored area of sex differences in receptor signaling has the potential to greatly impact our knowledge of pathophysiology of diverse diseases and to transform their related therapeutics.

Authorship Contributions
Wrote or contributed to the writing of the manuscript: Valentino, Bangasser, Van Bockstaele.

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