

Review

Corticotropin releasing hormone receptors: two decades later[☆]

Greti Aguilera^{a,*}, Maria Nikodemova^a, Peter C. Wynn^b, Kevin J. Catt^c

^a Section of Endocrine Physiology, Developmental Endocrinology Branch, NICHD, 10 Center Drive, MSC 1862, Bethesda, MD 20892, USA

^b Faculty of Veterinary Science, University of Sydney, PMB 3 PO Camden, NSW, Australia

^c Endocrinology and Reproduction Research Branch, NICHD, National Institutes of Health, Bethesda, MD, USA

Abstract

Hypothalamic corticotropin releasing hormone (CRH) regulates pituitary ACTH secretion and mediates behavioral and autonomic responses to stress, through interaction with type 1 plasma membrane receptors (CRHR1) located in pituitary corticotrophs and the brain. Although CRHR1 are essential for ACTH responses to stress, their number in the pituitary gland does not correlate with corticotroph responsiveness, suggesting that activation of a small number of receptors is sufficient for maximum ACTH production. CRH binding and hybridization studies in adrenalectomized, glucocorticoid-treated or stressed rats revealed divergent changes in CRH receptors and CRHR1 mRNA in the pituitary, with a reduction in receptor binding but normal or elevated expression of CRHR1 mRNA levels. Western blot analysis of CRHR1 protein in pituitary membranes from adrenalectomized rats showed unchanged receptor mRNA levels and increased CRHR1 protein, despite the binding down-regulation, suggesting that decreased binding is due to homologous desensitization, rather than reduced receptor synthesis. In contrast, decreased CRH binding following glucocorticoid administration is associated with a reduction in CRHR1 protein, suggesting inhibition of CRHR1 mRNA translation. The regulation of CRHR1 translation may involve binding of cytosolic proteins, and a minicistron in the 5'-UTR of the CRHR1 mRNA. It is likely that post-transcriptional regulatory mechanisms that permit rapid changes in CRH receptor activity are important for adaptation of corticotroph responsiveness to continuous changes in physiological demands.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Pituitary corticotroph; Corticotropin releasing hormone (CRH); Type 1 CRH receptor (CRHR1); CRHR1 mRNA; CRHR1 regulation; Translation

1. Introduction

The identity of the hypothalamic releasing factor responsible for the regulation of pituitary adrenocorticotropin hormone (ACTH) secretion remained elusive for several decades until the isolation and characterization of corticotrophin releasing hormone (CRH) from ovine hypothalamus [80]. The CRH peptide is produced in parvocellular neurons of the hypothalamic paraventricular nucleus and is secreted into the pituitary portal circulation from axonal terminals in the external zone of the median eminence [3]. Studies with synthetic CRH revealed that the neuropeptide is not only the major regulator of ACTH secretion, but also has actions in the brain that mimic the effects of stress on behavior and autonomic nervous system activation [36,87].

Other early studies following the characterization of CRH showed that its actions are mediated by a plasma membrane receptor coupled to the adenylyl cyclase/cAMP signaling

system [2,25]. Specific CRH receptors were first identified in rat pituitary membranes by binding studies using radioiodinated Tyr-oCRH [84]. Subsequent studies using radioligand binding to membranes, autoradiographic procedures, and cytochemical techniques led to the full characterization of CRH binding properties and the topographic distribution of CRH receptors in the pituitary and other tissues in several species [21,59,68,85]. Rapid progress in our knowledge of CRH receptor structure and physiology has occurred since the cloning of the human pituitary CRH receptor [14], with subsequent elucidation of the molecular structure of human and rodent receptors in pituitary, brain and other tissues [12,13,58,62]. This led to the identification of a second main receptor subtype, CRH receptor 2 (CRHR2), and later to the discovery of the CRH-like peptides, urocortins 1, 2 and 3 [46,66,81], which serve as specific ligands of type 2 CRH receptors. Type 1 CRH receptors (CRHR1) were found to be the main subtype in the pituitary corticotroph [12].

The number of CRH receptors in the pituitary changes markedly during alterations in HPA axis activity [1] and regulation of the number of CRHR1 receptors in the cell membrane may have an important role in cell responsiveness

[☆] DOI of original article: 10.1016/0196-9781(84)90174-8.

* Corresponding author. Tel.: +1-301-496-6964; fax: +1-301-402-6163.

E-mail address: Greti_Aguilera@nih.gov (G. Aguilera).

Brain and pituitary receptors for corticotropin releasing factor: Localization and differential regulation after adrenalectomy

P.C. Wynn, R.L. Hauger, M.C. Holmes, M.A. Millan, K.J. Catt, G. Aguilera

Peptides, 1984;5:1077–84

Abstract

Specific receptors for corticotropin releasing factor (CRF) were identified in two functionally distinct systems within the brain, the cortex and the limbic system. Autoradiographic mapping of the CRF receptors in the brain revealed high binding density throughout the neocortex and cerebellar cortex, subiculum, lateral septum, olfactory tract, bed nucleus of the stria terminalis, interpeduncular nucleus and superior colliculus. Moderate to low binding was found in the hippocampus, nucleus accumbens, claustrum, nucleus periventricularis thalamus, mammillary bodies, subthalamic nucleus, periaqueductal grey, locus coeruleus and nucleus of the spinal trigeminal tract. As in the anterior pituitary gland, CRF receptors in the brain were shown to be coupled to adenylate cyclase. However, in contrast to the marked decrease in CRF receptors observed after adrenalectomy in the anterior pituitary gland, CRF receptor concentration in the brain and pars intermedia of the pituitary was unchanged. The presence of CRF receptors in areas involved in the control of hypothalamic and autonomic nervous system functions is consistent with the major role of CRF in the integrated response to stress.

Keywords: Corticotropin releasing factor; Stress; Adrenalectomy; Localization

to CRH as shown for other systems [24]. Significant progress has been made in our understanding of the mechanisms of regulation of CRH receptors since the first characterization of CRH binding two decades ago [23,56,59]. Several recent studies have shown that the content of biologically active CRHR1 on the cell membrane, reflected in CRH binding and signaling responses, depends on receptor synthesis, post-translational processing and targeting to the membrane, and the rate of receptor desensitization and internalization following interaction with the ligand. This article briefly reviews the current knowledge of CRH receptor subtypes and their tissue distribution, and discusses the changes of CRHR1 in the pituitary during altered activity of the hypothalamic–pituitary–adrenal (HPA) axis and the mechanisms involved in this regulation.

2. Diversity of CRH ligands

Given the diverse actions of hormones of the hypothalamic–pituitary axis in co-ordinating responses to stress, it is not surprising that the CRH family of peptides, and their homologous receptor–effector systems, have developed a similar level of complexity. The identification of two CRH-like peptides, the 40 amino acid peptide sauvagine [53] and the 41 amino acid peptide urotensin 1 [45] that supplement the actions of CRH in amphibian species [76] and fish [54], respectively, suggested that additional CRH ligands remained to be identified in mammalian species. Subsequently, urocortin (now termed urocortin 1) was isolated from rat mid-brain [81], and has 63% homology with urotensin and 45% with CRH. More recently, two additional isoforms of urocortin, urocortin 2 [66] (also identified as stresscopin-related peptide [37]) and urocortin 3 [46] (stresscopin [37]) have been identified from human and mouse cDNA libraries, respectively. Together with urocortin 1, these peptides are distributed throughout many stress-sensitive central structures and numerous peripheral tissues including the pituitary, gastrointestinal tract, testis, immune tissues such as thymus and spleen, kidney, heart, adrenal, peripheral blood cells, muscle and skin [37,40,46,66]. This pervasive expression often coincides with the central and peripheral distribution of POMC, its constituent peptides, and melanocortin receptors [71,74], suggesting that these functionally related peptide families have a major role in coordinating the stress response of the organism.

3. Diversity of CRH receptors

Two major CRH receptor subtypes, encoded by different genes termed CRHR1 and CRHR2, and a non-membrane-associated CRH binding protein, have been identified [29]. Both plasma membrane receptors belong to the G protein-coupled receptor superfamily and stimulate adenylyl cyclase activity. The CRHR1 is the major subtype in the pituitary corticotroph, and mediates the stimulatory actions of CRH on ACTH secretion. CRHR1 are also located in cortical areas of the brain, cerebellum and limbic system. The CRHR2 has several splice variants that are located in sub-cortical areas of the brain and in the periphery [41–43,75]. An additional third receptor with unique ligand binding properties and tissue localization has been identified in catfish pituitary and urophysis [4].

Initial autoradiographic studies in brain sections using ¹²⁵I-Tyr-oCRH as the radioligand localized specific high-affinity receptors in two functionally distinct systems, the limbic system and the cerebral and cerebellar cortices. The highest limbic receptor concentration was found in the external plexiform layer of the olfactory bulb, with decreasing levels of expression in the amygdala, bed nucleus of the stria terminalis, lateral, intermediate and medial septal nuclei, nucleus accumbens and caudate putamen. Diencephalic

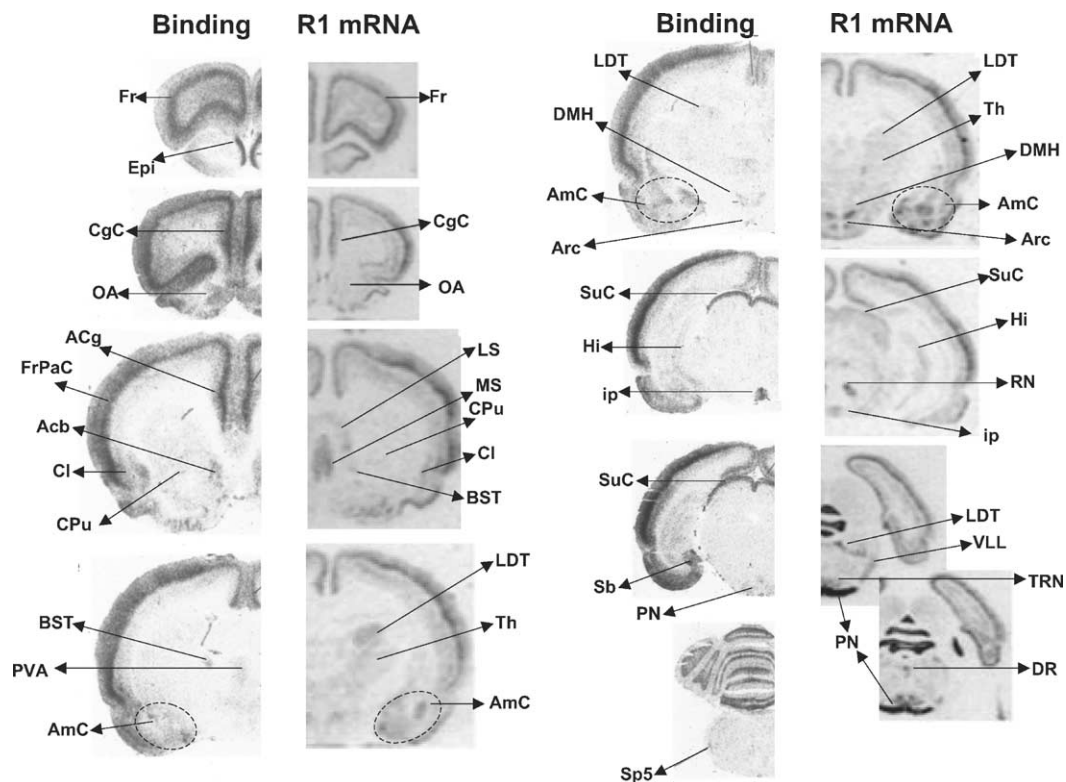


Fig. 1. Autoradiographic analysis of ^{125}I -Tyr-oCRH binding (Binding) and in situ hybridization analysis of CRHR1 mRNA (R1 mRNA) in rat brain sections. Abbreviations: Acb, nucleus accumbens; AmC, amygdala complex; Arc, arcuate nucleus; BST, bed nucleus stria terminalis; CgC, cingular cortex; Cl, claustrum; CPu, caudate-putamen; DMH, dorsomedial hypothalamic nucleus; DR, dorsal raphe; Epi, external plexiform layer of the olfactory bulb; Fr, frontal cortex; FrPaC, frontal parietal cortex; Hi, hippocampus; ip, interpeduncular nucleus; LTD, laterodorsal thalamic nucleus; LS, lateral septum; OA, anterior olfactory nucleus; PN, pontine nuclei; PVA, paraventricular thalamic nucleus; RN, red nucleus; Sb, subiculum; Sp5, spinal trigeminal nucleus; SuC, superior colliculus; Th, thalamic nuclei; TRN, tegmental reticular nucleus; VLL, ventral nucleus of lateral lemniscus.

nuclei expressing abundant receptors included the dorso-medial hypothalamic nucleus, the dorsolateral thalamic nuclei, supramammillary nuclei, the anterior paraventricular thalamic nuclei and the arcuate nucleus. CRH receptors were also expressed in a number of brain stem structures including the interpeduncular and pontine nuclei, the superior colliculus, inferior olive, dorsal tegmental nucleus, spinal trigeminal tract, locus ceruleus, and the nucleus of the solitary tract. Although this distribution is in general consistent with the distribution of CRHR1 mRNA in the brain and pituitary, there is not always a direct correlation between the two parameters (Fig. 1, Table 1). The lateral septum, posterior colliculus and sensory trigeminal nucleus display solely CRHR2, which has 70% sequence homology with the CRHR1 [14,48,83]. Although these receptor sub-types are co-located within the brain regions, there are some notable differences in the hypothalamus [14,48,83] that may be functionally significant. The distributions of CRHR1 and CRHR2 mRNAs, CRH binding sites and CRH binding protein are summarized in Table 1.

Although some initial reports described CRH binding in the median eminence, co-localized with CRH nerve endings [21], other studies in rodent and primate brain using radioli-

gand binding [52,86] did not confirm this finding under basal conditions. However, studies in rats have shown marked increases in CRHR1 mRNA and binding sites in the PVN following stress [49,51,55] (Fig. 2). CRHR1 expression after somatosensory stressors is confined to CRH-expressing cells in the parvocellular PVN [5]. Such CRHR1 expression in parvocellular CRH neurons could explain the presence of CRH binding in CRH terminals in the median eminence, especially if the animals had undergone stress during tissue collection [21]. In contrast to somatosensory stressors, osmotic stimulation induces CRHR2 expression in magnocellular neurons of the PVN and SON [5,49] (Fig. 2). CRHR1 expression in primates is similar to that in rodents. However, some differences in CRHR1 mRNA levels have been described, especially in the hypothalamus and locus ceruleus, where expression is lower or absent in primates [20]. In contrast, similar low levels of receptor expression have been described in both species when assessed by receptor binding assays.

The more recently discovered splice variants of CRFR2 display significantly different central localization patterns, at least in the rat. Whereas CRHR2 α mRNA is localized in the hypothalamus, hippocampus and lateral septum, CRHR2 β

Table 1

Distribution of CRF receptor binding, CRF receptors 1 and 2 mRNA and CRF binding protein mRNA/protein in the rat brain

Region	oCRH binding ^a	CRHR1 mRNA ^b	CRHR2 mRNA ^b	CRFBP mRNA/protein ^c
Cerebral cortex				
Prefrontal	**/**d	***		**
Anterior cingulate	**/**	***		
Frontoparietal (motor)	**/**	***		
Frontoparietal (sensory)	**/**	***		
Temporal, auditory area	—/**			
Subiculum	**			
Hippocampus	**	**	**	**
Entorhinal	—/**	**	**	
Basal telencephalon				
Olfactory bulb/tubercle	****			**
Lateral septal nucleus	**	*	****	
Medial septal nucleus		**	nd	
Nucleus accumbens	**			
Caudate putamen	*			*
Bed nucleus stria terminalis	***	**	**	**
Amygdaloid complex				
Anterior area	**			
Medial area	**	****	**	**
Basolateral area	***	****	nd	**
Clastrum	**			**
Diencephalon				
Paraventricular thalamic nuclei interior	*			*
Dorsolateral thalamic nuclei	*			
Supramamillary nuclei	**			**
Mamillary bodies				
Dorsomedial hypothalamic nuclei	**	***		**
Arcuate nucleus	*			**
Paraventricular nucleus	nd	nd	**	*
Periventricular nucleus	nd	*		**
Median eminence	nd			*
Brainstem				
Superior colliculus	**	***	*	**
Inferior colliculus				*
Sensory trigeminal			***	**
Interpeduncular nucleus	***	****	***	**
Periaqueductal grey	*			
Locus coeruleus	*	nd	nd	
Inferior olive	**			
Pontine nucleus	***	****	*	**
Nucleus of the solitary tract	*			**
Dorsal tegmental nucleus	**	****	nd	**
Spinal/sensory trigeminal tract	**		***	
Cerebellum				
Granular layer	****	****		*

Signals for binding and hybridization to mRNA: nd, not detectable; *, weak; **, moderate; ***, strong; ****, very strong.

^a Data adapted from Wynn et al. [86]. ¹²⁵I-Tyr-oCRH was used for the binding assay binding.

^b Data obtained from Chalmers et al. (1995), [12].

^c CRF binding protein expression data adapted from Potter et al. (1995) [62]. CRFBP mRNA and protein were co-expressed at all sites. Levels of expression are estimated from hybridization histochemistry and are not quantitative.

^d Binding intensity in cortical layers 1–3/layer 4.

mRNA is confined to structures in the brain stem and vasculature. In contrast, it is widely distributed in peripheral tissues including the heart, lung, skeletal musculature and gastrointestinal tract [47].

The biological functionality of CRH is further modified through interactions with a non-receptor CRH binding pro-

tein. Although this 37 kDa species was initially thought to modulate the functions of CRH during pregnancy [60], its wide distribution in rat brain and pituitary, and its differential affinity for CRH ligands, suggest that it may have a role in regulating CRH signaling centrally as well as in peripheral tissues [34]. This protein binds both CRH and urocortin

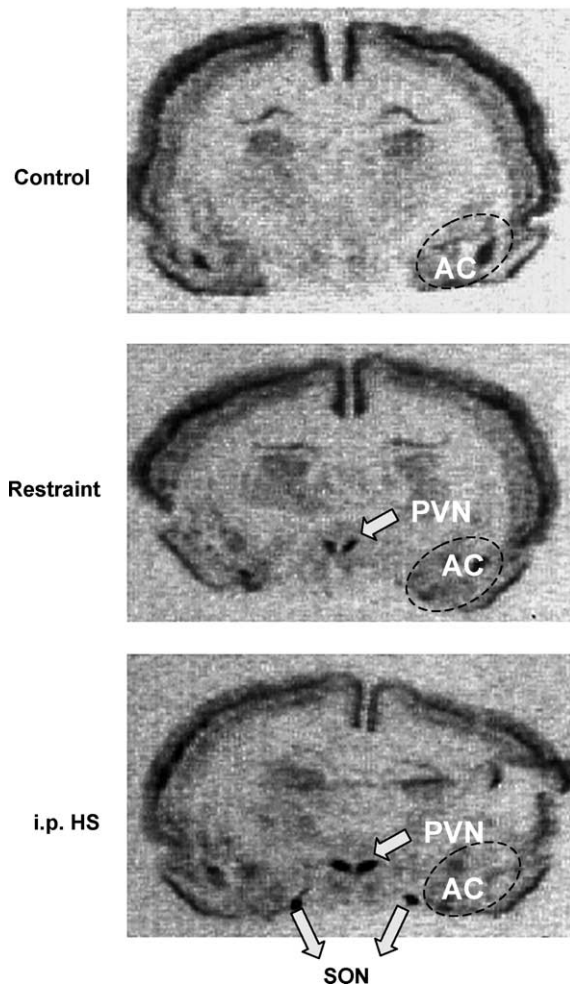


Fig. 2. In situ hybridization for CRHR1 mRNA showing changes in the PVN and SON in brain sections of rats in control conditions, or 3 h after 1 h of restraint (psychosomatic stress paradigm) or 4 h after a single intraperitoneal hypertonic saline injection (ipHS) (combination of painful and osmotic stimuli). CRHR1 mRNA is not detectable in the PVN or SON of control rats, it appears only in the parvocellular PVN after restraint, and in parvocellular PVN and magnocellular PVN and SON after ipHS. Abbreviations: AC, amygdala complex; SON, supraoptic nucleus.

with an affinity equal to or greater than that of the receptors, and blocks CRH-mediated ACTH release *in vitro*. The CRF binding protein and the CRF receptors are co-located broadly in cortical regions, in limbic structures and certain sensory relay nuclei (Table 1), suggesting that this binding protein may have a profound impact on the biological activity of CRH (or urocortin). Mouse models of CRH-BP over-expression or deficiency have shown changes in the HPA axis and in energy balance, and behavior consistent with the hypothesis that CRH-BP plays an important *in vivo* modulatory role by regulating levels of CRH and CRH-like peptides available to receptors in the pituitary and brain [70].

Studies employing CRFR1 and CRFR2 knock-out mice, and the use of selective CRHR antagonists, have provided important information on the functional significance of CRH

receptor sub-types in the brain. Whereas ablation of the CRFR1 results in a decrease in anxiety-like behavior and an impaired stress response [72,77,78], CRFR2-deficient mice show increased anxiety-like behavior and stress hypersensitivity [8,10,17]. However, it is possible that CRFR2 agonists have a role in adrenocortical activation, since double mutant mice (deficient in both CRFR1 and CRFR2) showed more marked atrophy of the adrenal fasciculata than that found in CRFR1-deficient mice [9].

The co-ordinated actions of both CRFR1 and R2 agonists, both at central and peripheral sites, appear to be critical in the mechanisms of regulation of energy metabolism. The co-expression of urocortin and CRFR2 in the arcuate nucleus probably suppresses feeding behavior since CRFR2 mutant knockout mice eat more, yet have a lower fat status while maintaining the same body weight [7]. Clearly, urocortin is acting in concert with the family of neuropeptides and afferent signals that fine-tune orexigenic signals within the hypothalamus that direct feeding behavior [11]. These mutant mice also display increased peripheral insulin sensitivity and sympathetic tone. Fine control of energy substrate distribution can be exerted through the actions of urocortin via CRFR2 receptors in endothelial cells of the peripheral vasculature [39,68], and also through the regulation of angiogenesis [9].

4. Regulation of CRHR1 and HPA axis activity

Under most experimental conditions there is a poor correlation between pituitary responsiveness and the number of CRH receptors in the anterior pituitary [1]. For example, following adrenalectomy there is marked down-regulation and desensitization of pituitary CRH receptors, with decreases in both receptor number and CRH-stimulated adenylate cyclase [87]. These changes are discordant with the increases in pituitary POMC mRNA and plasma ACTH levels observed in these conditions [18,65]. Such loss of CRH receptors is largely due to increased exposure of the pituitary to CRH and VP, as shown by the effects of osmotic minipump infusion of the peptides in normal and VP-deficient, Brattleboro rats [27,35]. Similarly, the increased ACTH responses to a novel stress observed in most chronic stress paradigms is associated with CRH receptor down-regulation and desensitization [1]. It is unlikely that decreases in pituitary CRH receptors account for the desensitization of the ACTH responses to some repeated stimuli, since similar or higher CRH receptor loss is observed in other chronic stress models with maintained ACTH response after repeated stress [1]. A discrepancy between changes in CRH receptors and pituitary responsiveness has also been described during chronic osmotic stimulation, a condition in which ACTH responses to a novel stress or CRH injection are decreased in the absence of any change in pituitary CRH receptor levels [16,27].

It is clear from this evidence that CRH receptor number is not a major determinant of corticotroph responsiveness,

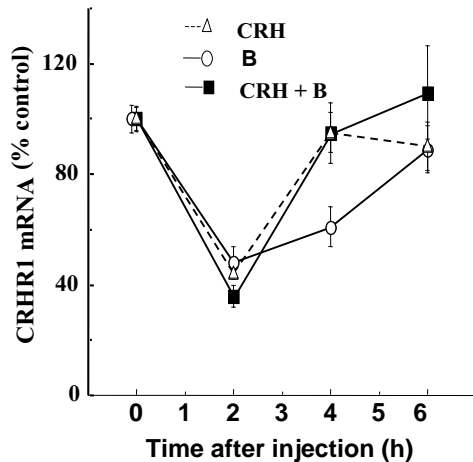


Fig. 3. Changes in CRH binding and CRHR1 mRNA during restraint stress. Repeated restraint for 1 h a day for 14 days (restraint \times 14) results in decreased CRH binding but increased CRHR1 mRNA in the pituitary. Acute restraint down-regulates CRHR1 mRNA in controls and also in repeatedly restrained rats. The rapid recover of mRNA levels is probably due to transcriptional activation of the CRHR1 gene. Data are expressed as percent of control values, represented by the dotted horizontal bars.

and that full ACTH responses can be achieved with partial receptor occupancy. However, the severely blunted ACTH responses observed after pharmacological or surgical elimination of CRH, and in CRH or CRHR1 knockout mice, indicate that the presence of CRH, and at least a small number of CRHR1, are required for the pituitary gland to respond to most stressors [59,79,82].

5. Regulation of CRHR1 mRNA levels in the pituitary gland

Alterations of the HPA axis cause dynamic changes in CRHR1 mRNA levels in the pituitary. However, as shown by the example in Fig. 3, there is a complete lack of correlation between CRH binding and CRH mRNA content, indicating that the number of active CRH receptors in the pituitary does not depend on the prevailing levels of mRNA. Acute stress causes biphasic changes in pituitary CRHR mRNA, with a decrease at 2 h followed by recovery to normal levels or an increase by 4 h after initiation of the stress. In some acute stress paradigms, such as immobilization, increases in CRHR mRNA are accompanied by increases in CRH binding (Fig. 3). In other stress models, such as hypertonic saline or lipopolysaccharide injection, there is a more prolonged decrease in CRHR mRNA, and CRH binding does not increase [6,64]. As shown in Fig. 3, during repeated stress CRHR mRNA levels decrease transiently after each stress exposure but are elevated again 24 h after the last stress episode, despite the concomitant CRH receptor down-regulation [51,64].

The mechanism regulating pituitary CRHR1 mRNA levels during stress is likely to involve increased exposure of the

pituitary corticotroph to glucocorticoids, CRH and VP. Although injection of CRH decreases pituitary CRHR mRNA after 2 h, it returns to basal levels by 4 h despite the persistent elevation of circulating CRH [57,64]. In contrast, exposure of pituitary cells to CRH *in vitro* leads to sustained decreases in CRHR mRNA levels [63]. This discrepancy may be due to different patterns of exposure of the cell to CRH, or to the absence of interaction with other factors present *in vivo*, such as glucocorticoids. In this regard, prolonged CRHR1 mRNA down-regulation has been observed after CRH injection in the absence of glucocorticoids in adrenalectomized rats [64]. Daily injection of low doses of CRH to mimic plasma levels in the range of pituitary portal levels increases CRHR1 mRNA and CRH binding [64]. In contrast, osmotic minipump administration of CRH leading to sustained increases of the circulating peptide causes CRHR down-regulation and desensitization [1].

Experiments in which intraperitoneal injections of CRH and VP are given simultaneously, and studies in VP-deficient Brattleboro rats, have shown that VP synergizes with the effect of CRH on CRH receptor down-regulation. These findings strongly suggest that increased VP:CRH ratios, and interaction between these regulators, are responsible for CRHR1 down-regulation during adrenalectomy and chronic stress [1]. In contrast to the facilitation of CRH receptor down-regulation by vasopressin, the peptide can upregulate CRHR mRNA levels *in vivo*, since it increases CRHR1 mRNA levels when given as single or repeated injections for 14 days [64]. However, *in vitro* incubation of cultured pituitary cells with VP decreases CRHR mRNA and potentiates the down-regulatory effect of CRH [63]. The differences between the effects of VP *in vivo* and *in vitro* suggest that the actions of the peptide depend on interactions between signaling by the vasopressin receptor and other factors.

Glucocorticoids cause prolonged decreases in CRH binding, *in vivo* and *in vitro* [15,30,69] but only transient reductions in CRHR1 mRNA [38,50,65], suggesting that glucocorticoids inhibit CRHR1 synthesis at the post-transcriptional level. Pituitary CRHR1 mRNA levels are not under tonic inhibition by glucocorticoids, since they are unaffected by long-term adrenalectomy [50,65] (Fig. 4). However, pituitary CRHR1 mRNA levels decrease following glucocorticoid administration and recover only when circulating glucocorticoids decline below stress levels [57], suggesting that the glucocorticoid surge contributes to the initial decrease in pituitary CRHR1 mRNA during stress.

On the other hand, stress and CRH injection cause sustained decreases in CRHR1 mRNA in adrenalectomized rats, suggesting that resting glucocorticoid levels are necessary for the recovery of CRHR1 mRNA (Fig. 5). Recent studies showing full restoration of CRHR1 mRNA levels after 4 h when CRH and corticosterone are given simultaneously indicate that the interaction between both regulators counteracts the inhibition by each regulator alone, and shortens the duration of the inhibitory phase (Fig. 5). Since CRH injection in intact rats increases circulating glucocorticoids, the

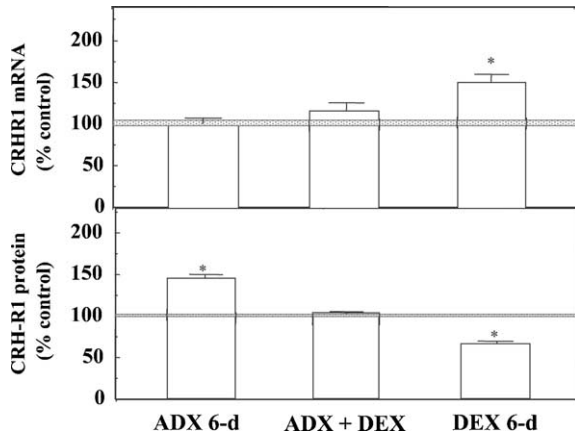


Fig. 4. Time course of the changes in pituitary CRHR1 mRNA following a single injection of CRH (1 μ g) or corticosterone (Cort, 2 mg.) or their combination. Data points are the mean \pm S.E. of the values obtained in situ hybridization in six rats per experimental group expressed as percentage change from vehicle-injected controls (basal). Basal values represent the mean of the pooled data obtained in vehicle-injected rats 2 or 6 h before decapitation ($n = 12$). * $P < 0.01$ vs. basal (time = 0).

combined effect of both regulators could explain the rapid recovery of CRHR1 mRNA. On the other hand, it is possible that feedback inhibition of CRH release at the median eminence following glucocorticoid injection [73] could contribute to the prolonged inhibitory action on CRHR1 mRNA.

Overall, CRHR1 mRNA levels in the pituitary are under the influence of glucocorticoids, vasopressin and CRH. The pituitary is exposed to changing concentrations of these regulators during alterations of HPA axis activity. Therefore, it is likely that the coordinated effects of CRH, VP and glucocorticoids are responsible for the changes in pituitary CRHR

mRNA levels during stress. It is also clear from these studies that the levels of CRHR mRNA do not parallel CRH binding in the pituitary, and that translational and post-translational mechanisms are likely to play a major role in determining the levels of CRH receptor expression in the pituitary.

6. Molecular mechanisms regulating CRHR levels

The dynamic changes in pituitary CRHR mRNA during repeated stress, with down-regulation followed by increases after each stress episode (Fig. 3), strongly suggest that activation of CRHR1 gene transcription is important for maintaining adequate CRH receptor levels. On the other hand, the dissociation between levels of CRH binding and CRHR1 mRNA levels during changes in HPA axis activity indicates that post-transcriptional events are probably important determinants of the pituitary CRH receptor content. It has been shown that CRH binding levels do not reflect the content of CRHR1 protein in pituitary membranes [56]. As illustrated in Fig. 5, two experimental conditions with opposite effects on HPA axis activity, namely adrenalectomy and glucocorticoid administration, are associated with CRH receptor loss and normal or elevated CRHR1 mRNA levels [65]. Western blot analysis of the CRHR1 protein revealed opposite changes in receptor content under the two experimental conditions (Fig. 5). In 6-day adrenalectomized rats, there was a significant increase in CRHR1 protein in spite of reduced CRH binding. This effect of adrenalectomy was prevented by glucocorticoid replacement. In contrast, the decrease in CRH binding following dexamethasone injection in sham ADX rats was accompanied by a reduction in CRHR1 protein. These data indicate that the decrease in binding during adrenalectomy is not due to a decrease in receptor synthesis, but that is probably caused by homologous desensitization and internalization [24,61]. On the other hand, glucocorticoids probably decrease binding by inhibiting CRHR1 mRNA translation and/or increase receptor degradation.

7. Desensitization of the CRHR1 receptor

The CRHR1 receptor, like many other GPCRs, undergoes desensitization and down-regulation following agonist-activated stimulation of receptor signaling [22]. Such homologous inhibition of receptor function serves to quench the signaling response initiated by the agonist-induced change in receptor conformation, even in the continued presence of the agonist. Desensitization occurs rapidly after agonist binding, and usually results from the binding of one or more of the seven G protein-coupled receptor kinases (GRKs) to the activated receptor, causing phosphorylation of serine/threonine residues in its carboxyl terminal domain and intracellular loops. This promotes the binding of one or more of the four β -arrestins to the receptor, which impedes its coupling to G protein(s) and thus impairs further

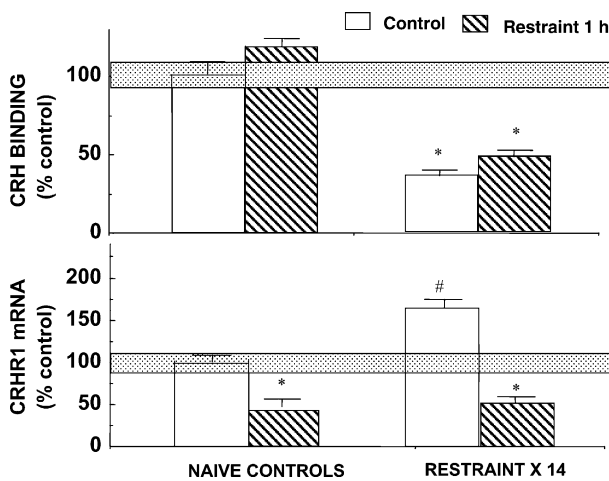


Fig. 5. Lack of correlation between CRHR1 mRNA, measured by in situ hybridization, and CRHR protein measured by Western blots, in pituitaries from rats subjected to adrenalectomy or dexamethasone administration for 6 days. Bars represent the mean and S.E. of the data in three experiments, expressed as percent change from the controls. The dotted horizontal bar represents the mean and S.E. of control values. * $P < 0.01$ lower than basal controls, # $P < 0.01$ higher than basal controls.

receptor-mediated signaling to downstream pathways. In contrast, receptor down-regulation occurs more slowly, and is most evident after hours *in vivo* [85].

CRHR1 receptors have been found to be rapidly desensitized in several cell types, including retinoblastoma Y79, neuroblastoma IMR-32, and transfected fibroblasts treated with high levels of CRF or urocortin [28]. Antisense studies have implicated GRK3 as a significant factor in the desensitization of CRHR1 receptors expressed in Y79 cells, and GRK3 expression is increased during receptor desensitization [19,20]. Furthermore, CRF treatment causes rapid dephosphorylation of CRHR1 receptors expressed in COS-7 cells, which express GRK2 but not GRK3 [33]. It appears that both GRK2 and GRK3 can participate in CRF receptor phosphorylation and desensitization in specific cell types.

In addition to the homologous desensitization caused by GRK-induced phosphorylation and arrestin binding in many agonist-activated GPCRs, agonist stimulation of receptor-activated signaling kinases, predominantly PKA and PKC, can mediate both homologous and heterologous desensitization in certain receptors [24]. However, although CRH action is associated with cAMP production and activation of PKA, this has not been implicated in desensitization of the CRHR1 receptor [32,67]. On the other hand, PKC-mediated heterologous desensitization of CRH action was observed in human myometrial cells [34], and the CRHR1 receptor contains PKC consensus sites in its C-terminal cytoplasmic domain [14].

In a recent report, the potential role of PKC-dependent phosphorylation in desensitization of the CRHR1 was investigated in human retinoblastoma and Y1 cells [31]. This study showed that PMA-induced activation of PKC in Y79 retinoblastoma cells, primarily the α and β isoforms, reduced CRH-stimulated cAMP production by 56%. However, inhibition of PKC did not affect CRH-induced receptor desensitization. The ability of PKC activation to stimulate receptor phosphorylation was demonstrated in transfected Y1 cells expressing the CRHR1 receptor. These findings are consistent with the PKC-dependence of oxytocin receptor-induced desensitization of CRH action on cAMP production in myometrial cells observed by Grammatopoulos and Hillhouse [34]. Taken together, these observations suggest that protein kinase C, specifically the α and β isoforms, has an important function in the phosphorylation and heterologous desensitization of the CRHR1 receptor. This raises the possibility that activation of other G_q -coupled receptors, especially those involved in stress mechanisms, could influence CRH-mediated neuronal responses in the central nervous system [31].

8. Translational regulation of CRHR1 by the 5'-UTR

The lack of correlation between CRHR1 mRNA and receptor protein suggests that CRHR1 mRNA translation is a site of regulation of CRHR1 synthesis during changes in HPA axis activity. However, little is known about the mech-

anisms regulating CRHR1 mRNA translation. There is evidence that structural characteristics of the 5'-untranslated region (5'-UTR) of mRNA have an important role in regulating translation [44]. In addition, binding of proteins to the 5'-UTR of the mRNA for a number of genes can influence translation [26,42]. Experiments using gel shift assays have shown binding of pituitary cytosolic proteins to the *in vitro* transcribed 5'-UTR of CRHR1 mRNA. In addition, protein binding to CRHR1 mRNA, but not to angiotensin AT₁ receptor mRNA, is increased by cytosol from adrenalectomized rats [89]. The ability of alterations of the HPA axis to specifically regulate the binding of protein complexes to the 5'-UTR of the CRHR1 mRNA suggests that RNA binding proteins are involved in the translational regulation of mRNA for CRHR1.

An additional mechanism of translational regulation involves the presence of minicistrons or upstream open reading frames in the 5'-UTR of mRNAs [44]. Computer analysis of the 5'-UTR of the CRHR1 mRNA reveals the presence of a short open reading frame encoding a putative 10 amino acid peptide. The influence of this upstream ORF on CRHR1 translation was studied in constructs containing the 5'-UTR of CRHR1, with or without an ATG to ATA mutation in the upstream ORF, and the main ORF of luciferase or CRHR1. Upstream mutation in luciferase constructs increased luciferase activity and luciferase protein when transfected into cell lines, compared with the native 5'-UTR. Transfection of CRHR1 constructs containing the upstream mutation increased CRH binding and CRH-stimulated cAMP production, without changes in CRHR1 mRNA levels. Mutation of the upstream ORF also increased CRHR1 protein when transfected into cells and in an *in vitro* translation assay, indicating that the upstream ORF inhibits CRHR1 translation [88]. The mechanism by which this occurs may involve translation of the upstream peptide, since the peptide showed the potential of being translated in studies using a fusion construct of the upstream ORF and green fluorescent protein. Also, the synthetic peptide inhibits *in vitro* translation of constructs containing the CRHR1 5'-UTR.

9. Conclusions

The hypothalamic peptide, corticotropin releasing hormone (CRH) and its receptors have an essential role in stress adaptation, by mediating behavioral, autonomic, and hormonal responses. The use of a variety of *in vivo* and *in vitro* procedures during the last two decades since the isolation and characterization of the peptide has led to a discovery of a family of CRH-related peptides with differential affinities for specific receptor subtypes. The topographic distribution of CRH and CRH receptors in the brain and peripheral tissues is consistent with the role of this family of peptides as coordinators of the integrated stress response. Marked progress has been also achieved in our understanding of the mechanisms regulating the levels of CRHR1 receptors

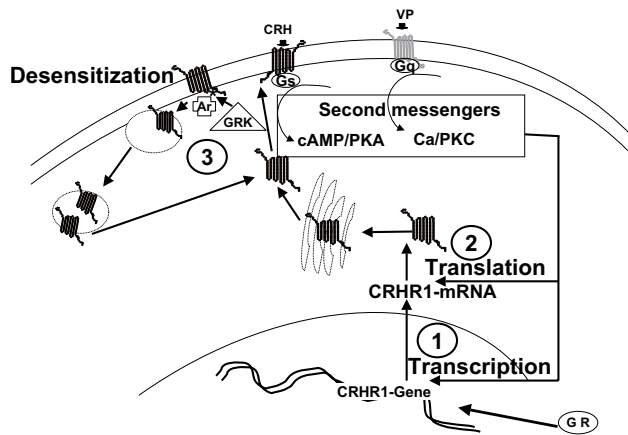


Fig. 6. Sites of regulation of the number of CRHR1 in the pituitary. The number of functional CRHR1 depends on receptor protein synthesis through transcriptional and translational activation (1 and 2), and receptor desensitization and internalization following interaction with the ligand (3). There is evidence for the roles of phosphorylation, G protein receptor kinase (GRK) and scaffolding proteins such as beta-arrestin (Ar) in the fate of CRHR1 following CRH binding. Active V1bR gene transcription (1) is required to maintain CRHR1 mRNA levels, but post-transcriptional regulation (2) appears to be a major determinant of receptor synthesis. Based on *in vivo* studies, it is likely that CRHR1 regulation involves signaling by CRH, VP and glucocorticoids. Abbreviations: VP, vasopressin; CRHR1, corticotropin releasing hormone type 1 receptor; Ar, β -arrestin; GRK, G protein-coupled receptor kinase; GR, glucocorticoid receptor.

during manipulations of the activity of the HPA axis. The relationship between the number of CRHR1 and corticotroph responsiveness indicates that a small number of receptors is sufficient for full ACTH responses, and that post-receptor events probably contribute to sustained ACTH responses in the presence of reduced CRH binding. Molecular studies have revealed that the regulation of CRHR1 synthesis occurs at transcriptional and post-transcriptional sites (Fig. 6). However, the divergent levels of CRH receptors and CRHR1 mRNA in the pituitary during alterations of the HPA axis argue for a major contribution of post-transcriptional mechanisms in CRHR1 regulation. This mode of regulation appears to permit the rapid changes in pituitary CRH receptor content that are necessary to adapt to the physiological demands during stress or other conditions leading to changes in HPA axis activity.

References

- [1] Aguilera G. Regulation of pituitary ACTH secretion during chronic stress. *Front Neuroendocrinol* 1994;15:321–50.
- [2] Aguilera G, Harwood JP, Wilson JX, Morell J, Brown JH, Brown JH, et al. Mechanisms of action of corticotropin releasing factor and other regulators of corticotropin release in rat pituitary cells. *J Biol Chem* 1983;258:8039–45.
- [3] Antoni FA. Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. *Endocr Rev* 1986;7:351–78.
- [4] Arai M, Assil IQ, Abou Samra AB. Characterization of three cat fish corticotropin-releasing factor receptors in catfish: a novel third receptor is predominantly expressed in pituitary and urophysis. *Endocrinology* 2001;142:446–54.
- [5] Arima H, Aguilera G. Vasopressinergic and oxytocinergic neurons of supraoptic and paraventricular nuclei co-express mRNA for type-1 and type-2 corticotropin releasing hormone receptors. *J Neuroendocrinol* 2000;12:833–42.
- [6] Aubry JM, Turnbull AV, Pozzoli G, Rivier C, Vale W. Endotoxin decreases corticotropin-releasing factor receptor 1 messenger ribonucleic acid levels in the rat pituitary. *Endocrinology* 1997;138:1621–6.
- [7] Bale TL, Anderson KR, Roberts AJ, Lee KF, Nagy TR, Vale WW. Corticotropin-releasing factor receptor-2-deficient mice display abnormal homeostatic responses to challenges of increased dietary fat and cold. *Endocrinology* 2003;144:2580–7.
- [8] Bale TL, Contarino A, Smith GW, Chan R, Gold LH, Sawchenko PE, et al. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat Genet* 2000;24:410–4.
- [9] Bale TL, Giordino FJ, Vale WW. A new role for corticotropin-releasing factor receptor-2: suppression of vascularization. *Trends Cardiovasc Med* 2003;13:68–71.
- [10] Bale TL, Picetti RR, Contarino A, Koob GF, Vale WW, Lee KF. Mice deficient for both corticotropin-releasing hormone receptor 1 (CRFR1) and CRFR2 have an impaired stress response and display sexually dichotomous anxiety-like behaviour. *J Neurosci* 2002;22:193–9.
- [11] Bray GA, York DA. The MONA LISA hypothesis in the time of leptin. *Rec Prog Horm Res* 1998;53:95–117.
- [12] Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol Sci* 1996;17:166–72.
- [13] Chang CP, Pearse RV, O'Connell S, Rosenfeld MG. Identification of a seven *trans*-membrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 1993;11:1183–95.
- [14] Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing factor receptor. *Proc Natl Acad Sci USA* 1993;90:8967–71.
- [15] Childs GV, Morrell JL, Niendorf A, Aguilera G. Cytochemical studies of CRH receptors in anterior lobe corticophs: binding, glucocorticoid regulation and endocytosis of [biotinyl-Ser¹]CRF. *Endocrinology* 1986;119:2129–36.
- [16] Chowdrey HS, Jessop DS, Patel H, Lightman SL. Altered adrenocorticotropin, corticosterone and oxytocin responses to stress during chronic salt load. *Neuroendocrinology* 1991;54:635–8.
- [17] Coste SC, Kesterson RA, Heldwein KA, Steven SL, Heard AD, Hollis JH, et al. Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. *Nat Genet* 2000;24:403–9.
- [18] Dallman MF, Akana SF, Scribner KA. Stress, feedback and facilitation in the hypothalamus pituitary adrenal axis. *J Neuroendocrinol* 1992;4:517–26.
- [19] Dautzenberg FM, Braun S, Hauger RL. GRK3 mediates desensitization of CRF1 receptors: a potential mechanism regulating stress adaptation. *Am J Physiol Regul Integ Physiol* 2001;80:R935–46.
- [20] Dautzenberg FM, Kilpatrick GJ, Hauger RL, Moreau JL. Molecular biology of the CRH receptors—in the mood. *Peptides* 2001;22:753–60.
- [21] De Souza EB, Perrin MH, Insel TR, Rivier J, Vale WW, Kuhar MJ. Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification. *Nature* 1983;224:1449–51.
- [22] Dieterich KD, Grigoriadis DE, DeSouza EB. Homologous desensitization of human corticotropin-releasing factor 1 receptor in stable transfected mouse fibroblast cells. *Brain Res* 1996;710:287–92.
- [23] Eckart K, Jahn O, Radulovic J, Radulovic M, Blank T, Stiedl O, et al. Pharmacology and biology of corticotropin-releasing factor (CRF) receptors. *Receptors Channels* 2002;8:163–77.
- [24] Ferguson SS, Caron MG. G protein coupled receptor adaptation mechanisms. *Semin Cell Dev Biol* 1998;9:119–27.

- [25] Giguere V, Labrie F, Cote J, Coy DH, Sueiras-Diaz J, Schally AV. Stimulation of cAMP accumulation and corticotropin releasing factor in rat anterior cells: site of glucocorticoid action. *Proc Natl Acad Sci USA* 1983;79:3466–9.
- [26] Goossen B, Caughman SW, Harford JB, Klausner RD, Hentze MW. Translational repression by a complex between the iron-responsive element of ferritin mRNA and its specific cytoplasmic binding protein is position-dependent in vivo. *EMBO J* 1990;9:4127–33.
- [27] Hauger RL, Aguilera G. Regulation of pituitary corticotropin releasing hormone (CRH) receptors by CRH: interaction with vasopressin. *Endocrinology* 1993;133:1708–14.
- [28] Hauger RL, Dautzenberg FM, Flaccus A, Liepold T, Spiess J. Regulation of corticotropin-releasing factor receptor function in human Y-79 retinoblastoma cells: rapid and reversible homologous desensitization but prolonged recovery. *J Neurochem* 1997;68:2308–16.
- [29] Hauger RL, Grigoriadis DE, Dallman MF, Plotsky PM, Vale WW, Dautzenberg FM. International Union of pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. *Pharmacol Rev* 2003;5:21–6.
- [30] Hauger RL, Millan MA, Catt KJ, Aguilera G. Differential regulation of brain and pituitary corticotropin-releasing factor receptors by corticosterone. *Endocrinology* 1987;120:1527–33.
- [31] Hauger RL, Olivares-Reyes JA, Braun S, Catt KJ, Dautzenberg FM. Mediation of corticotropin releasing factor type 1 receptor phosphorylation and desensitization by protein kinase C: a possible role in stress adaptation. *J Pharm Exp Therap* 2003;306:794–803.
- [32] Hauger RL, Smith RD, Braun S, Dautzenberg FM, Catt KJ. Rapid agonist-induced phosphorylation of the human CRF receptor, type 1: a potential mechanism for homologous desensitization. *Biochem Biophys Res Commun* 2000;268:572–6.
- [33] Henriot S, Dautzenberg FM, Kilpatrick GJ. Urocortin: slower dissociation than corticotropin-releasing factor for the CRF binding protein. *Eur J Pharmacol* 1999;376:321–4.
- [34] Hillhouse EW, Grammatopoulos DK. Control of intracellular signalling by corticotropin-releasing hormone in human myometrium. *Front Horm Res* 2001;27:66–74.
- [35] Holmes MC, Catt KJ, Aguilera G. Involvement of vasopressin in the down-regulation of pituitary corticotropin releasing factor receptors after adrenalectomy. *Endocrinology* 1987;121:2093–8.
- [36] Holsboer F. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonist to treat depression and anxiety. *J Psychiatr Res* 1998;33:181–214.
- [37] Hsu SY, Hsueh AJW. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin releasing hormone receptor. *Nat Med* 2001;7:605–11.
- [38] Iredale PA, Duman RS. Glucocorticoid regulation of corticotropin releasing factor 1 receptor expression in pituitary derived AT-20 cells. *Mol Pharmacol* 1997;51:794–9.
- [39] Jain V, Longo M, Ali M, George R, Saade GR, Chwalisz K, et al. Expression of receptors for corticotropin releasing factor in the vasculature of pregnant rats. *J Soc Gynecol Invest* 2000;7:153–60.
- [40] Kageyama K, Bradbury MJ, Zhao L, Blount AL, Vale WW. Urocortin messenger ribonucleic acid: tissue distribution in the rat and regulation in thymus by lipopolysaccharide and glucocorticoids. *Endocrinology* 1999;140:5651–8.
- [41] Kishimoto T, Pearce II RV, Lin CR, Rosenfeld MG. A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. *Proc Natl Acad Sci USA* 1995;92:1108–12.
- [42] Krishnamurthi K, Zheng W, Verbalis AD, Sandberg K. Regulation of cytosolic proteins binding cis elements in the 5' leader sequence of the angiotensin AT1 receptor mRNA. *Biochem Biophys Res Commun* 1998;245:865–70.
- [43] Kostich WA, Chen A, Sperle K, Largent BL. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the CRF₂. *Mol Endocrinol* 1998;12:1077–85.
- [44] Kozak M. Interpreting cDNA sequences: some insights from studies on translation. *Mammalian Genome* 1996;7:563–74.
- [45] Lederis K, Letter A, McMaster D, Moore G. Complete amino acid sequence of urotensin I, a hypotensive and corticotropin-releasing peptide from *Catostomus*. *Science* 1982;218:162–4.
- [46] Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, et al. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. *Proc Natl Acad Sci USA* 2001;98:7570–5.
- [47] Lovenberg TW, Chalmers T, Liu C, De Souza EB. CRF2 α and CRF2 β receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* 1995;136:4139–42.
- [48] Lovenberg TW, Liaw CW, Grigoriadis DE. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 1995;92:836–40.
- [49] Luo X, Kiss A, Makara G, Lolait SJ, Aguilera G. Stress-specific regulation of corticotropin releasing hormone receptors and receptor mRNA in the parvocellular and magnocellular hypothalamus of the rat. *J Neuroendocrinol* 1994;6:601–8.
- [50] Luo X, Kiss A, Rabadan-Diehl C, Aguilera G. Regulation of hypothalamic and pituitary corticotropin-releasing hormone receptor messenger ribonucleic acid by adrenalectomy and glucocorticoids. *Endocrinology* 1995;136:3877–83.
- [51] Makino S, Schulkin J, Smith MA, Pacak K, Palkovits M, Gold PW. Regulation of corticotropin releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. *Endocrinology* 1995;136:4517–25.
- [52] Millan MA, Jacobowitz DM, Hauger RL, Catt KJ, Aguilera G. Distribution of corticotropin-releasing factor receptors in primate brain. *Proc Natl Acad Sci USA* 1986;83:1921–5.
- [53] Montecucchi PC, Henschen A. Amino acid composition and sequence analysis of sauvagine, a new active peptide from the skin of *Phyllomedusa sauvagei*. *Int J Prot Peptide Res* 1981;18:113–20.
- [54] Morley SD, Schonrock C, Richter D, Okawara Y, Lederis K. Corticotropin-releasing factor (CRF) gene family in the brain of the teleost fish *Catostomus commersoni* (white sucker): molecular analysis predicts distinct precursors for two CRF's and one urotensin peptide. *Mol Mar Biol Technol* 1991;1:48–57.
- [55] Nappi RE, Rivest S. Ovulatory cycle influences the stimulatory effect of stress on CRH corticotropin releasing factor receptor messenger ribonucleic acid in the paraventricular nucleus of the female rat hypothalamus. *Endocrinology* 1995;136:4073–83.
- [56] Nikodemova M, Diehl CR, Aguilera G. Multiple sites of control of type-1 corticotropin releasing hormone receptor levels in the pituitary. *Arch Physiol Biochem* 2002;110:123–8.
- [57] Ochedalski T, Rabadan-Diehl C, Aguilera G. Interaction between glucocorticoids and corticotropin releasing hormone (CRH) in the regulation of the pituitary CRH receptor in vivo in the rat. *J Neuroendocrinol* 1998;10:363–9.
- [58] Palchoudhuri MR, Hauger RL, Wille S, Fuchs E, Dautzenberg FM. Isolation and pharmacological characterization of two functional splice variants of corticotropin-releasing factor-type 2 receptor from *Tupaia belangeri*. *J Neuroendocrinol* 1999;11:419–28.
- [59] Perrin MH, Vale WW. Corticotropin releasing factor receptors and their ligand family. *Ann NY Acad Sci* 1999;885:312–28.
- [60] Petraglia F, Florio P, Gallo R, Salvestrone C, Lombardo M, Genazzani AD, et al. Corticotropin-releasing factor-binding protein: origins and possible functions. *Horm Res* 1996;45:187–91.
- [61] Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. *Annu Rev Biochem* 1998;67:653–92.
- [62] Potter E, Sutton C, Donaldson C, Chen R, Perrin M, Lewis K, et al. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proc Natl Acad Sci USA* 1994;91:8777–81.
- [63] Pozzoli G, Bilezikjian L, Perrin M, Blount AL, Vale WW. Corticotropin releasing factor (CRF) and glucocorticoids modulate the expression of type 1 CRF receptor messenger ribonucleic acid in rat anterior pituitary cell cultures. *Endocrinology* 1996;137:65–71.

- [64] Rabadan-Diehl C, Kiss A, Camacho C, Aguilera G. Regulation of messenger ribonucleic acid for corticotropin releasing hormone receptor in the pituitary during stress. *Endocrinology* 1996;137:3808–14.
- [65] Rabadan-Diehl C, Makara G, Kiss A, Zelena D, Aguilera G. Regulation of pituitary corticotropin releasing hormone (CRH) receptor mRNA and CRH binding during adrenalectomy: role of glucocorticoids and hypothalamic factors. *J Neuroendocrinol* 1997;9:689–97.
- [66] Reyes TM, Lewis K, Perrin MH, Kunitake KS, Vaughan J, Arias CA, et al. Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. *Proc Natl Acad Sci USA* 2001;98:2843–8.
- [67] Roseboom PH, Kalin N. Persistent corticotropin-releasing factor (1) receptor desensitization and down-regulation in the human neuroblastoma cell line IMR-32. *Brain Res Mol Brain Res* 2001;92:115–27.
- [68] Schilling L, Kanzler C, Schmiedek P, Ehrenreich LH. Characterization of the relaxant action of urocortin, a new peptide related to corticotropin-releasing factor in the rat isolated basilar artery. *Br J Pharmacol* 1998;125:1164–71.
- [69] Schwartz J, Billestrup N, Perrin M, Rivier J, Vale WW. Identification of corticotropin releasing factor (CRF) target cells and effects of dexamethasone on binding in anterior pituitary using a fluorescent analog of CRF. *Endocrinology* 1986;119:2376–82.
- [70] Seasholtz AF, Burrows HL, Karolyi IJ, Camper SA. Mouse models of altered CRH-binding protein expression. *Peptides* 2001;22:743–51.
- [71] Smith AI, Funder JW. Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. *Endocr Rev* 1988;9:159–79.
- [72] Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikian LM, Gold LH, et al. Corticotropin releasing factor receptor-deficient mice display decreased anxiety, impaired stress response and aberrant neuroendocrine development. *Neuron* 1998;20:1093–102.
- [73] Spinedi E, Giacomani M, Jacquier MC, Gaillard RC. Changes in hypothalamo-corticotrope axis after bilateral adrenalectomy: evidence for a median eminence site of glucocorticoid action. *Neuroendocrinology* 1991;53:160–70.
- [74] Starowicz K, Przewlocka B. The role of melanocortins and their receptors in inflammatory processes, nerve regeneration and nociception. *Life Sci* 2003;73:823–47.
- [75] Stenzel P, Kesterson R, Yeung W, Cone RD, Rittenberg MB, Stenzel-Poore MP. Identification of a novel murine receptor for corticotropin-releasing hormone expressed in the heart. *Mol Endocrinol* 1995;9:637–45.
- [76] Stenzel-Poore MP, Heldwein KA, Stenzel P, Lee S, Vale WW. Characterization of the genomic corticotropin releasing factor (CRF) gene from *Xenopus laevis*: two members of the family exist in amphibians. *Mol Endocrinol* 1992;6:1716–24.
- [77] Steckler T, Holsboer F. Corticotropin-releasing hormone receptor subtypes and emotion. *Biol Psychiatry* 1999;46:1480–508.
- [78] Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, Stalla GK, et al. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin releasing hormone receptor. *Nat Genet* 1998;19:162–6.
- [79] Turnbull AV, Rivier C. Corticotropin-releasing factor (CRF) and endocrine responses to stress: CRF receptors, binding protein, and related peptides. *Proc Soc Exp Biol Med* 1997;215:1–10.
- [80] Vale WW, Rivier C, Brown MR, Spiess J, Koob G, Swanson L, et al. Chemical and biological characterization of corticotropin releasing factor. *Rec Prog Horm Res* 1983;39:245–70.
- [81] Vaughan J, Donaldson C, Bittencourt J, Perrin MH, Lewis K, Sutton S, et al. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature* 1995;378:287–92.
- [82] Venihaki M, Majzoub JA. Animal models of CRH deficiency. *Front Neuroendocrinol* 1999;20:122–45.
- [83] Vita N, Laurent P, Lefort S, Chalon P, Lelias JM, Kaghad M, et al. Primary structure and functional expression of mouse pituitary and human brain corticotropin releasing factor receptors. *FEBS Lett* 1993;355:1–5.
- [84] Wynn PC, Aguilera G, Morell J, Catt KJ. Properties and regulation of high-affinity pituitary receptors for corticotropin-releasing factor. *Biochem Biophys Res Commun* 1983;110:602–8.
- [85] Wynn PC, Harwood JP, Catt KJ, Aguilera G. Corticotropin-releasing factor (CRF) induces desensitization of the rat CRF receptor–adenylate complex. *Endocrinology* 1988;122:351–8.
- [86] Wynn PC, Hauger RL, Holmes MC, Millan MA, Catt KJ, Aguilera G. Brain and pituitary receptors for corticotropin-releasing factor: localization and differential regulation after adrenalectomy. *Peptides* 1984;5:1077–84.
- [87] Wynn PC, Harwood JP, Catt KJ, Aguilera G. Regulation of corticotropin-releasing factor (CRF) receptors in the rat pituitary gland. Effects of adrenalectomy on CRF receptor and corticotroph responses. *Endocrinology* 1985;116:1653–9.
- [88] Xu G, Rabadan-Diehl C, Nikodemova M, Wynn P, Aguilera G. Translational inhibition of corticotropin releasing hormone type 1 receptor by the 5' leader sequence. *Mol Pharmacol* 2001;59:485–92.
- [89] Wu Z, Ji H, Hassan A, Aguilera G, Sandberg K. Regulation of pituitary corticotropin releasing factor type-1 receptor mRNA binding proteins by modulation of the hypothalamic–pituitary–adrenal axis. *J Neuroendocrinol* 2004, in press.