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Physiology, pharmacology, and therapeutic relevance of urocortins in mammals: ancient CRF paralogs

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Abstract

Urocortins, three paralogs of the stress-related peptide corticotropin-releasing factor (CRF) found in bony fish, amphibians, birds and mammals, have unique phylogenies, pharmacologies, and tissue distributions. As a result and despite a structural family resemblance, the natural functions of urocortins and CRF in mammalian homeostatic responses differ substantially. Endogenous urocortins are neither simply counterpoints nor mimics of endogenous CRF action. In their own right, urocortins may be clinically relevant molecules in the pathogenesis or management of many conditions, including congestive heart failure, hypertension, gastrointestinal and inflammatory disorders (irritable bowel syndrome, active gastritis, gastroparesis, rheumatoid arthritis), atopic/allergic disorders (dermatitis, urticaria, asthma), pregnancy and parturition (preeclampsia, spontaneous abortion, onset and maintenance of effective labor), major depression and obesity. Safety trials for intravenous urocortin treatment have already begun for the treatment of congestive heart failure. Further understanding the unique functions of urocortin 1, urocortin 2 and urocortin 3 action may uncover other therapeutic opportunities.

Keywords

Urocortin 1 or urocortin 2 or urocortin 3 or stresscopin or stresscopin-related peptide; corticotropin-releasing factor or CRF or corticotropin-releasing hormone or CRH or corticoliberin; stress response; peptide; anxiety or depression; pregnancy; labor or parturitition; food intake or energy balance or obesity; inflammation or immune system; salt or fluid balance; cardiovascular or hypertension or heart failure; gastrointestinal motility or irritable bowel syndrome

1. Introduction

Since the isolation of corticotropin-releasing factor (CRF) in 1981 [284], three mammalian CRF-like paralogs have been identified. The first of these – urocortin 1 (Ucn 1) – was identified

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in 1995 for its cross-reactivity to antisera against suckerfish urotensin I, a fish peptide structurally related to CRF and now hypothesized to be an ortholog of mammalian Ucn 1. Ucn 1 was named for its similar primary structure and bioactivity to both urotensin I and CRF [289]. Because Ucn 1 exhibited greater affinity for and activation of type 2 CRF receptors (CRF_2) than did CRF, it was hypothesized to be a natural CRF_2 receptor ligand [253,289]. Ucn 2 and Ucn 3 prohormones, subsequently identified in 2001 [113,165,226], were recognized for their structural relation to both CRF and Ucn 1 preproteins, but named "urocortins" due to the predominant affinity of predicted mature peptides for the CRF_2 receptor. Although Ucn peptides share moderate sequence identity with one another and with CRF, each is phylogenetically distinct, with the CRF/Ucn peptide family resulting from multiple gene duplication events during evolution prior to the divergence of modern vertebrate fish from tetrapods [28,113,165]. Each peptide has a unique anatomical distribution under the control of different genes. Consequently, despite a structural family resemblance, the natural functions of Ucns and CRF in stress responses *in vivo* may differ significantly, as will be apparent in the current review.

1.1. Structure of Ucns, CRF and Related Peptides

The rat Ucn 1 cDNA was cloned from a library constructed from mRNA extracted from a portion of the rat midbrain that included the Edinger-Westphal (EW) nucleus in 1995 [289]. The gene codes for a 122 residue preprotein, with Ucn 1 contained in the carboxyl terminus. The Ucn 1 genes for several fish species (pufferfish, *Takifugu rubripes*; carp, *Cyprinus carpio*; goldfish, *Carassius auratus*; sucker, *Catastomus commersoni*; trout, *Oncorhynchus mykiss*; flounder, *Platichthys flesus*, sole, *Hippoglossoides elassodon;* and zebrafish, *Danio rerio* [28,36]), frog (*Xenopus laevis* and *Xenopus tropicalis*) [28], mouse [312], hamster (*Mesocricetus auratus*) [230], sheep (*Ovis aries*) [41], capuchin monkey (*Cebus apella*) [288], rhesus monkey (*Macaca mulatta*)(GenBank XM_001092536.1), dog (*Canis familiaris)*, cow (*Bos taurus*)(XM_618452, XM_596525) and human [81,312] also have been cloned or predicted and found to be similar across species. The human Ucn 1 gene resides on chromosome 2 (2p23-p21) and has two exons, with the coding region residing entirely in the second exon, like the CRF gene [81]. The Several possible transcription-factor binding sites have been identified by sequence homology in the Ucn 1 promoter, but few have yet been tested for actually controlling Ucn 1 transciption. Putative regulatory elements include a TATA box, GATA-binding sites, a CCAAT enhancer binding protein (C/EBP) transcription factor-binding site, a binding site for the POU domain transcription factor Brn-2, and a cyclic adenosine monophosphate (cAMP) responsive element (CRE) [312]. Four base pairs upstream of the CRE site, the Ucn 1 promoter contains a consensus half-site for glucocorticoid response elements (GRE), consistent with the ability of glucocorticoids to upregulate Ucn 1 mRNA synthesis [130]. The CRE is involved in both constitutive and forskolin-stimulated activity [312].

The murine and human cDNAs for Ucn 2 and Ucn 3 were identified by two groups from sequence homology searches of the mouse and human genomes, with the putative Ucn 2 peptide contained in the *C*-terminus of a 112 amino acid residue preprotein, and the putative Ucn 3 peptide encoded in a precursor deduced to span 161 residues [113,165]. The identity and existence of endogenous peptides derived from the Ucn 2 and Ucn 3 preproteins remains predicted, as mature peptides have not been isolated or sequenced from any species (but see [235]). Reflecting this uncertainty, one group predicted sequences other than Ucn 2 and Ucn 3 to be mature peptide products of the human Ucn 2 and Ucn 3 prohormones, stresscopin-related peptide and stresscopin, respectively. These alternatively predicted peptides are *N*-terminally extended analogs of the predicted Ucn 2 and Ucn 3 sequences, as shown in Figure 1. To explain this ambiguity, Ucn 3 prohormone sequences from several species (fish, cow, dog, chicken, frog) contain a prototypical dibasic cleavage sequence (arginine-arginine; RR)

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at the *N*-terminus that could result in a mature 40-amino acid sequence, as exemplified in the predicted peptide stresscopin. However, the dibasic arginine residues (RR) are not conserved in humans, rhesus monkey, or rodents, which led to the alternate prediction of a mature 38-amino acid peptide, cleaved following the threonine-lysine residues (TK) of the preprotein as exemplified in rodent, dog or human Ucn 3 (see Figure 1) [28]. For the Ucn 2 prohormone, the uncertainty relates to the presence of a potential N-terminal flanking cleavage site (serine-arginine) that would predict the 38-residue peptide Ucn 2 whereas an upstream site (threonine-arginine) might predict the 43-residue stresscopin-related peptide (see Figure 1). Even further complicating the identity and existence of a human Ucn 2/stresscopin-related peptide in humans, the human Ucn 2 prohormone lacks the proteolytic cleavage site (RR) seen in Ucn 2 prohormones from other species that should follow the putative C-terminal amidation donor glycine residue.

In addition to mouse and human sequences, Ucn 2 preproteins have also been identified in rat (GenBank accession: AY044835), chicken (*Gallus gallus)*, fish (*Tetraodon nigroviridi*, *Takifugu rubripes)*, dog [28], and rhesus monkey (RefSeq accession: XM_001097967.1), and Ucn 3 preproteins likewise in rat (XM_574076), rhesus monkey (XM_001104616.1), dog (XM_845862.1), chicken, fish (*Tetraodon nigroviridi*, *Takifugu rubripes*, *Danio rerio)*, and frog (*Xenopus laevis* and *Xenopus tropicalis*) [28]. The human genes for Ucn 2 and Ucn 3 reside on chromosomes 3 (3p21.3–4) and 10 (10p15.1), respectively [165,226]. The Ucn 2 promoter contains several GREs and is positively regulated by glucocorticoids, representing a molecular link between CRF_1-mediated HPA-axis activity and Ucn 2 responses to stress [57].

In terms of primary structure (see Figure 1), Ucn 1 resembles CRF as much as, or more, than it resembles Ucn 2 and Ucn 3. In contrast, Ucn 2 and Ucn 3 resemble one another more than they do CRF, a distinction coupled with their greater selectivity for the CRF_2 receptor that has led to their description as “type 2 Ucns” [318]. Thus, an ancient gene duplication event prior to the evolutionary divergence of teleost bony fishes from tetrapods is hypothesized to have resulted in separate “type 1 Ucn/CRF” (Ucn 1/CRF) vs. “type 2 Ucn” (Ucn 2/Ucn 3) lineages with subsequent gene duplication events giving rise to additional paralogs within each phylogenetic branch (see Figure 2).

1.2. Distribution of Ucns

1.2.1. Central Nervous System (see Table 1)

Ucn 1: In the brain, Ucn 1 has a restricted, subcortical, predominantly caudal distribution. The major site of brain Ucn 1 synthesis is the Edinger-Westphal nucleus (E-W). The prominent synthesis and expression of Ucn 1 in the E-W is well-conserved across rats [27,158,289], sheep [41], humans [114], monkeys [288] and frogs [156]. Recent double-label immunohistochemistry studies suggest that Ucn 1-expressing neurons in the E-W, a dorsal midbrain structure originally recognized for its role in oculomotor and pupillary control, may not be the same as those which control “classic” oculomotor E-W functions, as Ucn 1-immunoreactive neurons are not preganglionic cholinergic neurons [232,298]. Perhaps reflecting this, some evidence suggests additional functions for the E-W that may be subserved by urocortinergic neurons. These proposed, but not fully substantiated, functions include the regulation of food and water intake [297], behavioral responses to stressors [127,299], temperature homeostasis [12,204,251], nociception [120,153], motor control [231], vestibular function [137], and the effects of and motivation to consume alcohol [11,13]. Certain stressful stimuli (e.g., ether, lipopolysaccharide, and restraint, but not hyperosmotic or hemorrhagic stress) activate the E-W Ucn 1 system, demonstrated by Fos expression in Ucn 1-immunoreactive neurons or increased Ucn 1 mRNA. Chronic stress results in a partial habituation of Ucn 1 mRNA, but not Fos protein, responses to stress in the E-W [93,150,

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154]. On the other hand, conditions that chronically increase brain CRF activity have been observed to *reduce* E-W Ucn 1 activity [157], whereas conditions that lower CRF levels are associated with increased E-W Ucn 1 activity [245,299], raising the possibility of a long-term, inverse relation between CRF and E-W Ucn 1 systems in homeostatic stress responses.

Descending Ucn 1-LI-positive fibers of possible E-W origin are observed in (1) midbrain: substantia nigra, periaqueductal gray, interpenduncular nucleus, and red nucleus; (2) caudal midbrain/rostral pons: dorsal raphe nucleus, ventral tegmentum, basilar pontine nuclei, and parabrachial nucleus; (3) medulla: including the facial, lateral reticular, and spinal trigeminal nuclei, inferior olive, and the dorsal column nuclei, nucleus of the solitary tract (NTS) and area postrema; (4) cerebellum: in the flocculus and paraflocculus as well as deep cerebellar and vestibular nuclei [257]; and (5) spinal cord: throughout the spinal gray and, less so, in the dorsal and ventral horns. The most prominent *ascending* Ucn 1-LI-positive projection from the E-W origin targets the septal/preoptic region, robustly to the lateral septum (LS) and less so to the bed nucleus of the stria terminalis (BNST), globus pallidus and medial septal/diagonal band complex. Other ascending Ucn 1-LI-positive fibers innervate the hypothalamus, the thalamus and the rostral periaqueductal gray.

Validated secondary sites of brain Ucn 1 synthesis include the lateral superior olive, the supraoptic nucleus (SON) [27], the lateral hypothalamic area, and, most caudal, several brainstem and spinal cord motoneuron nuclei [27]. Possible additional sites of Ucn 1 synthesis include the mammillary nucleus of the hypothalamus, sphenoid nucleus, substantia nigra, tegmentum, periaqueductal gray (PAG), raphe, and vestibular nucleus [27,158,310]. Ucn 1-LI generally is scarce or absent in many regions in which its paralog, CRF, is prominent, including the external layer of the median eminence, the hypophysiotropic, dorsal medial parvocellular subdivision of the paraventricular nucleus of the hypothalamus (PVN), basal ganglia, amygdala, hippocampus, locus coeruleus (LC) and cerebral cortex [27,114,158,186].

Ucn 2: Ucn 2 also exhibits a restricted, subcortical expression in rodent brain [175,226,311]. Like Ucn 1, Ucn 2 mRNA is localized in the SON and magnocellular subdivision of the PVN as well as in brainstem motoneurons and the spinal cord. Unlike Ucn 1, Ucn 2 also has marked expression in the arcuate nucleus of the hypothalamus and the LC. The projection targets of Ucn 2 neurons are unknown. Non-neuronal Ucn 2 expression is present in the meninges, but not in glial cells [226].

Ucn 3: Ucn 3 exhibits the most rostral distribution of the Ucns, again expressed subcortically [113,165,167,175,291]. The three prominent sites of forebrain Ucn 3 synthesis are (1) the median preoptic nucleus of the hypothalamus, (2) a hypothalamic region bordered laterally by the fornix and medially by the PVN that extends rostrally into the posterior BNST, and (3) the dorsal medial amygdala. Less prominent forebrain sites of Ucn 3 synthesis are the dorsomedial hypothalamus, both magnocellular and parvocellular components of the PVN, a region dorsal to the SON, and the posterior cortical and amygdalohippocampal transition areas of the amygdala. In addition to peptide expression at sites of synthesis, Ucn 3-like immunoreactive fibers of unknown origin project heavily to the VMH and arcuate nucleus, and medial amygdala Ucn 3 neurons project to the ventral premammillary nucleus [40]. Ucn 3 fibers are scarcer in the SON, PVN and anterior, dorsomedial and lateral areas of the hypothalamus and are seen in the internal, but not external, zone of the median eminence [167]. Outside the hypothalamus, forebrain Ucn 3 fibers of uncertain origin are abundant in the LS, posterior BNST and the medial amygdala and scattered in the basomedial and posterior cortical nuclei of the amygdala and the ventral hippocampus. The topographical distribution of Ucn 3 in the LS differs from that of Ucn 1 with the former innervating ventral and intermediate aspects of the structure, and the latter innervating dorsal aspects [167]. Caudally, Ucn 3 cell bodies are found in the auditory complex, notably in the superior paraolivary nucleus, and scattered Ucn 3 fibers are present in

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the periacqueductal gray, superior and inferior colliculi, and the ventral lateral lemniscus [167] (see Table 1 for summary).

1.2.2. Periphery

Ucn 1: Ucns also are substantially distributed in the periphery [15,27,103,130,155]. Ucn 1 expression has been observed in adipose tissue [243], heart [145,193,197] (especially ventricles), and immunological tissue [14,15,22], including thymus [130], spleen [15,130], and skin [247,248], evident at the cellular level in lymphocytes, macrophages, fibroblasts [280], and mast cells [141], as well as in synovial cells from patients with rheumatoid arthritis [149, 280]. Ucn 1 also is present in the enteric nervous system of the duodenum, small intestine and colon [27,103], as well as in testis [130], kidney [130], adrenals (especially the medulla) [90], and, possibly, anterior (but not posterior) pituitary [115]. In the human pituitary, Ucn 1-like-immunoreactivity was coexpressed with growth hormone (77% of Ucn 1-positive cells) and, to a lesser degree, prolactin (22%), but negligibly with adrenocorticotropic hormone (ACTH) (<1%) [115]. Ucn1-like-immunoreactivity also has consistently been observed in parietal and oxyntic cells of the stomach [54,155], but the proportion of Ucn 1 actually synthesized by *gastric* tissue remains uncertain. Indeed, Ucn 1 is synthesized by lamina propria macrophages, components of the stomach's inflammatory mucosal immune system [53,187, 235,236]. Finally, Ucn 1-like-immunoreactivity is evident in human placenta and fetal membranes [21,213], produced by chorio-decidual cells [96], and is reportedly maintained at elevated levels in maternal plasma from 16 weeks of gestation through birth [64,85,87,96].

Ucn 2: In humans, Ucn 2 gene expression was observed in most peripheral tissues analyzed by polymerase chain reaction (PCR) analysis, with higher levels observed in heart, lung, muscle, stomach, adrenal and peripheral blood cells [55,113] and more recent identification in skin [246] and placenta and fetal membranes [119]. Survey of peripheral rodent tissue for Ucn 2 gene expression revealed high levels in skeletal muscle and skin, moderate levels in lung, stomach, adrenal, ovary, brown fat, spleen, thymus, and uterus, and lower or negligible levels in testes, kidney, liver, pancreas, white fat and intestine [55,311]. Unlike in humans, low Ucn 2 mRNA expression is seen in rodent heart or aorta [55,311]. Detailed studies of murine tissue confirmed that Ucn 2 is synthesized by cultured skeletal myocytes and that Ucn 2-like-immunoreactivity is present throughout epidermis and dermis regions of the skin [55]. In rat, Ucn 2-like-immunoreactivity also was seen in hypothalamus, β-endorphin-containing cells of the anterior/intermediate pituitary and adrenal medulla [311]. Expression of Ucn 2 mRNA in skin, but not skeletal muscle, is inversely related to circulating glucocorticoid levels, as manipulated by exogenous administration or adrenalectomy [55].

Ucn 3: Ucn 3 gene expression has been detected in adipose tissue [243], heart [265], and skin [165], albeit at levels considerably lower than those of Ucn 2. Ucn 3 also is present in thyroid, adrenals [90,263,265], pituitary, [265], β-cells of the pancreas [166], spleen [265], ovary [265], placenta and fetal membranes [119], kidney [113,165,265] and the muscularis mucosa of the gastrointestinal (GI) tract, notably in the stomach, small intestine, colon, and rectum but not esophagus [113,165,235].

1.3. Pharmacology of Ucns

As shown in Table 2, Ucn 1 has high affinity for every CRF binding site identified to date, including the CRF_1 and CRF_2 receptor families and the CRF-binding protein (CRF-BP) [25, 165,289]. This promiscuity differs from that of the type 2 Ucns (as well as stresscopin-related peptide and stresscopin), each which show selective CRF_2 affinity, and no or only moderate affinity for the CRF-BP in both mammalian [113,165,226,269,291] and non-mammalian vertebrates [28]. Two CRF receptor subtypes have been identified [50,62,148,169,171,207, 255,293], with catfish having a duplicate genomic analog of CRF_1 [7,7,49]. The subtypes are

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encoded by separate genes, share high sequency homology (~70%) differing predominantly at the *N*-terminus, and have unique tissue distributions and pharmacological affinity profiles, implying a diversity of function [20,169,255]. CRF receptors belong to the G-protein coupled, seven transmembrane domain receptor family (GPCR). Multiple splice variant isoforms have been observed for each receptor subtype family, and include both membrane-bound and soluble variants [59,71,171,209,215].

1.3.1. CRF_1 receptors—The CRF_1 receptor is a class B ("secretin-like") GPCR spanning ~415 amino acids [62,78,207]. At least eight alternatively spliced transcripts of CRF_1 have been identified in humans, three in rats, four in mice, and nine in hamsters [109,214–216]. However, only one of these splice variants is known to induce signal transduction in humans and rodents ($CRF_{1(a)}$), so the following information applies to that isoform. Ucn 1 exhibits reversible, saturable, high-affinity binding to CRF_1 receptors transfected in stable cell lines (K_i=0.2 nM). Indeed, Ucn 1 is ~3 fold more potent than CRF at binding to the CRF_1 receptor and 1–2.5 fold more potent than CRF in stimulating the production of cAMP from CRF_1 expressing cells (see Tables 2 and 3). Ucn 1's affinity for the CRF_1 receptor is determined by regions in the first, second and third extracellular domains of the receptor [208,304]. In contrast to Ucn 1, Ucn 2 and Ucn 3 have very low potencies to bind CRF_1 and thereby activate adenylate cyclase (Tables 2 and 3), and Ucn 3 also shows negligible potency to stimulate ACTH release *in vitro* ($\gg$1 μM), a CRF_1-mediated bioassay [165]. Unlike Ucn 3, which appears to show no functional activity whatsoever at CRF_1 receptors, Ucn 2 acts as a very low potency, but full agonist at CRF_1 receptors. For example, Ucn 2 showed maximum functional efficacy (100%) comparable to that of CRF (110%) and Ucn 1 (93%) to induce cAMP accumulation in AtT20 cells, but was much less potent than both peptides (EC_{50}'s=360, 1.3 and 1.1 nM, respectively) [110] (see also Table 3). The CRF_1 receptor can employ multiple signal transduction pathways when stimulated by Ucn 1 [109]. These include activation of adenylate cyclase with production of cAMP and activation of protein kinase A-dependent pathways; activation of phospholipase C with production of inositol-1,4,5-triphosphate which in turn activates protein kinase C-dependent and calcium activated pathways; MAP kinase-dependent pathways; nitric oxide production; and proximate interactions with calcium channels [32,83,109,201,289,302].

1.3.2. CRF_2 receptors—CRF_2 receptors also are class B GPCRs. To date, four major CRF_2 splice variants have been identified, including membrane-bound and soluble isoforms of $CRF_{2(a)}$, and membrane-bound $CRF_{2(b)}$ and $CRF_{2(c)}$ receptors. The $CRF_{2(c)}$ receptor has only been observed in human limbic neurocircuitry [151], and sub-variants of $CRF_{2(a)}$ and $CRF_{2(b)}$ also have been identified [109,199,281]. Membrane-bound CRF_2 receptors are respectively ~411, 431 and 397 residues in length, differing only in their extracellular *N*-terminal domains [44,151,171]. The 143 residue soluble $CRF_{2(a)}$ isoform was identified in mice, and results from a frame-shift deletion of exon 6 [59]. Unlike CRF, all Ucns bind with high affinity to membrane-bound CRF_2 receptors transfected in stable cell lines [165,226, 289] or to endogenously expressed CRF_2 receptors [111]. Human Ucn 1 and Ucn 2 are each ~1–2 orders more potent than CRF at binding to membrane CRF_2 receptors, with murine and human Ucn 3 (in rank order) only slightly less potent (Ucn 1=Ucn 2>Ucn 3>CRF) (Table 2). In contrast to membrane-bound CRF_2 receptors, the soluble $CRF_{2(a)}$ receptor unexpectedly shows high affinity for Ucn 1 and CRF (classically, CRF_1 ligands), and low affinity for Ucn 2 and Ucn 3 (Table 2) [59]. Neuroanatomical and functional studies suggest that, similar to the CRF-BP (see below), the soluble $CRF_{2(a)}$ receptor may curb CRF_1 signaling by competitively sequestering ligand.

The combination of high membrane CRF_2 potency and low CRF_1 potency makes type 2 Ucns much more *selective* CRF_2 agonists than Ucn 1. Ucn 2 (which is a full, albeit very low affinity, CRF_1 agonist) shows ~1000-fold greater functional selectivity for the CRF_2 than does Ucn 1. Ucn 3 does not show CRF_1-like agonism, making it the most selective, albeit not most potent,

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CRF_2 agonist (Tables 2 and 3). The CRF_2 receptor also can activate the mitogen-activated protein (MAP) kinase pathway, with some evidence suggesting that Ucn 3 may be less efficacious than Ucn 1 or Ucn 2 at engaging this signal transduction mechanism [32,56].

Chimeric CRF_2/CRF_1 receptor studies show that the strong *selectivity* of human Ucn 2 and Ucn 3 for the CRF_2 receptor is mainly determined by the extracellular domain of the CRF_2 receptor with an additional contribution of the juxtamembrane domain for Ucn 2 [110]. In contrast, the (weaker) selectivity of human Ucn 1 for the CRF_2 receptor is determined entirely by the juxtamembrane domain of the receptor, further underscoring different ligand CRF_2 binding determinants across the Ucns [110]. The high *potency* binding shared by Ucns depends on a stabilizing interaction with the juxtamembrane domain of the CRF_2 receptor that putatively involves the *N*-terminal peptide end [110]. Whereas the ability of Ucn 1 (and other agonists) to bind CRF_1 receptors is highly dependent on receptor coupling to G-proteins (2–3 orders greater affinity to coupled receptors), the affinity of Ucn 2 and Ucn 3 for CRF_2 is only mildly greater (<1 order) in the G-protein coupled state, and Ucn 1's CRF_2 affinity is insensitive to uncoupling [110], underscoring differential CRF_1 vs. CRF_2 binding determinants for Ucns.

1.3.3. CRF-Binding Protein (CRF-BP)—The CRF-BP is an evolutionarily conserved 37-kDa secreted glycoprotein that binds CRF with high affinity [26,198,300]. Cloned from human liver, rat cerebral cortex and mouse brain, CRF-BP cDNAs encode a ~322 residue protein. The mature CRF-BP protein is not membrane-associated, lacking prototypical transmembrane domains or a phosphatidyl inositol anchor signal. The CRF-BP has been hypothesized to limit CRF receptor agonist effects by sequestering secreted ligand and facilitating subsequent enzymatic degradation, thereby limiting peptide bioavailability [23,24,24,124,211,211,241, 241,317,317]. In contrast, it also has been postulated that the CRF-BP, like other binding proteins [84], may *enhance* the effects of bound ligand, by shielding peptide from metabolic degradation during diffusion to membrane-bound CRF receptors [139]. A more recent suggestion is that the CRF-BP may have signaling properties [45], some of which may depend on ligand/CRF-BP complexes [279]. Finally, the degree to which the Ucns themselves are sequestered by the CRF-BP under basal conditions would constitute a physiologically relevant reservoir that could be competitively "freed" by another CRF-BP ligand that otherwise has different direct pharmacological properties (such as CRF). Thus, the degree to which Ucns interact with the CRF-BP may have great biologic relevance.

Ucn 1 is approximately equipotent to CRF at binding to the CRF-BP in mammals (Table 2), less so in frogs (*Ki*=50.3 vs 4.1 nM for Ucn 1 vs. CRF) [28]. The region of Ucn 1 that has affinity for the CRF-BP (residues 4–28) differs from those responsible for its affinity for CRF receptors [109,123,256,300]. Ucn 1 is believed to be the predominant natural ligand for the CRF-BP in ovine brain and dissociates from the CRF-BP approximately twice as slowly as does CRF [107], potentially increasing the physiologic significance of Ucn 1/CRF-BP interactions. In contrast to CRF and Ucn 1, Ucn 3 does not appreciably bind to the human, rat or frog CRF-BP (Table 2, K_i>1 μM for frog) [28]. Again underscoring pharmacological differences between the type 2 Ucns, murine Ucn 2 *does* bind with moderately high affinity to the rat and frog, but not human, CRF-BP albeit slightly less potently than CRF/Ucn 1 (Table 2) [28,125,165].

1.4. Physiologic and Behavioral Effects of Ucns

Because CRF and Ucns are putative paralogs derived from a common ancestral gene [49], they are hypothesized to share complementary regulatory properties to create an integrated organism response to threats to homeostasis.

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1.4.1. Hypothalamic-pituitary-adrenal axis—Unlike CRF, peripheral Ucn 2 or Ucn 3 administration does not increase corticosterone secretion [291] (Fekete, ÉM and Zorrilla, EP, unpublished observations), consistent with the relative absence of CRF_2 on ACTH-secreting pituitary corticotroph cells [170]. However, intracerebroventricular (icv) or intravenous (iv) administration of Ucn 1 activates the pituitary-adrenal axis (as or more potently than CRF), via a CRF_1-dependent mechanism, stimulating ACTH release and proopiomelanocortin synthesis in pituitary corticotrophs [9,73,74,223,289]. However, Ucn 1 is not a likely physiologic regulator of the HPA axis. Unlike CRF-deficient mice [290], Ucn 1-deficient mice exhibit normal basal and stress-induced HPA hormone levels [292,296]. Similarly, unlike CRF antisera, peripheral administration of specific Ucn 1 antisera does not modify basal, stress-induced or adrenalectomy-induced ACTH levels [179,278]. Finally, unlike the distribution of CRF, Ucn 1-immunoreactive fibers are scarce in the PVN and the external layer of the median eminence under basal conditions [100,101,158].

It remains possible, however, that the type 2 Ucns modulate HPA-axis activity at the hypothalamic level in paracrine or autocrine fashion. Indeed, Ucn 2 and Ucn 3 mRNA are increased in the parvocellular PVN following immobilization/restraint stress [267,291], and hypothalamic Ucn 2 expression is increased by glucocorticoids [57]. More importantly, female mice deficient for Ucn 2 or for CRF_2 receptors recently were found to exhibit greater peak corticosterone (CORT) and ACTH levels at the circadian light→dark phase transition. Ucn 2 knockout mice showed greater hypothalamic magnocellular expression of AVP, which is known to augment ACTH secretion, while exhibiting normal stress endocrine responses to restraint and forced swimming. Thus, Ucn 2 via CRF_2 receptors may endogenously modulate basal HPA-axis circadian amplitude via an arginine vasopressin (AVP)-dependent mechanism (see Figure 3) [58].

Ucns also might directly modulate other adrenal functions (if not adrenocorticoal activity). Ucn 1 and Ucn 3, as well as CRF receptors, are observed in non-pathological adrenal glands, but at reduced levels in tumor cells of pheochromocytomas, adrenocortical adenomas and carcinomas [90,266], suggesting physiologic relevance or regulation of their expression. Ucn 2 may regulate catecholamine synthesis and release in the adrenal medulla, as, in PC12 cells, it induces noradrenaline release and phosporylation of tyrosine hydroxylase through protein kinase A and protein kinase A-Erk1/2 pathways, respectively [190].

1.4.2. Osmoregulation—Because of the presence of Ucn 1 and Ucn 2 in magnocellular neurons of the SON and the existence of Ucn 1 projections to the posterior pituitary, an osmoregulatory role for Ucns is hypothesized. Such a function would correspond well to the ancestral phylogenetic relation of the CRF/Ucn lineages to orthologous diuretic hormones in teleost fish, insects and other invertebrates [49]. Accordingly, salt loading, dehydration and hypophysectomy increase Ucn-like-immunoreactivity in magnocellular SON and PVN neurons, whereas food deprivation decreases Ucn- like-immunoreactivity in the SON (studies in which the antibody specificity for Ucn 1 vs. Ucn 2 is uncertain) [100–102]. Specific increases in SON Ucn 1 mRNA expression also have been observed following salt loading [118]. Chronic osmotic stimulation (by salt loading or water deprivation) increases CRF_2 mRNA levels in the SON and magnocellular PVN, potentially increasing sensitivity to resident Ucns [8]. Finally, female Ucn 2 KO mice recently were found to exhibit increased hypothalamic magnocellular AVP expression (Figure 3) and a blunted circadian regulation of water intake across the day, failing to show the typical difference in food/water ratios between light and dark phases which reflects a greater sensitivity to osmotic stress during the nocturnal/feeding phase [58]. Collectively, the findings support an endogenous role for Ucns 1 and 2 in the regulation of salt/water balance.

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Peripheral Ucns also may control body fluid homeostasis as a result of their potency to stimulate atrial natriuretic peptide release from cardiomyocytes [116], through a putative CRF_2 mechanism. Atrial natriuretic peptide, present in cardiac atrial tissue, has profound effects on salt and water homeostasis [105], as it reduces blood volume, acutely by sequestering plasma and longer term by promoting renal salt and water excretion. Atrial natriuretic peptide antagonizes the renin-angiotensin-aldosterone system at many levels [6]. Interestingly, this property of Ucns would reduce the preload or after-load on a compromised heart, to complement their direct vasodilatory effects, modulation of cardiac function and cardioprotection (see below) [74].

1.4.3. Cardiovascular function—Ucns (and CRF) are vasodilatory via arteriole and cardiac CRF_2 receptors when given intravenously in animal models [1,2,51,60,66,92,131, 203,223,268,289]. Conversely, CRF_2-deficient mice exhibit elevated mean arterial pressure suggesting an endogenous relaxant function of Ucn/CRF_2 interactions on vasculature *in vivo* [18]. The vasodilatory effects of Ucns are orthologous to the properties of urotensin I and other fish and invertebrate peptides of the CRF/Ucn superfamily that evolved to subserve osmoregulatory functions [49]. Vasodilatory effects of Ucn 2 in rat thoracic aorta are mediated by protein kinase A and MAP kinase signaling pathways [131], and relaxation of pulmonary arteries involves inhibition of a protein kinase C-dependent contractile mechanism [46]. Vasodepressor effects of Ucn 1 in rodents did not appear to involve activation of the nitric oxide/L-arginine pathway, prostanoid production, or K^+ channels and were not counteracted by compensatory vasoconstrictive mechanisms (e.g., angiotensin, endothelin) [2,92].

The *human* cardiovascular system also expresses high numbers of CRF_2 receptors [63,303]. Accordingly, both Ucn 2 and Ucn 3 produced potent, sustained, direct, endothelium-independent vasodilating effects in an *in vitro* human internal mammary artery model of endothelin-1 induced constrictions [303]. Ucn 1 also produced endothelium-dependent vasodilating effects in this model, putatively mediated (unlike *in vivo* rodent studies) by nitric oxide and, downstream, cyclic guanine 3′,5′ monophosphate-dependent stimulation of calcium-activated K^+ channels in vascular smooth muscle [63]. However, in humans, *in vivo* hemodynamic effects of Ucn 1 were not observed following acute bolus doses (i.v. 50 μg) that were sufficient to activate the HPA-axis [73,74]. Perhaps higher doses or CRF_2 selective ligands would produce such effects.

Complementing their hemodynamic effects, Ucns have cardioprotective and cardiovascular function-enhancing effects on compromised heart [37,196,220,237]. Systemic or *in vitro* Ucn 1 infusion has prolonged cardiac inotropic actions [202,268], increasing cardiac contractility, heart rate and aortic blood flow independent of changes in peripheral vascular resistance [19, 51]. Ucns also are cardioprotective when added to post-ischemic/hypoxic cardiomyocytes or to isolated intact heart during reperfusion after regional ischemia [33,35,47,126,161,163,164, 239,285]. For example, Ucn 1 promoted hemodynamic and bioenergetic recovery of isolated, paced rat hearts following post-ischemia reperfusion, effects associated with improved ventricular performance [239]. Ucn 2 and Ucn 3 reduced infarct size in isolated rat hearts after post-ischemia reperfusion [33] and also protected human heart from reperfusion injury [51]. In ventricular pacing large animal models of congestive heart failure, Ucn 1 and Ucn 2 similarly had palliative effects, the latter reducing left atrial pressure, brain natriuretic peptide and vascular resistance (see Figure 4). These changes were accompanied by normalizing decreases in circulating vasopressin, aldosterone, endothelin-1, and epinephrine levels, with corresponding increases in sodium and water excretion, indicating effective “unloading” of compromised cardiovascular/renal systems [37,220,221,223] (see also Figure 4).

The cardioprotective effects of Ucns are mediated by a CRF_2-dependent mechanism [19,33] and involve multiple signal transduction pathways. Ucn 1 increased synthesis, expression and

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translocation/activation of the protein kinase C epsilon isozyme in primary rat cardiomyocytes and in Langendorff perfused *ex vivo* heart. Ucn 1 no longer reduced apoptosis resulting from ischemia-reperfusion in isolated cardiomyocytes when peptide inhibitors of the isozyme were present, and the cardioprotective effects of Ucn 1 on whole heart were absent in protein kinase C epsilon-deficient mice [162,164]. Similarly, chelerythrine, a specific PKC inhibitor, eliminated Ucn 1-induced cardioprotection of isolated rat heart following ischemia-reperfusion [91]. Conversely, Ucn 1 reduced ischemia-induced increases in mRNA and protein expression of a calcium-insensitive phospholipase A2 enzyme (iPLA2) as well as levels of its toxic metabolite lysophosphatidylcholine in isolated cardiac myocytes [163,164]. Ucn 1, Ucn 2 and Ucn 3 cardioprotection also involves MAP kinase signaling, as each peptide phosphorylated Erk1/2-p42, 44 in neonatal cardiomyocytes and reduced post-reperfusion infarct size by a CRF_2 dependent mechanism, effects abolished by inhibitors of MEK1, Ras, or Raf-1 [32,34, 47,160,240]. Ucn 1 also increased synthesis of the mitochondrial K_{ATP} potassium channel subunit Kir 6.1. in cardiomyocytes, with general and mitochondrial-specific K_{ATP} channel blockers blocking the cardioprotective effects of Ucn 1 both in isolated cardiac cells and in intact heart [161,164]. Finally, both cardioprotective and (undesired) hypertrophic effects of Ucns also involve activation of a phosphatidylinositol-3 (PI-3) kinase/Akt-dependent pathway [47,48,126], which ultimately reduces Beclin-1 expression and resulting autophagic cell death in cardiomyocytes [285]. Importantly, some of Ucns' cardioprotective effects are dissociable from their hypertrophic effects, opening potential therapeutic avenues [48,72,225].

Supporting an *endogenous* compensatory response for Ucns in cardioprotection, Ucn 1, 2 and 3 are present in the heart [55,145,193,197,264], with Ucn 2 and Ucn 3 expression abundant in myocardium. Plasma Ucn 1-like-immunoreactivity is increased in human systolic heart failure [191] as well as in an experimental model of heart failure [52], and Ucn 1 expression is increased in diseased human heart [117,193]. Ucn 1 expression also increased more than 9-fold in viable, apoptotic-resistant human myocytes after surgical cardioplegic arrest-reperfusion for coronary bypass surgery [237,238]. Finally, Ucn 1 mRNA is increased in post-hypoxic cardiomyocytes, and isolated cardiomyocytes treated with CRF receptor antagonists or from CRF_2-deficient mice are more susceptible to ischemia-perfusion injury [19,35]. Endogenous Ucn/CRF-related peptides also may be involved in *hemodynamic* adaptations to heart failure, because intravenous infusion of a preferential CRF_2 receptor antagonist produced greater acute effects on arterial pressure, peripheral resistance and circulating renin and endothelin-1 levels in a pacing-induced, ovine model of heart failure than in the non-diseased state [222]. Finally, human venous endothelial cells synthesize and secrete Ucn 1 in response to inflammatory cytokines, such as tumor necrosis factor-α and interferon-γ. This response has been hypothesized to oppose damaging effects of oxidative stress because incubation with Ucn 1 suppressed angiotensin II-induced accumulation of reactive oxygen species in human umbilical vein endothelial cells [112].

1.4.4. Energy balance—Exogenous administration of Ucns promotes negative energy balance, by both increasing energy expenditure and decreasing food intake. The reviewed distributions of the Ucns support the hypothesis that they may regulate metabolism or food intake by interacting with CRF_2 receptors, including concordant hypothalamic expression in the VMH (Ucn 3), arcuate nucleus (Ucn 2, Ucn 3) and PVN (Ucn 1, Ucn 2) of the hypothalamus, lateral septum (Ucn 1, Ucn 3), and in the NTS of the caudal hindbrain (Ucn 1) [27,43,165, 167,170,226,287]. In addition, VMH CRF_2 mRNA levels vary directly with circulating leptin levels [104,174,194,227,272], potentially modulating sensitivity to catabolic Ucn action, and circulating leptin facilitates the entry of peripheral Ucn 1 into the central compartment [134, 135]. The presence of Ucns in the GI tract, glucoregulating tissue (e.g., pancreas, VMH) and adipose tissue also is consistent with a hypothesized peripheral role for Ucns in the short- or long-term regulation of energy balance.

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Energy expenditure: With respect to energy expenditure, Ucn 1 (i.c.v.) increases whole body oxygen consumption as measured by indirect calorimetry, and increases signs of sympathetic nervous system activity, including mean arterial pressure and colonic body temperature [76, 253]. The hypothalamus is a candidate site of metabolic action for Ucns, because intra-PVN Ucn 1 increases plasma leptin levels, induces BAT uncoupling protein-1 (UCP1) mRNA synthesis, and increases relative utilization of fat as an energy substrate [152]. Similarly, intra-PVN Ucn 1 blocked the ability of NPY to promote food intake or the preferential utilization of carbohydrates as an energy substrate [68]. Whether the energy expenditure-increasing effects of Ucn 1 are shared by the type 2 Ucns and mediated by CRF_2 receptors is unclear. Supporting a role for CRF_2 receptors in the body weight-reducing effects of Ucns, Ucn 1 promoted greater, more sustained reductions in body weight in CRF_1 knockout mice relative to their wild-type littermates despite achieving similar anorexia across genotypes [30]. In addition to these central actions of Ucns, peripheral activation of CRF_2 receptors in oxidative skeletal muscle also directly promotes thermogenesis via substrate cycling between *de novo* lipogenesis and lipid oxidation [252].

Food intake: With respect to food intake, peripheral administration of Ucn 1 or stresscopin reduces food intake in rodents (see Figure 5A for effects in fasted mice), possibly in part by slowing gastric emptying [10,113,294], and Ucn 1 suppresses operant responding for food reward [146]. Sustained intravenous infusion of Ucn 1 also reduced food intake in an experimental ovine heart failure model for 2 days, but with subsequent tolerance observed [223]. The receptor subtype mediating these actions remains uncertain.

Central infusion of Ucns also potently suppresses feeding in mammalian and non-mammalian vertebrates, effects shown to be at least partly CRF_2-mediated in studies that used selective agonists (see Figure 5B for effects in non-fasted rats) or antagonists, antisense knockdown of receptor expression, and knockout mice [28,67,77,195,206,253,316,318]. CRF_2 KO mice are constitutively hyperphagic on high-fat diets [17] or on 15% corn syrup-sweetened chow pellets (Consoli D., Diaz-Chaves Y., Monseingeon M., Corcuff J., Drago F., Vale W., Bale T., Koob G., Contarino A., Zorrilla E., and Tabarin A., unpublished observations). Unlike CRF_1 agonists, type 2 Ucns do not produce malaise, arousal or anxiety-like effects at the minimum central doses needed to reduce food intake in rats [77,121,195,206,226,282,316,318]. Also unlike ligands with CRF_1 affinity, the anorectic effects of i.c.v. type 2 Ucns are delayed approximately 2-6 hr in onset, again perhaps reflecting slowed gastric emptying (see below). The degree to which endogenous brain Ucns produce these effects under physiologic conditions remains unclear, because Ucn 1 and Ucn 2 deficient mice exhibited normal spontaneous food intake [58,292]. On the other hand Ucn 2 deficiency did blunt the anorectic effects of fenfluramine, suggesting a downstream role in serotonin's satiating effects [58].

In addition to the hypothesized anorectic role of central Ucns via the CRF_2 receptor, brain Ucn 1 also may reduce food intake via additional acute onset CRF_1-dependent mechanisms [318]. Brain CRF_1 stimulation suppresses feeding through a different behavioral mechanism than CRF_2 receptor activation, as evidenced by different time courses, effects on meal patterning and dietary self-selection [316,318]. Possible loci for endogenous Ucn 1-CRF_1 mediated anorexia include the dorsomedial nucleus of the hypothalamus, the parabrachial nucleus and other caudal hindbrain glucoregulatory sites. Ucn 1 infused into the fourth ventricle reduced intraoral sucrose solution intake even in chronically maintained decerebrate rats, supporting a hindbrain mechanism of anorectic action for brainstem Ucn 1 [70].

Ucn 3 is present in pancreatic islet β-cells, and secretion of Ucn 3-like immunoreactivity by MIN6 cells is increased by extracellular glucose [166]. *In vivo* and *in vitro* studies demonstrated that exogenous Ucn 3 increased glucagon and insulin levels, resulting in a net increase in blood glucose levels. Effects were abolished by pretreatment with a selective CRF_2 antagonist, and

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suggest an autocrine or paracrine regulation of glucose homeostasis by pancreatic Ucn 3 [166].

Human genetic studies also suggest a relation of CRF_2 receptors and, by association, Ucns to energy balance. Four genome-wide linkage analyses have revealed an association between energy balance-related endpoints, including body mass index (BMI) [3,306], type 2 diabetes [305], and lean body mass [42], with the portion of chromosome 7 that includes the CRF_2 gene (7p15–7p21) [180]. In addition, a study of early-onset (<10 years of age) obesity identified a single nucleotide mutation substitution (Val411Met) in the CRF_2 gene of a hyperphagic, severely obese 5-year old girl that was not evident in 140 alleles from control subjects. The heterozygous substitution also was observed in the hyperphagic proband's mother and maternal grandfather, both of whom also were obese.

1.4.5. Gastrointestinal motility and function—Stressors release CRF-related peptides which inhibit gastric emptying through brain-gut CRF_2 receptor systems [177,318]. For example, stress-induced gastric stasis is reversed by central or peripheral pretreatment with nonselective or selective CRF_2 receptor antagonists [106,143,177,183,262]. Similarly, nonselective or selective CRF_2 receptor antagonists, but not CRF_1 antagonists, block the ability of i.c.v., i.v., or i.p. administered Ucns/CRF to slow gastric emptying [61,142,177,189,229, 294]. Through vagal efferents, central infusion of CRF or Ucn 1 reduces antral gastric motility, inhibits high amplitude gastric contractions, and shifts duodenal activity from fasted to fed motor patterns [142]. Intracisternal infusion of Ucn 2 also suppresses gastric emptying, but unlike Ucn 1 and CRF, its effects are mediated by a *non-vagal*, central CRF_2-dependent alpha-adrenergic$_1$ receptor mechanism [69].

Parallel to the central pathways for stress-induced gastric stasis, peripheral CRF_2 receptor activation delays gastric emptying, with peripheral (i.v. or i.p.) administration of agonists with high (i.e., Ucn 1) or selective (i.e., Ucn 2) CRF_2 affinity delaying gastric emptying more potently than CRF (see Figure 6) [177,178]. Similar to central administration, peripheral Ucn 1 reduces antral gastric motility in fed rats and shifts gastric motor patterns from a fasted to fed state [142]. Ucn 1 also hyperpolarizes stomach smooth muscle [210]. Candidate substrates that mediate CRF receptor-induced gastric stasis include, centrally, the PVN and dorsal vagal complex, via an undefined subset of its descending autonomic efferents [294,295], and, peripherally, myenteric fibers of the enteric nervous system [54,142] and the gastric antrum, each which expresses CRF, Ucn 1, Ucn 2 and both CRF receptor subtypes [219]. Outside the stomach, CRF-like peptides also inhibit phasic contractions of the CRF_2-expressing ileum of the small intestine, an effect that, similar to stomach, was blocked by CRF_2, but not CRF_1, antagonists [218]. An endogenous role for Ucns in stress-induced changes in gastrointestinal motor function is also supported by the presence of Ucns in the PVN, NTS of the caudal hindbrain, GI tract and enteric nervous system. Consistent with this hypothesis, site-specific RNA interference knockdown of Ucn 2 (or CRF) expression in the terminal ileum increased diurnal fecal output under basal conditions, suggesting a physiologic role for Ucn 2 in the regulation of ileal motility [159].

Gastric Ucn 1 also may participate in the control of gastric acid secretion [54]. Ucn 1 colocalizes with tyrosine hydroxylase in parietal cells of the stomach, in proximity to CRF_2, but not CRF_1 receptors [54,155]. CRF_2 receptors, in turn, co-localize with H^+/K^+-ATPase the enzyme gastric proton pump, and somatostatin, which inhibits parietal cell activity and secretion of gastrin and histamine [54]. Perhaps accordingly, peripheral administration of nonselective CRF_2 receptor agonists inhibits gastric acid secretion and increases gastric mucosal blood flow.

In contrast to their inhibitory effects on gastric and ileal motility, diverse stressors stimulate colonic motor function, seen as increased colonic motility, decreased colonic transit time,

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defecation and watery diarrhea [259,261,318]. Central and peripheral administration of CRF_1, but not CRF_2, agonists also stimulates colonic motility, and selective CRF_1, but not CRF_2, antagonists attenuate stress- or CRF/Ucn 1-induced colonic hypermotility [177,318]. An apparent exception to this pattern of CRF_1 activation selectively stimulating colonic motility is that central murine Ucn 2 infusion also increased colonic motility. However, central administration of Ucn 3 (an even more selective CRF_2 agonist) did not similarly stimulate colonic motility, and actions of Ucn 2 could be reversed by a selective CRF_1 receptor antagonist. Thus, CRF_1 activation was still an essential (if not proximate) mediator of the colonic motor stimulating effects of i.c.v. Ucn 2 [177]. Candidate substrates for CRF_1-mediated stimulation of colonic motility include, centrally, the PVN and LC/Barrington nuclei that activate sacral parasympathetic nervous system activity, and, peripherally, the colonic myenteric nervous system, tissue in which Ucn 1 is expressed. Ucn 1 also was recently shown to increase duodenal contractile activity *in vitro*, an effect reversed by CRF_1, but nor CRF_2, antagonists [218]. Thus, endogenous Ucn 1 may partly mediate the colonic and duodenal motor stimulating effects of stress.

Peripheral CRF receptor signaling, perhaps initiated by Ucns, also modifies visceral nociception, in particular that related to painful gastrointestinal stimuli. For example, peripheral (i.p. or i.v.) or intrathecal administration of Ucn 2 or stresscopin, selective CRF_2 agonists, reduced behavioral and visceromotor responses to duodenal or colorectal distension [176,192]. Similarly, peripheral sauvagine (an amphibian CRF/Ucn-related peptide with high CRF_1/CRF_2 affinity) and Ucn 2, but not CRF, prevented experience-induced increases (i.e., sensitization) in visceromotor responses to colorectal distension. These palliative effects were reversed by CRF_2 antagonist pretreatment [182,184]. Peripheral antinociceptive effects of Ucns and Ucn-related peptides were not CRF_1-mediated, as i.p. CRF produced *pronociceptive* effects in the duodenal distension pain model via a CRF_1-dependent mechanism [192,259]. In fact, selective CRF_1 receptor antagonists reversed stress and experience-induced increases in visceromotor hyperalgesic-like responses to colorectal distension [181,182]. The actions of exogenous CRF and Ucns on GI function as well as the ability of selective CRF antagonists to modify similar effects of stress have led to a proposed involvement of endogenous Ucns (and CRF) in stress-related functional GI disorders, such as irritable bowel syndrome [258].

1.4.6. Immune function—As reviewed previously, Ucns are expressed in immunological tissue, including thymus, spleen, and/or skin, with Ucn 1 seen at the cellular level in lymphocytes, macrophages, fibroblasts, and mast cells. Exogenous Ucn 1 administration has palliative effects in experimental models of autoimmune encephalomyelitis [217], thermal injury-induced edema [277], and Crohn's disease [95], though these actions may reflect glucocorticoid, vasodilatory or cytoprotective (rather than direct immune) mediated mechanisms [4]. Still, converging lines of evidence suggest that immune-derived Ucns locally and directly modulate proinflammatory responses to perceived environmental insults, with the direction of this effect depending on the tissue or CRF receptor subtype [270].

For example, Ucn 1 mRNA is expressed in lamina propria macrophages, and Ucn 1-like-immunoreactivity is detected throughout the entire lamina propria layer of the intestine [187, 236]. Although intestinal lamina propria inflammatory cells are evident as early as 12–18 weeks of gestation, Ucn 1-like-immunoreactivity is not detectable until after birth [187]. This suggests that intestinal Ucn 1 activity may be regulated by changes in the intestinal milieu, such as passage of dietary factors or food-associated bacterial antigens. Ucn 1-like-immunoreactivity is further elevated in intestinal lamina propria macrophages of patients with ulcerative colitis, where it is hypothesized to have a proinflammatory effect via CRF_1 receptors [236]. Intraperitoneal administration of CRF_1 agonists, such as Ucn 1, also increases intestinal mucosal permeability to macromolecules [258,260]. Interestingly, a history of early trauma

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potentiates the effects of acute stress on intestinal mucosal dysfunction in adult rats, a response blocked by peripheral injection of a CRF receptor antagonist [258,260]. Chronic psychosocial stress also reduces intestinal host defense and initiates intestinal inflammation through a mast cell, putatively CRF_1 receptor dependent, mechanism [258,260]. In contrast to Ucn 1, Ucn 3 was hardly detected in lamina propria inflammatory cells in colonic mucosa, although it was detected in other gastrointestinal tissue, underscoring peptide specificity in the physiological roles of Ucns [235].

Other stress-related inflammatory conditions are also accompanied by locally increased Ucn 1 expression. In rheumatoid arthritis, Ucn 1-like-immunoreactivity and Ucn 1 mRNA are substantially elevated in synovium, and the number of Ucn 1-positive cells in synovia [280], including leukocytes and marcophages, is higher and significantly correlates with inflammation severity [149,280]. The same degree of synovial Ucn 1 activation is not observed in osteoarthritis [149]. Supporting a proinflammatory action of secreted Ucn 1, Ucn 1 stimulates IL-1β and IL-6 secretion by peripheral blood mononuclear cells *in vitro*, presumably via a CRF_1-mediated mechanism [149].

Some stress-related dermatological inflammatory conditions also have altered Ucn/CRF crosstalk between mast cells, neurons and keratinocytes [246–250,302]. Mast cells, which play a role in allergy and inflammation by releasing histamine, proteases (tryptase, chymase), proteoglycans, prostaglandin D2, leukotriene C4, and several multifunctional cytokines, are distributed widely in the skin. Mast cells recently were recognized to synthesize and secrete both CRF and Ucn 1 in response to psychosocial stress or immunoglobulin E receptor crosslinking [141,172,244,270,271]. Mast cells also express CRF receptors, activation of which leads to the release of cytokines and other pro-inflammatory mediators (e.g., vascular endothelial growth factor) and increased skin vascular permeability [38,39,82,270]. Finally hair follicles are both sources and targets of CRF/Ucn 1 lineage peptides with resulting effects on pigmentation [122,138]. Thus, it has been suggested that disorders such as atopic dermatitis, psoriasis [270], alopecia areata [31,136], and chronic urticaria [200] involve a stress-related precipitation or exacerbation of skin mast cell activation (and possibly other resident skin cells) via local CRF/Ucn 1–CRF receptor signaling.

The endometrium, myometrium, and outer decidua of the reproductive tract also contain mast cells [140,173]. Ucn 1-like-immunoreactivity is increased more than 10-fold in spontaneous abortion products (which include myometrium, fetal membranes and chorionic villi) from women with a history of multiple non-elective abortions relative to products from elective or non-habitual abortions [86,173]. Supporting the hypothesis that the increase in Ucn 1-like-immunoreactivity is related to mast cell activation, levels of tryptase, which constitutes 20% of total protein in mast cells, and IL-8, an abortogenic mast cell-derived cytokine, also are robustly elevated in habitual spontaneous abortions. Endometriosis also is associated with an increased number of activated mast cells in association with strong positive Ucn 1 immunostaining [140]. Normal endometrium, in contrast, shows low tryptase and Ucn 1 immunoreactivity [140]. Thus, Ucn 1 expression and mast cell activation, increased in inflammatory conditions of the reproductive tract, may correlate with increased risk for spontaneous abortion.

Increased Ucn 1-like-immunoreactivity and mRNA also were observed in lung airway epithelial cells of rats with experimental allergic asthma, where Ucn 1 increases pulmonary vascular permeability via a mast-cell dependent mechanism [307,308]. Ucn 1 elevations were reversed by effective glucocorticoid treatment. Finally, Ucn 1-IR was elevated in stomach biopsies from patients with active gastritis [53]. The stomach does not contain the rich CRF_1 distribution of the colon, but rather is rich in CRF_2 receptors [54]. Correspondingly, in contrast to the CRF_1-mediated proinflammatory effects of intestinal Ucn 1, stomach Ucn 1 has been

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hypothesized, via CRF_2 receptors, to protect or repair gastric mucosa from injury following noxious stimuli [53]. Consistent with this possibility, immunoreactive Ucn 1 increases further in treatment-responding, but not treatment-resistant, patients during disease regression. Perhaps underlying this relation, both Ucn 1 and Ucn 2 reportedly have short-onset pro-apoptotic effects on macrophages via CRF_2 receptors [276], and Ucn 1 suppressed lipopolysaccharide-induced TNF-α production by rat Kupffer cells *in vitro* ([4], but see [275]).

1.4.7. Reproductive function—In addition to resident and infiltrating mast cells, the human reproductive system itself expresses Ucn 1, CRF receptors and CRF-BP [89,196,242, 301]. In the ovary, Ucn 1 may suppress ovarian steroidogenesis [188], and Ucn 1 and Ucn 2 levels change dynamically in relation to the luteal phase [309]. In addition, as reviewed previously, Ucn 1-like-immunoreactivity is produced by choriodecidual and placental tissue and is elevated in maternal plasma from mid-gestation through birth [85,87,274]. Ucn 2 and Ucn 3 gene expression also were recently observed in trophoblast and maternal decidua and, especially in late pregnancy, fetal membranes [119].

Placental Ucn 1 appears to act as a relaxant on uteroplacental vasculature via local action at CRF receptors. Supporting the physiological relevance of this function, pregnant women with impaired uterine artery blood flow during mid-gestation exhibit significantly reduced circulating Ucn 1 levels in proportion to the degree of increased arterial resistance [85]. Similarly, placental explants from women with preeclampsia were deficient in their cGMP response to Ucn perfusion, a mechanism through which Ucns are hypothesized to produce vasodilative effects on fetoplacental circulation [133].

Reproductive Ucns also may stimulate uterine contractility or augment contractility from other stimuli (e.g., prostaglandins) [108,132,212]. Myometrium expresses both CRF_1 and CRF_2 receptors [88,242]. Ucns increase contractility via autocrine and paracrine CRF_2-mediated actions [132] and may facilitate degradation of extracellular matrices (and thereby rupture of fetal membranes) via their ability to increase production of matrix metalloproteinase-9 [168]. Consistent with this hypothesis higher Ucn 1 levels at the time of medical induction of post-term labor strongly predicts a shorter time to delivery (see Figure 7) [274]. Thus, Ucns may regulate placental vessel resistance to blood flow *and* augment uterine contractility, suggesting an important role in the physiology of pregnancy and parturition.

1.4.8. Anxiety- and depressive-related behavior—Central Ucn 1 administration shares many neurochemical and behavioral properties of i.c.v. CRF treatment, reflecting their pharmacological similarity. These include behavioral arousing properties in familiar environments and proconvulsant and anxiogenic-like effects [315]. The anxiogenic-like properties of central Ucn 1 infusion, mediated at least partly by CRF_1 receptors, have been shown in several paradigms, including the open field [185,317], elevated plus-maze [128, 185,254], light-dark box [185], defensive withdrawal [254] and social interaction tests [94, 233,234]. Ucn 1's effects on acoustic startle responding appear to differ from those of CRF, however, as exogenous Ucn 1 dampens rather than potentiates the acoustic startle response [128].

Quite unlike CRF_1 receptor agonists, i.c.v. administration of type 2 Ucns does not consistently have anxiogenic-like effects in rats [77,273,282,283,291]. For example, Ucn 3 did not increase anxiety-like behavior in the open field [291], elevated plus-maze [283,291], light/dark box [291], social interaction [313], or defensive burying tests [313], under conditions in which CRF_1 agonists produced anxiogenic-like changes. In fact, i.c.v. Ucn 3 acutely produced *anxiolytic-like* changes in the elevated plus-maze and light/dark box tests [283,291]. Ucn 2 (i.c.v.) also lacked anxiogenic-like effects in the rat open field and elevated plus-maze tests

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[282]. Rather, Ucn 2 had delayed, anxiolytic-like effects under high baseline anxiety conditions in the plus maze [282] and opposed the anxiogenic-like effects of CRF in the open field [291]. Finally, whereas CRF_1 agonists increased activity in familiar environments, type 2 Ucns had mild motor suppressing effects and opposed the activating effects of CRF [195,282,283, 316].

On the other hand, some studies have been interpreted as showing pro-stress-like effects of CRF_2 receptor activation in the lateral septum or dorsal raphe of rats [16,99,224], findings apparently incongruous with the reviewed effects of i.c.v. type 2 Ucn administration. Furthermore, exogenous i.c.v. administration of high doses of Ucn 2 (but not Ucn 3) to mice increased anxiety-like behavior in the plus-maze [205,206] and acoustic startle responses [228]. However, because Ucn 2 (unlike Ucn 3) can activate CRF_1 receptors at high doses [110] and displaces CRF_1 agonists from the CRF-BP [125], the role of CRF_1 vs. CRF_2 receptors in mediating these effects remains unclear. Overall, whereas CRF_1 receptor activation has known anxiogenic-like effects [314], conclusions regarding anxiety-related effects of central type 2 Ucn administration are not yet possible and may be brain site-specific.

Moreover, the *endogenous* anxiety-related roles of Ucns remain similarly unclear as one Ucn 1-deficient mouse model exhibited normal anxiety-like behavior and autonomic responses to stress [296], whereas another Ucn 1-deficient mouse model showed increased anxiety-like behavior on the plus maze and open field tests [292]. Recently generated Ucn 2 deficient mice also did not exhibit altered anxiety-like behavior in the plus maze, light/dark box or conditioned fear tests [58].

While the role of endogenous Ucns in anxiety-related behavior is unclear, accumulating evidence suggests a role for Ucn 2 in the regulation of *depressive*-related behavior, perhaps via modulation of serotonergic signaling by the dorsal raphe nucleus (DRN). The DRN densely contains CRF_2 and, less so, CRF_1 receptors [75,287]. Several studies indicate that CRF_1 activation inhibits 5-HT release, whereas CRF_2 activation has excitatory effects [97–99,147, 286]. Ucn 2, which is highly expressed in the locus coeruleus, may innervate the DRN via a known reciprocal connection between these two brain regions [144]. Supporting a role for Ucn 2-CRF_2 signaling in the DRN, intra-DRN administration of Ucn-2 dose-dependently increased 5-HT efflux in the basolateral amygdala [5] and led to “learned helplessness”-like behavioral changes at doses 100-fold lower than those required for CRF, a less potent CRF_2 agonist [99]. Most recently, Ucn 2 deficient female (but not male) mice were shown to exhibit greater antidepressant-like behavior in the forced swim and tail suspension tests (see Figure 8 [58]). The resistance of Ucn 2 null females to acquire forced swim immobility resulted from a persistence of swimming, rather than climbing [58], a behavioral profile linked to serotonergic acting antidepressants in rodents [79,80]. Interestingly, female, but not male, 5-HT_{1B}-deficient mice also selectively show reduced immobility in the forced-swim and tail-suspension tests [129], and other studies have seen a differential sensitivity of females to genetic deletion or polymorphisms of serotonergic modulators [29,65,129]. These findings support the hypothesis that Ucn 2-CRF_2 signaling modulates 5-HT function..

1.4.9. Hearing—Ucn 1 may be required for the development and maintenance of normal hearing. The distribution of Ucn 1-like-immunoreactive neurons within the margin of the lateral superior olive of the brainstem as well as the presence of Ucn 1 fibers and terminals in the inner spiral bundle of the organ of Corti suggests a role for Ucn 1 in audition [292]. Ucn-like-immunoreactivity appears in the rat organ of Corti at 8 days of age, present only in the inner hair-cell region, and not within the tunnel or in the outer hair cell region [292]. CRF_1 and CRF_2 receptors also localize to the lateral portions of the organ of Corti, and include other hair cells, Deiters cells, Henson cells and Claudius cells [292]. Supporting a functional role for Ucn 1 in hearing, Ucn 1-null mutant mice have shorter hair-cells and a higher response threshold

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in the auditory brainstem response examination than wild-type littermates [292]. Perhaps reflecting impaired hearing, a different murine model of Ucn 1 deficiency had a reduced acoustic startle response [296].

1.5. Concluding remarks

In sum, understanding of Ucns has emerged from the shadow cast by their paralog CRF, with each peptide having a unique phylogenetic history, gene, pharmacology, tissue distribution, and functions. In several organ systems, type 2 Ucns exert pharmacological effects superficially opposite to those induced by CRF_1 ligands, including possible anti-inflammatory properties, visceral antinociception, dearousal, possible anxiolytic-like activity, stasis of the gastrointestinal tract, and hypotension. However, it appears to be an oversimplification that Ucns are simply the *yang* to CRF's *yin*, as Ucns, especially but not exclusively Ucn 1, share several general properties with CRF (e.g., anorexia, increased energy expenditure/thermogenesis, CRF_1-mediated proinflammatory effects, roles in parturition and reproduction, as reviewed elsewhere [14,20,78,106,108,260,314,318]) and have distinct ones of their own (hearing, cardioprotection, hemodynamic and osmoregulatory actions). Even where apparently "opposite" pharmacological effects exist, it is not clear whether the actions reflect counter-regulatory actions of endogenous Ucns as opposed to self-regulating, negative feedback actions of CRF at CRF_2 receptors (e.g., anxiolytic-like behavior, dearousal) or indeed whether they even *are* physiologically opposing actions (e.g., *gastric* stasis vs. *colonic* hypermotility, *peripherally-induced* hypotension vs. *centrally-induced* hypertension). Also, because CRF has previously been implicated as playing roles in many of the domains reviewed for Ucns, a critical area of future research will be to clarify how each *endogenous* ligand singly, in combination or in opposition subserves particular *physiological* functions. For example, the role of CRF, but not Ucn 1, in endogenous HPA-stress responses was partly clarified by distinguishing the degree to which each ligand did (CRF) or did not (Ucn 1) have stress-regulated access (PVN->median eminence-> portal blood) to the anatomical targets that govern ACTH release (anterior pituitary corticotrophs) and which accordingly express the principal identified regulatory receptor (CRF_1 subtype). Analogously, further understanding of CRF/Ucn physiology and pathophysiology in the functional domains reviewed above will involve even closer study of how each natural ligand is differentially expressed and secreted in tissue-specific fashion in concordance with its putative cognate receptor(s).

Thus, in their own right, Ucns may be clinically relevant molecules in the pathogenesis, treatment or management of many conditions, including congestive heart failure, hypertension, inflammatory disorders (irritable bowel syndrome, active gastritis, rheumatoid arthritis), atopic/allergic disorders (dermatitis, urticaria, asthma), gastroparesis, pregnancy and parturition (preeclampsia, spontaneous abortion, onset and maintenance of effective labor), major depression and obesity. Safety trials for intravenous Ucn treatment have already begun for the treatment of congestive heart failure. Further understanding the unique functions of Ucn 1, Ucn 2 and Ucn 3 action may uncover other therapeutic opportunities.

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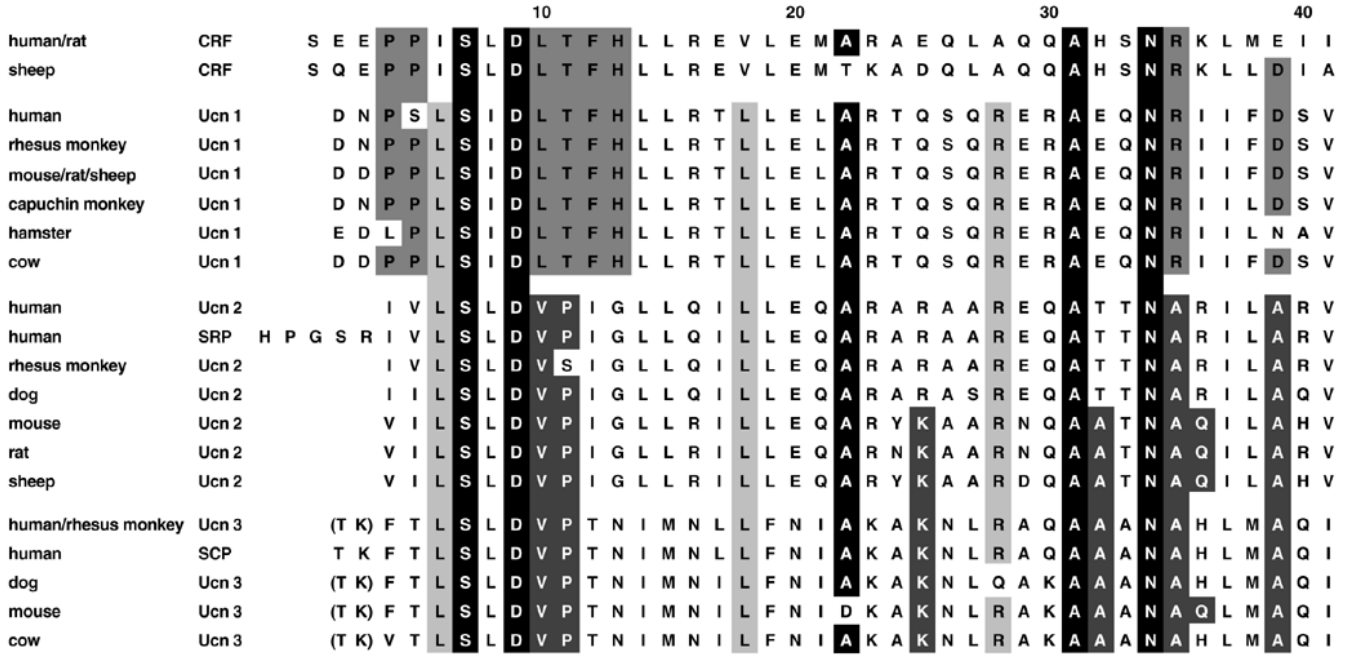

Figure 1.

Comparison of the primary structures of urocortin/corticotropin-releasing factor (Ucn/CRF) family mammalian peptides. Selected putative amino acid sequences for CRF and Ucn 1, 2 and 3 across human (*Homo sapien*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), rhesus monkey (*Macaca mulatta)*, capuchin monkey (*Cebus paella*), hamster (*Mesocricetus auratus*), sheep (*Ovis aries*), cow (*Bos taurus*), and dog (*Canis familiaris*). Boxed regions indicate sequence identity. Black fill with white letters indicates CRF superfamily homology (only 5 residues). Dark grey fill with white letters indicates selective "type 2 Ucn" (Ucn 2, Ucn 3) lineage homology (7 residues). Grey fill with black letters indicates selective type 1 Ucn/CRF lineage (Ucn 1, CRF) homology (8 residues). Light grey fill with black letters indicates pan-Ucn homology (3 residues). The threonine-lysine (TK) residues are shown parenthetically for Ucn 3 because the flanking dibasic arginine residue (RR) cleavage site (not shown) is not conserved in humans, rhesus monkey, or rodents. This leads to the prediction of a mature 38-residue peptide cleaved *after* the TK residues. For species in which the RR residues are present in the preprotein, a 40-residue peptide that includes the TK residues, orthologous to the alternatively predicted peptide stresscopin (SCP), might be a mature prohormone product. Similar uncertainty regarding the N-terminal proteolytic cleavage processing has led to the alternative prediction of stresscopin-related peptide (SRP), rather than Ucn 2, as the mature product of the Ucn 2 prohormone. The definitive identity of mature peptides derived from the Ucn 2 and Ucn 3 prohormones remains uncertain.

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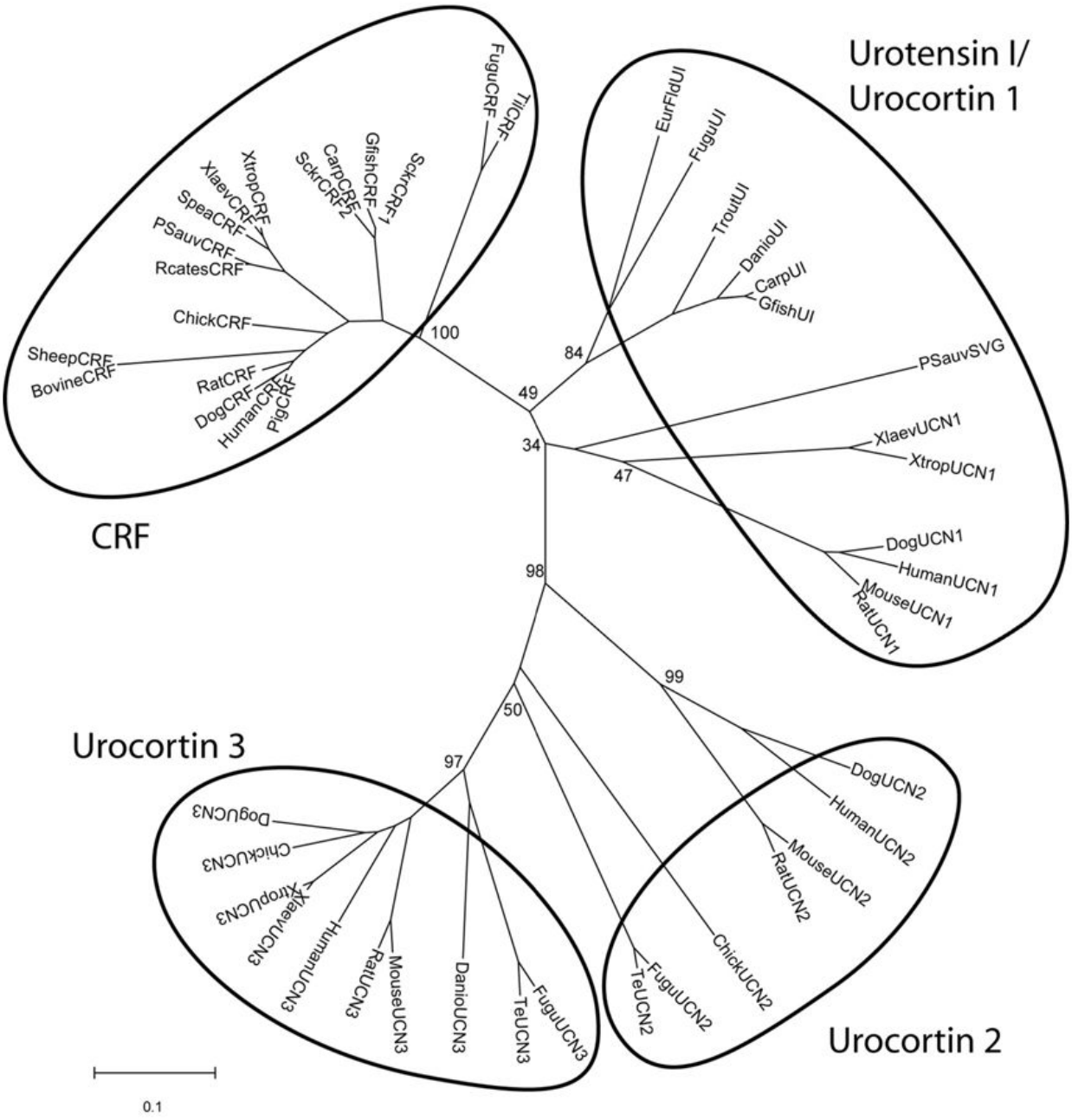

Figure 2.

Neighbor joining phylogenetic tree using p-distance of vertebrate CRF-like prohormone amino acid sequences. *Numbers* at branch nodes represent the confidence level of 1000 bootstrap replications. UI, Urotensin I; SVG, sauvagine; UCN1, urocortin 1; UCN2, urocortin 2; UCN3, urocortin 3; Carp, *Cyprinus carpio*; Danio, *Danio rerio*, zebrafish; EurFld, *Platichthys flesu*, European flounder; Te, *Tetraodon nigroviridi*, pufferfish; Fugu, *Takifugu rubripes*, pufferfish; Gfish, *Carassius auratus auratus*, goldfish; Xlaev, *X. laevis*, South African clawed frog; Xtrop, *X. tropicalis*; Rcates, *Rana catesbeiana*, North American bullfrog; Psauv, *Phyllomedusa sauvageii*; Sckr, *Catostomus commersoni*, sucker; Spea, *Spea hammondii*, Western spadefoot toad; Til, *Tilapia mossambicus; Trout, Oncorhynchus mykiss.*

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Prohormone	GenBank Accession # / ENSEMBL ID
CRF	
Bovine	NM_001013400
Carp	AJ317955
Chick	AJ621492
Dog	NM_001014278
Fugu	SINFRUG00000146091
Gfish	AF098629
Human	NM_000756
Pig	AF440229
Psauv	AY596828
Rat	NM_031019
Rcates	AB161633
Sckr1	S65264
Sckr2	x58784
Sheep	J00803
Spea	AY262255
Til	AJ011835
Xlaev	S50096
Xtrop	ENSXETG00000020294
UCN2	
chick	XM_425157.1
dog	ENSCAFG00000012466
human	NM_033199
mouse	AF331517
rat	NM_133385
Te	AL175143
UCN1 / UI / SVG	
carp UI	M11671
Danio UI	BX510372
dog UCN1	ENSCAFG00000004852
EurFld UI	AJ517171
Fugu UI	SINFRUG00000137751
Gfish UI	AF129115
human UCN1	NM_003353
mouse UCN1	NM_021290
Psauv SVG	AY943910
rat UCN1	NM_019150
Trout UI	AJ005264
Xlaev UCN1	AY596827

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Prohormone	GenBank Accession # / ENSEMBL ID
UCN3	
chick	BX930520.2
Danio	BX004864.7
dog	ENSCAFG00000005250
Fugu	AJ251323.1
human	NM_053049
mouse	AF361944
rat	XM_574076
Te	GSTENG00027885001
Xlaev	AY596826
Xtrop	ENSXETG00000016289

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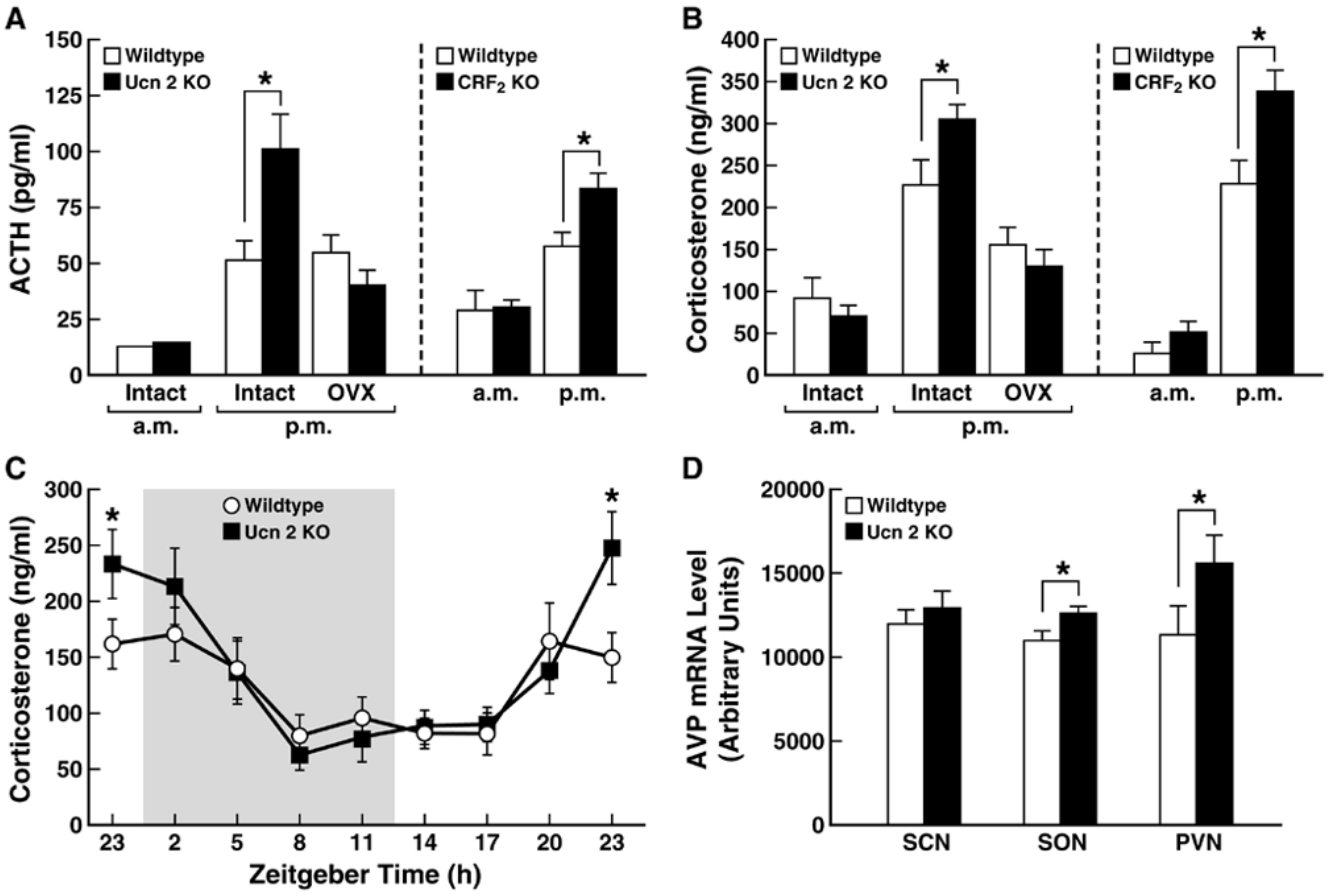

Figure 3.

Amplified circadian peak of basal hypothalamic-pituitary-adrenal (HPA)-axis activity in urocortin 2 (Ucn 2) or corticotropin-releasing factor type 2 (CRF_2) receptor-deficient female, adult mice. Panels A and B show plasma adrenocorticotropic hormone and corticosterone levels, respectively, in Ucn 2 or CRF_2 null (−/−) mutant mice and their respective wildtype (+/+) littermate controls. Nocturnal peaks in ACTH and CORT levels (p.m., -1hr dark onset) were greater in both mutant mice models, which did not differ in circulating nadir levels (a.m., -1 hr light onset). Effects of Ucn 2 deficiency were abrogated by ovariectomy (OVX). The amplified circadian amplitude of adrenocortical activity was confirmed by time course analysis in Ucn 2-deficient female mice (Panel C, shading indicates the 12-hr dark cycle), and may be related to their increased expression of arginine vasopressin in the supraoptic (SON) and paraventricular (PVN), but not suprachiasmatic nuclei (SCN) of the hypothalamus (Panel D). Data reflect *M*±SEM. **p*<0.05 vs. wildtype controls. Adapted from [58] with permission.

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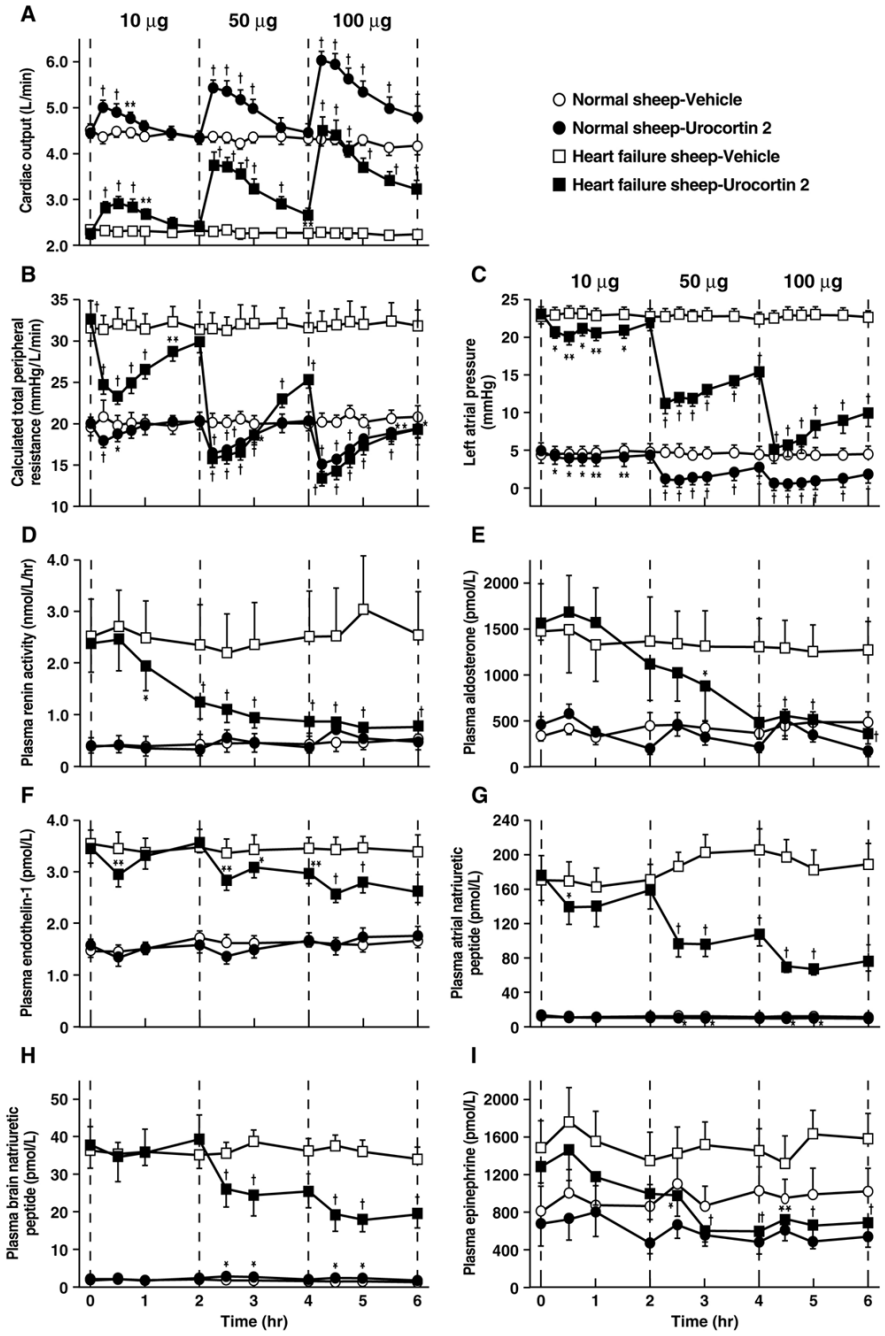

Figure 4.

Effects of bolus intravenous mouse urocortin 2 (Ucn 2) infusion on hemodynamic (Panels A–C) and cardiovascular-relevant hormonal responses (D–I) in sheep before or after induction of heart failure by 7 days of rapid (225 beats/minutes) left ventricular pacing, as compared to vehicle-infused (10 ml isotonic saline) controls. Ucn 2 tended to normalize all parameters in the heart failure model, while having lesser direct effects in healthy sheep. Data reflect *M* ±SEM. **P*<0.05, ***P*<0.01, *P*<0.001 vs. respective vehicle-treated controls. Adapted from [220] with permission.

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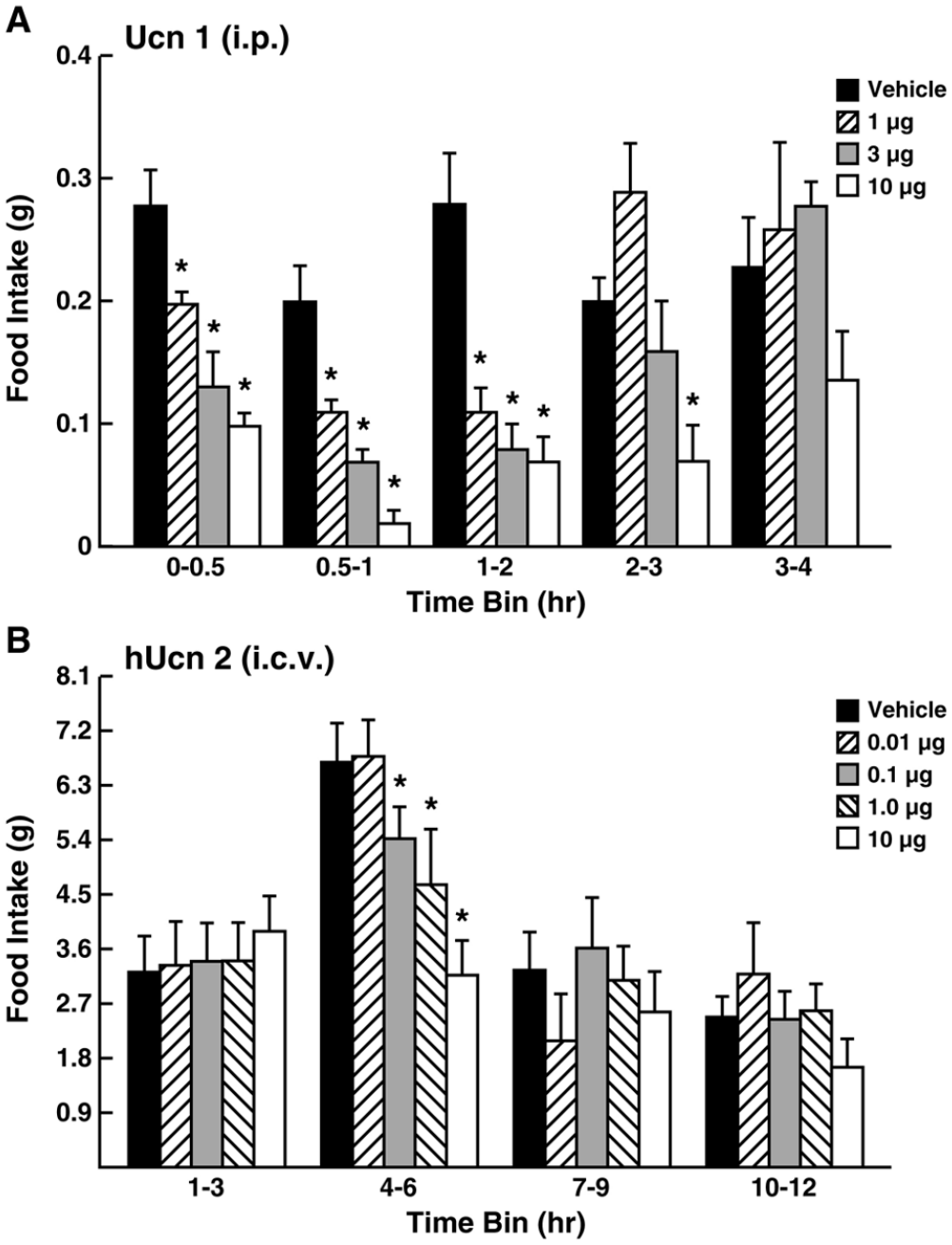

Figure 5.

Anorectic effects of urocortins on incremental food intake. Panel A shows acute-onset, dose-dependent anorectic effects of intraperitoneal (i.p.) rat urocortin 1 (Ucn 1) in previously fasted (18–20 hr) adult male mice, with refeeding intake monitored by intermittent (30, 1 hr) weighing. Panel B shows delayed-onset, dose-dependent anorectic effects of intracerebroventricular human urocortin 2 (hUcn 2) in non-food deprived adult male rats, with spontaneous nocturnal intake monitored by an automated precision-pellet system. Data reflect *M*±SEM. **p*<0.05 vs. vehicle-condition. Adapted from [294] and [121], respectively, with permission.

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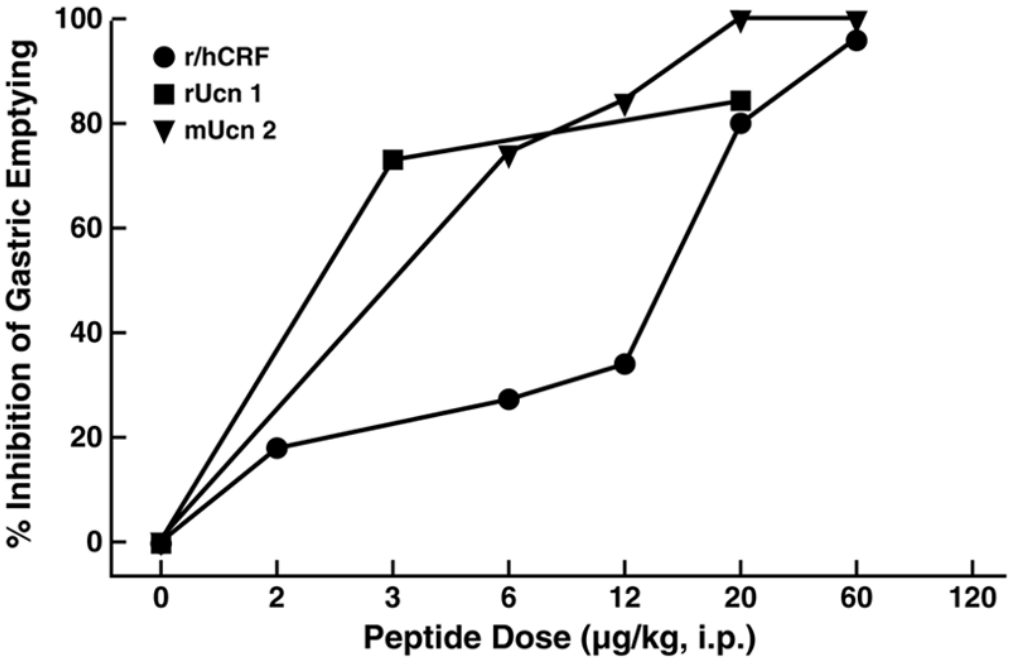

Figure 6.

Relative potency of intraperitoneal administration of urocortins (Ucn 1, Ucn 2) or rat/human corticotropin-releasing factor (r/hCRF) to inhibit gastric emptying in conscious mice. Graphs represent the % mean inhibition of gastric emptying of a solid nutrient meal in the 2 hr (mice) after peptide administration; r=rat, m=murine, h=human. Adapted from [177] with permission.

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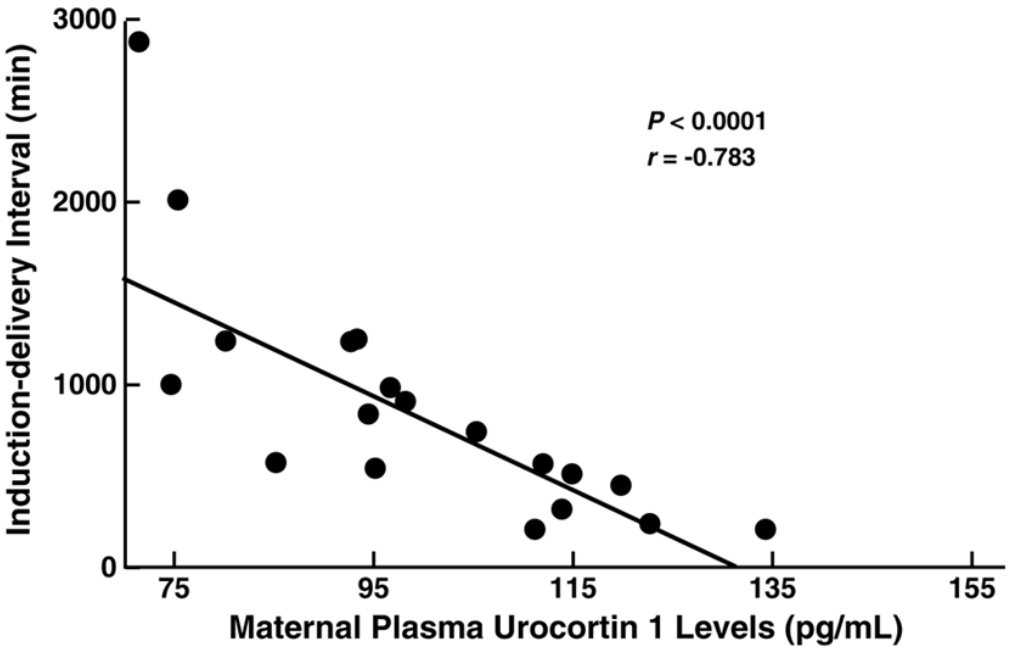

Figure 7.
Higher preinduction maternal plasma urocortin 1 levels correlate significantly with a shorter time to delivery following intravaginal prostaglandin labor induction in post-term pregnancies. Figure depicts scatterplot of individual observation with fit regression line. Adapted from [274] with permission.

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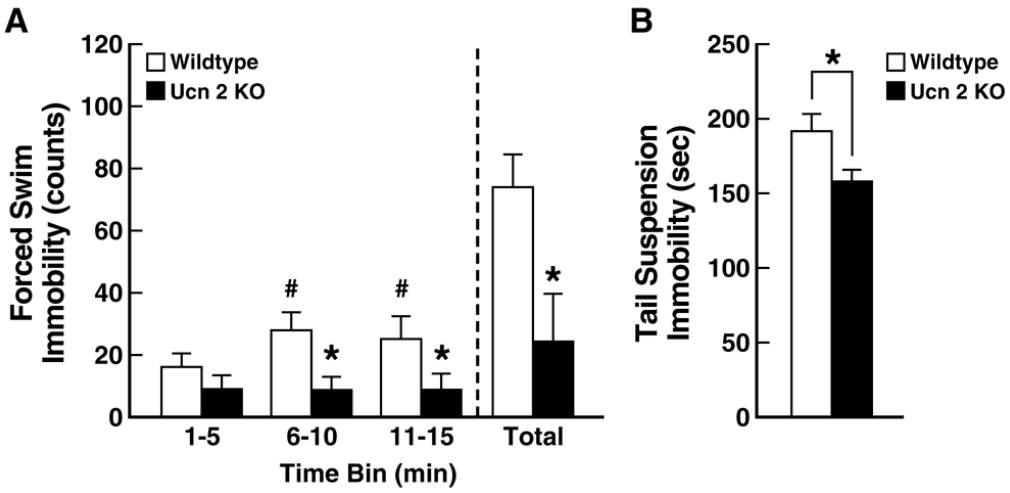

Figure 8.

Increased antidepressant-like behavior in female urocortin 2 (Ucn 2) null mutant mice. Panel A shows that female Ucn 2 null mice did not become increasingly immobile during a modified forced-swim test, unlike female wildtype (WT) mice. Behavioral analysis indicated that this resulted mainly from persistent swimming, a behavior linked to serotonergic acting antidepressants. Panel B shows that female Ucn 2 null mutant mice also were significantly less immobile in the tail-suspension test. Data reflect *M*±SEM. *$p<0.05$ versus WT mice; #$p<0.05$ versus 1–5 min time bin. Adapted from [58] with permission.

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Table 1

Semi-quantitative relation of prominent Ucn projection fields/expression to CRF receptor expression in rat brain

Region	Ucn 1	Ucn 2	Ucn 3	CRF_1	CRF_2
I. Forebrain					
Septum	++/+++	–	++++	–/+	++++
Lateral	+/++	–	–/+	+++	–
Medial/diagonal band complex					
Amygdala	+/++	–	–	–/+	–
Central nucleus	+	–	+++	+/++	++
Medial nucleus	+	–	+++	++	+++
Cortical nuclei	++	–	++	++	–
Amygdalohippocampal area					
Bed nucleus of the stria terminalis	++	–	+	++	–
Rostral	+	–	+++	+++	++
Posterior					
Globus pallidus	+/++	–	–	++	–
Substantia nigra	++	–	–	++/+++	–
Thalamus	++	–	+/++	+/++	–
Hypothalamus	+	+++	–/+	++	+
Supraoptic nucleus	–/+	+++	++	++	+
Arcuate nucleus	–/+	–	++++	–	+++
Ventromedial nucleus	++	–	+	+	+
Lateral hypothalamus	–/+	–	++++	+	?
Perifornical region	++	–	+	+	+
Lateral preoptic area	++/+++	–	+	++	+
Anterior hypothalamus	+++	–	+	+++	–
Suprachiasmatic nucleus	++	–	+/++	+/++	+
Dorsomedial nucleus	+/++	–	+++	–/+	+/++
Medial preoptic nucleus	+/++	+++	+/++	+/++	–/+
Paraventricular nucleus	++	–	++		–/+
Mammillary nuclei					
II. Brainstem					
Visual nuclei (sup. colliculus, anterior pretectal nucleus)	+++	–	+	+/++	–
Somatosensory nuclei (dorsal column)	++	–	–	++++	–/+
Auditory nuclei (cochlear nuclei, inf. colliculus, lateral superior olive)	++++	–	+/++	++/+++	–/+
Vestibular nuclei Visceral nuclei	++++	–	–	++	–
Solitary tract nucleus	++	–	–	–/+	++
Area postrema	++	–	–	–	++
Parabrachial nucleus	+/++	–	–	++/+++	–
Motor nuclei (oculomotor, facial, hypoglossal)	++/+++	+++	–	+/++	–
Periaqueductal gray	++/+++	–	+/++	+	+
Tegmental nuclei	++/+++	–	–	++/+++	–

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Region	Ucn 1	Ucn 2	Ucn 3	CRF_1	CRF_2
Red nucleus	+++	–	–	+++	–
Edinger-Westphal nucleus	++++	–	–		
Pontine nuclei	++	–	–	++/+++	–
Raphe nuclei Dorsal Median	++/+++ +/++	– –	–/+ –	+ ++	+++ ++
Locus coeruleus	+/++	+++	–	–/+	–
III. Cerebellum					
Deep nuclei	++	–	–	+++	–
Cortex	+/++	–	–	++/+++	–

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Table 2

Binding properties of select CRF/Ucn receptor ligands

	CRF_1 (K_i) (nM)	CRF_2 (K_i) (nM)			CRF-BP (K_i or IC_{50}) (nM)	
Peptide	**CRF_1**	**$CRF_{2(a)}$**	**s$CRF_{2(a)}$**	**$CRF_{2(b)}$**	**hCRF-BP**	**rCRF-BP**
r/hCRF	0.95	15 (9–19)	23	13.5 (10–17)	0.21	0.54
rUcn 1	0.32 (0.16–0.32)	1.5 (0.8–2.2)	6.6	0.62 (0.41–0.62)	0.10	0.98
hUcn 1	---	0.42 (0.27–0.57)	---	0.44	---	
mUcn 2	>100	0.58 (0.16–2.1)	113	0.66 (0.25–0.66)	“No appreciable”	4.4
hUcn 2	>100	0.17 (0.15–1.7)	---	0.50 (0.073–0.50)	“No appreciable”	---
mUcn 3	≫100	9.6 (5.0–14.2)	>200	1.8	“No appreciable”	>2,000
hUcn 3	≫100	2.7 (1.3–21.7)	---	7.9 (2.3–13.5)	“No appreciable”	---

Values represent median (range) from mean binding affinities reported in [56,59,110,125,165,226,289]. Values were combined across receptor species (human, rat, mouse), across which large systematic differences were not discerned, but differences across species were evident for CRF-BP. For peptides, r=rat, h=human, m=mouse, CRF=corticotropin-releasing factor, and Ucn=urocortin, CRF-BP=CRF-binding protein. All values reflect inhibitory binding constants (K_i's) except for rat CRF-BP values, which reflect half-maximal inhibitory concentrations (IC_{50}'s).

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Table 3

Functional potency of select CRF/Ucn receptor ligands

	CRF_1 (EC_{50}) (cAMP, nM)	CRF_2 (EC_{50}) cAMP, nM)	
Peptide	**CRF_1**	**$CRF_{2(a)}$**	**$CRF_{2(b)}$**
r/hCRF	1.3 (0.3–1.9)	15 (3.1–19)	5.8 (1.6–15)
rUcn 1	0.55 (0.1–0.9)	0.53 (0.063–1)	0.18 (0.087–0.84)
hUcn 1	1	0.42 (0.27–0.57)	0.32 (0.19–0.44)
mUcn 2	>100 (19– >100)	0.21 (0.14–0.9)	0.20 (0.05–0.43)
hUcn 2	>100 (>100–360)	0.17 (0.15–0.26)	0.25 (0.027–0.42)
mUcn 3	>1,500 (>1000– >1500)	7.1 (0.073–14.2)	0.46 (0.081–0.83)
hUcn 3	>1,000 (>1,000– >1,000)	1.3 (0.16–2.7)	2.3 (0.12–2.7)

Values represent median (range) from mean functional potencies reported in [56,110,125,165,226,289]. Values were combined across receptor species (human, rat, mouse), across which systematic differences were not discerned. For peptides, r=rat, h=human, m=mouse, CRF=corticotropin-releasing factor, and Ucn=urocortin.