Sex differences in stress responses: a critical role for corticotropin-releasing factor

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Abstract

Rates of post-traumatic stress disorder, panic disorder, and major depression are higher in women than in men. Another shared feature of these disorders is that dysregulation of the stress neuropeptide, corticotropin-releasing factor (CRF), is thought to contribute to their pathophysiology. Therefore, sex differences in responses to CRF could contribute to this sex bias in disease prevalence. Here, we review emerging data from non-human animal models that reveal extensive sex differences in CRF functions ranging from its presynaptic regulation to its postsynaptic efficacy. Specifically, detailed are sex differences in the regulation of CRF-containing neurons and the amount of CRF that they produce. We also describe sex differences in CRF receptor expression, distribution, trafficking, and signaling. Finally, we highlight sex differences in the processes that mitigate the effects of CRF. In most cases, the identified sex differences can lead to increased stress sensitivity in females. Thus, the relevance of these differences for the increased risk of depression and anxiety disorders in women compared to men is also discussed.

Keywords Anxiety · Arousal · Attention · Corticotropin-releasing hormone · Depression · Post-traumatic stress disorder · Sexual dimorphism

Introduction

Psychiatric disorders, such as post-traumatic stress disorder (PTSD), panic disorder, and major depression, affect many individuals worldwide [1–3]. These disorders are considered stress-related because stressful life events are associated with their onset and severity [4–7]. Indeed, risk for developing panic disorder and depression is related to the number of life stressors the patient experiences [4, 5, 8]. PTSD is, by definition, preceded by exposure to a traumatic event [9]. In addition to sharing stress as an etiological factor, these disorders are also sex-biased, being more common in women than in men [10–13]. This sex bias is observed across cultures [14–16], suggesting that sociocultural factors cannot fully explain sex

differences in disease prevalence. New research from non-human animal studies is revealing biological factors that can increase female vulnerability to stress and stress-related pathology [17, 18]. Here, we focus on sex differences in the stress neuropeptide, corticotropin-releasing factor (CRF), and highlight a multitude of CRF-driven mechanisms that can alter responses to stress in males versus females and potentially contribute to the higher rates of stress-related psychiatric disorders in women.

During a stressful event, CRF activates the hypothalamic-pituitary-adrenal (HPA) axis when its release from the paraventricular nucleus (PVN) of the hypothalamus causes the anterior pituitary to stimulate the secretion of adrenocorticotrophic hormone (ACTH). ACTH, in turn, acts on the adrenal cortex to produce glucocorticoids (e.g., cortisol in primates and corticosterone in many rodent species). Glucocorticoids mobilize energy stores through glucose metabolism while suppressing the immune system and reproduction [19–21]. Glucocorticoids then act on the PVN and pituitary to terminate the HPA axis response [22, 23]. Along with initiating this endocrine limb of the stress response, CRF acts centrally via CRF₁ and CRF₂ receptors in brain stem and forebrain regions to initiate autonomic, behavioral, and cognitive responses to stress [24–26]. Activation of these responses by CRF in the

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short term facilitates coping with environmental challenges. However, inappropriate or persistent release of CRF is linked to pathology. In fact, individuals with PTSD and depression show elevated levels of CRF in cerebrospinal fluid (CSF), with successful treatment being able to reduce these high CRF levels [27–30]. CRF levels in CSF are thought to reflect extrahypothalamic release of CRF and do not correlate well with plasma cortisol levels [31], which may explain why elevated CRF in CSF is found in patients with both PTSD and depression, conditions associated with low and high levels of cortisol, respectively [32, 33]. In addition to changes in CSF measures of CRF, alterations in CRF and CRF₁ receptor binding are also observed in the brains of patients with PTSD and depression [27, 34–36]. Additionally, genomic studies have identified single-nucleotide polymorphisms on the genes for CRF and the CRF₁ receptor that are linked to the development and/or severity of PTSD, panic disorder, and depression [37–40]. These studies implicate CRF dysregulation as an etiological contributor to several psychiatric diseases. Given that these disorders occur more frequently in women than in men, sex differences in CRF function could contribute to sex-biased psychopathology.

Historically, female rodents have been excluded from most basic neuroscience studies, including those investigating stress responses [41]. One reason for this underrepresentation of females is that studying female rodents has been considered challenging because of their short estrous cycle and the fact that ovarian hormones can induce neuronal plasticity (e.g., [42, 43]). However, recent studies have demonstrated that data from female rodents are no more variable than data from male rodents [44, 45]. Thus, although cycle effects are important to consider, excluding female subjects due to concerns about increased variability is not a valid approach. Instead, comparing males to females can, in some cases, reveal risk factors for disease. Here, we detail how incorporating female rodents into studies examining CRF function is revealing important clinically relevant sex differences.

Sex differences in CRF-mediated responses to stress

Stressful events activate CRF-producing neurons in several brain regions and there is evidence for sex differences in this activation. For example, acute restraint stress activates more CRF neurons in regions of the hypothalamus and bed nucleus of the stria terminalis (BNST) in adult female than in male rats [46]. The downstream effects of stressor exposure on CRF function are also different in males and females. Specifically, in adult mice, females are more sensitive to a chronic variable stress manipulation and this increased sensitivity is linked to epigenetic regulation of the CRF signaling pathway in the nucleus accumbens [47]. In contrast to the effects of stress in

adulthood, maternal stress early in gestation increases stress responsivity only in male offspring, an effect that is associated with epigenetic modifications to the CRF gene [48]. Certainly, more studies including females are needed. Yet collectively, these results indicate that stressor exposure can initiate sex-specific responses via regulation of CRF, and these effects differ between the sexes depending on the developmental time point.

Other research has focused on sex differences in the effect of CRF administration or CRF overexpression on anxiety-related behaviors. Initial studies evaluating the effects of CRF on behavior used male rodents and found that central administration of CRF in males evokes anxiety-related behaviors, including burying (a defensive behavior), headshakes (an arousal-related behavior), and grooming (a self-directed behavior thought to be soothing) [37, 49, 50]. We extended this work to examine CRF-evoked behaviors in both male and female rats. CRF increased all of these behaviors in both sexes, but females groomed more than males [51]. Females groomed the most when CRF was administered at the stage of their estrous cycle when ovarian hormones were highest, suggesting that these ovarian hormones potentiate the effect of CRF on grooming [51]. Finally, this sex difference in grooming was associated with ovarian hormone regulation of CRF-activated functional connectivity networks, highlighting a mechanism by which sex differences in the effects of CRF can be established at the network level [51, 52].

CRF-overexpressing (CRF-OE) mice have been used to demonstrate sex differences in the developmental effects of CRF hypersecretion. Specifically, mice with forebrain CRF overexpression during development displayed anxiety-related behavior in adulthood, an effect that was particularly pronounced in females [53]. When given a second stressor in adulthood (exposure to predator odor), only male mice with CRF overexpression early in development displayed avoidance [54]. One caveat to this result is that predator odor induced high levels of avoidance in females, regardless of a history of CRF overexpression [54]. Collectively, these studies suggest that under some circumstances, CRF increases anxiety-like behavior more in females than in males. Nevertheless, males are not unaffected by CRF and in fact may be more sensitive to a second stress hit after exposure to high levels of CRF early in development.

CRF can alter cognitive processes [55–59]. Although most of the research has focused on males (reviewed in [57]), our laboratory assessed in both sexes the effect of central CRF administration on attention. We specifically tested sustained attention, which is the ability to continuously monitor situations for intermittent and unpredictable events [60]. CRF dose-dependently impaired sustained attention in both male and female rats. However, the effect of CRF on sustained attention in females was dependent on the estrous cycle [60]. When females were in diestrus, where ovarian hormones are

low, CRF profoundly disrupted attention. However, when females were in cycle phases characterized by elevated levels of ovarian hormones, CRF had no effect on attention [60]. Thus, in this example, ovarian hormones are hypothesized to be protective against the negative impact of CRF on attention. Whether CRF regulates other cognitive processes differently in male versus female rats warrants further study.

Studies on sex differences in the effects of CRF administration in non-human primates and humans have focused on CRF's regulation of the HPA axis. Specifically, an intravenous CRF challenge increases cortisol levels more in female than male rhesus monkeys and common marmosets [61, 62]. This sex difference is linked to dihydrotestosterone, which reduces CRF-stimulated cortisol release in male monkeys [63]. Although these findings suggest sex differences in CRF sensitivity, the CRF challenge in marmosets did not increase ACTH levels more in females than males, suggesting sex differences in the adrenal response to ACTH in this species [62]. In healthy humans, an intravenous CRF challenge increases ACTH levels more in women than men, suggesting heightened sensitivity to CRF in women [64]. These studies do provide evidence that, in primates, the HPA axis of females is more sensitive than that of males to the effects of CRF. Whether sex differences in CRF sensitivity are also present in regions that regulate anxiety and cognition remains to be determined in primates.

Sex differences in CRF receptors

As noted, CRF can differentially alter behavior in males versus females. The fact that behaviors change following the administration of CRF indicates that sex differences are mediated by postsynaptic processes. Indeed, there is evidence for sex differences in CRF receptor density, expression, distribution, trafficking, and signaling in certain brain regions (Fig. 1). Evidence for sex differences in CRF receptors first comes from binding studies. Specifically, CRF₁ receptor binding in regions of the amygdala and cortex is higher in adult female rats, while CRF₂ receptor binding is higher in regions of the amygdala and hypothalamus in male rats [65, 66]. Interestingly, many of these changes in binding emerge following puberty, implicating pubertal hormone surges in these sex differences [65, 66].

Sex differences in receptor binding can be driven by changes in receptor number. Although the regions in the binding study were not directly assessed for sex differences in receptor levels, the dorsal raphe (DR) has been. In the dorsal and ventrolateral portions of the DR, CRF₁ receptor expression is increased in female compared to male rats, while in the ventrolateral DR, CRF₂ receptor expression is also higher in females than males (Fig. 1a) [67]. Unlike in rats, sex differences in CRF₁ receptor expression are not found in the DR of mice, but there are sex differences in CRF₁ receptor distribution

Fig. 1 Depiction of sex differences in CRF receptors. CRF receptors are in green and CRF is in blue. **a** Sex difference in CRF receptor expression. **b** Sex difference in the localization of CRF receptors on different cell types. **c** Sex difference in CRF receptor trafficking. **d** Sex difference in CRF receptor coupling and signaling. β , β -arrestin2; PKA, protein kinase A

[68]. Specifically, the CRF₁ receptor co-localizes with DR parvalbumin neurons more in male than in female mice (Fig. 1b) [68]. Given that the levels of CRF₁ receptor mRNA are comparable in both sexes [68], CRF₁ receptors must co-localize with a cell type different from parvalbumin neurons in females, but the identity of that cell type remains unknown. Sex differences in the types of neurons preferentially regulated by CRF could lead to different behaviors. In fact, this sex difference in CRF₁ receptor distribution is associated with increased anxiety in males following local administration of CRF into the DR [68]. Sex differences in the distribution of CRF receptors are also found in hippocampal CA1 dendrites [69]. In CA1, female rats have more CRF receptors in delta opioid receptor-containing dendrites than males [69]. These structural sex differences could lead to sex differences in interactions between CRF and endogenous opioids.

In addition to sex differences in CRF receptor distribution in different types of neurons, we identified sex differences in CRF₁ receptor localization within neurons in the locus coeruleus (LC)-arousal center. During a stressful event, CRF is released into the LC where it binds to CRF₁ receptors [70–72]. This receptor activation causes LC neurons to increase their firing rate, thereby releasing norepinephrine into the forebrain to increase arousal [70–73]. Typically, activation of this circuit increases alertness to facilitate responding to stressors. However, overactivation of the circuit can lead to the dysregulated state of hyperarousal, which is characterized by restlessness, lack of concentration, and disrupted sleep [74, 75]. One cellular mechanism that compensates for excessive CRF release is receptor internalization. During internalization, β -arrestin2 binds to the CRF₁ receptor, initiating its trafficking from the plasma membrane to the cytosol where the receptor can no longer be activated [76–80]. In male rats, acute swim stressor exposure causes β -arrestin2 to bind to the CRF₁ receptor, an effect accompanied by CRF₁ receptor internalization in LC dendrites [81, 82]. However, β -arrestin2 binding and internalization are not observed following exposure to swim stress in female rats [82]. Further, studies in CRF-OE mice with overexpression throughout their lifespan revealed a similar pattern of CRF₁ receptor internalization in LC dendrites of males, but not females (Fig. 1c) [83]. This lack of internalization in females may render their LC neurons more sensitive to conditions of excessive CRF release. In fact, LC neurons of CRF-OE females fire three times faster than those of males [83], an effect that would lead to increased arousal in CRF-OE females.

CRF₁ receptors also activate different intracellular signaling pathways in male and female rodents [84, 85]. CRF₁ receptors are G protein-coupled receptors that preferentially bind Gs to activate the cAMP-PKA signaling pathway [77, 86–88]. CRF₁ receptors are more highly coupled to Gs in females than males [82]. Accordingly, overexpression of CRF induces greater cAMP-PKA signaling in female than in male mice [84, 85]. In the LC, this increased CRF₁ receptor signaling through the cAMP-PKA pathway in females is associated with increased sensitivity to CRF [84]. Thus, a stressful event could increase arousal more in females than males because female CRF₁ receptors signal more through the cAMP-PKA pathway that activates LC neurons.

Interestingly, male CRF₁ receptors may preferentially signal through a different pathway. As mentioned above, their CRF₁ receptors more readily bind β -arrestin2 than those of females [82]. In addition to initiating internalization, β -arrestin2 also can activate signaling cascades that are often distinct from pathways activated by G proteins [89–91]. Using a phosphoproteomic approach in CRF-OE mice, we found increased phosphorylation of β -arrestin2-mediated signaling pathways (e.g., Rho signaling) in CRF-OE male mice [85]. Collectively, these results suggest a model of sex-biased

CRF₁ receptor signaling, such that this receptor signals more through β -arrestin2-mediated pathways in males and more through Gs-mediated pathways in females (Fig. 1d) [92–94]. Different signaling pathways induce distinct cellular consequences, leading to different physiological responses, some of which may increase the risk for certain types of pathology. Therefore, sex differences in signaling could bias males versus females towards different diseases. In fact, an unexpected finding from our phosphoproteomic studies was that overexpression of CRF increased the phosphorylation of proteins in Alzheimer's disease pathways more in female than male mice [85]. Using a mouse model of Alzheimer's disease pathology, we found that CRF overexpression increased amyloid plaque formation to a greater degree in females than males [85]. Taken together, these results suggest that sex-biased CRF receptor signaling is an important, yet underexplored, mechanism by which sex differences in risk factors for diseases ranging from psychiatric to neurodegenerative are established.

Sex differences in CRF expression and the regulation of CRF effects

Sex differences in CRF levels are also found in certain brain regions. CRF expression in the PVN has been found to be higher in female than male rodents in some [95–97] but not all studies [98]. Given that CRF in this region initiates the HPA axis, increased CRF expression in the PVN of females may explain why levels of corticosterone are higher in female than male rodents [99, 100]. The sex difference in hypothalamic CRF is linked to ovarian hormones in females, as levels of CRF are highest at the phases of the estrous cycle characterized by high ovarian hormones [96]. Evidence that this effect is driven by estradiol comes from a study in rhesus monkeys in which estradiol treatment of ovariectomized females increased CRF expression in the PVN [101]. Outside of the PVN, increased CRF expression has been reported by some to be elevated in the central nucleus of the amygdala in female relative to male rats [95], although this sex difference is not always observed [96]. Similarly, CRF immunoreactivity is stronger in female than male rats in the fusiform, but not in the oval nucleus of the BNST [98].

Excess CRF expression in females was recently associated with increased anxiety [102]. Li and colleagues (2016) found that oxytocin interneurons in the medial prefrontal cortex of both male and female mice release CRF-binding protein (CRFBP), which binds free CRF reducing its bioavailability, thereby inhibiting CRF's effect on its receptors [103]. Despite the release of CRFBP in both sexes, oxytocin interneurons mitigated the anxiogenic effect of CRF only in males [102]. The lack of an effect in females was attributed to their higher levels of CRF expression, which are thought to exceed the capacity of CRFBPs to prevent CRF from inducing anxiety [102]. Interestingly, in the pituitary, CRFBP expression is

higher in females than in males, perhaps to compensate, at least in part, for higher levels of CRF in the PVN [95–97, 104]. When considered together, these studies point to sex differences in CRF expression and CRFBP efficacy as potential contributors to sex differences in stress responses.

CRFBP is one mechanism for reducing the effect of CRF. However, there are other processes involved in CRF regulation, and new research has found a sex difference in another such mechanism. As noted, CRF activates LC neurons and females are more sensitive to this effect [82, 105]. This LC activation is tempered by the release of endogenous opioids that bind to μ -opioid receptors (MORs) [106, 107]. The ability of a MOR agonist to reduce CRF activation of LC neurons was greater in male compared to female rats [108]. This sex difference was linked to decreased female MOR expression in the LC [108]. These findings indicate that during stress, the ability of endogenous opioids to promote the recovery of the LC arousal circuit is decreased in females. When considered with the aforementioned sex differences in CRF₁ receptors that render female LC neurons more sensitive to CRF, these findings indicate that arousal in females is more responsive to stress and less quick to subside after stressor exposure, which would predispose females to hyperarousal. If similar mechanisms are found in humans, it may explain why women are more likely to suffer from disorders with hyperarousal features, such as PTSD and depression.

In addition to sex differences in mechanisms that regulate the downstream consequences of CRF, there is emerging evidence for sex differences in the receptors that regulate neurons that produce CRF. CRF neurons express NMDA receptors, suggesting glutamatergic regulation of these cells [109]. Knocking out *Grin1* subunits of the NMDA receptor results in a loss of NMDA function, and mice genetically modified so that *Grin1* is deleted specifically from their CRF-containing neurons have been produced [110–112]. These mice display increased fear expression and social withdrawal if they are male [111, 112]. However, female mice are unaffected by this loss of NMDA receptor function in CRF neurons [111]. Thus, glutamatergic regulation of CRF neurons via NMDA receptors appears sex-specific. Given that the receptor subtype(s) on CRF neurons that mediate these stress-related behaviors in females remains unknown, certainly, further investigation into sex differences in the afferent control of CRF neurons is required.

Implications

CRF function has mostly been characterized in male subjects, but when females are included, studies reveal several important sex differences. First, CRF-producing neurons are regulated by different types of receptors [111]. Moreover, within CRF neurons, expression of CRF is reported to be higher in

females than males in some brain regions, an effect that can overcome the ability of CRFBP to buffer the effects of CRF on anxiety in females [102]. Further, at the receptor level, there are sex differences in receptor expression, distribution, trafficking, and signaling, and many, but not all, of these sex differences have been linked to increased female CRF sensitivity [67, 68, 82, 83, 85]. Finally, there are sex differences in the processes that regulate CRF's effects, such as increased CRFBP in the pituitary of females [104] and reduced MOR regulation of CRF-induced neuronal activation in females [108]. Most of these sex differences translate into enhanced CRF efficacy in females and may help explain why women are more likely to suffer from disorders characterized by CRF dysregulation, including PTSD, panic disorder, and major depression [10–13].

How these sex differences in CRF function are established remains largely unknown. There is evidence that, in some cases, circulating ovarian hormones play a role [51, 60, 96, 113, 114]. These hormones may directly regulate the expression of CRF because its promotor contains putative estrogen response elements [115]. Membrane estrogen receptors that initiate intracellular signaling cascades also can regulate CRF neurons. For example, estradiol increases the excitability of CRF neurons in the PVN via the activation of the putative Gq-coupled membrane estrogen receptor [116]. The effect of CRF on postsynaptic neurons can also be regulated by membrane estrogen receptors, such as the G protein-coupled estrogen receptor 1 (GPER1), which can form a heterodimer with CRF receptors [117]. Although the cellular consequences of this interaction remain unknown [117], this receptor heterodimerization likely alters intracellular signaling. It is important to note, however, that not all sex differences are regulated by circulating ovarian hormones. For example, sex differences in CRF₁ receptor function in the LC are still apparent in rats gonadectomized in adulthood [82, 105]. This result indicates that circulating hormones do not play a role, but rather that this receptor sex difference results from organizational effects of hormonal surges in development or the different complement of genes on sex chromosomes. In fact, not only can circulating levels of estradiol regulate CRF in the hypothalamus [101], but perinatal estradiol exposure masculinizes adult hypothalamic CRF gene expression [118, 119]. This result highlights how organizational effects of gonadal hormones can lead to the sexual differentiation of CRF circuits. As more sex differences are identified in the effects of CRF, additional studies will be needed to determine the factors that establish sex differences in CRF function.

In conclusion, the field is just beginning to include female subjects and already sex differences have been found in almost every aspect of CRF function. Not only can many of these sex differences increase female vulnerability to certain disorders, but they suggest that pharmacotherapies targeting

aspects of CRF function may work differently in men and women. More broadly speaking, it seems unlikely that the CRF system is unique in its sexual differentiation because CRF and CRF receptors are similar to other neuropeptides and receptors. It is therefore likely that as more investigators compare male and female brains, extensive sex differences beyond the CRF system will be reported, thereby shedding light on a multitude of mechanisms that can bias males and females towards different pathologies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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