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Corticotropin Releasing Hormone: Therapeutic Implications and Medicinal Chemistry Developments

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Abstract—Corticotropin releasing hormone (CRH, sometimes known as CRF) is an endogenous 41 amino acid peptide that has been implicated in the onset of pregnancy, the 'fight or flight' response, in addition to a large number of physiological disorders. Recently, medicinal chemists have developed a number of potent and selective compounds that show promise in a vast array of therapeutic uses. Herein we review the current status of research.

Introduction

Corticotropin releasing hormone (CRH), a 41-residue peptide (Fig. 1) originally isolated from ovine hypothalamus based on its ability to stimulate adrenocorticotropin (ACTH) and β-endorphin release from cultured anterior pituitary cells,[1] is the principal neuroregulator of the basal and stress-induced secretion of ACTH, β-endorphin, and other proopiomelanocortin-related peptides from the anterior pituitary.[2] In addition to its endocrine role in the regulation of the hypothalmic–pituitary–adrenal axis, CRH seems to be implicated in a variety of other central and peripheral functions including food intake,[3] thermoregulation,[4] reproduction,[5] inflammation,[6] and cardiovascular function.[7] CRH exerts its effects by binding to high affinity membrane receptors which are coupled to Gs-protein, resulting in increased intracellular levels of cAMP,[8,9] especially in response to stress.[10,11] CRH is thought to exert its actions on the corticotrope via protein kinase A and protein kinase C.[12] The release of ACTH by CRH causes a release of adrenal glucocorticoids, which is the final stage of the hypothalmic–pituitary–adrenal axis.[13] It has an important role in the immune system in mediating stress responses. CRH has been linked to many central nervous system (CNS) disorders, anorexia nervosa, Alzheimer's disease and inflammatory disorders such as arthritis. In the brain, CRH produces a wide spectrum of autonomic and electrophysiological effects. It activates the sympathetic nervous system with consequential increases in epinephrine, norepinephrine and glucose levels, increased heart rate and mean arterial blood pressure. CRH can also have an opposite effect and cause vasodilation, which reduces blood pressure.

Classification of receptor subtypes

The mediation of CRH is via CRH receptors, several of which have been characterised recently (CRH_1, $CRH_{2\alpha}$, $CRH_{2\beta}$, $CRH_{2\gamma}$, CRF-BP),[13,14] but it is only recently that the presence of $CRH_{2\beta}$[15] and $CRH_{2\gamma}$ in humans[14] has been reported, along with CRH_1, $CRH_{2\alpha}$ and CRH-Binding Protein (CRH-BP). CRH_1 is a 415 amino acid protein that is 80% homologous across species (human, mouse, rat). The $CRH_{2\alpha}$ and $CRH_{2\beta}$ receptors are 411 and 431 amino acids, respectively, with $CRH_{2\alpha}$ being 71% identical to the CRH_1 receptor. $CRH_{2\beta}$ differs from $CRH_{2\alpha}$ by the replacement of the first 34 amino acids by 54 different amino acids.[13] The smallest receptor, CRH-BP, is only 322 amino acids with the rat sequence 85% homologous to the human version.[16]

The CRH_1 and CRH_2 receptors are similar to G-coupled transmembrane proteins and are distributed in the pituitary, cerebellum and cerebral cortex. CRH-BP is

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	1	5	10	15	20	25	30	35	40
Ovine/Carpine CRH	S	Q	E	P	P	I	S	L	D	L	T	F	H	L	L	R	E	V	L	E	M	T	K	A	D	Q	L	A	Q	Q	A	H	S	N	R	K	L	L	D	I	A	NH_2
Bovine CRH	----	-----	-----	-----	-----	-----	---N-	-----	--NH_2
Human/Rat CRH	-E--	-----	-----	-----	--AR-	E----	-----	---ME	-INH_2
Porcine CRH	-E--	-----	-----	-----	--AR-	E----	-----	---ME	$NFNH_2$
Sucker CRH	-E--	-----	-----	-----	--AR-	E----	-----	--MME	-FNH_2
Frog Sauvagine	--G-	-----	-SLE-	--KMI	-IE-Q	EKEK-	--AN-	-L---	$TINH_2$
Carp Urotensin	NDD-	---I-	-----	--NMI	--ARN	ENQRE	--GL-	--Y--	$EVNH_2$

Figure 1. CRH family of peptides.

primarily found in the liver, placenta and brain.[16] There is no significant overlap in the distribution of these receptors in the body, leading to the conclusion that each receptor type has distinct functional roles. The role of CRH-BP in the liver is to facilitate the removal of circulating CRH in the peripheral plasma, which is at elevated concentrations throughout pregnancy.[11,17]

Recently, three forms of placental CRH have been discovered. A high molecular weight form ($M_w > 30{,}000$), a medium molecular weight form ($M_w \sim 7500$) and the 41 residue peptide.[18] When the large form binds to receptors in ovine pituitary cells, it dissociates into the 41 peptide moiety, while still stimulating ACTH. Upon binding, CRH forms an α-helical conformation at the receptor. These α-helices are highly amphiphilic in nature.[18,19] Studies by Rivier et al.[19–22] indicated that the helical nature of CRH ranges from 20% in solution to 80% upon binding to a substrate, which is an unusual occurrence for a hormonal peptide. The helix folds with hydrophobic and hydrophilic regions separated on opposite sides of the helix, though it is unknown whether this occurs before or after binding to a receptor. The first eight residues of the peptide are important for receptor activation while the hydrophobic side chains on residues (5–19) are important for activation and binding. Residues (20–41) in the C-terminal region are more responsible for the formation of the helical structure than binding.[21]

Biological effects of CRH

There is evidence to suggest that CRH has a role in a number of CNS disorders, e.g., anxiety and depression. Clinical studies showed that there is a possibility that hyperactivity of the brain's CRH circuits may contribute to the symptomatology of depressive illnesses. This is supported by pre-clinical studies both in rodents and non-human primates.[27] Additionally, excessive secretion of endogenous CRH potentially inhibits food consumption in experimental animals, and similar pathophysiological characteristics abounding in anorexia nervosa support a hypothesis that CRH plays an active role.[27]

Several studies have provided evidence in support of alteration of CRH in Alzheimer's disease. There are data which suggest reductions in CRH concentration and reciprocal increases in CRH receptor density in the cortex of the brain. A large proportion of the total CRH is complexed with CRH-BP and is therefore unavailable for receptor activation.

Recent evidence suggests that CRH has a direct pro-inflammatory action in rat models of inflammation and arthritis. Moreover, clinical studies indicate an enhanced expression of immunoreactive CRH in situ in synovium from patients with rheumatoid arthritis.

Strategies to counter the deleterious effects associated with high levels of CRH include: inhibition of CRH synthesis and secretion, inactivation of CRH or antagonism of the effects of CRH through receptor blockage. Where low levels of CRH are present, there is a requirement for compounds that act to dissociate CRH from the CRH–(CRH-BP) complex. This will act to selectively increase synaptic levels of free CRH in the brain and have obvious use as potential therapeutic agents in the treatment of conditions associated with low levels of CRH.

Placental/pregnancy

Recently, the regulation of CRH secretion has been linked to the length of pregnancy, termed a 'biological clock'.[23,24] In healthy non-pregnant women, the concentration of CRH in the peripheral blood circulation is extremely low (practically undetectable). During the second and third trimesters of pregnancy CRH levels rise exponentially, to a maximum at parturition, and then fall rapidly back to normal levels after birth (usually 2 days). CRH concentrations can be up to 1000

times greater than the normal level in humans.[25,26] Only the other higher apes (Gorillas, Gibbons) show similar changes in CRH during pregnancy.[27]

In a study of 168 subjects (from the John Hunter and Mater Misericordiae Hospitals, Newcastle, Australia; see Fig. 2), CRH plasma concentrations were estimated every 2–4 weeks after 24 weeks gestation, until 2 days postpartum. It was discovered that CRH levels began to rise many weeks before the onset of labor, and that the levels of CRH were higher in women who had premature births.[25,26] The results of this study were initially rejected because the sample size was deemed too small for statistical significance (there were only 11 preterm births). It was later shown, in conjunction with other studies,[23] that there is statistically significant difference in the concentrations of CRH in the peripheral plasma between women who delivered preterm and those who delivered full term. In those mothers that gave birth preterm, in comparison to those who would achieve full term, at the same week of gestation, there was an increase in CRH concentration up to 25%.

Seventy percent of neonatal mortality occurs as a result of preterm deliveries (delivery before 37 weeks). These infants are 40% more likely to die in their first few weeks, and are more susceptible to other morbidities such as cerebral palsy, blindness, deafness and respiratory illness.[25,27] Neonatal care is one of the most expensive health care budget items in terms of cost per patient, and yet there is currently no accurate test to predict preterm labour. The current system of preterm labour prevention is largely unsuccessful. It relies upon screening processes that identify if a pregnant woman falls into a risk category. Such screening processes include studying the family medical history, blood pressure, and abnormalities detected via ultrasound. If initial analysis is confirmed after further testing, tocolytic drugs are provided to try to prevent premature birth. Unfortunately, the later into a pregnancy diagnosis of risk of premature birth is discovered, the less likely tocolytic drugs will have any benefit.[25]

Preterm birth can be classified into three categories. The first 30–45% comprise of spontaneous premature rupturing of the fetal membranes. The next 25% of preterm deliveries are due to elected birth as a result of deterioration of the intrauterine environment. The final

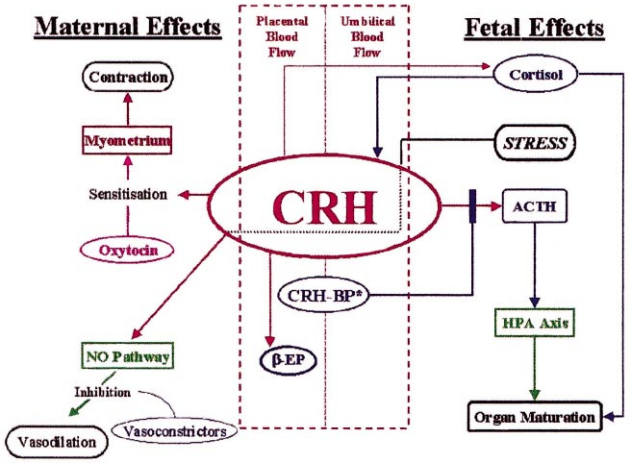


Figure 3. Some of the important roles that CRH plays in regulating chemical and physical responses during pregnancy. Red arrows (red arrow) indicate CRH stimulating a response or synthesis of other substrates (indicated in blue). Physiological effects are indicated by a black box. Green boxes indicate complicated biochemical pathways. *CRH-BP binds to CRH throughout pregnancy and inhibits the synthesis of ACTH. This effect drops markedly as term is approached.

30–45% of preterm births result from premature labour. This last category represents the group in which research into preterm birth prevention may have the greatest success.[28] The most favourable predictor of premature labour so far has been the measurement of plasma CRH levels. The creation of drugs that can be administered to regulate the changes in CRH concentration over the course of a pregnancy would be a substantial step towards the prevention of preterm labour.

CRH production is typically from the hypothalamus, in response to stress, where it is transported via the hypophysial portal system to the anterior pituitary. The production of CRH in pregnant women is primarily localised in the cytotrophoblastic and syncytiotrophoblastic cells of the placenta. A corresponding exponential increase in the concentration of ACTH has not been observed.[13,16,29] The absence of ACTH is accounted for by the presence of CRH-binding protein (CRH-BP) in the placenta, which binds CRH before it can stimulate ACTH release. The concentration of CRH-BP is relatively constant during most of the pregnancy. Closer to

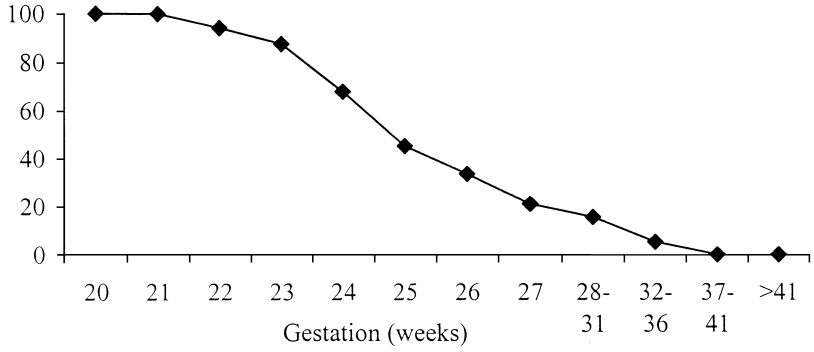


Figure 2. Perinatal mortality, John Hunter Hospital (Newcastle, Australia, 1991–1994).

term, the concentration of CRH-BP drops elevating free CRH concentration.

Though the mechanisms by which CRH acts are currently unknown, a few models have been developed to understand its mode of action throughout the term of a pregnancy. CRH is secreted to both maternal and fetal circulations (in a 10:1 ratio[30]), giving rise to the possibility that the mother and child have the capacity to initiate labour.[17] CRH stimulates the production of ACTH which in turn stimulates the development of the fetal pituitary–adrenal axis, which is involved in the maturation of fetal organs, especially the lungs.[17]

A reason for preterm labour may be due to fetal stress, consequently producing increased concentrations of the steroid cortisol (a glucocorticoid), which promotes maturation of critical fetal organs. Cortisol is normally a negative feedback inhibitor of the synthesis and secretion of ACTH in the pituitary, though in pregnant women fetal cortisol production serves to increase CRH production in the placenta.[29] Glucocorticoids (also catecholamines) returning to the placenta from umbilical blood also promote a positive feedback loop for the secretion of CRH (Fig. 3). CRH is also suspected to precipitate labour by sensitising the myometrium to the effects of oxytocin.[26,31] The myometrium, the wall of the uterus, is comprised of bundles of smooth muscle fibres that are composed largely of collagen. Shortening of these muscles results in tension (contraction).[27] Oxytocin causes contraction of the myometrium. The number of oxytocin receptors in the myometrium increases 6-fold from conception to midterm, another 12-fold before term, and then doubles during labour.[27] Oxytocin is administered intravenously to promote the onset of labour for pregnancies longer than 41 weeks.

A study by Quartero[32] on changes in contracticility of the myometrium in vitro discovered that in the presence of CRH the responsiveness of oxytocin almost doubles than if CRH is not present. CRH does not cause contraction if present on its own (Fig. 4). Additionally, when the myometrium was primed with CRH a short time before the introduction of oxytocin, there was an increased response, than in the absence of priming.

Changes in blood flow in the placental–fetal circulatory system are important because it needs to selectively control the amounts of autacoids circulating in the umbilical blood for proper fetal development.[33] The effects of vasodilation (causing low blood pressure) usually predominate due to the absence of nerves in the placenta, and the presence of vasodilators (regulator of placental vascular tone).[34]

CRH is a potent vasodilator[33] and increases in concentration when there is an increase in fetal distress as part of a mechanism to maintain adequate blood flow (Fig. 3). Complications that cause fetal distress include pregnancy induced hypertension (PIH), intrauterine fetal growth retardation and pre-eclampsia.[35,36] Pre-eclampsia and fetal growth retardation reduce output of nitric oxide (NO) and prostacyclin, and increase the production of thromboxane-A_2 and endothelin-1. The former have a vasodilatory effect while the latter promote vasoconstriction. The latter result in an increased sensitivity to other vasoconstrictors, vascular coagulation and increasing the feto-placental resistance to blood flow and growth.[33]

There is some controversy over the role that CRH plays in vasodilation. It is thought that CRH initiates

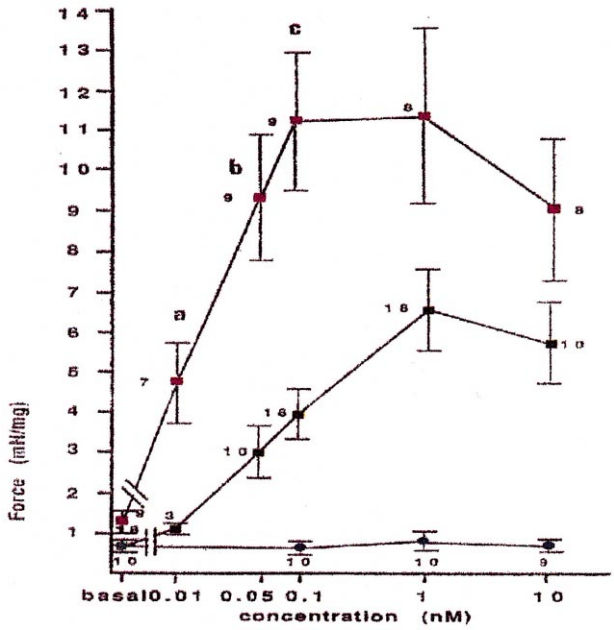


Figure 4. Dose–response curves obtained for CRH (green box), oxytocin (blue circle), and oxytocin in the presence of 1 nM CRH (red square). The number of interventions at each point, mean values and SEM, are plotted. One way analysis of variance indicated CRH and oxytocin/1 nM CRH to be significantly different from basal (CRH: $P < 0.001$, oxytocin/1 nM CRH: $P < 0.001$). Oxytocin concentrations of ≤ 0.1 nM (red square) were significantly different from CRH ($^{a}P = 0.012$, $^{b}P = 0.0019$, $^{c}P = 0.0032$). Data adapted from ref 32.

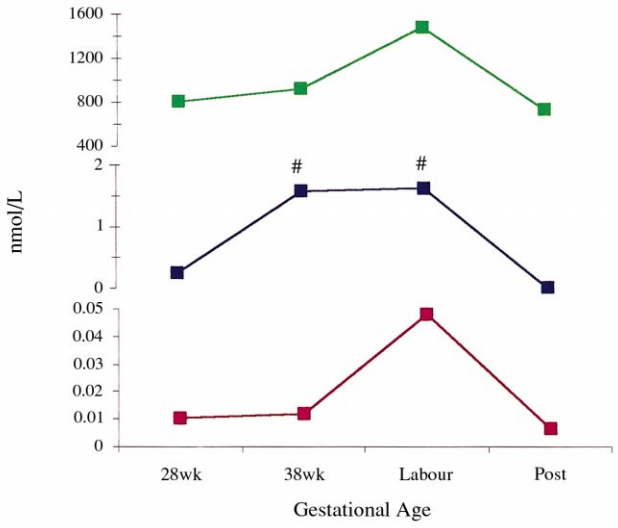


Figure 5. Profiles of maternal plasma CRH, β-EP and cortisol during pregnancy and parturition. Concentrations (mean±SEM) of plasma CRH (blue square), β-EP (red square), and cortisol (green square) rise with gestational age and decline by postnatal day 2 (Post). Within each hormone, all points are significantly different except those marked with a #. Data adapted from ref 37.

vasodilation by indirectly stimulating the synthesis of NO (a potent endogenous vasodilator) that is released from endothelial cells (Fig. 3).[33] Though NO has been postulated to regulate CRH, a study by Roe et al. on the regulatory role of NO on the bidirectional release of CRH between the mother and the fetus produced conflicting results.[30,36]

Fetal stress damages endothelial cells, so it has been hypothesised that because there is a decrease in NO production, CRH concentrations increase to compensate for the drop in vasodilatory action. This effect may also enhance CRH's delivery to other target sites in the placenta (e.g., myometrium).[35] Furthermore if CRH is not able to counteract the effects of fetal stress (restore blood flow), it may cause parturition.[30]

Another effect of CRH is to stimulate the release of beta-endorphin (β-EP) (Fig. 3). It is synthesised by both the pituitary and placenta, though most placental circulation appears to originate from the pituitary.[38–40] β-EP concentration rises during pregnancy and falls dramatically at parturition, following the same changes in concentration as CRH (Fig. 5).[39] Women with larger falls in β-EP concentration at parturition were shown to have a negative childbirth experience,[39] giving weight to the hypothesis that β-EP is released because of the acute stress of childbirth.[38] However, it is unknown whether β-EP also has a regulatory effect on fetal development.[40,41]

While the concentration of CRH is higher in the maternal plasma than the fetus, β-EP concentrations are similar in both. Though it is unknown how CRH and β-EP are dependent upon one another, they have proven to be both effective when studying compounds with potential agonist/antagonist activity. The inhibition or stimulation of β-EP, caused by a test compound, can be used as a secondary indicator of accuracy.[42]

Medicinal Chemistry of CRH

There have been reviews that present short summaries of the then available agonists and antagonists of CRH receptors.[16,43] This section reviews the current status of both peptidic and non-peptidic agonists and antagonists of CRH. It is also noted that the terms CRH (corticotropin releasing hormone) and CRF (corticotropin releasing factor) are interconvertable, and for consistency, we have used the term hormone in this review.

Peptide antagonists and agonists

The peptidic nature of CRH allows study of variations in the hormone itself as a lucrative source of potential agonists and antagonists as well as allowing insights into the action of the hormone itself. For example, it was found in 1992 that residues 1–4 were not required for binding[21] (with this finding confirmed the following year[44]) and that the smallest hormone fragment with CRH comparable activity is CRH(5–41).[21] The entire C-terminal is required for activity, with all alanine residues with higher potency found in this region. These results arose from an alanine replacement series, the results of which are summarized below:

- Alanine replacement series[21] (ovine CRH studies):
  - *No activity*—Ile^{6}, Leu^{8}, Leu^{10}, Phe^{12}, Leu^{14}, Leu^{38} (these residues are mostly conserved throughout the CRH family)
  - *Significant loss of activity*—Ser^{7}, Asp^{9}, Thr^{11}, His^{13}, Leu^{15}, Arg^{16}, Val^{18}, Leu^{19}, Met^{21}, Lys^{23}, Leu^{27}, Arg^{35} (most of these residues, or at least their function, are also conserved)
  - *Equipotent*—Pro^{5}, Glu^{17}, Glu^{26}, Gln^{29}, Gln^{30}, Asn^{34}, Lys^{36}, Leu^{37}
  - *Increased activity*—Glu^{20}, Thr^{22}, Asp^{25}, His^{32}, Ser^{33}, Asp^{39}, Ile^{40}

Hernandez et al. confirmed that Ala substitutions at 22 and 32 increased potency of CRH 3–4 times, and additionally at position 41.[45]

Numerous studies have investigated the presence of D-residues in the peptide with the thought that the increase in potency on the introduction of a D-amino acid may be due to stabilisation of β turns.[44] For example, it is reported that D-substitutions at positions 12 and 20 lead to an increase in potency, whereas all other positions cause a decrease or total loss of potency.[21] Other groups also report that D-Phe^{12} CRH is twice as potent as native CRH and that D-His^{13} CRF has no activity at all.[44] However, a 2-fold increase in potency arose from D-residue replacement at positions 22 or 32, while a 2-fold *de*crease in potency arose from replacement at position 31.[46] Further available information states that [D-Phe^{12}, Nle^{21,38}, Cα-MeLeu^{37}] hCRH(12–41) is more potent and longer acting than CRH and that [D-Phe^{12}, Nle^{21,38}]h/rCRH(21–41) is 15 times more potent than α-helical CRH.[45]

Another study showed an activating region at residues 4–8 and the main binding region at residues 9–41.[44] Further, replacement of Met^{21,38} by Nle leads to an increase in potency. Conclusions drawn include that increasing the hydrophobicity, acidity or basicity at position 12 reduces the potency.[44] An aromatic side chain at position 12 has been reported to be advantageous.[47] Basic residues are also preferred at position 36.[44] The hydrophilic residues of Asp^{9}, Thr^{11}, His^{13}, Gln^{30} and Arg^{35} appear significant and there is a large loss in activity when Ser^{7}, Arg^{16}, Glu^{17} and Asn^{34} are replaced.[47]

In a series of single point alterations, Beyerman et al. found CRH agonist effects down to a concentration of 0.3 nM with changes in Arg^{16}, Ala^{31}, Arg^{35} and amino acids in the N-terminal (5–14) region.[47] They also noted that deletion of the N-terminal sequence 1–8 led to CRH antagonists, and reiterated the importance of residues 4–8 for receptor activity. Residues in non-conserved regions (21–29, 32–33, 36–41) can be replaced without significant loss of activity.

Astressin

Astressin {cyclo(30–33)[D-Phe^{12}, Nle^{21}, Glu^{30}, Lys^{38}, Nle^{38}]hCRH(12–41)}[46] is a potent antagonist with low

intrinsic activity, high receptor affinity and a high solubility in aqueous solution.[52] It has a K_i value of 2 nM[48] and has been injected into the CSF at low doses (1–10 μg)[49] inhibiting surgical stress induced inhibition of gastric emptying, which shows that CRH causes stress-related alterations in gastrointestinal function. The potency of this antagonist can be increased (D-His^{32} doubles the effect) or decreased (D-2Nal^{32} and D-Glu^{32} astressin are 5 times less potent than astressin).[46] From such results, Koerber et al. suggested that the C-terminal residues (9–41) of the CRH molecule are mostly responsible for binding and that an increase in the binding in this region will increase the potency of the agonist/antagonist.[46]

Non-helical analogues of astressin are also considerably less potent. One idea to increase the activity is to increase the stabilisation of the CRH antagonist via insertion of α-helix inducing residues. This also introduces the concept of constraining the peptide to increase the potency of the agonist/antagonist. For example, cyclo(20–23)[D-Phe^{12}, Glu^{20}, Lys^{23}, Nle^{21,38}]hCRF(12–41) and cyclo(20–23)[D-Phe^{12}, Glu^{20}, Orn^{23}, Nle^{21,38}] hCRF(12–41) are, respectively, 3 times and 2 times more potent than the parent compound and [D-Phe^{12}, Nle^{21,38}]r/hCRF(12–41) is 18 times more potent than α-helical CRH.[50] Further selected examples are illustrated in Table 1.

Conformationally restrained analogues can be achieved in a number of ways. The introduction of salt bridges which specifically stabilise the α-helix has been studied via a systematic scanning of the hCRH$_{(9–41)}$ or hCRH$_{(12–41)}$ sequences with an $i–(i+3)$ bridge consisting of the Glu-Xaa-Xbb-Lys scaffold, which has been shown to maintain or enhance α-helical structure.[50] From this series, seven analogues were obtained which were either equipotent or up to 3 times more potent than their assay standard.

Covalent constraints (e.g., side chain-to-side chain lactam rings) also stabilise α-helical conformations.[51] Further results from this paper include:

- residues Ser^1, Glu^{2,3}, Ser7, and Thr^{11} form a polar patch as do residues Glu^{25}, Gln^{26,29,30} and Ser^{33}
- there is a highly charged patch on the N-terminal axial face consisting of Asp^9, Glu^{12}, His^{13}, Arg^{16}, Glu^{20}, and Arg^{23}
- there are two hydrophobic stretches, Ile^6, Leu^{10,14}, Val^{18}, Ala^{22} and Leu^8, Phe^{12}, Leu^{45}, Leu19, Ala^{22}
- deletion of residues 1–7 leads to a 5-fold decrease in activity compared to deletion of residues 1–6
- cyclisation at positions 30–33 holds analogues in a favourable conformation.

Table 1. Selected examples of conformationally restricted CRH peptide analogues with a range of potencies[a]

Structure	IC_{50} (nM)	K_i (nM)
[D-Phe^{12}, Nle^{21,38}]hCRF(12–41)	25.4	56
α-Helical CRF(9–41)	373.7	17
Cyclo(30–33)[D-Phe^{12},Nle^{21,38},Glu^{30},Lys^{33}] hCRF(12–41)	1.0	2.0
Cyclo(30–33)[D-Tyr^{12},Nle^{21,38},Glu^{30},Lys^{33}] hCRF(12–41)	7.0	2.0
[D-Phe^{12}, Nle^{21,38}, Glu^{30}, Lys^{33}]hCRF(12–41)	264.2	525
hCRF	0.027	6.9
[Ac-Pro^4,D-Phe^{12}, Nle^{21,38}]hCRF(4–41)	0.012	1.6
c-(30–33)[Ac-Pro^4,D-Phe^{12},Nle^{21,38},Glu^{30}, Lys^{33}] hCRF(4–41)	0.006	1.1
[Ac-Pro^4,D-Phe^{12}, Nle^{21,38}, Glu^{30}, Lys^{33}] hCRF(4–41)	0.008	4.2

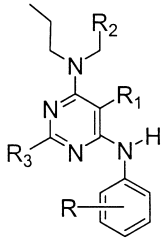
[a]Data taken from ref 48.

Table 2. Peptide CRH receptor agonists[a]

	Mean adenylate cyclase EC_{50} (nM)	
Peptide	CRH_1	$CRH_{1\alpha}$
Urocortin	0.8	0.18
Urotensin I	6.3	1.5
Sauvagine	3.4	1.4
r/hCRH	3.5	13.2
oCRH	9.7	61.9

[a]Table adapted from ref 43. Sauvagine is the equivalent hormone in frogs, and urotensin I from sucker fish.

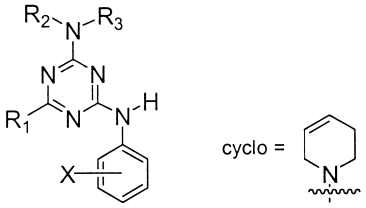
Table 3. Selected pyrimidine analogues as CRH antagonists[a]



R_1	R_2	R_3	X	K_i (nM)
H		Me	2,4,6-Cl_3	30
Me		Me	2,4,6-Cl_3	2.3
Et		Me	2,4,6-Cl_3	3.8
Me	Et	Me	2,4,6-Cl_3	2.5
H		Me	2,4,5-Cl_3	253
H		Me	2,4,6-Me_3	1390
Cl		Me	2,4,6-Cl_3	1.7
Br		Me	2,4,6-Cl_3	2.0
H		H	2,4,6-Cl_3	> 10,000

[a]Data reported represent duplicate determinations with experiments repeated two or three times.[53]

Table 4. Selected triazine analogues as CRH antagonists[a]



R_1	R_2	R_3	X	K_i (nM)
Me	$PhCH_2CH_2$	Me	2,4,6-Me_3	2100
Me	cyclo*	cyclo*	2,4,6-Me_3	1050
Me	nBu	nBu	2,4,6-Me_3	490
Me	$PhCH_2$	nBu	2,4,6-Me_3	1050
Me	nPr	nPr	2,4,6-Me_3	130
Me	nPr	cycloPr-CH_2	2,4-$(MeO)_2$	8000
Et	nPr	cycloPr-CH_2	2,4,6-Me_3	> 10,000
Me	nPr	cycloPr-CH_2	2,4-Me_2	> 10,000
Me	Et	cycloPr-CH_2	2,4,6-Me_3	2260
Me	nBu	cycloPr-CH_2	2,4,6-Me_3	1930
Me	nPent	cycloPr-CH_2	2,4,6-Me_3	No activity
Me	isoPent	cycloPr-CH_2	2,4,6-Me_3	4800
Me	benzyl	cycloPr-CH_2	2,4,6-Me_3	2250
Me	H	cycloPr-CH_2	2,4,6-Me_3	No activity
Me	CH_2CHCH_2	CH_2CHCH_2	2,4,6-Me_3	115
Me	cycloPr-CH_2	nPr	2,4,6-Me_3	57
Me	Et	$PhCH_2$	2,4,6-Me_3	1470
Me	nPr	$PhCH_2$	2,4,6-Me_3	490
Me	H	4-CF_3PhCH_2	2,4,6-Me_3	1690
Me	CH_2CHCH_2	CH_2CHCH_2	2,6-Me_2	> 10,000
Me	cycloPr-CH_2	nPr	2,6-Me_2	> 10,000
Me	Et	$PhCH_2$	2,6-Me_2	> 10,000
Me	nPr	$PhCH_2$	2,6-Me_2	> 10,000
Me	H	4-CF_3PhCH_2	2,6-Me_2	> 10,000
Me	CH_2CHCH_2	CH_2CHCH_2	2,6-Et_2	5700
Me	cycloPr-CH_2	nPr	2,6-Et_2	> 10,000
Me	Et	$PhCH_2$	2,6-Et_2	1700

[a]Data reported represent duplicate determinations with experiments repeated two or three times.[54]

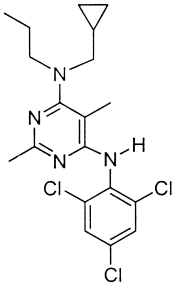


Figure 6. CRH antagonist showing binding affinity of 2 nM.

In a later publication, this same group investigated the possibility of increased antagonist activity after reduction of the amide bonds at the N-terminal, or by subtle alterations of those residues' side chains.[52] After the synthesis and evaluation of a range of peptides with the amide bond reduced (CH_2NH) between residues 6 and 9 in $oCRH_{(5-41)}$, they concluded that neither the individual amide linkages between 6–11 and 12–13, or a carbamide moiety in place of the side chain of Ser^7 led to CRH antagonist.

Other peptide CRH receptor agonists have been reviewed and discussed previously.[43] These are summarised in Table 2.

Non-peptide ligands as CRH antagonists

In recent years, increasing numbers of small molecular weight ligands have emerged as CRH antagonists. Early reports published structures with claims of antagonist activity, but no K_i values or biological data were reported, despite the appearance of patents.[16,43] Since these early reports, a plethora of compounds, with associated antagonist data, has appeared.

For example, numerous substituted pyrimidines and substituted triazine compounds as antagonists have been reported, examples of which are illustrated in Tables 3 and 4.[53,54]

For both these series of compounds, optimisation occurred using rapid microscale synthesis. Consequently, an additional 365 derivatives were synthesised and evaluated for their biological activity.[55] Arising from these studies was an antagonist with a binding constant of 2 nM (see Fig. 6).

These compounds were synthesised with the intention of being used for depression and anxiety related disorders, mediated through the CRH_1 receptor.

Another group also interested in depression and anxiety disorders synthesised a series of over sixty structurally similar compounds to those above, based upon a lead derived from a screening of their chemical library.[56] Their strategy was to synthesise compounds with substituent variations in two key structural components (aryl ring and pyrimidine ring) and to synthesise the analogous triazine compounds. From these analogues,

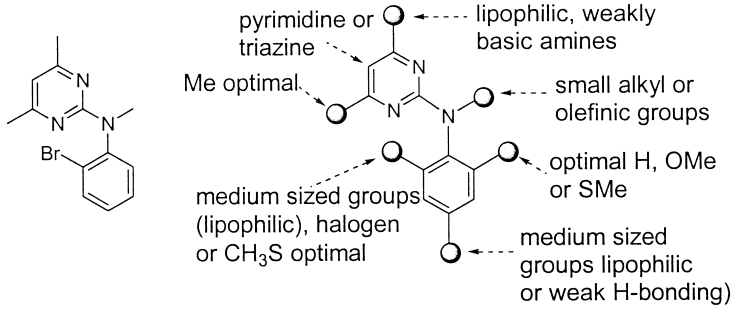


Figure 7. Lead compound and pharmacophore generated from SAR studies of derivatives synthesised. Adapted from ref 56.

they proposed a pharmacophore. The initial lead compound, and an outline of this proposed pharmacophore, are illustrated in Figure 7.

These studies produced compounds with measured K_i ($hCRH_1$) values < 10 nM. A further aim was to improve the pharmacokinetic profile of these compounds without reducing CRH binding affinity.

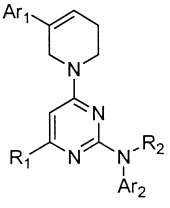
Other pyrimidine-based structures have been synthesised and tested (see Tables 5 and 6).[57] These antagonists were designed targeting the treatment of psychotic disorders including depression and anxiety.

Structure–activity relationship (SAR) analysis of the derivatives in Table 4 reveals that a halogen substituted phenyl substituent as Ar_1 shows the highest affinity, while the equivalent in Table 5 requires a 2-methylphenyl substituent. In both series, Ar_2 is best represented by a 2MeS-4-*i*-Pr-phenyl substituent, and it is essential that R_1 is a methyl group. It is the ethyl group that shows the highest affinity for R_2. These CRH antagonists were developed for stress related studies.[57]

A recent study[58] has reported two compounds that are selective and competitive CRH_1 receptor antagonists, and exhibit anxiolytic and antidepressant-like properties. These structures are highlighted in bold in Table 6.

This study also tested some of their better compounds in behavioural studies against a 'standard' CRH_1 antagonist. These results are summarised in Table 7.

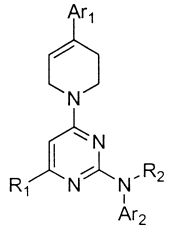
Table 5. Tetrahydropyrimidinopyrimidine analogues as CRH receptor antagonists



Ar_1	Ar_2	IC_{50} (nM)
Ph	2-Br-4-*iso*Pr-Ph	89.0
3-F-Ph	2-Br-4-*iso*Pr-Ph	96.1
4-F-Ph	2-Br-4-*iso*Pr-Ph	82.3
3-Cl-Ph	2-Br-4-*iso*Pr-Ph	180
4-Cl-Ph	2-Br-4-*iso*Pr-Ph	359
3-F-Ph	2-MeS-4-*iso*Pr-Ph	82.3
4-F-Ph	2-MeS-4-*iso*Pr-Ph	27.6
3-Cl-Ph	2-MeS-4-*iso*Pr-Ph	97.7
4-Cl-Ph	2-MeS-4-*iso*Pr-Ph	155
3,4-F_2-Ph	2-MeS-4-*iso*Pr-Ph	335
3,5-F_2-Ph	2-MeS-4-*iso*Pr-Ph	82.3
3,4-Cl_2-Ph	2-MeS-4-*iso*Pr-Ph	> 1000
2-Me-Ph	2-MeS-4-*iso*Pr-Ph	12.7
2-Et-Ph	2-MeS-4-*iso*Pr-Ph	27.6
2-*iso*Pr-Ph	2-MeS-4-*iso*Pr-Ph	44.1
3-Me-Ph	2-MeS-4-*iso*Pr-Ph	70.4
4-Me-Ph	2-MeS-4-*iso*Pr-Ph	70.4
4-MeO-Ph	2-MeS-4-*iso*Pr-Ph	112

Conformationally restricted analogues of the type of compounds shown above have also been developed. Schultz et al. have synthesised a potent, selective non-peptidic CRH antagonist (CP-154,526), that quickly enters the CNS, and may have uses for depression and

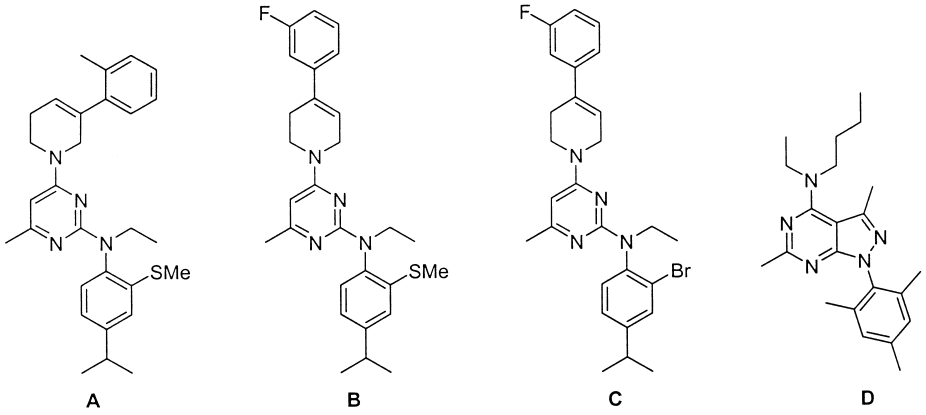
Table 6. Tetrahydropyrimidinopyrimidine analogues as CRH receptor antagonists[a]



R_1	R_2	Ar_1	Ar_2	IC_{50} (nM)
Me	**Et**	**3-F-Ph**	**2-Br-4-*iso*Pr-Ph**	**22.1**
Me	Et	4-F-Ph	2-Br-4-*iso*Pr-Ph	79.3
Me	Et	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	24.8
Me	Et	4-Cl-Ph	2-Br-4-*iso*Pr-Ph	38.9
Me	Et	4-Br-Ph	2-Br-4-*iso*Pr-Ph	242
Me	Et	3,4-F_2-Ph	2-Br-4-*iso*Pr-Ph	50.0
Me	Et	3,4-Cl_2-Ph	2-Br-4-*iso*Pr-Ph	67.3
Me	Et	2-Me-Ph	2-Br-4-*iso*Pr-Ph	133
Me	Et	3-Me-Ph	2-Br-4-*iso*Pr-Ph	65.8
Me	Et	4-Me-Ph	2-Br-4-*iso*Pr-Ph	62.9
Me	Et	2-MeO-Ph	2-Br-4-*iso*Pr-Ph	242
Me	Et	3-MeO-Ph	2-Br-4-*iso*Pr-Ph	139
Me	Et	4-MeO-Ph	2-Br-4-*iso*Pr-Ph	82.4
Me	Et	thiophen-2-yl	2-Br-4-*iso*Pr-Ph	52.2
Me	Et	furan-2-yl	2-Br-4-*iso*Pr-Ph	44.5
Me	Et	napth-2-yl	2-Br-4-*iso*Pr-Ph	> 1000
Me	Et	napth-3-yl	2-Br-4-*iso*Pr-Ph	> 1000
Me	Et	3-F-Ph	2-Br-4-*tert*Bu-Ph	572
Me	Et	3-F-Ph	2-I-4-*iso*Pr-Ph	27.6
Me	Et	3-F-Ph	2-Br-4-Me_2N-Ph	38.5
Me	**Et**	**3-F-Ph**	**2-MeS-4-*iso*Pr-Ph**	**10.5**
Me	Et	3-F-Ph	2-MeS-4-*tert*Bu-Ph	248
Me	Et	3-F-Ph	2-EtS-4-*iso*Pr-Ph	226
Me	Et	3-F-Ph	2-*iso*PrS-4-*iso*Pr-Ph	> 1000
Me	Et	3-F-Ph	2,4-$(MeO)_2$-Ph	187
Me	Et	3-F-Ph	2,4,6-Me_3-Ph	73.9
Me	Et	3-Cl-Ph	2-Br-4-Me-Ph	142
Me	Et	3-Cl-Ph	2-Br-4-*n*Pr-Ph	359
Me	Et	3-Cl-Ph	2-Br-4-*tert*Bu-Ph	> 1000
Me	Et	3-Cl-Ph	2-Br-4-Me_2N-PH	55.9
Me	Et	3-Cl-Ph	2-MeS-4-*iso*Pr-Ph	20.1
Me	Et	3-Cl-Ph	2-Me-4-Et_2N-Ph	> 1000
Me	Et	3-Cl-Ph	2,4-$(MeO)_2$-Ph	359
Me	Et	3-Cl-Ph	2,4,6-Me_3-Ph	129
Me	CH=C–CH_2	3-F-Ph	2-Br-4-*iso*Pr-Ph	32.3
Me	cycloPr-CH_2	3-F-Ph	2-MeS-4-*iso*Pr-Ph	153
Me	CH=C–CH_2	3-F-Ph	2-MeS-4-*iso*Pr-Ph	89.0
Me	CH=C–CH_2	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	96.2
Me	cycloPr-CH_2	3-Cl-Ph	2-MeS-4-*iso*Pr-Ph	153
Me	CH=C–CH_2	3-Cl-Ph	2-MeS-4-*iso*Pr-Ph	51.5
Me	Me	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	292
Me	*n*Pr	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	138
Me	*n*Pent	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	> 1000
Me	*iso*Bu	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	811
Me	CH_2=C–CH_2	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	242
Me	CH=C–CH_2	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	31.3
Me	CH=C–CH_2	3-Cl-Ph	2-MeS-4-*iso*Pr-Ph	112
H	Et	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	> 1000
*iso*Pr	Et	3-Cl-Ph	2-Br-4-*iso*Pr-Ph	> 1000

[a]Entries highlighted in bold are reported to exhibit selective and competitive antagonism at the CRH_1 receptor.[58]

Table 7. Behavioural effects of some pyrimidinal CRH antagonists[a]



Behaviour	Minimal effective dose (mg/kg, po)			
	A	**B**	**C**	**D**
Stress-induced anxiogenic-like behaviour in rats	1	10	10	10
Stress-induced anxiogenic-like behaviour in mice	0.1	3	10	10
CRH-induced anxiogenic-like behaviour in rats	NT[b]	0.3	1	3
Spontaneous locomotor activity in mice	NT	>100	>100	>100
Passive avoidance task in rats	NT	>100	>100	>100

[a]See Tables 4 and 5.
[b]NT, not tested.

anxiety.[59] Table 8 shows this compound's effect against a variety of targets, along with the structure of the compound itself.

Investigations into the bound conformation of these small ligands include a combined X-ray crystal analysis, NMR structure determination analysis and semi-empirical calculations to establish probable binding conformations.[60] As a starting point, the assumption was made that the ligands would bind in a conformation energetically close to a global minimum. It was shown that calculated conformations were observed both in solution and in the crystal form, and that the low energy conformations were significantly separated from other, higher energy conformers. It is interesting to note that, in a reference to unpublished results, it was reported that the anilinopyrimidine ligands do not compete with the peptide agonist, and it is therefore of little value to use models of bound natural agonists as leads in the design of new anilinopyrimidines. However, from the reported conformational studies, a series of twenty three designed, conformationally locked, mimetics were made (for a general structure, see Fig. 8), with antagonist values of down to 1 nM (K_i).

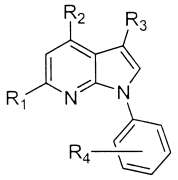


Figure 8. General structure of conformationally locked antagonists.

In an extension of this study, over 100 examples of these conformationally locked derivatives were synthesised, with numerous examples showing activity of <5 nM (K_i). Examples of parent structures are illustrated in Figure 9.

Pharmacokinetic studies were also undertaken (rat and dog) and experiments were undertaken to establish which compounds had properties suitable for potential therapeutics.[61]

Other structural studies related to small ligand antagonists of CRH involve the development of pharmacophore based on some of the anilinopyrimidines and triazines mentioned earlier. Some insights into structure–activity were given, including a clustering of features within one region. Validation of the hypotheses included correlations with newly reported antagonists[62] (Fig. 10).

To date, there has been only one published report of non-peptidic agonists of CRH_1[63] (Fig. 11). These compounds show remarkable similarity in structure to some of the known CRH_1 antagonists; however, it remains to be shown which molecular features are necessary for selective agonist or antagonist activity.

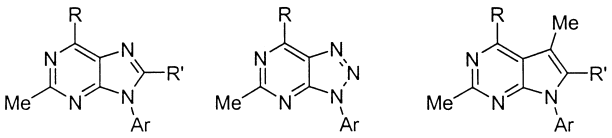


Figure 9. Parent structures of conformationally locked antagonists.

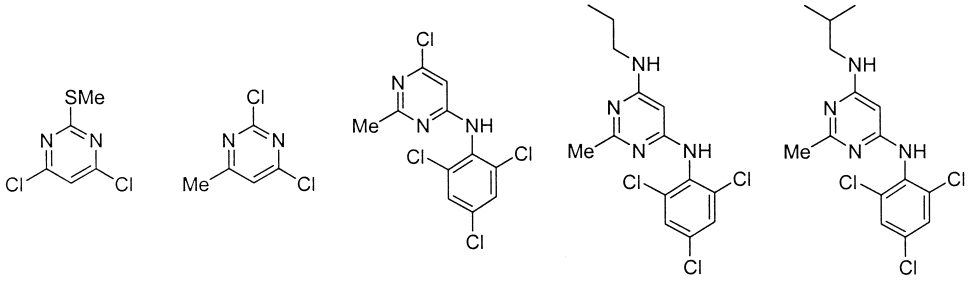


Figure 10. Non-peptide ligands as CRH antagonists.

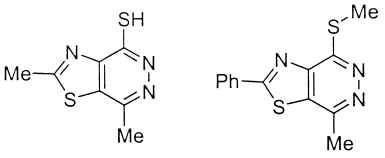
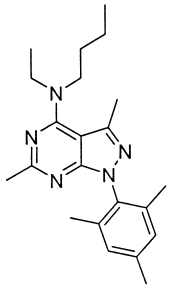


Figure 11. Non-peptidic CRH_1 agonists.

Table 8. Conformationally restricted CRH_1 antagonist CP-154,526 and its effects; the binding constant of α-helical CRH is also presented for comparison



CP-154,526

Tissue	CP-154,526 K_i (nM)	α-helical CRH K_i (nM)
IMR32	2.7	31
Rat pituitary	1.4	30
Guinea pig pituitary	2.9	26
Bovine pituitary	3.5	9.3
Rat cortex	5.7	33
Guinea pig cortex	7.5	51
Dog cortex	6.0	12
Marmoset cortex	9.3	85

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