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Review

# The corticotropin-releasing factor (CRF) family of peptides as local modulators of adrenal function

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**Abstract.** Corticotropin-releasing factor (CRF), also termed corticotropin-releasing hormone (CRH) or corticoliberin, is the major regulator of the adaptive response to internal or external stresses. An essential component of the adaptation mechanism is the adrenal gland. CRF regulates adrenal function indirectly through the central nervous system (CNS) via the hypothalamic-pituitary-adrenal (HPA) axis and via the autonomic nervous system by way of locus coeruleus (LC) in the brain stem. Accumulating evidence suggests that CRF and its related peptides also affect the adrenals directly, *i.e.* not through the CNS but from within the adrenal gland where they form paracrine regulatory loops. Indeed, CRF and its related peptides, the urocortins (UCNs: UCN1, UCN2 and UCN3), their receptors CRF type 1 ($CRF_1$) and 2 ($CRF_2$) as well as the endogenous pseudo-receptor CRF-binding protein (CRF-BP) are all expressed in adrenal cortical, medullary chromaffin and resident immune cells. The intra-adrenal CRF-based regulatory system is complex and depends on the balance between the local concentration of CRF ligands and the availability of their receptors.

**Keywords.** Corticotropin-releasing hormone, Urocortin, CRF receptor, CRF-binding protein, adrenal, catecholamine, pheochromocytoma, macrophage.

## 1. Components of the system

**A. Corticotropin-releasing factor (CRF).** CRF, a 41-amino acid peptide, also called corticotropin-releasing hormone (CRH) or corticoliberin, is the major regulator of the adaptive response to internal or external stressors [1–3]. CRF regulates adrenal function indirectly through the central nervous system (CNS) via the hypothalamic-pituitary-adrenal (HPA) axis. In the hypothalamus, the parvocellular neurons of the paraventricular nucleus (PVN) respond to central and peripheral stimuli by expressing and secreting CRF. The parvocellular neuronal endings form an anatomical structure at the bottom of hypothalamus, the media eminence, from which CRF is released into the portal circulation, which connects the hypothalamus to the anterior pituitary lobe. In the anterior pituitary, CRF stimulates expression of the proopiomelanocortin (POMC) gene in

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corticotroph cells. POMC is subsequently cleaved to adrenocorticotropin hormone (ACTH), which is secreted into the systemic circulation and reaches the adrenal cortex, where it stimulates the synthesis and secretion of cortisol from the middle cortical zone, the zona fasciculata. Simultaneously, several parvocellular CRF neurons innervate sympathetic neurons in locus coeruleus (LC) in the brain stem. At the same time, the sympathetic system innervates all components of the HPA axis. In this way the two major stress axes coordinate their response to stress. Multiple published reports now suggest that the interaction between the PVN (CRF) and the LC (norepinephrine) also takes place at a lower (peripheral) level within the adrenal gland itself. Indeed, CRF is also expressed in cortical and medullary cells within the adrenal gland, forming a regulatory intra-adrenal circuit parallel to the PVN and LC/sympathetic nervous system (SNS).

**B. The urocortins (UCNs).** The first CRF-related neuropeptide to be identified, the 40 amino acid-long urocortin 1 (UCN1), was isolated from a rat midbrain library screened with a urotensin probe [4]. The first component of its name is derived from the homology UCN1 exhibits to the teleost hormone urotensin I, a fish neuropeptide present in the urophysis, a caudal neurosecretory organ of the common suckerfish *Catostomus commersoni*, while the second component stems from its homology to CRF. Indeed, UCN1 shares 63% homology to teleost fish urotensin I, 45% homology to rat/human CRF and 35% homology to a non-mammalian CRF-like peptide, sauvagine, a 40-amino acid peptide derived from the skin of the Phyllomedusa frog, which lives in South America. Like CRF, UCN1 can stimulate ACTH production from anterior pituitary corticotrophs *in vitro* and *in vivo*. UCN1 is more potent than CRF with regard to other biological effects, including suppression of appetite, but is less potent in generating anxiety. A few years after the identification of UCN1, two additional mammalian CRF-like peptides were isolated. Urocortin 2 (UCN2; stresscopin-related peptide) is a 38-amino acid peptide with an amidated C-terminus that was first predicted in mouse from a gene encoding a 112-amino acid protein [5, 6]. Human urocortin 3 (UCN3; stresscopin) was identified later and found to have high homology (>80%) to UCN2 [7]. In the brain, UCN2 is mainly expressed in the magnocellular part of the PVN in hypothalamus, in the LC and in the facial motor nucleus. UCN3 was identified due to its similarity to a CRF-like peptide identified in the Japanese puffer fish *Takifugu rubripes* [7] and is expressed in several brain regions, including the hypothalamus and structures of the limbic system.

**C. The CRF receptors.** The biological effects of CRF and UCNs are mediated by two distinct receptors, CRF receptor type 1 ($CRF_1$) and 2 ($CRF_2$), which belong to the class B subdivision of the G protein-coupled receptor superfamily of brain-gut neuropeptides [8–12]. Two separate genes encode the CRF receptors [13]. $CRF_1$, a 415-amino acid protein, exhibits high affinity towards CRF and UCN1 but low affinity towards UCN2 and no affinity towards UCN3. The $CRF_1$ receptor is mainly expressed in CNS and the anterior pituitary. Indeed, the $CRF_1$ receptor is expressed in the entire paraventriculo-infundibular system, septum, bed nuclei of stria terminalis nucleus accumbens, in the cerebral cortex and the limbic system (in amygdala and hippocampus) [14, 15]. The $CRF_2$ receptor shares 70% sequence homology to $CRF_1$ and is expressed mainly in extra-CNS sites. In CNS it is expressed in hypothalamus (mainly in the paraventriculo-infundibular system) and in the limbic system (mainly in the amygdala) [15]. The $CRF_2$ receptors exhibit high affinity towards UCNs and no affinity towards CRF. Activation of the $CRF_2$ receptor suppresses multiple metabolic functions including feeding in fasted mice [16], heat-induced edema and gastric emptying [17]. $CRF_2$ has two splicing variants in rodents and three splicing variants in humans. The $CRF_2$alpha receptor is mainly expressed in hypothalamus and other areas of the brain, while $CRF_2$beta is principally expressed in peripheral tissues including the adrenals [18, 19]. In contrast to mice lacking $CRF_1$, $CRF_2$-deficient mice exhibit enhanced anxious behavior [16, 17, 20]; indeed, $CRF_2$ receptors mediate a central anxiolytic response, opposing the general anxiogenic effect of CRF mediated by the $CRF_1$ receptor [21]. Pharmacological inhibition of CRF receptors has attracted the interest of researchers as a potential therapeutic tool in treating depression. The structures and biological effects of multiple new synthetic CRF receptor antagonists have been reported [13, 22, 23].

**D. The CRF-binding protein (CRF-BP).** Finally, CRF-BP, a 37-kDa glycoprotein, is a soluble protein that binds CRF. It was first identified in a soluble form in human plasma. The human and rat CRF-BP cDNAs encode highly homologous glycoproteins 322 amino acids long [24]. CRF-BP has been detected in anthropoid primates and rodents but not in evolutionarily earlier species, suggesting that a complex regulatory mechanism involving CRF-BP developed later in evolution [24–26]. In the circulation and in the interstitial spaces, CRF-BP binds to either CRF or

**Table 1.** Expression of CRF, UCNs and their receptors $CRF_1$ and $CRF_2$ in human adult adrenals.

| | Medulla | Cortex | | |
|---|---|---|---|---|
| | | reticularis | fasciculata | glomerulosa |
| CRF | ++ | ++ | ++ | ++ |
| UCN1 | +++ | + | + | + |
| UCN2 | + | +++ | + | ++ |
| UCN3 | + | ++ | ++ | ++ |
| $CRF_1$ | +++ | + | + | − |
| $CRF_2$ | + | ++ | ++ | + |

CRF, corticotropin-releasing factor; UCN, urocortin.

UCNs with high affinity [25, 27]. In primates, the CRF-BP gene is expressed in several tissues including liver, placenta and brain [28, 29]. By binding to CRF and UCNs, CRF-BP reduces their bioavailability and prevents their binding to the biologically active CRF receptors, thus controlling in a fast and transient manner the concentration of the available ligands.

## 2. The intra-adrenal CRF-based circuits

**A. CRF peptides in human adult adrenals.** The adrenal gland develops early in embryonic life from neuroectoderm, but its structure is not completed until after birth. The fully developed adult adrenal is comprised of the cortex and the medulla (Fig. 1). The medulla primarily consists of chromaffin cells as well as resident macrophages, vascular endothelial cells and the neuronal axes of the innervating pre-ganglionic autonomic neurons. The cortex, which is responsible for steroidogenesis, surrounds the medulla and consists of three zones: glomerulosa, fasciculata and reticularis. Shortly after the isolation of hypothalamic CRF by Vale et al. in 1981 [1], CRF was shown to be expressed in the adrenal gland of all mammals studied, including rodents, cattle, dogs and cats [30–32]. Indeed, it is now more than 20 years since the presence of CRF and specific CRF-binding sites were first identified in human adrenal glands [32, 33]. One decade later and following the isolation of the CRF-homologous peptides (UCN1–3) and identification of their receptors ($CRF_1$, $CRF_2$) and the CRF pseudo-receptor CRF-BP, it was realized that most if not all CRF-related peptides and their receptors are also expressed in the adrenals, forming an elaborate intra-adrenal circuit (Table 1) [6, 34, 35]. It should be noted here that CRF peptides in the adrenal gland do not exclusively derive from adrenal cortical or medullary cells but also from sympathetic pre-ganglionic neurons, resident immune cells and other locally present cells [26, 36, 37]. For example, in elegant studies using electron microscopy, the release of CRF peptides within the adrenal gland was documented following direct electrical stimulation of splachnic sympathetic neurons [38–40]. Recently, several studies using immunohistochemistry and *in situ* hybridization in human adrenals produced data regarding the distribution of CRF-, UCN- and $CRF_1$- and $CRF_2$-producing cells in the adrenals (Fig. 1a). More specifically, CRF, the preferential endogenous $CRF_1$ ligand, was found to be expressed throughout the gland, *i.e.* in all three cortical layers as well as in the medulla, while the $CRF_1$ and $CRF_2$ ligand UCN1 is expressed mainly in chromaffin cells in the medulla (Table 1) [18, 41]. On the other hand, the preferential $CRF_2$ receptor ligand UCN2 is expressed in all three cortical zones but barely in human adrenal medulla [18]. Finally, the selective $CRF_2$ receptor ligand UCN3 appears to be expressed in human adrenals [42]. Expression analysis revealed co-localization of the ligands and their respective CRF receptors: $CRF_1$ and UCN1 are both expressed in the medulla, $CRF_2$ and UCN2 are found in the cortex, and $CRF_2$ and UCN3 are co-localized in more than 85% of adrenocortical cells [18, 41]. These findings suggest that a $CRF_1$ receptor/ligand system may be active in the medulla, while a parallel $CRF_2$ receptor/ligand system may work in the cortex.

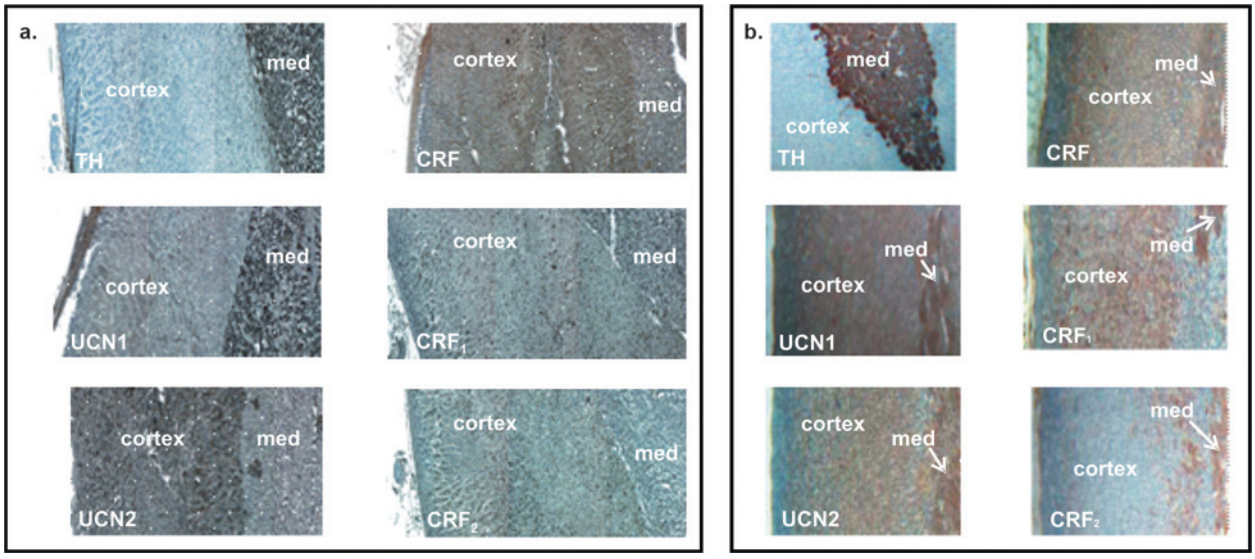


**Figure 1.** Expression of CRF, UCN1, UCN2 and their receptors $CRF_1$ and $CRF_2$ in human and rat adrenals. (*a*) Paraffin-embedded sections of human adrenal gland were stained with antibodies specific for tyrosine hydroxylase (TH), UCN1, UCN2, CRF, $CRF_1$ or $CRF_2$; Chromaffin cells of the adrenal medulla were positive for TH. UCN1 and $CRF_1$ receptor were mainly expressed in the medulla, while UCN2 and $CRF_2$ receptor were mainly expressed in the cortex. CRF was abundantly expressed throughout the adrenals. (*b*) In sections of rat adrenal gland, TH-positive cells were exclusively localized in the medulla. UCN1, UCN2, CRF, $CRF_1$ and $CRF_2$ were strongly expressed in the medulla, zona fasciculata and zona glomerulosa and weakly expressed in the zona glomerulosa.

**B. CRF peptides in human fetal adrenals.** During gestation, the adrenal gland changes dramatically according to the endocrine needs of the body. Thus, in humans and higher primates, the fetal adrenal cortex is comprised primarily of the fetal zone, which expresses the P-450 17α-hydroxylase-C17,20-lyase (P-450c17) enzyme essential for the production of the C19 androgen dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEAS), which serve as requisite precursors for placental estrogen production. During the late stages of gestation, two additional zones develop: a "transitional zone" which expresses the 3β-hydroxysteroid dehydrogenase-isomerase (3βHSD) and P-450c17 enzymes for the production of cortisol, and a "definitive zone" which expresses 3βHSD but not P-450c17 for the production of aldosterone. These developments depend on ACTH from fetal pituitary, which induces the expression of 3βHSD and P-450c17 [43]. The adrenal medulla appears to mature during the end of the second trimester, as documented by the onset of catecholamine production [44]. Indeed, the average volume of medullary cells increases between 24–39 weeks of gestation, indicating tissue differentiation [45]. Several published reports have addressed the biological significance of adrenal CRF peptides in fetal adrenal development and their interaction with ACTH. It now appears that the pattern of expression of CRF and UCNs in fetal adrenals parallels the developmental changes (Table 2) [46]. In human fetal adrenals, UCN1, UCN3 and the $CRF_1$ and $CRF_2$ receptors appear to be expressed in both fetal and definitive zones of the adrenal cortex of the conceptus [41, 47]. The levels of UCN1 peak just before birth, approximately during the $39^{th}$ week of gestation [48]. Following birth, UCN1 is also expressed in the adrenal medulla of the newborn. It is of interest that the selective $CRF_2$ receptor agonist UCN3 appears to be expressed by both fetal and definitive adrenal zones, a finding suggesting its importance in the function of fetal adrenals. The receptor itself, $CRF_2$, appears to be detectable, although at relatively low levels, in the fetal zone from the $20^{th}$ to the $37^{th}$ week of gestation [41]. In contrast, $CRF_1$ is strongly expressed in both fetal and definitive zones from the $20^{th}$ to the $37^{th}$ week of gestation [49]. It should be noted, however, that the apparent molecular weights of $CRF_{1\alpha}$ and $CRF_{2\alpha}$, the isoforms of $CRF_1$ and $CRF_2$ in the adrenals, is higher compared to those reported for rat pituitary and human myometrium, most probably because of a higher degree of glycosylation or the expression of slightly different transcripts [47, 50].

**C. CRF peptides and their receptors in canine, bovine, sheep, monkey and rodent adrenals.** CRF is present in rodent, bovine and canine adrenals. More specifically, immunohistochemical analysis revealed the presence of CRF-immunoreactive cells in the adrenal medulla of dogs clustering in the vicinity of blood vessels and near the border between adrenal medulla and cortex (Table 3). The cortex appears to be devoid of any CRF-positive cells [38]. Similar findings have been

**Table 2.** Expression of CRF, UCNs and their receptors $CRF_1$ and $CRF_2$ in human fetal adrenals.

| | 16th week | 20th week | 37th week | 39th week | Newborn |
|---|---|---|---|---|---|
| CRF | nt | nt | nt | nt | nt |
| UCN1 | – | Fetal zone | Fetal zone<br>Definitive zone | Fetal zone<br>Definitive zone | Medulla |
| UCN2 | nt | nt | nt | nt | nt |
| UCN3 | – | Fetal zone | Fetal zone<br>Definitive zone | Fetal zone | nt |
| $CRF_1$ | – | Definitive zone<br>(high levels) | Definitive zone<br>(high levels) | nt | nt |
| $CRF_2$ | – | Definitive zone<br>(low levels) | Definitive zone<br>(low levels) | nt | nt |

CRF, corticotropin-releasing factor; UCN, urocortin; nt, not tested; (–), no expression.

reported in bovine adrenal medulla [39, 51]. Binding sites for CRF are present in bovine chromaffin cells in culture [52], while immunohistochemical analysis revealed CRF-like immunoreactivity in bovine adrenal medulla [53]. In sheep adrenals, CRF is present in clusters of darkly beaded axon-like fibers over medullary cells at the medullary-cortical interface or over islands of medullary cells surrounded by cortex or rays of medullary cells extending into the cortex [37]. CRF receptors have been demonstrated in the rhesus macaques and cynomolgus monkey adrenal medulla and sympathetic ganglia (Table 3) [54–56]. $CRF_1$ has also been isolated from the tree shrew Tupaia belangeri with a PCR-based approach. In addition, weak expression of $CRF_1$ mRNA has been documented in the adrenal gland of tree shrew [57]. Among rodents, a more detailed analysis has been performed in the rat (Table 4, Fig. 1b). CRF binding sites were originally detected in rat adrenal medulla [52]. Cultured rat adrenal chromaffin cells also express and release immunoreactive CRF [58]. The presence of UCN1 and CRF has been demonstrated in rat adrenal medulla [59], while Oki and co-workers measured immunoreactive UCN1 in whole rat adrenal preparations [60]. A more recent study from our laboratory demonstrated strong expression of UCN1 throughout the adrenal medulla as well as in the cortical fasciculate and glomerulosa zones but weak expression in the reticularis zone [18]. CRF was present throughout the rat adrenal medulla. $CRF_1$ and $CRF_2$ were clearly expressed in the medulla, weakly expressed in the cortical fasciculate and glomerulosa zones and barely expressed in zone reticularis. While all CRF peptides and their receptors were also detectable in the cortex, the intensity of staining was definitely less than that in medulla, with the exception of the zona glomerulosa [18]. UCN2-positive cells were detected in the medulla of rat [61], while another study demonstrated equal distribution in medulla and the cortical fasciculate zone [18]. Furthermore, high concentrations of immunoreactive UCN3 were found in the whole rat adrenal gland [62]. Finally, the transcript and protein of CRF-BP is also present in rat adrenal medulla [28]. UCN2 and low levels of $CRF_1$ mRNA are expressed in the adrenal of mice.

CRF-BP is expressed in adrenals [28]. In rodents, CRF-BP is mainly localized in the adrenal chromaffin cells (Table 4). The presence of CRF-BP in the adrenals suggests that this gland possesses a complex CRF-based regulatory circuit composed of all known CRF neuropeptides and their receptors. The simultaneous expression of CRF-BP by CRF-producing cells, as described in the brain, is retained by the chromaffin cells of the adrenals during their transition from neuronal to endocrine function early during development. Other peripheral tissues of non-neuronal origin, such as the heart and the lymphoid organs [29], in which expression of the CRF system may serve a different role, do not exhibit simultaneous CRF and CRF-BP expression. Altered expression of adrenal CRF-BP as a result, for example, of a malignant transformation process, may lead to changes in the

**Table 3**. Expression of CRF, UCN2 and their receptors in the adrenal glands of various species.

| Species | CRF | UCN2 | CRF receptors | Reference |
|---|---|---|---|---|
| Canine | Medulla | nt | nt | [38] |
| Baboon | Fetal definitive zones | nt | nt | [47] |
| Sheep | Medulla fibers in the medulla | nt | nt | [37] |
| Monkey | nt | nt | Medulla sympathetic ganglia | [56] |
| Tree shrew | nt | nt | $CRF_1$ in adrenal gland | [57] |
| Bovine | Medulla | nt | nt | [53] |
| Bovine | nt | nt | Medulla | [52] |

CRF, corticotropin-releasing factor; UCN, urocortin; nt, not tested.

**Table 4.** Expression of CRF, UCN1, UCN2, their receptors CRF1 and CRF2 and CRF-BP in rat adult adrenals.

| | Medulla | Cortex | | | Nerve fibers |
|---|---|---|---|---|---|
| | | reticularis | fasciculata | glomerulosa | |
| CRF | +++ | – | ++ | +++ | + |
| UCN1 | +++ | + | ++ | +++ | nt |
| UCN2 | +++ | ++ | ++ | – | nt |
| $CRF_1$ | +++ | + | ++ | ++ | nt |
| $CRF_2$ | +++ | + | ++ | ++ | nt |
| CRF-BP | ++ | + | + | + | nt |

CRF, corticotropin-releasing factor; UCN, urocortin; CRF-BP, CRF binding protein; nt, not tested; (–), no expression.

concentration of free, bioavailable local CRF and to a subsequent induction of its effects. Thus, it is of interest to compare CRF-BP expression in normal and tumoral adrenals, especially since differences in UCNs and CRF receptor expression levels were recently described in adrenal neoplasm [41].

**D. CRF peptides in the PC12 cell model.** This complex CRF-containing paracrine system appears to be preserved in adrenomedullary tumors, including the PC12 rat pheochromocytoma cell line. CRF, UCN1 and UCN2 as well as their receptors, $CRF_1$ and $CRF_2$, are all expressed in PC12 cells [18]. It has been shown that during the differentiation of PC12 cells into neuron-like cells, a process induced by nerve growth factor, a parallel induction of CRF-BP expression takes place [28]. CRF-BP expression is regulated by CRF itself, glucocorticoids and cytokines, as shown in neuronal cells and other cell types [27]. In agreement with these reports, the expression of adrenal CRF-BP appears to be under the positive control of protein kinase A (PKA) effectors (cAMP and CRF), glucocorticoids and interleukin-6 (IL-6) [28]. It is possible that CRF-BP mediates some of the adrenal effects of these factors. For instance, CRF-BP induction via activation of IL-6 receptors that are present in this cell line [63, 64] can contribute to the role of IL-6 in the differentiation of PC12 cells to a neuronal phenotype via gp130 [65] and the blockade of PC12 cell apoptosis following serum deprivation [66]. On the other hand, parallel to CRF-BP induction, glucocorticoids increase CRF production by pheochromocytoma cells [58]. The observation that stimuli that enhance CRF expression also provide resistance to its effect by simultaneously inducing CRF-BP expression suggests the existence of a micro-regulatory mechanism of CRF bioavailability within the adrenal gland, a phenomenon common in other tissues.

**Table 5.** Functions of CRF, UCN1 and UCN2 in the adrenals.

| Functions | CRF | UCN1 | UCN2 |
|---|---|---|---|
| Induction of enzymes responsible of catecholamine synthesis | ↑TH [18]<br>↑DBH, PNMT [92] | ↑TH [18, 115] | ↓TH (human) [18]<br>↑TH (rat) [18] |
| Catecholamine release | ↑NE, E, D [18, 76, 77] | ↑NE, E, D [18] | ↑NE, E, D (human, rat) [18]<br>↑NE (rat) [90] |
| Catecholamine synthesis | ↑NE, E, D [58, 88, 18] | ↑NE, E, D [18] | ↑E (human) [18]<br>↑NE, D (rat) [18] |
| Adrenal sympathetic nerve activity | ↑Adrenal sympathetic nerve activity [76] | nt | nt |
| Steroidogenesis | ↑Cortisol [34, 40, 49]<br>↑DHEA [83]<br>↑DHEAS [81, 82]<br>↑Corticosterone [40, 78] | ↑DHEA [83] | nt |
| Second messenger signaling | ↑Calcium ion levels via PKA, PKC [94, 95]<br>↑p38MAPK [93] | ↑cAMP levels [89] | PKA-Erk1/2 [90] |
| Trophic effect | Trophic effect on chromaffin cells [79] | nt | nt |
| Apoptosis | ↑Apoptosis via Fas ligand [93] | nt | nt |

CRF, corticotropin-releasing factor; UCN, urocortin; NE, norepinephrine; E, epinephrine; D, dopamine; DBH, dopamine beta-hydroxylase; PNMT, phenylethanolamine N-methyltransferase; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; TH, tyrosine hydroxylase; nt, not tested.

## 3. Modes of CRF action on the adrenals

Even though hypothalamic CRF is the major regulator of adrenal function via ACTH, very little is known about the physiological importance of intra-adrenal CRF, its related peptides and their receptors in adrenal function. Here we present what is known so far about the direct and indirect effects of CRF peptides on adrenal function (Table 5).

**A. Hypothalamic CRF can affect adrenals through either the HPA axis or SNS.** CRF is a major regulator of both the HPA axis and the autonomic nervous system. Thus, from the point of view of an adrenal gland, CRF can affect the adrenals via the HPA axis or via the hypothalamus-LC (brain stem)-peripheral SNS. Indeed, a CRF-expressing population of hypothalamic neurons project directly into the LC, modulating the activity of these neurons and thus integrating hormonal, autonomic and behavioral responses to stress [67–72]. It should be noted that noradrenergic neurons from LC and the peripheral sympathetic nerves respond to a variety of stress-related stimuli simultaneously, which results in coordinated norepinephrine secretion from central and peripheral SNS sources, including the adrenal medulla [73]. The effect of hypothalamic CRF on LC has been shown in multiple experimental protocols including microdialysis [73]. The effect of CRF is mediated via the $CRF_1$ receptors in the noradrenergic perikarya of LC [23]. The receptor is internalized following exposure to CRF or stress [71]. The synthetic $CRF_1$ receptor antagonist DMP695 abolishes activation of LC noradrenergic neurons by CRF in anesthetized rats [74]. Centrally administered CRF elevates blood pressure and accelerates heart rate via SNS [75] and sympathetic efferent nerve activity to the adrenals [76]. At the same time, it results in an acute increase in the plasma levels of noradrenaline and adrenaline in the systemic circulation. These elevations are abolished by centrally administered indomethacin, an inhibitor of cyclooxygenase, in rats [77], supporting the involvement of active metabolites of brain arachidonic acid in the CRF-induced activation of the central sympathoadrenomedullary outflow in rats.

**B. CRF peptides directly affect adrenal steroidogenesis.** Expression of all CRF peptides and their receptors in the adrenal suggests that complex CRF-based circuits exist within human and rodent adrenals. The circuits may control adrenal cortical and medullary function as well as the level of their interaction. Exogenous CRF enhances adrenal steroidogenesis in hypophysectomized calves [40], implying that the

effect of CRF is direct and not via ACTH. Furthermore, CRF stimulates cortisol production by fetal adrenocortical cells *in vitro* [49]; more specifically, exposure of adrenocortical cells to CRF elevates the mRNA levels of steroidogenic acute regulatory protein and of the enzymes needed to produce cortisol, *i.e.* 3β-hydroxysteroid dehydrogenase type II, 21-hydroxylase and 11β-hydroxylase [49]. The direct stimulatory effect of CRF peptides on cortisol synthesis is mediated by the $CRF_1$ receptor. In addition, CRF enhances the adrenal response to ACTH; if a submaximal dose of CRF is combined with sub-effective doses of ACTH, a marked dose-dependent increase in corticosterone release is observed in rat adrenals *in vitro* [49, 78]. Exogenous CRF also has a trophic effect on the adrenal medulla of hypophysectomized rats [79]. It has been reported that the stimulatory effect of CRF on cortisol production may need the presence of chromaffin cells [34]. It should be noted that in human adrenals, the cortical zones and the medulla are closely interwoven, thus coming into close cellular contact, the prerequisite of paracrine effects.

**C. Placental CRF directly affects fetal adrenal steroidogenesis.** Under normal conditions, CRF levels in the systemic circulation are too low to affect the adrenals or, for that matter, any other tissue with CRF receptors. However, during the third trimester of pregnancy, the levels of CRF in the maternal and fetal circulation increase exponentially, peaking during the last 2 to 3 weeks before delivery. This CRF derives from the placenta, which is a real factory in producing CRF. It is possible that the increasing production of cortisol by the maturing fetal adrenal induces placental CRF expression [80]. Placenta-derived CRF as well as UCN1 stimulate the production of DHEAS by the fetal zone of human adrenals via the $CRF_1$ receptor, which is expressed in the fetal zone [81–83]. Indeed, CRF induces the transcription of cytochrome P450 cholesterol side-chain cleavage and 17α-hydroxylase/17,20 lyase in fetal adrenal cells. It should be noted here that DHEAS is the principal substrate for estrogen synthesis by the placenta [84]. Increased estrogen production by the placenta accelerates the process of parturition in humans. It is of interest that glucocorticoids, while suppressing the expression of CRF in hypothalamus and most peripheral sites of CRF expression, enhance placental CRF production as mentioned above [80]. This appears to be a late evolutionary development via which placenta and fetal adrenals compose a mutually boosting circuit, resulting in higher estrogen production by the placenta, accelerating parturition.

Although non-primate mammals express the genes of several hypothalamic neuropeptides in their placenta, they do not express that of CRF [85]. Thus, it appears that this may represent a unique adaptation mechanism that has evolved exclusively in anthropoid primates [86]. Even within the anthropoid primates, there are at least two patterns of placental CRF expression over gestation: monkeys differ from great apes (and humans) in that they exhibit a mid-gestational peak of placental CRF expression. The functional significance of the differences between most mammals and primates as well as between monkeys and great apes (and humans) is not yet understood. It should be noted here that the fetal zone in the adrenals, its product DHEAS, and the placental production of CRF and estrogens appears to be of paramount importance mainly in apes and in humans. Alterations in placental CRF expression are associated with placental malfunction, accelerated aging and preeclampsia [87].

**D. CRF peptides directly affect adrenal catecholamine release.** Early on in the study of the interaction between CRF and the peripheral SNS, the physiology appeared simple and straightforward. Indeed, CRF induced catecholamine secretion from adrenal chromaffin cells *in vitro* [58, 88]. Subsequently, we have found that while $CRF_1$ receptor agonists rapidly induce catecholamine secretion, $CRF_2$ receptor agonists suppress it, an effect that appears to be mediated by actin filament reorganization [18]. Furthermore, a discrepancy became apparent between human and rat adrenal chromaffin cells in culture. Based on these data, we now hypothesize that the modulation of adrenal catecholamine secretion and synthesis by the intra-adrenal CRF-based circuits is a complex but highly flexible system depending on the concentration of the different ligands and the availability, sensitivity or desensitization of locally existing $CRF_1$ and $CRF_2$ receptors. Such changes in the expression of the receptors and their ligands provide plasticity to the system, allowing faster adaptation to a host of internal and external factors.

**E. CRF peptides directly affect catecholamine synthesis.** CRF peptides also affect *de novo* catecholamine synthesis. More specifically, UCN1 induces tyrosine hydroxylase (TH) expression via cAMP in rat adrenomedullary PC12 cells [89], while UCN2 induces TH phosphorylation via the PKA-Erk1/2 pathway [90]. We have found that both $CRF_1$ and $CRF_2$ agonists induce *de novo* catecholamine synthesis via induction of TH [18]. Thus, prolonged exposure of the adrenal glands to stress peptides (a condition occurring during chronic stress) may result in increased TH expression levels, leading to elevation of stored adrenaline in the secretory vesicles of adrenal medul-

lary cells, thus augmenting the magnitude of the catecholaminergic response to stressors [18]. Indeed, chronic stress increases TH and phenylethanolamine N-methyltransferase (PNMT) activities and mRNA levels in the adrenal medulla [91]. The continuous administration of CRF results in a significant increase in DBH (dopamine beta-hydroxylase) and PNMT activities in the adrenal glands. The pattern of the increase in the DBH response to various doses of CRF does not relate to that of plasma corticosterone, suggesting that the effect of CRF on these adrenal enzymes is independent of its effect on the HPA axis [92].

Expression of CRF peptides and receptors between humans and rodents appears relatively similar, supporting the use of rodents as experimental models for studies that can be applied in humans. Nevertheless, in functional studies the response to CRF receptor ligands is not always the same across species. According to our recently published work, it appears that in human chromaffin cells, $CRF_2$ signals suppress *de novo* catecholamine synthesis, while in the rat $CRF_2$ induces it. This discrepancy may reflect differences in the chronic response to stressors, which is more profound in rodents. Hence, results from rodent animal models should be interpreted cautiously.

**F. Other effects of CRF peptides on adrenal chromaffin cells.** CRF augments serum deprivation-induced PC12 cell apoptosis via the $CRF_1$ receptor [93]. Induction of apoptosis involves activation of p38MAPK and Fas ligand [93]. Stimulation of Fas ligand as well as that of multiple other events induced by CRF in adrenomedullary cells depends on the elevation of intracellular calcium levels. CRF promotes calcium ion influx and calcium ion mobilization from intracellular stores via PKA and protein kinase C (PKC) pathways in PC12 cells [94]. Furthermore, CRF differentially regulates PKC-isoenzyme phosphorylation via $CRF_1$, an effect that is apparently physiologically relevant, since blockade of conventional PKC phosphorylation abolished the biological effects of CRF in PC12 cells [95].

## 4. The role of CRF in the interaction between adrenals and the immune system

Crosstalk between the immune system and the HPA axis has been reported at various levels. Circulating cytokines influence the HPA axis either by affecting the secretion of CRF in the hypothalamus or by acting on the adrenals, altering the synthesis and secretion of catecholamines and cortisol [96–98]. In addition, locally produced cytokines affect the function and survival of cells in the HPA axis [99]. Furthermore, the immune system and the adrenal gland itself interact in a bi-directional way [100–102]. Rat and human adrenals have a population of resident macrophages distributed throughout the cortex and medulla [102–104]. Adrenal resident macrophage-derived prostaglandin E2 (PGE2) binds to adrenal chromaffin cells, induces release intracellular calcium stores and stimulates calcium influx through voltage-independent channels, modulating catecholamine release [105]. Earlier work from our laboratory demonstrated that macrophages, like adrenal chromaffin cells, express CRF and the UCNs, which affect the synthesis and secretion of prostaglandins and pro-inflammatory cytokines [106–108]. Thus, CRF and UCNs that are locally expressed by adrenal cells can affect production of these factors by resident macrophages in the adrenals, which can subsequently influence the synthesis of adrenal products. It should be noted here that cytokines reach the adrenals either through systemic circulation or by local production by resident macrophages. Moreover, adrenal medullary cells themselves possess the ability to produce cytokines, making their interaction with immune cells vastly more complex [64, 109]. The role of CRF peptides in immune system-adrenal crosstalk can, therefore, be separated into three categories: a) the effects of cytokines on adrenal CRF expression and adrenal function, b) the effects of CRF peptides on cytokine production from adrenal cells and c) indirect effects of adrenal CRF peptides on cytokine expression.

**A. Cytokines affect the adrenal CRF-based system.** During times of infectious challenge, the immune system stimulates the production of cortisol via interleukin-1 (IL-1) and tumor necrosis factor alpha (TNFα), which stimulate the expression and secretion of hypothalamic CRF in a direct manner [110–113]. The expression of the CRF gene is up-regulated through nuclear factor kappa B (NF-κB) and cAMP-response binding protein (CREB) [114, 115] and is suppressed by REST/NRSF, which binds to the RE-1/NRSE element in the CRF intron [116]. IL-1β and TNFα activate NF-κB and CREB. These cytokines also stimulate adrenal chromaffin cell CRF [88].

**B. Effect of CRF peptides on cytokines.** CRF peptides can affect the immune system directly, in a paracrine mode of action, or indirectly via catecholamines or by inducing the production of potent systemic immunomodulators (e.g. cortisol, DHEA). CRF induces the expression of the pro-inflammatory cytokines IL-1, IL-6 and TNFα from activated macrophages in a direct manner by promoting Toll-like receptor 4 (TLR4) expression

[106, 108]. This effect depends on signals from both $CRF_1$ and $CRF_2$. In contrast, UCNs promote apoptosis in macrophages [107], suggesting that local secretion of UCNs in the adrenal may eliminate resident macrophages. CRF peptides also affect mast cells and T cells. CRF and UCN1 induce histamine and IL-6 secretion from mast cells [117, 118]. Among splenocytes, comprised mainly of B cells and to a lesser degree T cells and macrophages, UCN2 promotes interleukin-10 (IL-10) production and suppresses interferon-γ (IFN-γ) and TNFα secretion, initiating an anti-inflammatory effect [119] that may oppose the action of CRF. Absence of CRF results in decreased TNFα and IL-1β production by splenocytes [120]. In thymocytes, absence of CRF results in reduced NF-κB activity and TNFα production, suggesting that it participates in positive regulation of T cell activation [121]. CRF peptides also affect chemokine expression and prostaglandin production by immune and non-immune cells. Thus, $CRF_2$ signals induce interleukin-8 (IL-8) and macrophage chemoattractant protein 1 (MCP-1) secretion from epithelial cells [122] and PGE2 secretion from macrophages [123], while CRF (also via $CRF_2$) induces IL-8 production by mast cells [124]. It is likely that CRF and/or the UCNs may promote IL-8 and MCP-1 production by adrenal cells, contributing to the infiltration of the adrenal by immune cells. Moreover, PGE2 secreted by resident macrophages may also affect local CRF production as reported to be the case for endometrial cells [125].

**C. Systemic immune effects.** Experiments using cocultures of adult human adrenocortical and chromaffin cells exposed to CRF resulted in induction of cortisol production, an effect inhibited by the $CRF_1$ antagonist antalarmin [34]. CRF and UCN1 (via $CRF_1$) also induce DHEAS synthesis from fetal adrenocortical cells [49]. DHEAS and DHEA suppress pro-inflammatory cytokines and promote the induction of anti-inflammatory mediators from different types of immune cells including macrophages and T cells, preventing inflammation and atherosclerosis as well as autoimmune disorders [126, 127]. Work from our laboratory has shown that CRF induces catecholamine secretion from adrenal chromaffin cells [58]. Catecholamines also affect immune cells by suppressing inflammatory responses via macrophages. More importantly, adrenergic receptor signals induce a Th2-type response in T cells and activate B cells [128, 129].

## 5. CRF peptides in adrenal disorders

### A. Eutopic and ectopic CRF as a cause of Cushing's syndrome

**Definition of Cushing's syndrome.** Cushing's syndrome is defined as the clinical and biochemical consequences of prolonged exposure of tissues to high levels of glucocorticoids [130, 131]. The causes of Cushing's syndrome can be exogenous, *i.e.* following long-term therapeutic (iatrogenic) or factitious administration of glucocorticoids or of ACTH, or it can be endogenous, due to chronic hypersecretion of cortisol from the adrenal cortex. Endogenous Cushing's syndromes can be either ACTH-dependent or ACTH-independent. ACTH-dependent Cushing's syndrome may be due to (a) overproduction of ACTH from corticotroph cell tumors (the corticotropinomas) of the anterior pituitary gland [132, 133], (b) excessive production of ACTH from ectopic sources [134] or, rarely, (c) ectopic CRF production from tumors, including CRF-producing pheochromocytomas [135–137]. The majority of cases of ACTH-dependent Cushing's syndrome are due to corticotropinomas (classically referred to as Cushing's disease). Cushing's disease can be also associated with bilateral macronodular adrenal hyperplasia, with nodules visible on central tomography (CT) and magnetic resonance imaging (MRI), which rarely become autonomous, *i.e.* producing cortisol independently of ACTH [130, 131]; this is called transitional Cushing's syndrome. On the other hand, ACTH-independent Cushing's syndrome results from adrenals that have become autonomous due to either benign or malignant adrenocortical tumors. Carney complex causes ACTH-independent Cushing's syndrome characterized by autonomously functioning pigmented adrenocortical tumors (micronodular adrenal disease).

**Cushing's syndrome resulting from eutopic production of CRF.** Eutopically produced CRF (*i.e.* CRF from the parvocellular neurons of the PVN in the hypothalamus) can cause Pseudo-Cushing's syndrome. Pseudo-Cushing's is a chronic hypercortisolemic state due to a hyperactive hypothalamus and is mainly the result of major somatic or emotional chronic stresses due to alcoholism, alcohol withdrawal, depression (particularly melancholic depression), panic disorders or psychotic syndromes [138–141]. The initial pathophysiological defect is localized above the level of the hypothalamus. It is hypothesized that the production of CRF in the PVN is augmented due to the relentless bombardment of the hypothalamus with stressful cortical or limbic stimuli.

The adrenal enlargement found in depressed patients further supports this hypothesis.

**Cushing's syndrome resulting from ectopic production of CRF.** Cushing's syndrome resulting from ectopic CRF production from several types of tumors may not be so rare as previously thought. Indeed, a growing list of tumors has been associated with ectopic CRF production high enough to cause Cushing's syndrome: this list includes bronchial, thymic or mediastinal carcinoids, small cell and squamous cell carcinomas of the lung, islet cell tumors of the pancreas, medullary carcinomas of the thyroid, prostatic cancers, intrasellar choristomas and Ewing's sarcomas [132–136, 142–149]. It should be noted that at autopsy, pituitaries of these patients show nodular ACTH cell hyperplasia instead of corticotroph adenomas [145], and they are polyclonal in origin compared to the monoclonality of corticotropinomas [132]. In rats, implantation of tumor cell lines that express CRF resulted in Cushing's syndrome characterized by corticotroph cell hyperplasia, confirming the hypothesis drawn from patients' specimens [150, 151]. Finally, it has been proposed that exposure of dispersed pituitary corticotroph cells to extracts from tumors suspected of secreting CRF and causing ectopic Cushing's syndrome may serve as an additional diagnostic tool to confirm the pathogenetic role of ectopic CRF [143].

### B. CRF and the pheochromocytomas

**Definition of pheochromocytoma.** Pheochromocytomas are relatively rare catecholamine-secreting tumors derived from chromaffin cells. Tumors that arise outside the adrenal gland are termed extra-adrenal pheochromocytomas, or paragangliomas. The clinical presentation is that of hypertension and cardiac arrhythmias due to excessive catecholamine production. Normal chromaffin cells and the pheochromocytomas are notorious producers of multiple neuropeptides.

**The CRF/UCN system in pheochromocytomas.** CRF, the UCNs and the $CRF_1$ and $CRF_2$ receptors are all present in normal chromaffin cells as well as in pheochromocytomas [41, 58, 89, 90, 152]. Chromaffin-derived CRF peptides affect the production of catecholamines from the adrenal medulla via paracrine regulatory loops. Chromaffin-derived CRF peptides also affect the production of steroids by the adrenal cortex and stimulate DHEA production by the nearby located zona reticularis [153]. The quantities of CRF peptides produced by pheochromocytomas may also be high enough to exert systemic effects on pituitary corticotroph cells, producing Cushing's syndrome via "ectopic" pheochromocytoma-derived CRF.

**Pheochromocytoma-produced CRF as a cause of ectopic Cushing's syndrome.** Pheochromocytomas may cause Cushing's syndrome by ectopically producing ACTH or CRF. Pheochromocytoma-derived CRF can cause Cushing's syndrome by two mechanisms: (a) high levels of ectopically produced CRF may reach the anterior pituitary corticotrophs in concentrations high enough to be biologically effective, causing chronic stimulation and finally hyperplasia of the corticotrophs, resulting in Cushing's disease with elevated blood ACTH [147, 154–157]; or (b) the pheochromocytoma-produced CRF can act locally in a direct paracrine mode of action, stimulating CRF receptors on the cortical cells themselves and causing Cushing's syndrome with low blood ACTH concentrations [137, 158].

## 6. Adrenal CRF and UCNs and their receptors: lessons from knockout mice

The generation of mice with genetic mutations or deletion of genes has been an important tool to study the *in vivo* role and functions of many genes and their involvement in human diseases [159]. However, because such mice are deficient in the specific gene product during their entire fetal and postnatal life, compensatory mechanisms likely develop in response to the deficiency, which may further alter the phenotype of these mice. Thus, conclusions regarding normal physiology or the pathology of knockout mice must be drawn cautiously.

**A. $Crf^{-/-}$ mice.** The recent generation of CRF-deficient ($Crf^{-/-}$) mice has proven an important tool to study the role of the CRF system in the development and function of the adrenal gland. $Crf^{-/-}$ mice were produced by targeted gene disruption in embryonic stem (ES) cells [160, 161]. The gene-targeting vector used was constructed such as to replace the entire pre-proCRF coding region with a neomycin resistance cassette. $Crf^{-/-}$ mice born from heterozygous matings have normal viability and are indistinguishable from their $Crf^{+/+}$ littermates in all parameters tested, including size, activity and general behavior [160]. They are fertile and have normal longevity despite their very low basal glucocorticoid levels [160]. Detailed examination of the development and function of the HPA axis showed that $Crf^{-/-}$ fetal mice appear to have normal pituitary histology and POMC mRNA expression [162]. However, adrenal size, expression of mRNA for StAR (a rate-

limiting enzyme in the biosynthesis of glucocorticoids) and blood corticosterone levels are significantly lower in $Crf^{-/-}$ fetuses compared to $Crf^{+/+}$ fetuses [162]. These data suggest that although fetal CRF is not required for the development of corticotrophs and the onset of expression of pituitary POMC mRNA, it is necessary for the normal development and function of the fetal adrenal gland. The absence of fetal CRF causes poor adrenal growth, diminished corticosterone secretion and, therefore, impaired pulmonary development [162].

Paradoxically, $Crf^{-/-}$ mice have normal behavioral responses to stress, which are attenuated by CRF antagonists [163]. Therefore, a possible role for UCN1 in regulating the behavioral stress response in $Crf^{-/-}$ mice as a result of the development of compensatory mechanisms was speculated. UCN1 mRNA expression was found only in the Edinger-Westphal nucleus (EW) of wild-type (WT) and $Crf^{-/-}$ mice [163]. Interestingly, UCN1 mRNA expression was up-regulated 2- to 3-fold in $Crf^{-/-}$ mice compared to WT mice, an effect that was not due to glucocorticoid insufficiency, since corticosterone supplementation in $Crf^{-/-}$ mice did not affect the levels of UCN1 mRNA expression in the EW of knockout mice [164]. Furthermore, in contrast to WT mice, restraint-induced stress did not affect UCN1 mRNA expression in the EW of $Crf^{-/-}$ mice. Since the EW is not connected to any brain regions known to be involved in the behavioral responses to stress, and the normal physiological role of UCN1 in the EW is unclear, UCN1 expressed in this site is unlikely to mediate stress-induced behaviors and to compensate for the lack of CRF in other brain regions.

Adult $Crf^{-/-}$ mice have low basal corticosterone production and impaired glucocorticoid responses to various stressors [160, 165]. Histological examination of the adrenal gland showed that adult $Crf^{-/-}$ mice have a markedly atrophic appearance of the zona fasciculata, the area primarily responsible for the production and secretion of glucocorticoids [160].

CRF is also thought to be important for the normal function of the adrenomedullary catecholaminergic system via activity within the brain as well as by activation of the HPA axis. It has been shown that hypophysectomy prevents the immobilization-induced increase in adrenal PNMT gene expression [91, 166], and central CRF antagonist administration blocks the increase in plasma epinephrine induced by central CRF injection in rats. In addition, CRF mRNA [31] and peptide [58, 167] are found in the adrenal medulla. $Crf^{-/-}$ mice clearly showed a marked decrease in basal and restraint-induced plasma epinephrine levels compared with those in WT mice [168]. Furthermore, basal and restraint-induced adrenal PNMT mRNA and enzyme activity levels in $Crf^{-/-}$ mice were lower than those in WT mice [168]. These data demonstrate that CRF deficiency leads to impaired PNMT gene expression and enzyme activity levels and to impaired epinephrine synthesis and secretion in the adrenal medulla, possibly due to impaired adrenocortical corticosterone production [168].

**B. $Crf1^{-/-}$ mice.** The recent generation of CRF1-deficient mice has provided important clues as to which biological effects are mediated by this receptor. Similar to $Crf^{-/-}$ mice, $Crf_1^{-/-}$ offspring from homozygous matings die within the first day of life. Neonatal mortality is a result of combined maternal/fetal glucocorticoid deficiency, which leads to inadequate lung maturation and can be reversed by the prenatal maternal administration of corticosterone [21, 169]. In accordance with $Crf^{-/-}$ mice, mice deficient in $CRF_1$ also have a marked agenesis of the zona fasciculata and lower corticosterone production compared with their WT littermates. This finding further supports the hypothesis that $CRF_1$ is the receptor that mediates the effects of CRF in pituitary. In addition, together with the findings in $Crf^{-/-}$ mice, these data suggest that both CRF and $CRF_1$ are required for normal adrenal development and adrenal responsiveness to physiologic stressors. The function of the adrenal medulla of $Crf_1^{-/-}$ and $Crf^{-/-}$ mice is similarly impaired. Thus, basal epinephrine levels in $Crf_1^{-/-}$ mice are less than 50% those of WT littermates, and PNMT mRNA levels in $Crf_1^{-/-}$ mice are only 25% the level of their WT controls [170]. Histological examination revealed that chromaffin cells in $Crf_1^{-/-}$ mice exhibit marked depletion in epinephrine-storing secretory granules, which was not completely normalized by ACTH treatment, suggesting that $CRF_1$ is required for normal chromaffin cell structure and function and that deletion of this gene is associated with a significant impairment of epinephrine biosynthesis.

**C. $Crf_2^{-/-}$ mice.** The recent generation of $CRF_2$-deficient mice as well as mice with deletion of both $CRF_1$ and $CRF_2$ has further proven the hypothesis that $CRF_1$ mediates the effects of CRF in the HPA axis. Targeted inactivation of $CRF_2$ is not accompanied by alterations in the structure or function of the adrenal gland (either cortex or medulla): $Crf_2^{-/-}$ mice have normal adrenal size and structure as well as normal basal corticosterone secretion [16, 17, 20]. However, $Crf_2^{-/-}$ mice show a rapid increase in plasma glucocorticoid concentrations following stress and early decline compared with WT mice [16, 171], suggesting that $CRF_2$ might mediate inhibitory actions of CRF on adrenocortical function.

## 7. Conclusions

Compelling evidence supports a significant role of the CRF family of peptides in the adrenals, not only through their well-known activity via the HPA axis but also as local regulators of a plethora of adrenal functions. The expression of the peptides and their receptors in the different cell subsets that comprise the adrenal reveals a complex intra-adrenal circuit that may act either independently or in concert with exogenous stimuli. The net effect of the CRF-based homeostatic system on adrenal function depends on the local availability of the various ligands and the number of their receptors, including the pseudo-receptor.


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