

# The role of CRH in behavioral responses to stress

Gennady N. Smagin<sup>a,\*</sup>, Stephen C. Heinrichs<sup>b</sup>, Adrian J. Dunn<sup>a</sup>

<sup>a</sup>Department of Pharmacology and Therapeutics, Louisiana State University Health Sciences Center, Shreveport, LA, 71130-3932, USA

<sup>b</sup>Boston College, Psychology Department, 140 Commonwealth Avenue, Chestnut Hill, MA 02467, USA

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## Abstract

Corticotropin-releasing hormone (CRH) and urocortin in the central nervous system affect behavior and can enhance behavioral responses to stressors. The action of CRH-related peptides is mediated through multiple receptors that differ markedly in their pharmacological profiles and anatomical distribution. Comparative pharmacology of CRH receptor agonists suggests that CRH, urocortin, sauvagine and urotensin consistently mimic, and CRH receptor antagonists consistently lessen, functional consequences of stressor exposure. Recently, important advances have been made in understanding the CRH system and its role in behavioral responses to stress by the development of specific CRH receptor antagonists, application of antisense oligonucleotides and development of transgenic mice lacking peptides and functional receptors. This review summarizes recent findings with respect to components of the CRH system and their role in stress-induced behavioral responses. © 2001 Elsevier Science Inc. All rights reserved.

*Keywords:* Corticotropin-releasing factor; Urocortin; Antagonists; CRH receptors; Antisense; Stress; Behavior

## 1. Introduction

Corticotropin-releasing hormone (CRH) is a mediator of endocrine, autonomic and immune responses to stress [15, 20,55,83] and it has been suggested that CRH may also coordinate autonomic and behavioral responses to stress, including anxiety-like behaviors, food intake, arousal, learning and memory [15,19,29,37,41,74]. Our understanding of the physiology of the CRH system and its response to stress has been significantly advanced in the last five years. Receptors mediating the action of CRH have been identified, cloned and their distribution in the brain and peripheral organs has been characterized [10,44,65]. Urocortin, a newly discovered peptide of the CRH family has been identified in the brain and peripheral organs of mammals [18,34,84] and it has been suggested that there may be other endogenous agonists(s) for CRH receptors [86].

Several reviews has been recently published, discussing the role of CRH, CRH-like peptides and CRH receptors in behavior [1,30,36,38,59,73]. The purpose of this article is to highlight recent findings on the role of different components of the CRH system in the behavioral responses to stress.

\* Corresponding author. Tel.: +1-318-675-7665; fax: +1-318-675-7857.

E-mail address: gsmagi@lsuhsc.edu (G.N. Smagin).

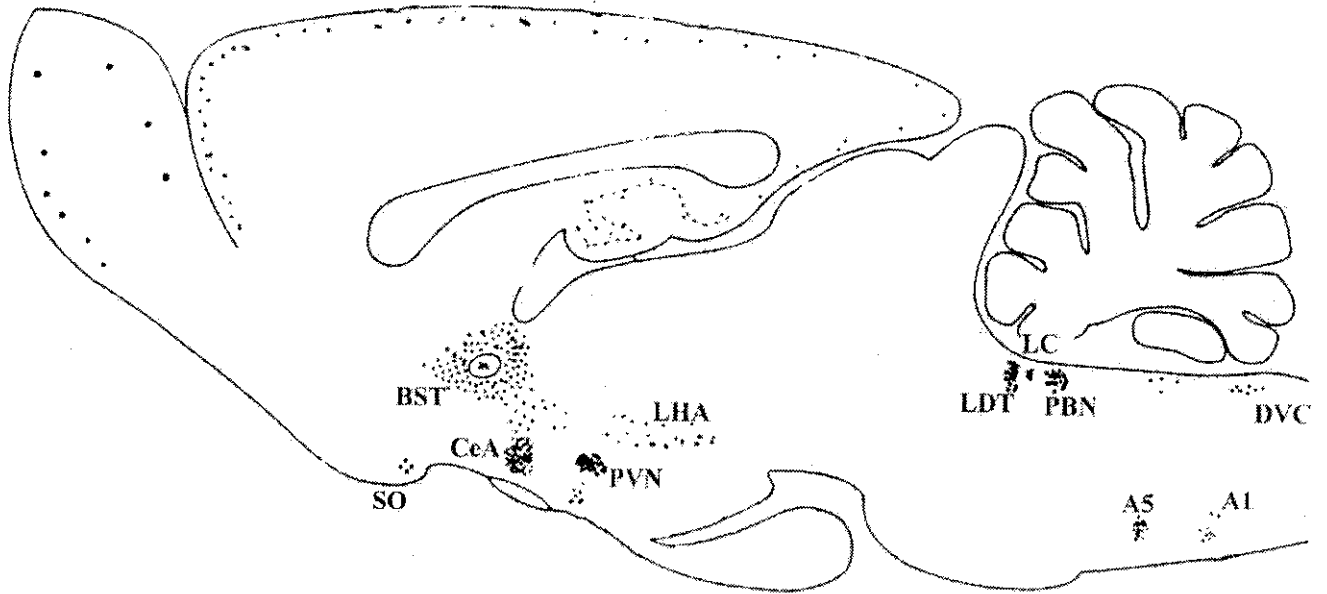
## 2. The CRH system

### 2.1. Peptides

The anatomy of the CRH system has been described in detail [66,67,75] and is summarized in Figure 1. High densities of CRH-immunoreactive neurons have been found in the paraventricular nucleus of the hypothalamus (PVN), the major locus of CRH-containing cell bodies. Hypothalamic CRH is released into portal vessels and is carried in the blood to the anterior pituitary from which it activates the release of ACTH [83] triggering the activation of the hypothalamo-pituitary-adrenocortical (HPA) axis. Extrahypothalamic CRH-containing neurons have been found in the central nucleus of the amygdala, the bed nucleus of the stria terminalis, the lateral hypothalamic area, the parabrachial nucleus, the hippocampus, the nucleus accumbens, and the cerebellum [49–51,64,66,75].

Recently, Vaughan et al. [84] discovered another member of the CRH family in mammals and named it urocortin (UCN), because the peptide is related to urotensin (63% sequence identity) and CRH (45% sequence identity). The major sites of UCN mRNA expression in the rat brain are the Edinger-Westphal (EW) nucleus, with lesser amounts in the lateral superior olive, the hippocampus, the basal ganglia, the medial septum, the PVN, and the

# CRH



# UCN

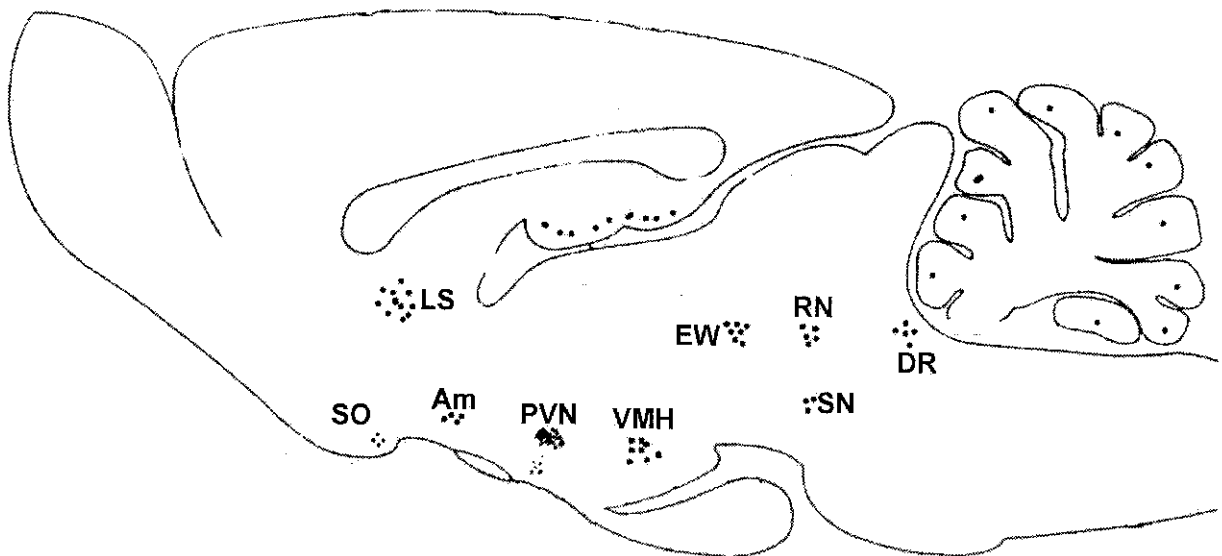


Fig. 1. Comparison of the brain distribution of CRH- and UCN-immunoreactive cells in the rodent brain. Data from [2,40,90,91]. All abbreviations are from Paxinos and Watson rat brain atlas [56].

Table 1  
Relative affinities (K<sub>i</sub>, nM) of CRH and UCN for CRH receptor subtypes expressed by stably transfected CHO cells

Peptide	Human CRH <sub>1</sub>	Rat CRH <sub>2α</sub>	Mouse CRH <sub>2α</sub>
r/h CRH	0.95	13	17
hUCN	0.41	1.8	1.5
rUCN	0.16	0.58	0.41

Data from [18].

lateral hypothalamus [84,89]. Immunohistochemical studies using an antibody specific for urocortin, found UCN-like immunoreactivity in the supraoptic, ventromedial hypothalamic nuclei and the PVN, the dorsal tegmental nucleus, the dorsal raphe nuclei and the substantia nigra. The most abundant UCN-immunoreactive (UCN-ir) perikarya were found in the EW nucleus [40,91]. In another study, only a few UCN-ir neurons were found in the hypothalamus [53], but a dense fiber network was found in the lateral septal area. However, no fibers were observed in the medial eminence or the pituitary [53]. A summary of the distribution of UCN-containing cell groups is shown in Figure 1. Table 1 represents relative affinities of peptides from the CRH family for CRH receptor subtypes.

## 2.2. Receptors

The structure of the cDNA encoding the human pituitary CRH receptor has been characterized by Chen et al. [13]. It encodes a 415-amino acid protein (designated as the CRH<sub>1</sub> receptor). CRH<sub>1</sub> receptors have also been identified in the rat brain [11,57]. Lovenberg et al. [44] identified a rat brain cDNA clone that encodes a second member of CRH receptor family. The CRH<sub>2</sub> receptor gene encodes a protein (CRH<sub>2α</sub>) of 411 amino acids and has 70% identity with the rat CRH<sub>1</sub> receptor over the entire coding region. An additional splice variant of the CRH<sub>2</sub> receptor with a different N-terminal domain, encodes a 431-amino acid protein, has been identified and designated the CRH<sub>2β</sub> receptor. CRH<sub>1</sub> and CRH<sub>2α</sub> receptors clearly have different tissue distributions [45]. Prominent expression of the CRH<sub>2α</sub> receptor was found in the lateral septum, the ventromedial nucleus of the hypothalamus, and several amygdaloid nuclei. CRH<sub>2α</sub> receptor mRNA was not detected in the neocortex and cerebral cortex, in contrast to the high levels of CRH<sub>1</sub> receptor expression in these regions. Similarly, CRH<sub>2α</sub> receptor expression was almost undetected in the pituitary lobes, where CRH<sub>1</sub> receptor expression is high [10,45]. A third splice variant of CRH<sub>2</sub> receptor, named CRH<sub>2γ</sub>, has been recently identified in human amygdala [39]. The data on distribution of CRH receptor mRNA and protein [10,58,59,61] are summarized in Table 2.

## 3. CRH system and stress

The role of CRH in endocrine and physiological responses to stress has been comprehensively reviewed [20, 36,38,55,81]. The distinct distributions of CRH and UCN in the rat brain, discussed earlier, suggest that these two peptides have distinct physiological roles, but these have not yet been established and will require further studies. When administered into the brain, UCN mimics endocrine responses to stress, elevating plasma ACTH and decreasing plasma LH concentrations [81]. Several reports have indicated that UCN is not a significant mediator of ACTH release in response to foot-shock or adrenalectomy because these effects are not prevented by antibodies to UCN that do not recognize CRH [48,82]. In a recent study in CRH knockout mice, normal and CRH-deficient mice had very similar distributions of UCN mRNA, as determined by *in situ* hybridization [86]. There was no ectopic UCN mRNA expression in CRH-deficient mice in areas that normally express CRH. To test further the hypothesis that UCN is involved in neuroendocrine reaction during stress, we studied UCN and CRH mRNA expression in animals subjected to restraint stress. Male Sprague Dawley rats were restrained in commercial plastic devices for 1 hour and decapitated immediately after stress. Brain areas (hypothalamus and midbrain, containing the EW nucleus) were dissected, total mRNA was extracted and processed for RNase protection assay to determine the concentration of UCN and CRH mRNA. As shown in Fig. 2, one hour of restraint significantly increased UCN mRNA levels in the hypothalamus and midbrain. Levels of mature CRH mRNA were not affected in either brain region. It has been shown previously that an increase in mature CRH mRNA occurs in the hypothalamus 90–120 min after the onset of restraint, whereas the heteronuclear CRH mRNA (hnRNA) is significantly increased 10–30 minutes after the onset of restraint [31]. In other studies, it was shown that UCN-like immunoreactivity is increased in response to a physiological stressor (salt loading) [24,25]. Using *in situ* hybridization, it has been found that UCN mRNA expression in the EW nucleus was increased approximately 3-fold after 3 hours of restraint [88]. Chronic glucocorticoid treatment blocked the restraint-induced rise of UCN mRNA. In CRH-deficient [knockout (KO)] mice, UCN expression in the EW nucleus was up-regulated 2- to 3-fold compared with that in wild-type mice. This up-regulation was not due to a lack of glucocorticoid inhibition, because glucocorticoid supplementation did not alter UCN expression [88]. Since the EW nucleus is not known to project to any brain regions believed to play a role in stress-related behavior. The data suggest that the EW nucleus may regulate some aspects of autonomic nervous system function, as well as sensory inputs. Although the EW nucleus might play a role in mediating stress-induced behaviors through an indirect pathway, an entirely novel behavioral pathway must be postulated to accommodate the possibility that UCN in the

Table 2  
Distribution of CRH binding sites in the brain

Anatomical region	CRH <sub>1</sub> mRNA	CRH <sub>1</sub> protein	CRH <sub>2</sub> mRNA	CRH <sub>2</sub> protein
<b>Telencephalon</b>				
Olfactory bulb	++++	++++		
Neocortex	++++	++++		
Lateral septum			++++	
Medial septum	+++	++	±	
Bed nucleus of the stria terminalis	++	++	++	++
CA1 hippocampal area	++	+	++	
Dentate gyrus	++	+	++	
Basolateral nucleus of the amygdala	++	++	±	
Medial nucleus of the amygdala	++	+	++	
Posterior cortical nucleus of the amygdala			+++	
<b>Diencephalon</b>				
Anterior hypothalamic area	+	+		
Periventricular hypothalamic nucleus	+	+		
Paraventricular hypothalamic nucleus (PVN)	+	+++	++	
Supraoptic nucleus	+	++	+++	
Dorsomedial hypothalamic nucleus	+++			
Ventromedial hypothalamic nucleus	+		++++	
<b>Mesencephalon</b>				
Substantia nigra	++	++		
Edinger-Wesphal nucleus (EW)	++++	+		
Nucleus raphe dorsalis	+	+	++	
Central gray	++	++		
Red nucleus	++++	++++		
<b>Pons/medulla</b>				
Dorsal tegmental nucleus	++++	+++		
Sensory trigeminal nucleus			+++	++
Reticular pontine nucleus	++	++		
Cuneate nucleus	++	++		
Cerebellum	+++	++++		

The hybridization signal for CRH<sub>1</sub> and CRH<sub>2</sub> mRNA is expressed as –, undetectable; +, weak; ++, moderate; +++, strong; and +++++, very strong. The immunohistochemical staining is expressed as ±, undetectable, +, weak; ++, moderate; +++, strong; +++++, very strong.

EW nucleus mediates stress-induced behaviors [86,88]. Since there were no differences in behavioral responses to stress in CRH KO and WT mice, UCN in the EW nucleus compensated for the lack of CRH through an indirect pathway to induce behaviors that perfectly mimic those seen in WT animals. Therefore, if UCN mediates stress-induced behaviors in CRH-deficient mice through descending projections from the EW nucleus, it seems likely that this pathway also mediates stress-induced behaviors in WT animals. On the other hand, if UCN is not involved in mediating behavior in either genotype, then an unidentified CRH-like molecule may participate in stress-induced behaviors [86,88].

#### 4. Behavioral effects of CRH family receptor agonists

Administration of CRH or urocortin into the central nervous system of a rat, mouse or primate produces a wide variety of behavioral effects, and the behavioral pharmacological profile resulting from exogenous administration of

these neuropeptides depends on the baseline state of arousal of the animal [20,35]. In non-stressed animals under low arousal conditions, CRH or urocortin produce a dose-dependent behavioral activation that includes increases in locomotor activity, rearing and grooming when rats are tested in a familiar environment [33]. Motor activation is not observed following systemic administration of CRH and is not blocked by hypophysectomy or pretreatment with dexamethasone, suggesting that this effect of CRH is mediated by actions in the central nervous system independent of the pituitary adrenal axis [84]. CRH can modulate learning and memory, either enhancing or impairing retention in a dose and site specific manner [37,60]. Other reported effects of CRH receptor agonists include suppression of food intake [29], enhancement of acoustic startle reactivity [14] and facilitation of defensive burying [17].

When animals are exposed to a more stressful environment, the profile of the behavioral effects of exogenously administered CRH and urocortin changes to reflect an enhanced behavioral response to stress [33,70]. The same doses that produce marked behavioral activation in a famil-

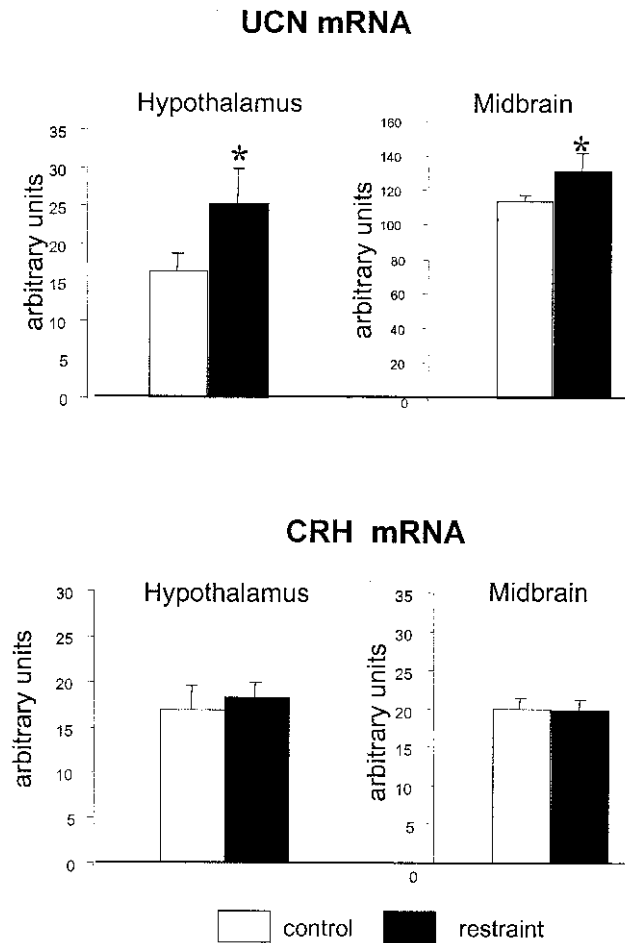


Fig. 2. The effect of one hour restraint on UCN and CRH mRNA in the hypothalamus and midbrain. RNase protection assay was carried out using kits (RPAII<sup>™</sup>) from Ambion (Austin, TX) according to the suggested protocol. For *in vitro* transcription, <sup>32</sup>P-labeled antisense rat UCN, CRH and  $\beta$ -actin RNA probe were synthesized using T7 RNA polymerase. Sense RNA was used in the RPA for further quantification of tissue mRNA levels. 20  $\mu$ g of total sample RNA (obtained by using TRIZOL (Gibco-BRL) extraction method according to the company's protocol), were hybridized overnight with the gel-purified probe at 45°C. RNA-RNA hybrids were digested in RNase A/T1 digestion buffer for 30 min. The reaction was stopped with inactivation buffer and protected fragments were precipitated. Precipitated fragments were denatured and separated on the gel. The gel was dried and directly exposed to a Phosphorimaging screen for 24–48 hours. Values (means  $\pm$  SEM) were standardized to arbitrary units. \*Significantly different from control ( $P < 0.05$ ,  $n = 8$ ).

iar environment produce behavioral suppression in a novel, presumably stressful environment [77]. CRH and urocortin also exert anxiogenic-like properties as shown by inhibition of exploration in several paradigms, including the open field, the elevated plus-maze, and the light-dark test [52]. The stress-like effects of CRH clearly have aversive properties in that CRH can produce both taste and place aversions [3,9]. Thus, exogenously administered CRH and urocortin produce behavioral activation, enhance behavioral responses to stress, and produce a stress-like behavioral state that is potentially aversive [38].

## 5. Comparative pharmacology of CRH receptor agonists

Comparative behavioral pharmacology studies have evaluated the family of CRH receptor agonist peptides with the goal of identifying diversity and functional selectivity of CRH systems and receptors (Table 3). Mixed receptor (CRH<sub>1</sub>/CRH<sub>2</sub>) agonist peptides native to the rat, CRH and urocortin, as well as sauvagine and urotensin have been administered in rodent models of exploratory, feeding and anxiety-like behavior [7]. All available studies report common features of CRH agonist administration such as appetite suppression, locomotor activation and anxiogenic-like behavior (Table 3). Most of these reports also describe subtle differences in CRH receptor agonist potency or efficacy in select paradigms. For example, exogenous urocortin administered centrally reduces food intake more potently than CRH in both fasted and *ad libitum* feeding paradigms [72]. It appears premature, however, at the present time to attribute any agonist action to specific CRH receptor subtypes [6,33] although the existence of distinct agonist lineages does suggest the evolution of within-system differences in functionality [43].

The role of CRH receptors in stress-related behaviors has been assessed using various approaches, by blocking the receptors using specific and nonspecific receptor antagonists; by downregulating expression of the receptor protein using antisense oligonucleotides, and by producing mice lacking specific receptors.

The first CRH receptor antagonist described was alpha-helical CRH<sub>9-41</sub> ( $\alpha$ hCRH<sub>9-41</sub>) [62]. Other peptide CRH receptor antagonists became available later, such as D-Phe<sup>12</sup>,Nle<sup>21,38</sup>, ( $\alpha$ MeLeu<sup>37</sup>) CRH<sub>12-41</sub> (abbreviated as D-Phe CRH<sub>12-41</sub>), a more potent antagonist of CRH receptors than  $\alpha$ hCRH. Astressin (cyclo(30–33){D-Phe<sup>12</sup>, Nle<sup>21,38</sup>, Glu<sup>30</sup>, Lys<sup>33</sup>}hCRH<sub>12-41</sub>) is significantly more potent in inhibiting ACTH secretion when administered peripherally than any of the other analogs [46]. However,  $\alpha$ hCRH<sub>9-41</sub>, D-Phe CRH<sub>12-41</sub> and astressin bind to both subtypes of CRH receptor (Table 4) and thus do not distinguish the sites of action of CRH-like peptides.

## 6. Behavioral Effects of CRH Family Receptor Antagonists

Compelling evidence for a role of endogenous CRH-like neuropeptides in behavioral responses to stressors comes from the demonstration of anti-stress actions of CRH receptor antagonists [20]. Central administration of  $\alpha$ hCRH<sub>9-41</sub> has been shown to reverse the attenuation of feeding induced by stress in rats [26], and to attenuate stress-induced fighting in rats [79] suggesting that both the suppression and activation in behavior associated with stressors may involve endogenous CRH systems. In mice,  $\alpha$ hCRH<sub>9-41</sub> reversed the suppression in exploratory behavior produced by re-

Table 3  
Comparative Efficacy of CRH Receptor Agonists in Rodent Behavioral Models

Peptide	Behavioral Effects	Investigators
CRH, sauvagine	Sauvagine produced larger and longer lasting suppression of nocturnal feeding than CRH	[22]
CRH, sauvagine, urotensin	Sauvagine was more potent than CRH or urotensin in producing anxiogenic-like behavior	[7]
CRH, urocortin, urotensin	Urocortin and urotensin were more potent in reducing deprivation-induced feeding than CRH	[72]
CRH, urocortin, urotensin	Urocortin and urotensin were less effective than CRH in producing anxiogenic-like behavior	[72]
CRH, urocortin	CRH and urocortin were equally potent and effective in stimulating anxiogenic-like and exploratory behavior	[52]
CRH, sauvagine, urotensin, urocortin	All peptides increased motor activity, reduced feeding and induced anxiogenic-like behavior in a similar manner	[33]

straint stress [5], and in rats produced a more rapid emergence from a small dark enclosure into a large open field and more exploration of the unfamiliar open field [78]. Subsequent studies have shown that CRH receptor antagonists are very effective in reversing the decrease in exploration of the open arms of an elevated plus-maze caused by exposure to a variety of stressors including restraint, swim stress, ethanol withdrawal and social stress [28].

An intensive screening of chemical libraries has yielded a group of compounds with specific affinity for CRH<sub>1</sub> receptor subtypes (Table 4). CP-154,526 (butyl-ethyl-(2,5-dimethyl-7-[2,4,6-trimethylphenyl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl)amine) was found by Pfizer Inc. [68]. Intracerebroventricular (icv) administration of this compound blocked the effect of CRH on the acoustic startle reflex, used as an indicator of fear and anxiety [68]. CP-154,526 has also been reported to have antidepressant effects in rats exposed to inescapable foot-shock [47]. NBI27914 (2-methyl-4(N-propyl-N-cyclopropanemethylamino)-5-chloro-6-(2,4,6-trichloroanilino)pyrimidine), was synthesized by Neurocrine Biosciences, Inc [12]. Antalarmin (N-butyl-N-ethyl-(2,5,6-trimethyl)-7-[2,4,6-trimethylphenyl]-7H-pyrrolo[2,3-d]pyrimidin-4-yl-amine) was synthesized from 2,4,6-trimethylaniline [85]. It has been reported that antalarmin can suppress CRH-induced ACTH release *in vitro* and *in vivo*, although it is only effective *in vivo* for brief mild stressors [85]. Despite its behavioral

effects, antalarmin had no effect on the elevations of ACTH and corticosterone induced by inescapable shock [16].

We have tested the involvement of CRH<sub>1</sub> receptors in anxiety-like behaviors induced by restraint. Behavioral activity of the selective CRH<sub>1</sub> antagonist, NBI27914, was evaluated using the elevated plus-maze and defensive withdrawal behavior. Each animal received a subcutaneous injection of NBI27914 (5 mg/kg) or vehicle one hour prior to a five minute test in the elevated plus maze. Administration of NBI27914 significantly increased the time animals spent on the open arms of the maze and the number of entries, suggesting anxiolytic properties of this compound (Figure 3). Defensive withdrawal was conducted in rats familiar with the behavioral apparatus. Behaviors scored during a 10 min session included latency to emerge from the darkened chamber in the open field; time spent inside and outside of the chamber and locomotor activity (moving, rearing). Animals were injected with NBI27914 or vehicle one hour prior to restraint and briefly restrained (20 min) in plastic restrainers. Restraint induced defensive withdrawal with an increase in the time spent in the enclosed chamber, and a decrease in exploratory behavior (rear). Pretreatment of animals with NBI27914 (5 mg/kg), significantly attenuated the effects of restraint on behavior, reducing the mean and total time animals spent in the chamber, and the number of entries (Figure 4). The data suggest that CRH<sub>1</sub> receptors are

Table 4  
Pharmacological binding characteristics of CRH receptor antagonists

Compound	K <sub>i</sub> (nM), CRH <sub>1</sub> receptors	K <sub>i</sub> (nM), CRH <sub>2</sub> receptors	Reference
αhCRH <sub>9-41</sub>	40	96.2	[2]
D-Phe-CRH <sub>12-41</sub>	30	24	[2]
Astressin	2.0		[23]
CP154,526	2.7	>10	[68]
CRA1000	15.7 <sup>C</sup>	>100000 <sup>C</sup>	[54]
NBI27914	1.7	Devoid of activity, data not shown	[12]
Antalarmin	1.3–1.9 <sup>D</sup>	Did not antagonize <sup>E</sup>	[85]
Antisauvagine-30	1.4 <sup>A</sup>	153.6 <sup>B</sup>	[63]

<sup>A</sup> K<sub>d</sub> displacing [<sup>125</sup>I-Tyr<sup>0</sup>]SvG;

<sup>B</sup> K<sub>d</sub> displacing [<sup>125</sup>I-Tyr<sup>0</sup>]oCRH;

<sup>C</sup> IC<sub>50</sub> for <sup>125</sup>I-ovine CRH binding to pituitary and heart membranes;

<sup>D</sup> <sup>125</sup>I-oCRH binding in pituitary, cerebellum and frontal cortex homogenates, e.g. tissues expressing mostly CRH<sub>1</sub> receptors.

<sup>E</sup> Inhibition of <sup>125</sup>I-oCRH binding to heart homogenates, tissue, predominately expressing CRH<sub>2</sub> receptors.

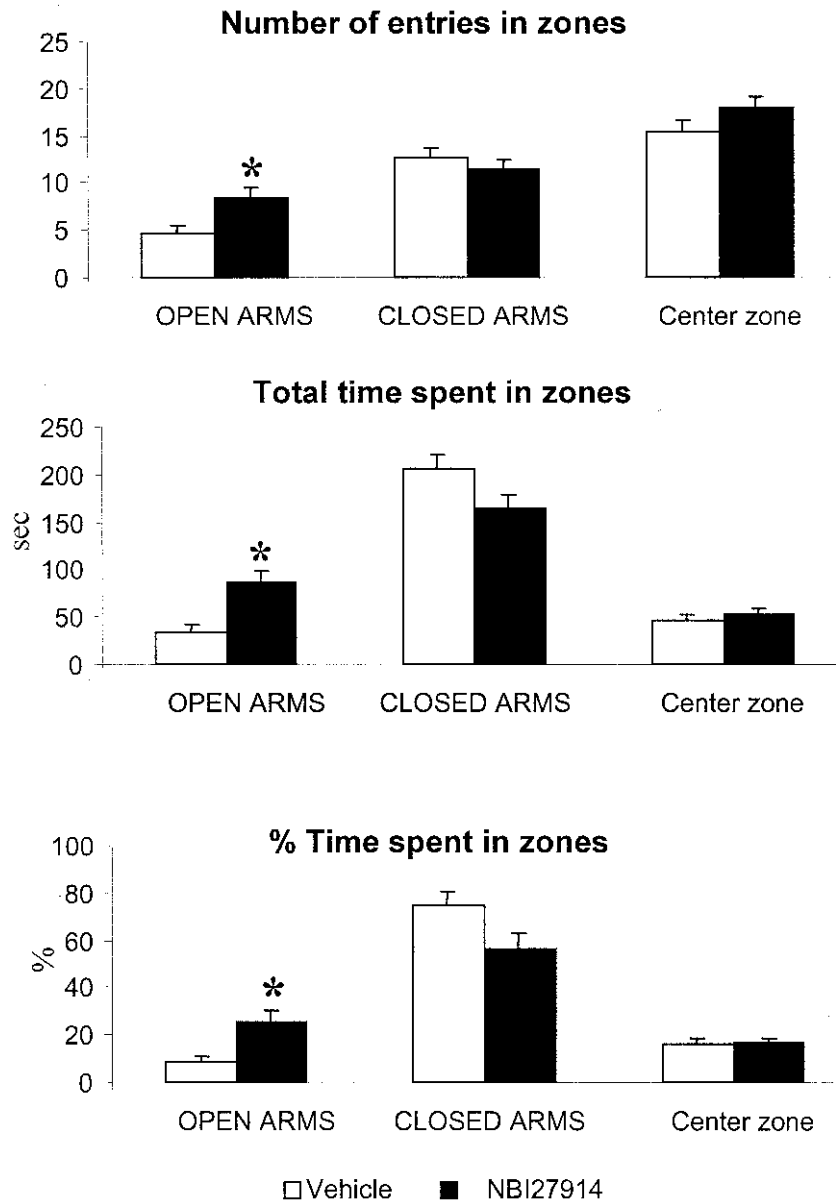


Fig. 3. The effect of the CRH<sub>1</sub> antagonist, NBI27914, on anxiety-like behavior in the elevated plus-maze. Each animal received a subcutaneous injection of NBI27914 (5 mg/kg) or vehicle one hour prior to testing and was tested for 5 min in the elevated plus-maze. \*Significantly different from vehicle-injected animals, ANOVA followed by LSD test ( $P < 0.05$ ).

involved in the stress-induced behavioral changes observed in animal models of anxiety.

CRH has been implicated in the withdrawal and relapse syndromes for a variety of drugs of abuse. Administration of the selective CRH<sub>1</sub> antagonist CP-154,526 prior to naltrexone significantly decreased many of the somatic signs of opiate withdrawal [32]. Anti-stress efficacy of CP-154,526 has also been examined in a paradigm of stress-induced relapse to drug seeking in cocaine- and heroin-trained rats [69]. Rats were first trained to self-administer heroin or cocaine and then responding for intravenous administration of drug solution was extinguished by substitution of saline. A footshock stressor reliably reinstated extinguished cocaine- and heroin-taking behavior and retreatment with CP-

154,526 significantly attenuated the reinstatement effect of the stressor in both heroin- and cocaine-trained rats. CP-154,526, administered in the absence of the footshock stressor, did not affect extinguished drug seeking. These results highlight an important and selective role for CRH or urocortin working through the CRH<sub>1</sub> receptor in the expression of drug withdrawal symptoms and vulnerability to stress-induced relapse.

Recently, CRA1000 (2-[N-(2-methylthio-4-isopropylphenyl)-N-ethylamino]-4-[4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridin-1-yl]-6-methylpyridimidine) and CRA1001 (2 [N-(2-bromo-4-isopropylphenyl)-N-ethylamino]-4-[4-(3-fluorophenyl)-1,2,3,-tetrahydropyridin-1-yl]-6-methylpyrimidine), new CRH<sub>1</sub> selective receptor antago-

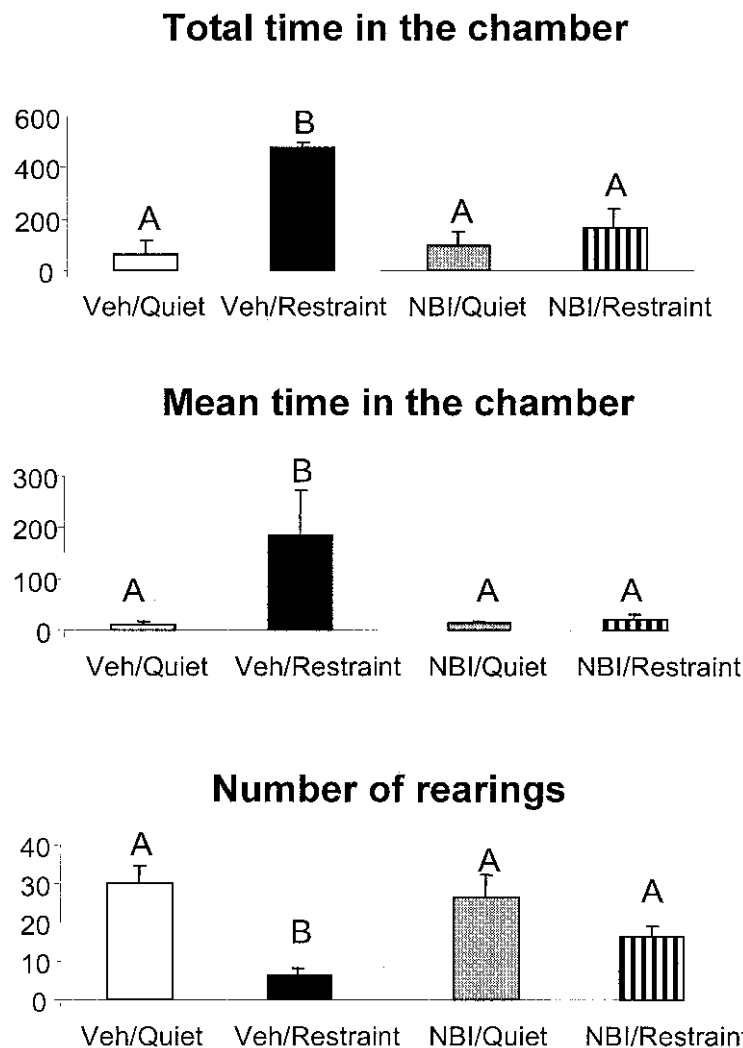


Fig. 4. The effect of NBI27914 on restraint-induced defensive withdrawal in rats. Animals were injected with NBI27914 (NBI) or vehicle (Veh) one hour prior to restraint and restrained for 20 min in plastic restrainers. Behavior was scored for 10 min. Values that do not share common superscript are significantly different from one another (two-way ANOVA, followed by LSD test,  $P < 0.05$ ).

nists have been described [54]. In studies in mice, CRA1000 and CRA1001 administered orally reversed the swim stress-induced reduction of the time spent in the light area in the light/dark box. These compounds also reversed CRH-induced reduction of time spent in open arms in the elevated plus-maze [54].

The functional significance of CRH<sub>2</sub> receptors remains unclear. Because of the lack pharmacological tools, such as potent CRH<sub>2</sub> receptor agonists and antagonists, studies of the role of these receptors in behavioral responses was initially carried out using alternative technologies, such as antisense oligonucleotides. Chronic administration of phosphorothioate substituted 18-mer antisense oligonucleotides designed to bind to the area surrounding the ATG codon of both the CRH<sub>1</sub> and CRH<sub>2</sub> receptor mRNA downregulated the expression of CRH<sub>1</sub> and CRH<sub>2</sub> receptors [27]. Downregulation of CRH<sub>2</sub> receptors using antisense oligonucleotides directed against the CRH<sub>2</sub> receptor mRNA did not affect anxiety-like behaviors [27,42]. Furthermore, there

were no effects on general locomotor activity in an open field or on the spatial learning in a Morris water-maze [42]. However, CRH<sub>2</sub> receptor antisense treatment selectively affected performance of rats in the forced swim test without influencing other behaviors, suggesting a role for CRH<sub>2</sub> receptors in coping behavior in stressful situations [42].

Recently, a highly specific antagonist, [D-Phe<sup>11</sup>,His<sup>12</sup>] Sauvagine<sub>(11–40)</sub>, directed against CRH<sub>2β</sub> receptors has been developed. This antagonist has been called antisauvagine-30 (anti-Svg-30) [63]. It binds to CRH<sub>2β</sub> receptors with a higher affinity (109:1) than to CRH<sub>1</sub> receptors [63]. In context- and tone-dependent fear conditioning of mice, the enhanced retention caused by injections of CRH into the dorsal hippocampus before training was blocked by astressin, but not by anti-Svg-30 [60]. In contrast, the impairment of fear conditioning observed after intraseptal application of CRH was mediated by CRH<sub>2</sub> receptors, as indicated by the ability of both astressin and anti-Svg-30 to block this effect.

Generation of CRH<sub>1</sub> receptor deficient mice has been

reported by two groups. Timpl *et al.* [80] generated a mouse with a truncated protein instead of functional CRH<sub>1</sub> receptor, unable to activate adenylyl cyclase. Smith *et al.* [71] replaced the last 12 amino acids of the first extracellular domain, which resulted in a non-functional CRH<sub>1</sub> receptor protein. Both groups obtained similar results in behavioral tests. When tested in the light-dark box, mice lacking CRH<sub>1</sub> receptors showed less anxiety-related behavior. In the elevated plus-maze, CRH<sub>1</sub> receptor-deficient mice visited and spent more time in the open arms of the apparatus, indicating a reduced anxiety response [71]. In another test, mice were subjected to a forced alcohol-drinking procedure and tested under withdrawal conditions in the light-dark box. During withdrawal, CRH<sub>1</sub> receptor-deficient mice showed a lower latency to enter the lighted compartment that wild type mice made more entries and spent more time within the lighted compartment [80].

To examine the role of CRH in behavior, extensive studies have been performed using CRH-deficient mice (CRH-KO). Published results demonstrate that CRH-deficient mice display normal behavior in multiple categories, including learning, locomotion, responsivity to startle, and pain sensitivity [21,76,86,87]. They also exhibit a normal increase in anxiety-like behavior after restraint or CRH administration in the brain.

We studied the effect of IL-1 on exploratory behavior in the multicompartiment chamber (MCC). The MCC has nine separate compartments, each with the floor and a wire stimulus just below the floor level. The mice lick, chew, or sniff the stimulus, and the mean time to do this was scored as stress-sensitive measure [4]. Wild type (WT) and CRH-KO mice were briefly restrained (15 min), injected with mL-1 (100 ng i.p., 30 min before observation), or CRH (20 ng i.c.v.) and behaviors were observed in the MCC during 30 min session. As shown in Fig. 5, restraint, i.p. administration of IL-1 $\beta$ , and i.c.v. CRH all produced significant decreases in the mean time per contact with the stimulus, and there was no effect of genotype, suggesting that CRH is not mediator of these behaviors [21,36].

## 7. Perspectives for future research

The evidence reviewed above is insufficient for us to understand the role of the CRH system in the behavioral responses to stress. Recent advances have made it clear that the “CRH system” is no longer confined to one peptide ligand, but is composed of at least two (and possibly more) related peptides, and at least two receptors. It is important that both CRH and UCN are active on both known CRH-receptor subtypes, even though their relative affinities for the receptor subtypes (CRH<sub>1</sub> and CRH<sub>2</sub>) differ. On the basis of their findings, Steckler and Holsboer [73] suggested that CRH<sub>1</sub> receptors may be more concerned with cognitive aspects of behavior, including attention, executive functions, emotions, and possibly, learning and memory,

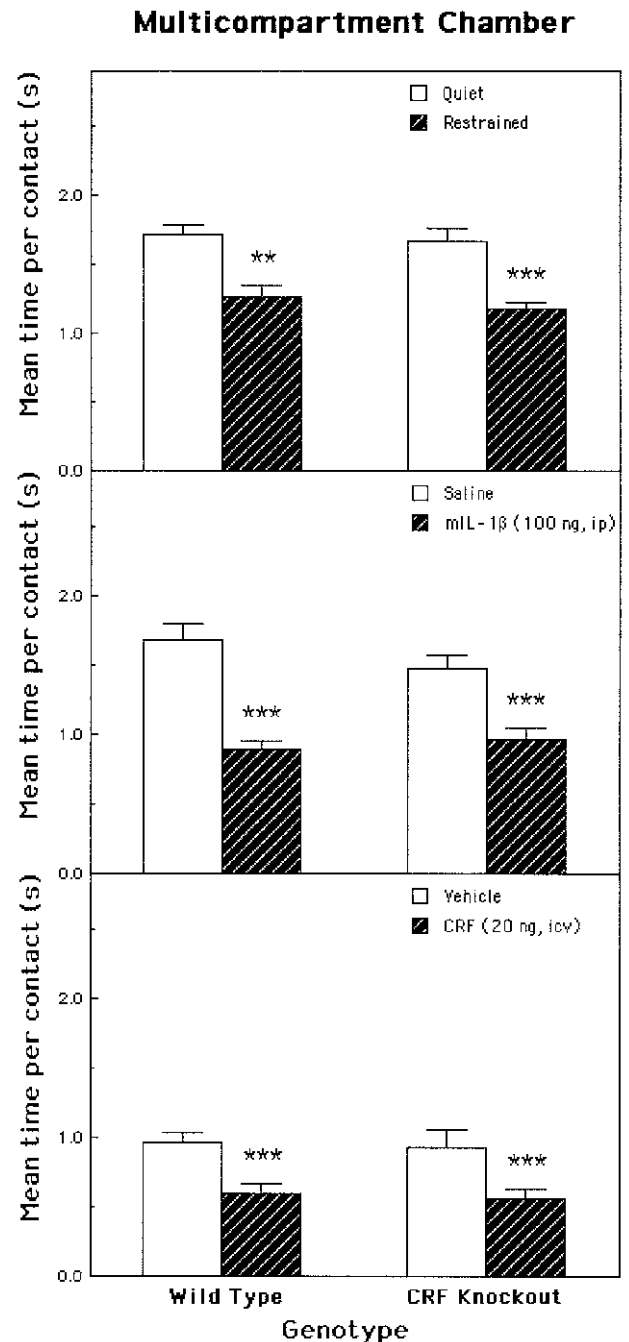


Fig. 5. The effect of restraint, interleukin-1 and CRH on behavior in the multicompartiment chamber in wild type (WT) and CRH knockout (KO) mice. Mice were restrained for 15 min or left quietly in their home cages (top panel); injected ip with 100 ng mL-1 $\beta$  or saline (middle panel); or injected icv with aCSF or 20 ng of CRH (bottom panel). Animals were placed in the chamber immediately following restraint, 30 min after IL-1, or 10 min after CRH. Behaviors were scored for 30 min. \*\*Significantly different from control animals (ANOVA followed by LSD test). Data are from Dunn *et al.* [21] and Weninger *et al.* [86].

whereas CRH<sub>2</sub> receptors primarily influence processes necessary for survival, including feeding, reproduction and defense. Our understanding of the interactions of the various components of the CRH system is currently limited because

of the lack of adequate pharmacological tools, such as highly selective CRH<sub>1</sub> agonists and CRH<sub>2</sub> agonists and antagonists, and because we may not yet have identified all the ligands and receptors.

Studies of mice lacking a functional gene for CRH failed to confirm earlier speculation on the role of CRH in behavior, because the CRH knockouts exhibited minimal alterations in their behavior and in their responses to stressors and to the administration of CRH and UCN [21,76,86]. However, the evidence based on CRH-receptor antagonists (and to a lesser extent from CRH-receptor knockouts is so strong, that it has been suggested that another peptide or peptides acts on CRH receptors to elicit behavioral responses [86]). It is not necessarily the case that such other peptides function only in the absence of CRH. The role of the UCN remains to be assessed. Its expression was not changed in CRH knockout mice, but its mRNA was upregulated by stressful treatments more in CRH knockouts than in wild type mice [86]. A clearer assessment awaits the development of UCN-KO mice, and it may be particularly revealing to study double (CRH + UCN) knockouts, if they can survive.

Thus the new findings have not fundamentally changed the belief that brain CRH is an important mediator of behavior, and especially of stress and anxiety responses. The concept has merely been extended to the CRH family of peptides, and not surprisingly has revealed that the regulatory mechanisms are significantly more complex than originally conceived.

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