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Keywords

Corticotrophin Releasing Hormone, CRH, Corticotrophin Releasing Factor, CRF, GPCR receptor, Drug Design, CMMB

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Corticotropin Releasing Hormone - a GPCR Drug Target

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Abstract

Corticotrophin Releasing Hormone (CRH) is a primary hormone in the fight or flight response targeting a membrane bound G-protein coupled receptor (GPCR). Many people worldwide stand to benefit by the development of CRH agonists and antagonists for the treatment of anxiety and depression, with additional therapeutic targets including Alzheimer's, pain and the prevention of premature birth: so why the delay in development? In this review, we will discuss not only CRH, related proteins, receptors and ligands, but some of the obstacles that have arisen, as well as strategies being pursued to overcome these problems in the pursuit of this GPCR targeted therapeutic.

Several key proteins influence the complex and intrinsic regulation of CRH, including its receptors (CRHR), of which 3 types have been categorised, CRHR₁, CRHR₂, CRHR₃, each containing active and inactive splice variants. Additionally, the CRH binding protein (CRHBP) is believed to moderate the effects of CRH at the receptor, whether it is as a molecular mop, or a delivery vessel, or both, is still being investigated.

Homology based receptor modelling is a technique that has only recently become available with the crystallisation of bovine rhodopsin (a GPCR),[1] and the application of this technique to the CRH receptors is still in the early stages of development. Therefore, the medicinal chemist has previously had to rely on ligand-based strategies, specifically, the development of pharmacophores. Thus, an extensive number of both CRH peptide analogues and small ligands that show nanomolar antagonism have been developed with SAR libraries being integral to the iterative drug design process.

Keywords: Corticotrophin Releasing Hormone, GPCR receptor, Drug Design

Abbreviations

CRH	Corticotropin Releasing Hormone
CRF	Corticotropin Releasing Factor
GPCR	G-protein Coupled Receptor
CRHR	Corticotropin Releasing Hormone Receptor
CRHBP	Corticotropin Releasing Hormone Binding Protein
CNS	Central Nervous System
HPA axis	Hypothalamus-Pituitary-Adrenal axis
GHRH	Growth Hormone Releasing Hormone
ECD	Extracellular Domain
TMD	Transmembrane Domain
ICD	Intracellular Domain
IC	Intracellular
3D	Three Dimensional
A.A.	Amino Acid
BP	Binding Protein
IGF-I	Insulin-Like Growth Factor-I
mRNA	messenger RNA
G _s	Guanine Nucleotide Stimulatory Proteins (G-protein)
TM	Transmembrane
cAMP	cyclic AMP
ACTH	Adrenocorticotrophin
PKA	Protein Kinase A
PKC	Protein Kinase C
MAPK	mitogen-activated protein kinase
hCRH	Human Corticotropin Releasing Hormone
oCRH	Ovine Corticotropin Releasing Hormone
rCRH	Rodent Corticotropin Releasing Hormone
aCRH	Amphibian Corticotropin Releasing Hormone
fCRH	Fish Corticotropin Releasing Hormone
HIV	Human Immunodeficiency Virus
PVN	Hypothalamic Paraventricular Nucleus
AVP	Arginine Vasopressin
gp120	glycoprotein 120
AD	Alzheimer's disease
CSF	Cerebrospinal Fluid
SAR	Structure-Activity Relationship
α -hel	α -helical
IC ₅₀	Concentration required for 50% Inhibition
rCRHBP	Rat Corticotropin Releasing Hormone Binding Protein
K _i	Inhibitory Binding Constant
clogP	Calculated Octanol-Water Partition Coefficient

Introduction

There are approximately 800 different G-protein coupled receptors, GPCRs distributed throughout the human body,[2] that are intrinsically involved in the control of the majority of the bodies basic functions. They are the largest known family of signal-transducing molecules.[3] GPCRs are typified as membrane bound receptors, containing seven highly conserved hydrophobic transmembrane α -helices, which coordinate to a heterotrimeric G protein unit, thus deriving the name GPCR. In 1999 up to a quarter of the top 100 selling drugs targeted GPCRs,[4] it has also been estimated that greater than 40% of all current drugs are targeted at GPCRs.[3] Therefore, the potential for drug design and development targeting GPCRs is immense, despite it being currently notably underdeveloped. This review focuses on the history of one example that has undergone considerable development, corticotrophin releasing hormone (CRH) (also known as corticotrophin releasing factor (CRF)), its GPCR, antagonists and agonists and their relationship with regards to therapeutic intervention.

CRH

Corticotrophin Releasing Hormone (CRH) was isolated and characterised in 1981.[5-10] It is a 41 amino acid polypeptide (Fig. 1a),[5-14] formed from the 196 amino acid CRH precursor by the cleavage of the C-terminus.[5] The CRH peptide can be segmented into three functional parts as shown in Fig. 1a. Residues 1-16 are believed to be important for agonist binding and receptor activation.[15] Residues 17-31 contain the CRH-binding protein binding (CRHBP) site and control the structural conformation of the protein,[15] while 32-41 are vital for receptor binding.[15] The 3D structure of this peptide is not

fully established but is believed that the central section of the peptide is α -helical, and that both terminal ends are relatively unstructured, however there is evidence that the C-terminus may form another α -helix, at least when bound to the receptor.[7, 16]

The CRH family of peptides and receptors has been identified in a large variety of species, from mammals to fish and amphibians, but most importantly humans. CRH has wide distribution throughout the CNS and periphery, including the reproductive system.[9, 10, 12] CRH is the key regulator of the hypothalamus-pituitary-adrenal (HPA) axis.[6, 8, 9, 13] The HPA axis influences a multitude of vital bodily functions, the driving force being the variety of stress responses. Therefore the systems influenced by alterations in CRH encompass a large and diverse cross section including cardiovascular regulation, respiration, appetite control, glucose metabolism, immune function, cognitive and motor behaviour, and reproduction. It is through the interaction of CRH with its receptors that it elicits these effects. With such widespread influences throughout the body, it is logical that any imbalances or errors within the system are attributed or connected to a multitude of disorders. Some that are discussed later include neurodegeneration, anxiety, depression, and Alzheimer's disease.

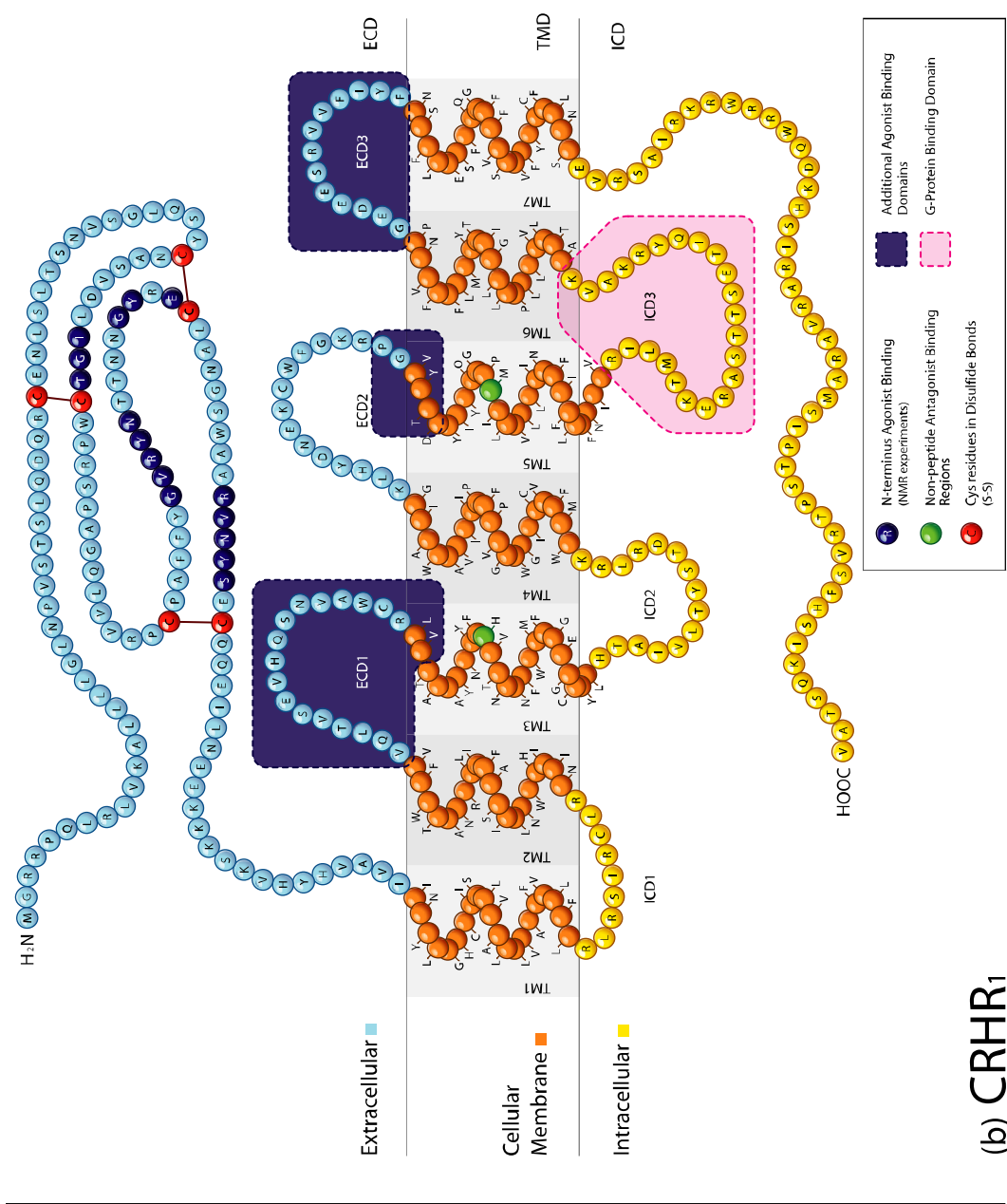


Figure 1: (a) The CRH peptide, showing the various binding regions of the peptide. (b)

The CRH receptor 1 (CRHR₁), the extracellular domain (ECD), transmembrane domain

(TMD), and intracellular domain (ICD). The peptide binding (determined from NMR binding and mutation studies), non-peptide binding sites and G-protein binding sites are also highlighted. Note the NMR binding studies were performed on CRHR₂ but have been extrapolated here for diagrammatic purposes.[14, 17-19]

CRH Receptor (CRHR)

The CRH receptor is a membrane bound G-protein coupled receptor (GPCRs) (Fig (1b)), subclass B,[5, 7, 13, 16, 20] distributed throughout the brain and the periphery. Class B G-proteins are a unique class of G-protein known as the secretin-vasointestinal peptides;[3] there are 15 known receptors in this class.[16] GPCRs are categorised by their nucleotide and amino-acid sequence homology, native ligands, genes, and N-terminus.[3, 21] Class B specifically are activated by neuropeptides and peptide hormones.[3] Included in the class B GPCRs are calcitonin, parathyroid hormone, glucagon-like peptide, and GHRH, some of the applications, current and potential include osteoporosis and Type II diabetes.[16, 22]

There are three types of CRH receptors that have been identified. CRHR₁ and ₂ share 70% homology on the amino acid level and are coded for by separate genes.[19, 23] The general structure of a CRHR and GPCRs in general consists of a N-terminus and 3 loops in the extracellular domain (ECD), 7 hydrophobic α -helical transmembrane domains (TMD), and 3 loops and the C-terminus in the intracellular domain (ICD) (Fig (1b)).[16] The ECD region is the initial region for ligand binding and possesses the greatest variation between the receptor subtypes.[23] The N-terminus region has only 40% homology between CRHR₁ and CRHR₂. The TMD and ICD regions have greater than

80% homology, while the third IC loop is identical between all CRH receptors and is responsible for the G-protein interaction, as highlighted in Fig (1).[5, 7, 20]

CRHR₁

Primarily found in the brain,[5, 6, 11, 12] CRHR₁ is composed of 415 amino acids (α splice variant). It has 8 variants; α , β , c, d, e, f, g, h, however α is believed to be the only active form.[20, 23]

From mutant and chimera studies of CRHR₁, it has been shown that the N-terminus controls peptide ligand binding to the receptor [7, 11, 24] by binding to the C-terminus of the ligand. The ECD1 and 3 and the ECD2-TMD5 junction are also believed to be involved in binding.[7, 14, 18, 19] It is believed that the N-terminus of CRH also binds the TMD with weak affinity and it is this interaction that is responsible for activation of the receptor, however specific residues/domains for this interaction are not known. Mutation studies exchanging residues His199 (TMD3) and Met276 (TMD5) of CRHR₁ with residues from CRHR₂ showed 100 fold decrease in affinity for the non-peptide antagonist NBI 27914; thus it has been postulated that TMD3 and 5 are responsible for non-peptide ligand binding.[7, 19] Therefore, the extracellular domain being responsible for the initial peptide binding and non-peptide ligands are inserted into the membrane bound helix bundle.[7]

CRHR₁ is the primary receptor responsible for the normal responses to stress; it is the primary receptor for CRH and thus a key regulator of the HPA axis. Therefore, it plays an integral role in an array of processes, making it a major target for CRH antagonist therapeutic intervention.

CRHR₂

Unlike CRHR₁, CRHR₂ has three active variants, α , β , γ . The α and β versions are larger, consisting of 411-413 and 413-438 amino residues respectively, and are found throughout the CNS and the periphery, while γ is only 397 amino acids in length and found only in humans solely in the brain.[6, 9, 11, 24]

While CRHR₁ is involved in the normal response to stress, it is believed that CRHR₂ is involved in the fine-tuning of these responses, it regulating the peripheral stress responses, such as metabolism, vasculature, and muscular responses. It also appears to counteract much of the CRHR₁ responses, possibly in a regulatory role to avoid over stimulation.

In 2004 a proposed 3D structure of the mouse CRHR_{2 β} N-terminus region was published based upon NMR structure studies.[17] They concluded that there were two pairs of antiparallel β -sheets and three conserved disulfide bonds that construct the tertiary assembly of the N-terminus. Also identified were amino acid residues that appear to be integral in peptide ligand binding, which were compared to those already identified through mutagenesis studies. This tertiary structure has also been demonstrated for CRHR₁. [16, 25]

CRHR₃

CRHR₃ is the newest of the CRH family of receptors identified and thus far has only been identified in the catfish.[5, 12, 23, 26] It is notably similar to CRHR₁ and CRHR₂ with sequence homology of 85% and 80% respectively.[26] As it has not been identified in

humans and thus is not a potential therapeutic target, it shall not be further examined in this review.

Table 1 summarises the properties, location role and the natural ligands that bind to the CRH receptors and the CRHBP.

Table 1: Comparison of the properties of the peptides that bind CRH; CRHR₁, ₂, ₃ and CRHBP.

	A. A. length	Variants	Primary Location	Ligand Binding	Role
CRHR₁	415(α), (145-444)	α -h	Brain and pituitary	CRH, Urocortin I, Sauvagine, Urotensin	Regulate the stress responses (HPA axis)
CRHR₂	411(α), (397-431)	α - γ	CNS, periphery, brain, heart, vasculature	Urocortin I-III, Sauvagine, Urotensin	Homeostasi s/fine tuning of stress response
CRHR₃	428	-	Catfish brain	CRH	-
CRHBP	322	-	Circulation, brain	CRH, Urocortin, Urotensin I	CRH storage, delivery, clearance

CRH Binding Protein (CRHBP)

Binding proteins (BPs) have been reported for a growing number of hormones throughout the body. Hormones such as insulin-like growth factor-I (IGF-I) and growth hormone have BPs believed to play an essential role in the action and regulation of these hormones.[27] Associated with all CRH function is a CRH binding protein (CRHBP). This water-soluble free protein is 37 kDa in size [11, 20, 27] and consists of 322 amino acid residues [6, 8] and is therefore smaller than the receptors. CRHBP has been identified in both mammalian and non-mammalian vertebrates, including humans, mice, rats, sheep, fish, amphibians (frogs), and birds.[27] The binding protein is highly conserved between species, with 321-324 amino acids across all species.[27] It is highly conserved with 85% homology between human and rat CRHBP [8] and it has been suggested that it is a phylogenetically ancient protein with extensive structural and functional conservation.[27]

Human CRHBP is distributed in plasma, amniotic fluid, synovial fluid, placenta, pituitary, and brain.[27] Rodent and ovine CRHBP however, has not been found in the plasma and in these species CRH mRNA has only been identified in the brain and pituitary.[27] CRH and CRHBP are both expressed in many common regions within the brain, as is the CRHR and CRHBP.[27]

There are 10 cysteine residues located in the protein that form 5 consecutive disulfide loops when folded, and are essential for binding CRH.[27, 28] The greatest sequence diversity is found at the two terminus ends,[27] leaving the core of the BP (residues 40-300) with a high sequence homology of 73% across the species; human, rat, mouse, sheep and frog.

In contrast to the receptors of CRH, the non-membrane bound CRHBP is found throughout the plasma and the CNS, and has 10 fold higher affinity for CRH compared to the receptors.[6, 29] It is estimated that between 60-90% of total CRH in the human brain is complexed with the BP.[29] Investigations into the affinity of various fragments of hCRH or related peptides for CRHBP indicate that residues 9-28 are crucial for ligand binding.[30]

Currently the function of the BP is poorly understood. It is theorised that the binding protein acts as a buffer to regulate the concentration of free CRH and CRH-like peptides, and thus their influence upon the HPA axis.[6, 27] An additional theory is that the binding protein may assist in the clearance of CRH from the body,[27] while yet another suggests that it may act as a CRH transport complex, protecting it (and like peptides) from degradation and assisting in delivery.[27] A further theory is that CRHBP may have its own membrane bound receptor and is mostly associated with the membrane and not free, but this is as yet unsubstantiated.[27, 31] Adding to the debate regarding the function of the BP, is the frog CRHBP, identified as a thyroid-associated hormone connected to the metamorphic phase of tadpole maturation to frog.[27, 32] In summary, the role and function of the CRHBP is not yet fully understood.

Receptor Binding and Signalling

Activation of the CRH signalling pathway can be divided into two areas; receptor binding and pathway signalling. A hypothesis was proposed for the mechanism of binding for class B GPCRs by peptide agonists (such as CRH) involving a two-domain process (Fig (2)).[7, 15-17, 22] The N-terminus of the receptor initially binds the carboxyl terminus of

the peptide; this then initiates rearrangement of the receptor with the insertion of the peptide agonist amino terminus into the transmembrane helix bundle and activation of the receptor (Fig. (2), Step 1 and insert).[13, 16, 17]

The CRHR in the activated form binds the G-protein: guanine nucleotide stimulatory proteins (G_s) (Step 2, Fig. (2)). G_s a heterotrimeric G-protein, consists of three subunits, G_α - G_β - G_γ , and at least 28 different G_α subunits have been identified.[21]

The G-protein trimer is associated with the inactive GDP. Upon interaction with an activated receptor, the GDP is converted to GTP and binds to the G_α subunit (Fig. (2), step 3), the G_α -GTP complex then dissociates from G_β - G_γ and the separated parts proceed to activate a multitude of different 2nd messenger pathways (Fig. (2), step 4).[21] One point to note is that receptors do not have to be activated for coupling with G-proteins, and this coupling can in fact promote ligand binding.[16, 17]

Non-peptide CRH ligands (antagonists), have a different binding mode to the peptide ligands. It appears that the small molecules have no interaction with the ECD, but insert themselves directly into the TM domain (Fig. (1)), specifically shown to have affinity for residues 199 and 276 in TM2 and TM5 respectively.[7, 19] After insertion of the non-peptide ligand into the helix bundle, the ligand allosterically blocks the 2nd domain insertion of the peptide ligand binding, thus acting as antagonists to CRH and its associated ligands.[13, 16, 17]

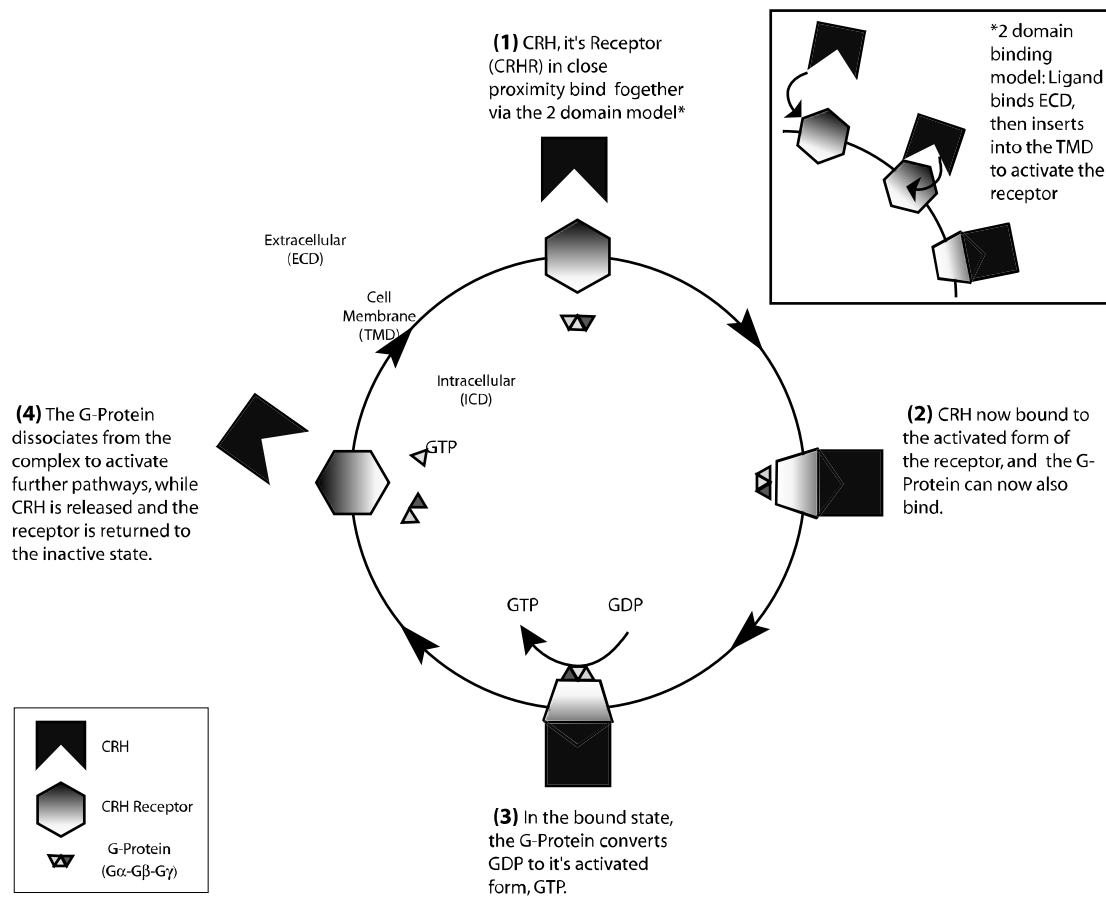


Figure 2: The CRH signalling/binding cycle. Details of the step-wise binding and activation of the CRH peptides are shown, including the 2 domain binding model.[16]

Therefore, the signalling from the activated receptor and G-protein initiates the G-protein activation stimulation of adenylyl cyclase that in turn activates protein kinase A (PKA) and other cAMP pathways. They can stimulate adrenocorticotrophin (ACTH), β -endorphin and other proopiomelanocortin related peptides from the anterior pituitary,[8] protein kinase C (PKC), protein kinase B/Akt, p44/p42 and p38 mitogen-activated protein kinase (MAPK), intracellular Ca^{2+} , nitric oxide synthase, guanylate cyclase, prostaglandins, steroidogenic enzymes, FasL production and apoptosis.[12, 33] As a result the activation

of the CRH receptor has a multitude of different potential influences upon cells that are intrinsic to cellular, and bodily functions.

Natural Ligands

CRH is the endogenous ligand for the CRH receptors, however over time an increasing number of natural ligands have been identified for CRHR₁ and CRHR₂. At least five CRH-like agonist ligands have been recognised across a variety of species, Urocortin I, II, III, Urotensin I, and Sauvagine (Table 2).

Urocortin I, II and III have been identified in numerous species (Table 2), from mammals to fish.[5] Urocortin I was discovered in 1995, followed by II and III in 2001,[34, 35] these three peptides may share the same name but they have very different binding affinities. Urocortin I is non-selective between the CRH receptors, slightly favouring CRHR₁, while Urocortin II and III are selective for CRHR₂. [15] It has been theorised that CRH is the endogenous ligand for CRHR₁ and Urocortin is the endogenous ligands for CRHR₂ (particularly II and III).[7] Both Urocortin II and III seem to influence appetite, acting as a suppressant rather than playing a role in the stress response,[34] and Urocortin III has been linked to glucagon and insulin secretion.[35] Human Urocortin II, also known as Stresscopin has been identified from the human genome, however it appears to lack the processing genes to produce it, there is conjecture as to the entire identity of this gene.

Initially identified in fish, Urotensin I has since also been identified in mammals. Similar to CRH, it is a 41 amino acid peptide chain but is reported to only share 50% homology

(Table 2). Urotensin is a non-specific ligand with high affinity for CRHR₁ and CRHR₂, but uniquely has little affinity for the BP.[29]

Sauvagine is a linear amphibian ligand of 40 amino acid length.[36] While being highly potent for both CRH receptors it does bind with higher affinity to CRHR₂, it is 5-10 times more potent for these receptors than CRH.[6, 8, 37] It has been suggested that there is still a sauvagine like ligand in mammals that is yet to be discovered that would be the native ligand for CRHR₂ rather than Urocortin.[38]

The only CRHR₁ selective natural ligand identified is ovine CRH (oCRH). Table 2 shows all other ligands possessing good affinity for both or favour CRHR₂ binding.[15, 39] While not found in humans, this ligand has the closest homology to CRH, therefore it potentially provides a useful tool for investigating both receptor locations and for refining our understanding of the differences in ligand-receptor interaction for the two CRH receptors.

Table 2: Comparison of CRH and other natural ligands and their receptor selectivity. The oCRH is the only peptide selective for CRHR₁, Urocortin II and III are selective for CRHR₂, there is no peptide selective for only the BP. Human=h, rodent=r, ovine=o, amphibian=a, fish=f [5, 30, 35, 39]

Ligand	Animal	A.A.	% Homology	Binding Affinity		
		Residues	to hCRH	CRHR ₁	CRHR ₂	CRHBP
hCRH	h	41	100	High	Medium	High
oCRH	o	41	83	High	Low	Low
Sauvagine	a	40	48	High	High	Medium
Urocortin I	r, h, o	40	44	High	High	High
Urocortin II	h, r, f	38	34	Low	High	High

Urocortin III	h, r, f	38	30	Low	High	Low
Urotensin	f	41	50	High	High	Low

CRH in Disease and Therapeutic Application

CRH has a broad spectrum of influences upon the human body and therefore has direct implications for the intervention in a wide-ranging number of disease states. For example, one of the many side effects of HIV-1 infection is neurodegeneration. This condition has been largely attributed to increased activation of the HPA axis. It is believed the HIV viral coat protein gp120 acts at the hypothalamic paraventricular nucleus (PVN) to stimulate CRH and arginine vasopressin (AVP) production and thus stimulation of the HPA.[40]

There are multiple neurological diseases proposed as potential drug targets for CRH agonists and antagonists including anxiety, depression, Alzheimer's disease, and post traumatic stress disorder, to name a few.[41-44]

It has been proposed from rat models that a CRHR₂ agonist may serve as an anxiety therapeutic. In contrast to CRHR₁ that induces anxiogenic properties, CRHR₂ was found to stimulate anxiolytic properties. By targeting CRHR₂ for anxiety, it has been suggested that side effects would be minimised due to the few known biological roles it plays in control of the body's equilibrium.[41] It has also been suggested that CRHR₁ antagonists

would also have low side effects with respect to the treatment of depression. CRHR₁ antagonists have been found to exert their effect only during high stress situations, when the HPA axis and CRH are activated.[42] Thus, it is expected that these antagonists would have minimal effects and would be observed only during stress.

During high chronic stress situations, cortisol levels are elevated for prolonged periods. Cortisol is believed to be toxic to neurons with long-term exposure and the damage caused is believed to impair the ability to form memories thus inducing post-traumatic stress disorder. As CRH is one of the primary stimulating hormones in the production of cortisol, a CRH antagonist is a potential treatment to assist in the recovery from trauma.[43]

Alzheimer's disease (AD) is typically characterised by the formation of the destructive amyloid β -peptide,[44] and decreased levels of CRH in CSF fluid.[29, 43] It is reported that CRH prevents cell death caused by amyloid β -peptide and thus could slow or prevent the onset of Alzheimer's disease.[44] Ligands capable of displacing CRH from its binding protein, thus increasing free CRH, may have potential therapeutic application and have been shown to increase spatial learning and memory in rats.[29, 44]

The CenterWatch Drugs in Clinical Trials Database (USA) reports four CRH related drugs have recently reached clinical trials;[45] two are peptide-based whilst the other two are non-peptide small molecules. Xerecept, which is the trade name for the synthetic preparation of CRH, is the most advanced, reaching phase III trial in 2004. It is to be tested for the treatment of peritumoral cerebral oedema resultant from brain tumours (brain swelling due to brain tumours), utilising the vasoconstrictive properties of CRH after trauma injury.[45] Urocortin II a CRHR₂ selective agonist was placed in phase I

clinical trials in 2004 for the treatment of congestive heart failure. It exhibits hypotensive and anxiolytic effects via CRHR₂, and is located in the cardiovascular system, and is expected to move into phase II early in 2005. There are reports (2002-3) of 2 separate CRHR₁ non-peptide antagonist in phase I clinical trials for depression and anxiety, NBI-34041 and SB723620 (NBI-37582) although the outcome of these trials is unclear.[45]

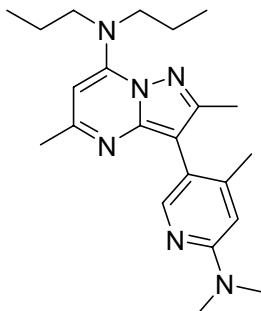


Figure 3: CRHR₁ non-peptide antagonist R121919 (NBI 30775) underwent phase II clinical trials in 1999.

Phase II clinical trials were carried out on the CRHR₁ antagonist R121919 (NBI 30775), shown in Figure (3), a water-soluble pyrazolopyrimidine,[46] as an antidepressant in 1999. The results of the 24 patient trial appear to be positive for CRH antagonists as viable antidepressants. The drug showed an improvement in the symptoms of depression and an increase again at the cessation of treatment.[47] Weight gain and leptin levels were also monitored due to the known role of CRH in weight control and no effect was observed.[48] Although not definitive, R121919 also presented some evidence of stabilising effects on sleep patterns of major depressive patients.[49] Liver toxicity was observed at high dosages of R121919, reversible elevations of liver enzymes were observed in a parallel study with healthy controls, thus halting all further testing with R121919.[46, 50] It was concluded that CRHR₁ was a promising antidepressant target,

with minimal side effects observed, and the liver toxicity is believed to be a result of that specific compound and not the mechanism of action;[50] thus other CRHR₁ are not expected to show such toxicity. It is worth noting that no placebo was used in the trial.[16]

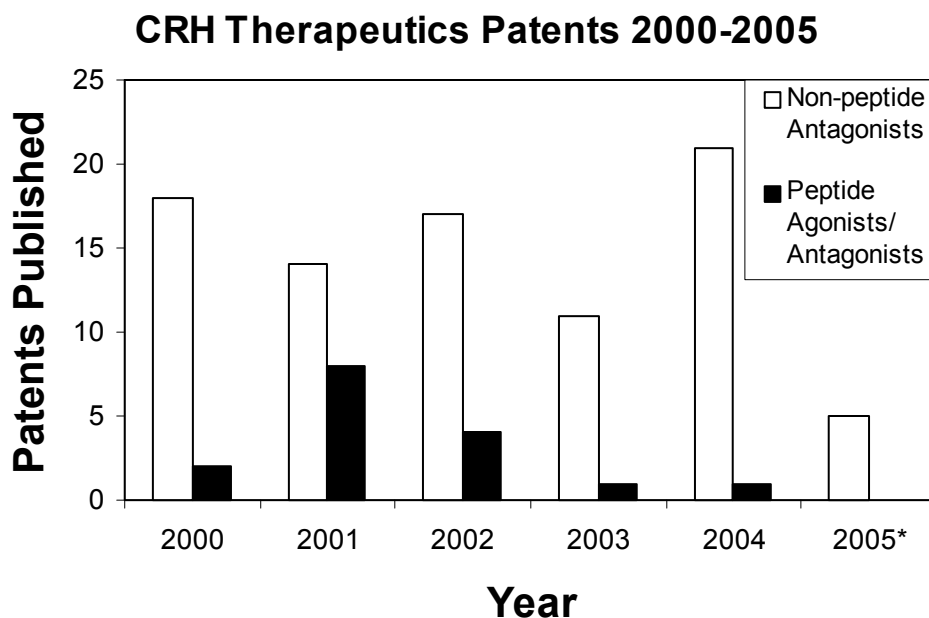


Figure 4: Non-Peptide and Peptide agonist and antagonist patents published since 2000.

(* As of 11th May 2005.)

Since 2000, numerous patents have been published for potential CRH targeted therapeutics (Fig (4)). The majority of these have been targeted at CRHR₁ and in particular non-peptide antagonists. The year 2004 was the most prolific year thus far for non-peptide CRH antagonist patents; unfortunately, the consistent turnover of compounds/patents (over 100 since the year 2000) still has not seen a successful therapeutic reach the market.

Antagonists Drug Design for CRH

As CRH targets a GPCR, it presents a difficult challenge to design selective receptor antagonists. Several techniques have been adopted in an attempt to develop highly active, specific and novel antagonists for this drug target. There are two types of antagonists, peptide and non-peptide, both types have had compounds reach at least phase I clinical trials and are relevant potential therapeutics.

The peptide antagonists are largely based upon the natural ligands of the CRH receptors. The main advantage of this type of antagonist is its ability to mimic the endogenous ligand and integrate into the body's systems. They have been developed from structure-activity relationships (SAR), mutant, chimeric, and substitution studies.

Non-peptide antagonists have also been extensively published. Considering the little information known about the receptor-binding site for these compounds, work in this area has been successful with numerous sub-nanomolar affinity compounds published over the last 10 years. These compounds have been developed initially using library screening and now in combination with SAR work.[6-10, 51-53]

Peptide Antagonists

All of the non-natural peptide ligands, both agonists and antagonists, have been developed from SAR work and derivatisation of the natural ligands. A large variety of alterations from chain shortening/lengthening, residue substitution, methylation, acylation, radiolabelling, cyclisation, and confinement to helical configuration (via a lactam bridge), have been performed in an attempt to develop new, more potent ligands.

The potential for CRH to form a helix at the C-terminal, theorised to be the active form of CRH, was the motivation for the development of the α -hel-CRH₍₉₋₄₁₎,[7] the first CRH

antagonist developed.[54] A more active derivative, astressin, {cyclo(30-33)-[D¹²Phe, Nle^{21,38}, Glu³⁰, Lys³³]r/h CRH₁₂₋₄₁} is also α -helical, utilising a lactam ring to constrain the peptide and is reported to be about 32 times more potent than α -hel-CRH₍₉₋₄₁₎ (Table 3) for CRHR₁. [54] It is believed that astressin may have neuroprotective properties that would be useful in treating ischemic damage.[55] Interesting to note is the inverse affinity these ligands have for the binding protein.

Most of the peptide antagonists developed have been synthesised from natural ligands with shortening of the N-terminus region. This supports the hypothesis previously discussed,[17] that ligand binding occurs in a two-step model with the carboxyl terminus of the ligand binding, and then the amino terminus inserting into the transmembrane domain to activate the receptor. The absence of the amino terminus end of the antagonists means inability to initiate the activation of the receptor but effectively blocks the endogenous ligand from binding the receptor.[16, 17]

Numerous CRH natural ligands have now been radiolabelled. Human and ovine CRH, Urocortin and Sauvagine have all been labelled with ¹²⁵I and used in radio assays to investigate receptor distribution, antagonist binding and ligand receptor interactions.[56-58] Urocortin has also been labelled with tritium.[57] All of the radiolabelled compounds have reported minimal effects on binding and appear to be useful tools in investigating this complex hormonal system.

Table 3: Receptor affinity (IC₅₀ (nM)) of human CRH and Sauvagine, both of which are native CRH ligands, and comparison with some synthesised peptide antagonists. NB: no binding observed (to rCRHBP).[39, 59]

	CRHR₁	CRHR₂	CRHBP
hCRH	1.6	42	0.54
Sauvagine	0.52	0.92	57
α-hel-CRH	61	4.3	1
Anti-sauv-30	370	0.30	NB
astressin	11	5.2	90

CRHR₁ and ₂ are not the only targets for CRH related antagonists. The CRHBP has not been as widely targeted however, at least one patent for peptide and/or non-peptide antagonists was published in 1996.[31] The therapeutic is for the treatment of Alzheimer's disease utilising a CRHBP antagonist, with the premise to block CRH from the BP thus increasing the levels of free CRH as described.[31] Ligands such as hCRH(6-33) have been designed with high specificity for binding to the CRHBP over CRHR.[27, 30] For example, a comparison of the CRF-BP ligand inhibitor, r/hCRF(6-33) with CRH in mice and rats showed a reduced food uptake observed in mice.[60]

Non-Peptide Antagonists

In 1991 the first CRH non-peptide antagonist patent was published describing a series of pyrazolones.[6, 9] The progression from then was slow until 1996,[61-63] but subsequently hundreds of CRH non-peptide antagonists have been published. Despite the great number of these compounds, none have made it onto the pharmaceutical market. A possible explanation of this can be shown by a timeline of antagonist development (Figure (5)). Until 2002, only two structural scaffolds had emerged, derivatives of those

developed in 1996. From 2002 onwards a much greater diversification has occurred accompanied by further derivatisation and optimisation of the current scaffolds, but no clearly successful drugs. The need is evident for new structural diversification if a drug is to be developed due to the problems that are plaguing the current leads.

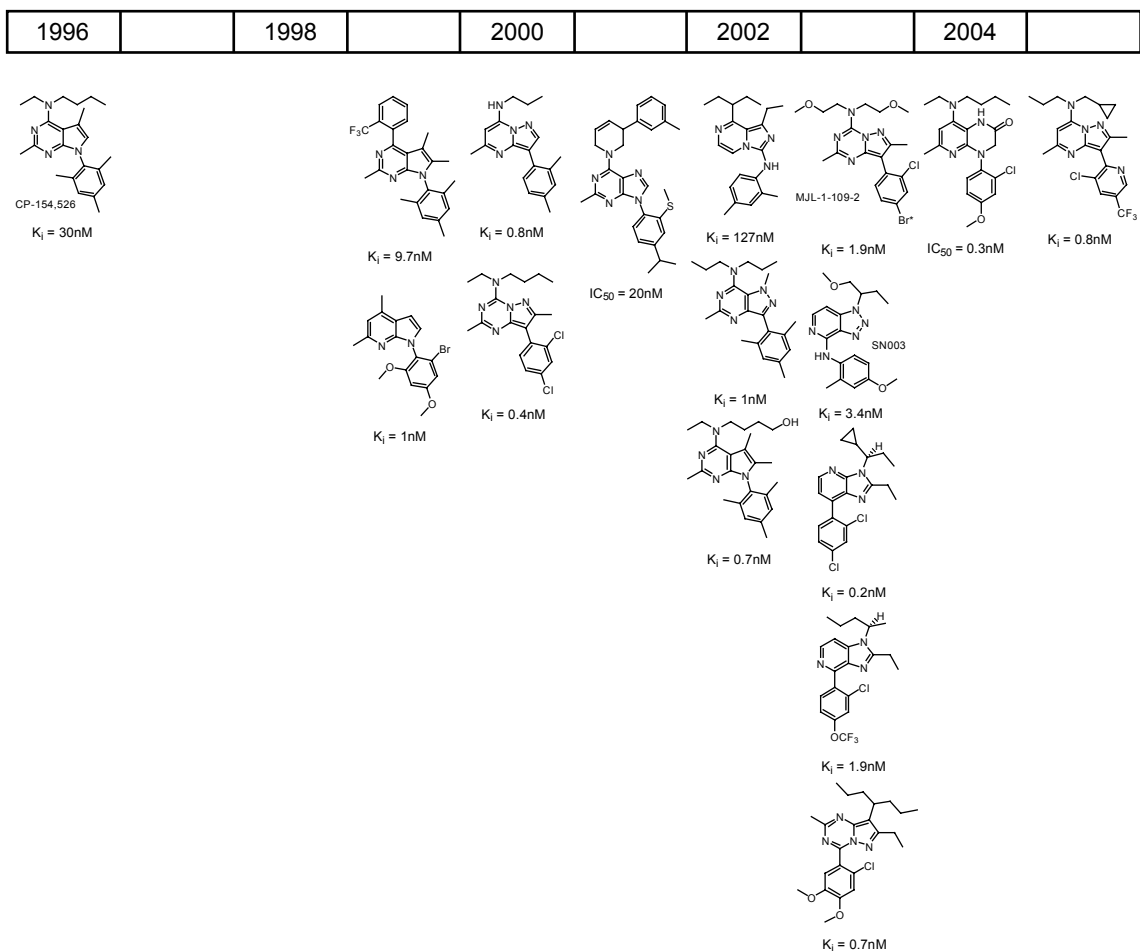
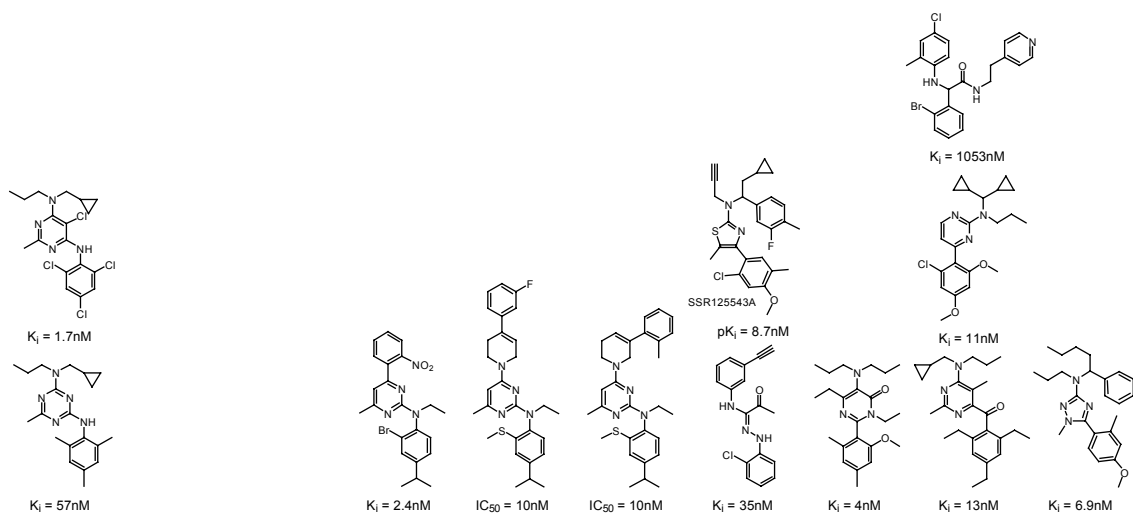


Figure 5: Timeline depicting examples of published non-peptide CRH antagonist development.[6, 61, 62, 64-84]

Although biological activity is prominent, with many sub-nanomolar derivatives available, substantial problems prevail and include poor bioavailability, toxicity, lack of structural diversity, and potentially low specificity (high receptor distribution) and thus high possibility of side effects.[16, 85]

Solubility is a considerable issue with CRH non-peptide antagonists. The hydrophobic nature of both the membrane bound GPCRs and the hypothesised binding domain within the transmembrane region means that antagonists need to be largely hydrophobic - the majority have a calculated LogP (clogP) (octanol-water partition coefficient) greater than 3. With such hydrophobic compounds, the most difficult challenge is maintaining reasonable bioavailability to ensure they dissolve into the circulatory system and are also able to cross the blood brain barrier (the primary target for most potential applications). Thus far, in an attempt to overcome these difficult problems, attention has turned to increasing the number of heteroatoms, particularly in the aromatic rings and utilised ionisable derivatives.

As discussed previously toxicity has been a problem for at least one non-peptide antagonist, R121919. While this toxicity is believed to be independent from the compounds mode of action at the receptor and thus not likely to extend to other antagonists, this is still yet to be confirmed in human trials. This liver toxicity was also only observed at high doses.[50]

CRH is still a relatively new drug target (of the last 10 years) and as relatively little detail is known of GPCRs and specifically the CRH receptor binding site, this poses a difficult therapeutic target for drug design and development. As a result, there is little structural diversity contained within small ligand antagonists leading to a restriction on available

information but has allowed for detailed SAR of the compounds. Figure (6) shows some examples of the molecules and the three key regions required for activity for the small molecule antagonists of CRHR₁, (A), (B), (C). The lower region, (A), is the most highly conserved and is a substituted aromatic moiety, mostly with a 2,4 or 2,4,6 substitution pattern. It is believed that this ring is angled into the plane in comparison to section (B).[7, 9, 10, 78] The middle region (Fig. (6) (B)) consists of a 5 or 6 atom aromatic heterocyclic core and has become diversified in recent years with the most widely investigated being the pyrimidine based core structures;[65] these rings can be fused to additional rings,[62, 86] have additional heteroatoms, and there has also been examples with 5 membered rings and linear conjugated heteroatoms.[71, 72] It appears most importantly for this region that one of the heteroatoms is a nitrogen (H-bond acceptor) and is located 2-3 bonds away from the aromatic ring of region (A). The third region (Fig. (6) (C)) is the most diversified region, the only consistency maintained being its hydrophobic nature. Many of the substituents in this region connect to region (B) via a weakly basic tertiary amine.

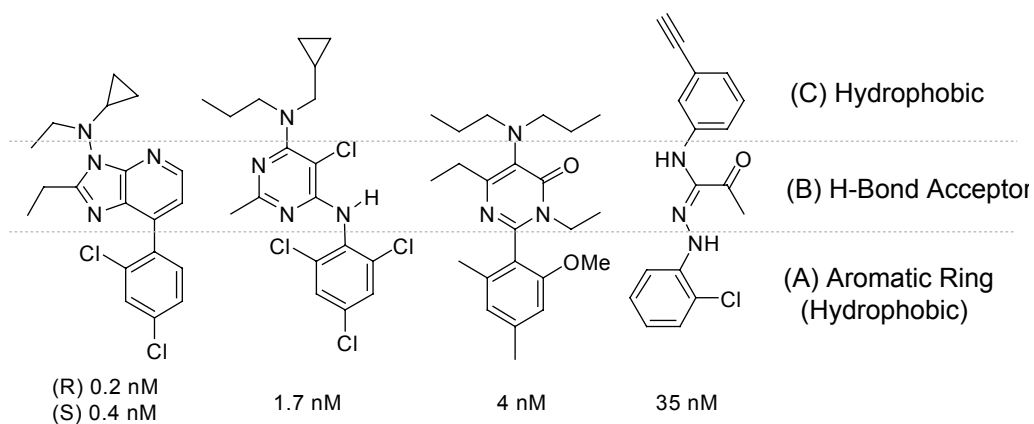


Figure 6: Structure-activity relationships for CRH non-peptide antagonist. There are three regions (A, B, and C) that is necessary to consider.[61, 71, 73, 87]

The potential side effects due to the wide spread action and location of CRH receptors is a relevant issue. From the clinical trial of R121919, it appears that CRHR₁ antagonists are promising drugs with little if any side effects.[47-50] From the reports, the drugs had no adverse effects on hormonal systems (including the HPA axis),[50] weight gain [48] or sleep patterns, if anything stabilised them to normal at the higher doses.[49] This little influence on the multitude of homeostatic systems regulated by CRH can be attributed to three key points. The CRH systems are highly controlled with negative feedback and backup systems in place to minimise disturbances; it has been shown that there is no effect on the HPA system in rodents under normal conditions.[20] The CRHR₁ is located in the brain and pituitary and does not directly influence the body's periphery, thus eliminates those side effects. Finally CRH has the greatest influence on the bodies homeostasis when stressed, and therefore non-peptide antagonists are unlikely to exhibit much influence except under stressed conditions; this theory has been tested in rodents.[20]

As noted earlier, antagonists of the CRHBP could have potential applications for treating Alzheimer's disease. Currently there is only one patent for non-peptide CRHBP antagonists published, WO9851312.[88] These compounds are thiadiazinedione dioxides, Figure (7), are structurally unique to any of the other CRH non-peptide antagonists and reportedly increase levels of free CRH in the brain. Further information describing these molecules as therapeutics is not reported.

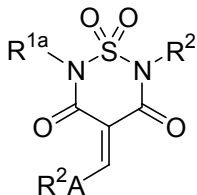


Figure 7: CRHBP non-peptide antagonists for the treatment of Alzheimer's disease. R^1 , R^{1a} = alkyl, arylalkenyl, furylalkenyl, thienylalkenyl, pyrrolylalkenyl. A = S, NH, monocyclic or bicyclic heterocyclic group containing 1 or more nitrogen atoms in which A is bound through a nitrogen atom. R^2 = H, alkyl, cycloalkyl, aryl

The Future of Drug Design Development for CRH

SAR has been the most successful technique to date for targeting this specific GPCR, for both peptide and non-peptide antagonists. While the peptide agonists and antagonists have had some success through SAR studies and development, it is the small molecule non-peptide antagonists that are of most interest as potential therapeutics. There has been a greater structural diversification in recent years of these compounds, however it has proven elusive to combine good pharmacokinetics with good activity. With the increasing demand to diversify the structural basis of the antagonists, alternative techniques are being pursued.

Due to the absence of a definitive 3D structure of a GPCR until recently, computer-aided ligand based design has been one of the few tools available to medicinal chemists. Limited ligand-based pharmacophore work has been published for CRH.[85], [89] Ligand-activity pharmacophores are a 3D representation of the theorised key elements in receptor-ligand interaction extrapolated from correlations between known structure and

activity data. This work confirmed the hydrophobic nature of the molecules and the importance of a hydrogen bond acceptor and the angular positioning of the aromatic ring to the heterocyclic core. There have been significant limitations in applying computer-aided pharmacophore development programs until recently; while these hypothesis were able to predict good activity they gave a large number of false positives as they were unable to consider any negative steric interactions.

With the elucidation of the first GPCR (rhodopsin) to be crystallised,[1, 90, 91] homology modelling has come to the forefront as the leading technique for GPCR based drug design, and is under investigation for its application to CRH. At this stage it has limitations; Rhodopsin itself is a class A member of the GPCR superfamily while CRH is class B,[21] these two classes of GPCRs differ in amino acid and gene sequence, mechanism of activation and types of activators, and initial sequence alignment comparisons. Comparison of the two receptors show that the key binding domains for peptides of the receptors appear to be significantly longer in length for CRH [7] and are also positioned in the most flexible regions (N-terminus and ECD). The recent publication of the NMR structure of the N-terminus greatly assists in the understanding of these differences.[17] Additionally, observations of the molecular structure of the non-peptide antagonists of both classes of GPCR would suggest unique and independent binding domains. Class A molecules display an array of potential ligand-receptor interactions while the class B CRH molecules suggest largely hydrophobic interactions.[16] This method offers enormous possibilities for the future development in drug design for CRH, however, it still has many obstacles to overcome.

Therapeutic Application and Limitations

It would appear that the evolution of CRH as a therapeutic target is entering an exciting stage in its development. The non-peptide antagonist, R121919, and a number of others, reaching clinical trials, has legitimised this target as a very viable and promising area of research, particularly in the area of depression.

Although peptide agonists and antagonists have numerous applications as potential therapeutics, significant limitations exist including the crossing of membrane barriers (particularly the blood brain barrier), and peptide metabolism, both integral for therapeutic application. These limitations are reflected by the lower number of patent applications.

The far more lucrative non-peptide antagonists continue to give hope to the rise of potential therapeutic. Finally with diversification of the core units and improving solubility it appears that soon a break-through compound capable of balancing the hydrophobic nature of the receptor binding site and the hydrophilic demands of bioavailability might soon be attained.

CRH related diseases are very broad and varied. Although the distinction between the receptors and their vastly different signalling and effects is clearly defined, the possibility of such an influential target not having wide ranging side effects must be carefully examined. The intricacy and complexity of CRH signalling and the HPA axis may afford a solution to this or compound the problem. The HPA axis has numerous feed-back and backup utilities in place designed to minimise any disturbance to the body's homeostasis. From the various knockout mice created we can observe that they are all viable and often no side effects are observed unless under stressed conditions, when the majority of the

applications of the drugs would be desired. Despite these aspects, the expectation that therapeutics based on CRH antagonists and agonist will soon emerge is high.

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