The Prevention of Preterm Labour – Corticotropin Releasing Hormone Type 1 Receptors as a Target for Drug Design and Development

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Abstract: The role of the corticotropin releasing hormone in the onset of labour and the subsequent medicinal chemistry implications of CRH antagonists for the prevention of premature birth, and identification of the CRH type 1 receptor as the target for this drug design, are reviewed here.

Keywords: Corticotropin Releasing Hormone (CRH), Premature Birth, Antagonists.

INTRODUCTION

Preterm labour is the outcome of 10% of all pregnancies and accounts for 70% of all neonatal morbidity and mortality [1,2]. Even though tocolytic and corticosteroid techniques have been used to inhibit labour and reduce the consequences of prematurity on the foetus, they have not reduced the rate of preterm birth [2,3,4].

Human parturition is characterised by a complex interplay of autocrine/paracrine signalling [5]. One such chemical in this interplay is corticotropin releasing hormone (CRH), a 41 amino acid peptide originally isolated from ovine hypothalamus. It is the principle neuroregulator of the basal and stress-induced secretion of ACTH, β-endorphin, and other propiomelanocortin-related peptides from the anterior pituitary. CRH is found in the hypothalamus, pituitary and placenta. Recently it has been found that women who experience preterm labour have a higher concentration of plasma CRH compared to gestationally matched women with full term pregnancies [6].

The corticotropin releasing hormone as a drug design target for the medicinal chemist has attracted a notable amount of attention with respect to the treatment of depression, anxiety and other neuropsychiatric disorders [7,8,9]. However, with the recent reporting of the importance of CRH in the onset of labour and the ability of antagonists of the CRH type 1 receptors to delay the onset of labour in sheep model studies [10,11], the development of CRH type 1 receptor antagonists as potential treatments for premature birth have taken on a new direction and significance. We have recently reviewed the status of CRH antagonists themselves and their medicinal chemistry implications within the field of premature birth [12]. Of great importance to drug design and development by the medicinal chemist is an intimate knowledge of the targets involved. Here we review the interplay of CRH in labour progression, and the status of the CRH type 1 receptor as a target for the treatment of premature birth, through drug design and development of small ligand antagonists.

PREMATURE BIRTH

Premature birth is defined by the World Health Organization as birth at less than 37 weeks of completed gestation, full term as birth between 37 and 42 weeks of gestation [13]. Premature birth affects approximately 1 in 10 pregnancies and accounts for at least 75% of all neonatal mortalities, excluding those related to congenital malformations [14]. Infants born prematurely are 40 times more likely to die during their first few weeks, and those that survive have an increased risk for developing morbidities such as deafness, blindness, cerebral palsy, respiratory illness and other complications [15].

A large-scale study carried out in the United Kingdom and Ireland of extremely premature infants, born between 20 and 25 weeks of gestation, found that 79% were stillborn or died before admittance to a Neonatal Intensive Care Unit (NICU) [16]. Of the 811 infants in this study admitted to an NICU, 60% died in hospital. Of those discharged; 2% died, 23% were classified as having severe disability at 30 months (defined as one likely to put the child in need of physical assistance to perform daily activities), 25% had other disabilities at 30 months, and 49% were found to have no disabilities at 30 months. No relation between the pattern of morbidity and either gestational age or occurrence of multiple birth was found [16].

Over the past 30-40 years, there has been a substantial decrease in the rate of neonatal mortalities due mainly to the development of neonatal intensive care units, yet no decrease in the rate of premature birth itself. Neonatal survival has been shown to improve most significantly with gestational age, as shown in Fig. (**1**) [15].

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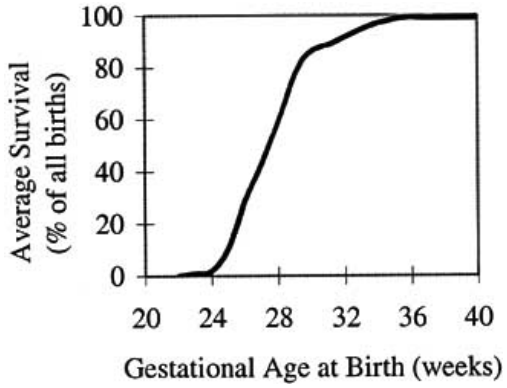

Fig. (1). Neonatal survival (28 days of life) increases according to gestational age at birth. Data derived from institutions in Britain, Europe, and Scandinavia [4].

This increase in survival is accompanied by a dramatic increase in the cost of caring for these babies, with neonatal care one of the most expensive items in health care budgets, in terms of cost per patient [15]. In the US alone, it has been estimated to cost $US 5 to 6 million annually [1]. Medication and therapy for a disabled child results in a constant financial burden and the need for special education classes and additional resources to overcome learning difficulties can also contribute to the costs of caring for children born prematurely [17].

Even though survival of preterm infants has increased, there has not been a decrease in the rate of premature birth in the last 30 years [15]. This is mainly due to the lack of understanding of the underlying mechanisms precipitating parturition and thus a lack of appropriate technologies for diagnosis and treatment. Parturition involves structural and functional changes of the myometrial, cervical and foetal membranes that are essential for labour and delivery to occur [18].

CURRENT PREVENTION

The current system of prevention is largely unsuccessful, as no accurate test exists to predict the early onset of parturition. Current techniques rely on a screening program to identify those at high risk of giving birth prematurely, modification of their specific determinants of risk, and, if necessary, drug treatment. Tocolytic drug therapy is employed to delay labour, and corticosteroids are used to improve foetal outcome by promoting organ maturation [15].

Risk scoring tests, such as the commonly used Creasy system, calculate a risk index for individual pregnant women where socio-economic, medical history and lifestyle factors are weighted [15]. Some clinical factors associated with preterm labour are shown in **Table 1** [14]. The sensitivity of such risk tests, however, ranges between 25 and 50%, meaning that at least half of the women that give birth prematurely would not be identified as having a high risk and thus not targeted for preventative treatment [15].

Table 1. Clinical Factors Associated with Preterm Labour

Maternal Factors	Low socio-economic status; Non-white race; Maternal age ≤ 18 or ≥ 40 years; Smoking; Low pregnancy weight; Substance abuse
Maternal History	Previous history of preterm delivery; Previous history of a second-trimester abortion.
Uterine Factors	Uterine volume increased; Uterine anomalies; Trauma.
Infection	

Early identification of preterm risk is crucial to successful tocolytic therapy, with the effectiveness of these agents reduced if labour has already been initiated [15]. While the efficacy of tocolytic agents, such as magnesium sulphate, ritodrine and terbutaline have long been debated, their use has been associated with a delay of labour of 24 to 48 hours [14]. A study by Guinn and associates (1998), found that treatment of preterm women with terbutaline did not prolong gestation compared to those treated with a placebo, even when it was administered by pump to allow continuous low dosing and instant administration of medication when uterine contractions developed. Therapy also had to be aborted in several cases due to the development of side effects [19].

The many contraindications and side effects of tocolytic therapy limit their use for treatment of preterm labour [14]. Ritodrine, the most common drug used to delay preterm labour, is poorly tolerated and causes maternal hypotension and significant maternal and foetal tachycardia [14].

Delay of labour allows the administration of corticosteroids, which have been shown to improve foetal outcome by reducing intraventricular haemorrhage, respiratory distress syndrome, and mortality, even when therapy lasts less than 24 hours. Optimal benefits, however, begin after 24 hours of corticosteroid therapy and last a week [15]. This, as well as the limitations to their use, highlights the need for tocolytic agents that delay labour for a longer period. It should be noted that while the combined use of these agents may improve foetal outcome, therapy with tocolytic and corticosteroids does not reduce the incidence of preterm labour [14].

Much research is currently being conducted into the usefulness of biological markers for the prediction of preterm pregnancy, since hormones and other biological substances encounter concentration changes during pregnancy, particularly at parturition.

Continued gestation is desirable for 30-45% of preterm pregnancies that result from spontaneous idiopathic preterm labour. The development of agents to prevent preterm labour would be of most benefit to these pregnancies. The other 55-70% of preterm pregnancies result from foetal or maternal compromise, which makes the continuation of pregnancy undesirable [12].

CORTICOTROPIN-RELEASING HORMONE (CRH)

Research into the mechanisms underlying human pregnancy have revealed that corticotropin-releasing hormone (CRH) is a key signal and potential regulator for the onset of birth [18]. It is produced in the hypothalamus and placenta during pregnancy and has been implicated in a variety of central and peripheral functions including behaviour, food intake, thermoregulation, inflammation, reproduction, cardiovascular function, gastrointestinal secretion, motility, and transit [12,20].

Corticotropin-releasing hormone (CRH) is a 41 amino acid peptide, Fig. (**2**) [20,21,22], synthesised primarily in the hypothalamus and then transported *via* the portal system to the anterior pituitary, where it regulates the release of adrenocorticotropin (ACTH) [20], β-endorphin and other propiomelanocortin-derived peptides [23]. These peptides are released from the anterior pituitary gland into the general circulation and work primarily by binding to the CRH receptor [24]. ACTH stimulates secretion of glucocorticoids from the adrenal gland and is the principle regulator of the hypothalamic-pituitary axis (HPA) [25].

H-Ser-Gln-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu

-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu

-Met-Thr-Lys-Ala-Asp-Gln-Leu-Ala-Gln-Gln

-Ala-His-Ser-Asn-Arg-Lys-Leu-Leu-Asp-Ile

-Ala-NH_2

Fig. (2). The amino acid sequence of CRH [21].

CRH forms a secondary structure α-helical amphipathic molecule in solution and on binding to the receptor [20,22]. The first 8 residues are important for activation, whereas the hydrophobic side chains from amino acids 15-19 are crucial for activation of the receptor as well as its binding, the remainder of the C terminus is involved in binding and is required in its entirety for full potency [20]. The C-terminal region is responsible for the formation of the α-helix [20]. For further details on the secondary structure of CRH see previous review [12].

Hypersecretion of CRH in the brain is associated with depression, anxiety-related disorders, and anorexia nervosa, whereas a deficit of CRH in the brain, due to dysfunction of CRH neurons, is associated with Alzheimer's disease [26,27], Parkinson's disease [28], and Huntington's disease. Overproduction of CRH at synovial joints is thought to contribute to rheumatoid arthritis [28]. It also mediates other responses including inflammatory disorders such as rheumatoid arthritis [29], food intake [30], thermoregulation, various behavioural responses, cardiovascular function [21], stroke, tumours and from an ectopic centre, Cushing's syndrome [29].

CRH has also been discovered in extra-hypothalamic sites such as the human placenta [31]. Hypothalamic and placental CRH are identical in structure and bioactivity, but differ in their regulation. Glucocorticoids inhibit the production of hypothalamic CRH but stimulate the production of placental CRH, establishing a positive feedback loop during pregnancy that results in elevated levels of CRH, ACTH and cortisol, a glucocorticoid important for foetal lung maturation [32]. The secretion of placental CRH is also stimulated by prostaglandins, cytokines and catecholamines and inhibited by nitric oxide [18].

The processing of CRH from its precursor also differs in the hypothalamus and placenta. Like most hormones, CRH is synthesised from a pro-hormone, pro-CRH, and released post-translation by the action of endopeptidases. After signal peptide removal and *C*-terminal amidation to yield pro-$CRH_{27\text{-}194}$, endoproteolytic cleavage at two sites (between pairs of basic amino acids at residues 124-125 and 151-152) produces three products: the *N*-terminal product, $CRH_{27\text{-}124}$; the mid-portion fragment; pro-$CRH_{125\text{-}151}$; and *C*-terminal $CRH_{1\text{-}41}$ [33]. In the hypothalamus, pro-CRH undergoes immediate post-translational processing with subsequent storage of mature $CRH_{1\text{-}41}$. In the placenta, however, most CRH exists as unprocessed pro-CRH, with very little in the form of mature $CRH_{1\text{-}41}$, except in pre-eclampsia [33], a complication of pregnancy characterised by hypertension and proteinuria.

CRH RECEPTORS

CRH binds to the CRH receptor family, consisting of a seven transmembrane domain receptor Fig (**3**) [23,24]. The two main receptor isoforms, CRH_1 and CRH_2, are part of the class II (secretin-type) G-protein coupled receptor (GPCR) super family [26,34]. When the CRH ligand binds to the CRH_1 or CRH_2 receptor, the signal is activated and conducted *via* a Gs-coupled [34], adenylate cyclase second messenger system [27]. This messenger system is present in brain, pituitary and spleen tissue [35]. Treatment of placental and foetal tissues with CRH does not activate adenylate cyclase but does increase inositol phosphates (IP_3, IP_2, IP) suggesting that CRH receptors can couple to different G proteins in different tissues [36].

The N-terminus contains the sequence responsible for high affinity binding of the agonist and antagonist [34]. The CRH receptor has five predicted glycosylation sites at the N-terminus whose function are currently unknown, Fig (**3**) [27,34]. The C-terminus appears to be needed for G-protein activation even though the G-protein is coupled to the residues in the first intracellular loop. CRH receptors are down regulated upon continual exposure to CRH due to phosphorylation state of the C-terminal cytoplasmic tail serine/theronine domain, which may regulate the internalization of the CRH antagonist [34]. Both CRH_1 and CRH_2 receptors are serine/threonine rich (CRH_1: AA 396-413) and contain potential protein Kinase A/C phosphorylation sites in the 1st loop and the carboxyterminus tail, Fig (**3**) [27].

The CRH_1 receptor is made up of 415 amino acids, Fig. (**3**) [27,34]. CRH_1 is primarily found in the cerebellum, cerebral cortex [21], and sensory relay structures as well as localisation in placental and foetal tissue [27]. Within the

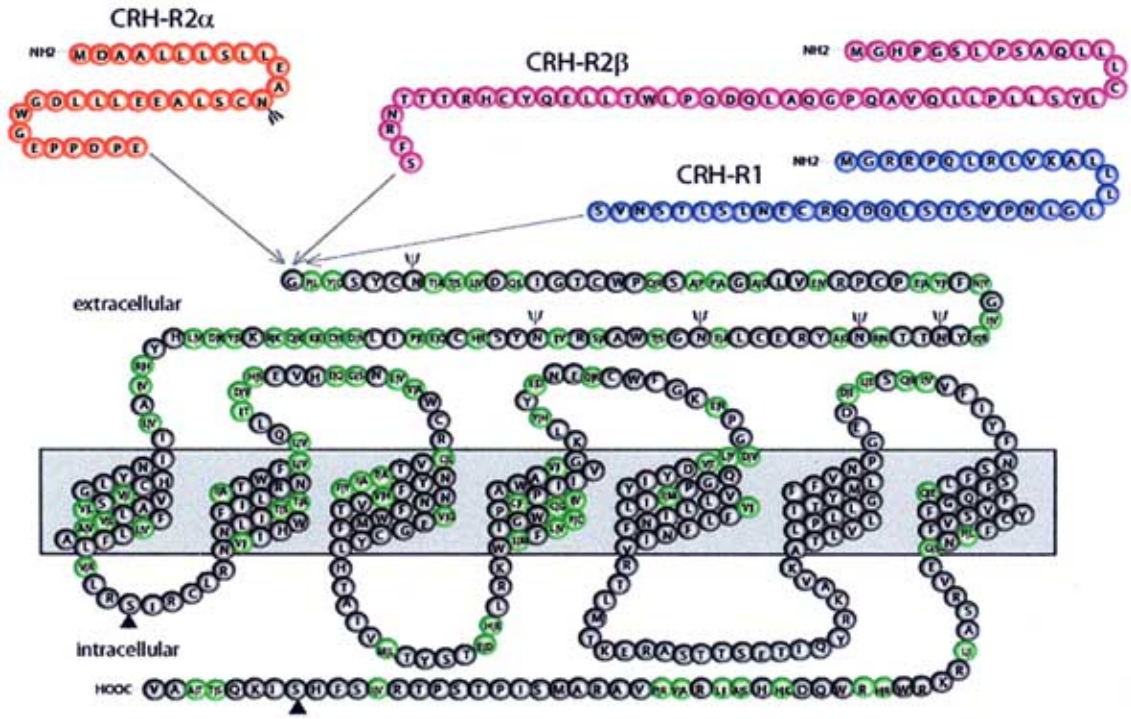

Fig. (3). The amino acid sequence of corticotropin releasing hormone CRH_1, $CRH_{2\alpha}$ and $CRH_{2\beta}$ receptors. The receptor is shown with the seven predicted transmembrane domains. The main receptor shown is CRH_1 and the arrows indicate where $CRH_{2\alpha}$ and $CRH_{2\beta}$ diverge at the N- terminus (shown in red and purple respectively). Amino acids that are shaded in green are those that differ between CRH_1 and CRH_2 receptors. Potential N-glycosylation sites are marked by ψ and potential sites of protein kinase C phosphorylation are denoted by ▲. Figure adapted from reference [27].

pituitary gland CRH_1 is found in both anterior and intermediate areas and is believed to be responsible for regulation of ACTH levels [27].

The CRH_2 receptor sub-family has a different sequence, tissue distribution and pharmacological profile to the CRH_1 receptor but has high structural homology to the CRH_1 receptor [24,27]. These receptors are mainly localised in the sub cortical structures [27]. CRH has a lower affinity for CRH_2 receptors than CRH_1 [21].

There are three spliced variants of the CRH_2 receptor. These isoforms are the $CRH_{2\alpha}$, $CRH_{2\beta}$ and $CRH_2\gamma$ receptors. The $CRH_{2\alpha}$ receptor is a 411 amino acid protein [21] and is 71% homologous to the CRH_1 receptor but has a different N-terminal sequence [27], which is involved in the ligand-receptor interaction [37]. In humans $CRH_{2\alpha}$ is predominantly expressed in the hippocampus, heart, skeletal muscle, hypothalamus, septum and cortex [37]. $CRH_{2\beta}$ is a 431 amino acid protein that differs from $CRH_{2\alpha}$ at the N terminus [21]. It is found mainly in rat heart, lung and sketetal muscle aswell as having limited distribution in the septum, hippocampus and amygdala [38].

The third isoform of the CRH_2 receptor is $CRH_2\gamma$ [37]. $CRH_{2\gamma}$ is expressed in the central nervous system (CNS) in a similar pattern to $CRH_{2\beta}$ but follows the pharmacology of the $CRH_{2\alpha}$ receptor [37]. $CRH_{2\gamma}$ is mainly expressed in the septum and hippocampus and weakly in the amygdala. $CRH_{2\gamma}$ is weakly detectable in the lung but absent in the heart and skeletal muscle [37]. The lack of overlap in the tissue localisation of CRH_1, $CRH_{2\alpha}$ and $CRH_{2\beta}$ suggests a distinctive functional role for each receptor [21,27]. However, the role different receptors may play in illness is still undergoing investigation and intensive research [24,26].

CRH binding protein (CRH-BP) is a 322 amino acid protein [40], with a MW of 37kDa [41]. It is produced by the liver, placenta and brain [41]. CRH-BP has a large influence on the bioactivity of CRH and seems to protect the anterior pituitary gland, but not the hypothalamus, from over stimulation by excess concentrations of placental CRH [23,41,42]. It circulates in the plasma, binding excess hormone, particularly during pregnancy when placental CRH is secreted into maternal and foetal plasma. When the CRH-BP complex is formed it is subject to rapid clearance from the blood [43], more rapid than other glycoproteins its size [42].

Concentrations of both CRH_1 receptors in reproductive tissues [36,44] and circulating CRH-BP [27], change during pregnancy and labour, thus providing further support to the theory that CRH has an important role in gestation and birth.

CRH - A ROLE IN PREGNANCY

Placental CRH is secreted into both the maternal and foetal circulations, in a 10:1 ratio [12]. Studies have shown

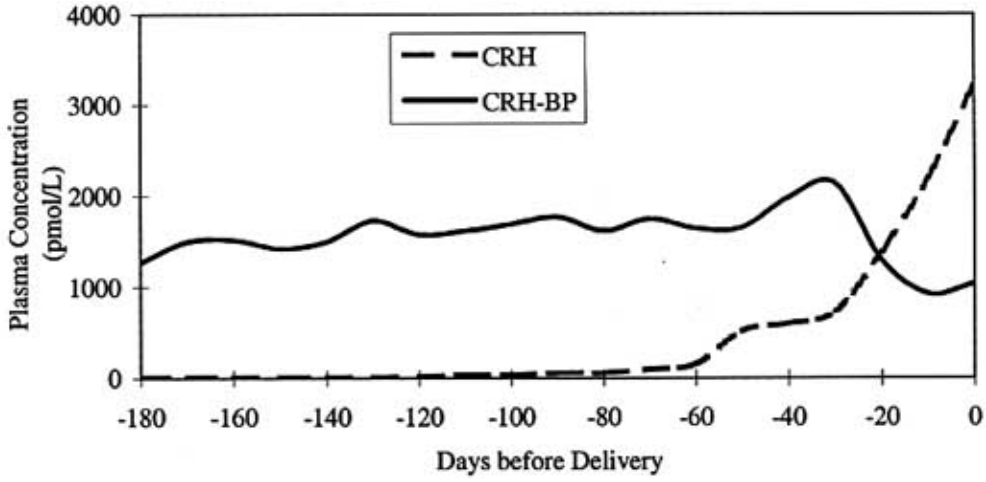

Fig. (4). Comparison of the molar concentrations of CRH and CRH-BP in maternal plasma during the final 180 days of gestation in pregnancies ending in spontaneous labour [17].

that the concentration of placental CRH secreted into the maternal plasma increases exponentially during pregnancy, climaxing at birth, Fig. (**4**). Human CRH levels are normally less than 2 pmols, but during pregnancy may exceed 10 times this level [45]. Concentrations of CRH are maintained during pregnancy by CRH-BP, which binds CRH with high affinity. The level of this binding protein decreases at approximately 36 weeks of gestation resulting in a dramatic increase in maternal plasma CRH leading to the onset of structural and functional changes in gestational tissues, in preparation for parturition [18]. CRH-BP levels return to non-pregnant levels within 5 days postpartum [23].

CRH is secreted from the syncytiotrophoblast and intermediate trophoblast layer of the placenta during pregnancy [41,46]. It is also secreted from the epithelium and subepithelial cells of the amnion and the amniotic epithelium of the umbilical cord [29].

As pregnancy progresses into the second trimester, CRH increases exponentially towards term [47], rises dramatically during labour [23] and declines quickly following parturition [29]. This rise in CRH blood plasma levels is mirrored in the amniotic fluid [29].

There is an extensive network of CRH receptors in the myometrium and these receptors are up regulated to a high affinity receptor in the pregnant myometrium [48]. A simplistic diagram showing the major physiological effects of CRH is shown in Fig. (**5**). During most of pregnancy, the smooth muscle of the uterus remains relaxed while the cervix, kept firm by collagen fibres, seals the bottom of the uterus. Progesterone, a placental steroid hormone secreted into the maternal plasma, maintains this structure of the uterine and cervical tissues [10]. Oestrogen, another placental hormone, opposes these effects and promotes contractility by stimulating the production of contractile agents and

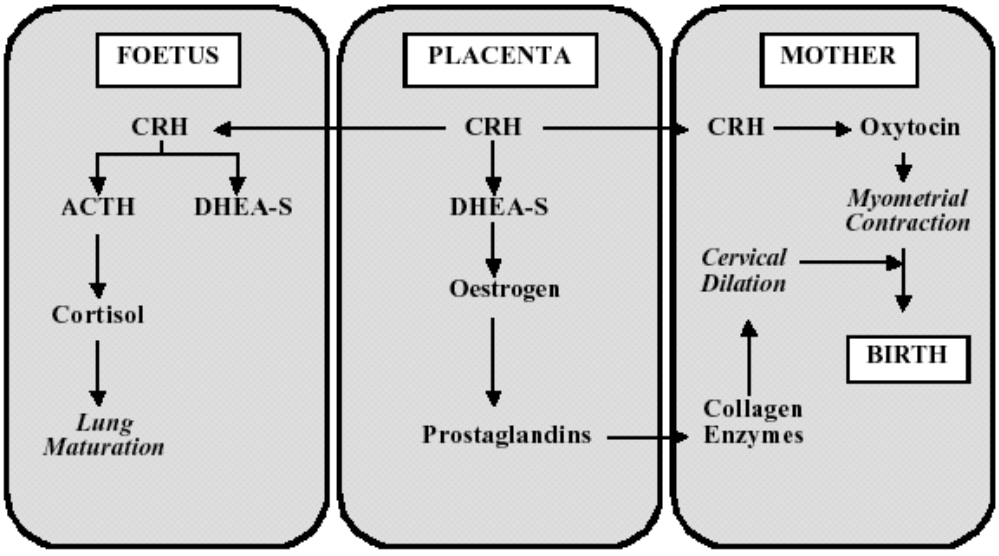

Fig. (5). Corticotropin releasing hormones role in parturition [10,47].

expression of specific receptors and gap junctions during parturition. The production of oestrogen in the placenta is, however, reliant on the ability of CRH to stimulate production of its precursor, dehydroepiandrosterone sulphate (DHEA-S) [10]. Progesterone inhibits the activity of CRH, presumably by prohibiting the propagation of the positive feedback loop that exists between CRH, ACTH, and cortisol [49].

The absence of progesterone receptors in the placenta means progesterone must compete for glucocorticoid receptors. Progesterone binds with a higher affinity to these receptors, effectively inhibiting cortisol binding and propagation of the feedback loop, thus inhibiting CRH secretion [49]. It is believed however that secretion of placental CRH into the foetal circulation allows propagation of the feedback loop in the foetus. Accumulation of cortisol would produce effective concentrations that would displace progesterone from placental glucocorticoid receptors, counteracting its actions and stimulating CRH secretion. The establishment of the positive feedback loop in the placenta, and a significant drop in circulating CRH-BP, results in the exponential increase of CRH seen in maternal and foetal plasma in the later stages of gestation [49].

Foetal cortisol production is then responsible for foetal lung maturation in preparation for birth [10]. Placental CRH and foetal ACTH stimulate the production of DHEA-S which is then converted to oestrogen in the placenta. Oestrogen stimulates the production of prostaglandins $F_{2\alpha}$ and E_2, which in turn maintain CRH synthesis in the placenta [50], as well as enzymes that digest collagen fibres, promoting dilation of the cervix. Oestrogen also stimulates the formation of gap junctions between myometrial (uterine muscle) cells, which allows coordination of contractions, and increased expression of oxytocin receptors in the myometrium. Oxytocin is a maternal steroid hormone that stimulates myometrial contraction [50]. A positive feedback loop results with continued secretion of oxytocin until contractions are strong and close enough to lead to birth [10]. The action of this hormone, as well as that of prostaglandins, is potentiated in the presence of CRH but CRH has no contractile activity of its own [50].

CRH is also a potent vasodilator, producing hypotensive effects, and is thought to have an important role in the regulation of foetal-placental vascular tone. Increased CRH production is a possible compensatory response to increased placental vascular resistance [51]. This is associated with impaired availability of oxygen and endogenous substrate, restriction of foetal growth and subsequently high perinatal mortality [23].

Elevated levels of CRH can be neurotoxic to parts of the brain responsible for learning and memory, resulting in impaired foetal responsiveness to novel stimulus. These regions of the brain are also rich in CRH receptors, suggesting a role for CRH in foetal neurological development [52].

Rodent placenta does not produce CRH and CRH does not affect parturition in these animals. Foetal rodents do synthesize CRH in localised brain areas as well as express CRH_1 and CRH_2 receptors in late gestation, indicating that the foetal nervous system is a plausible target for extra-hypothalamic CRH. Administration of CRH to pregnant rodents results in infants with developmental anomalies [52].

Low concentrations of CRH have been associated with development of depression and antisocial behaviour during pregnancy, particularly in pregnant adolescents [53,54]. It is suspected that reduced sensitivity and expression of CRH receptors during the postpartum period, due to hyperactivity of the HPA axis by placental CRH during pregnancy, may persist postpartum and reduce adrenal allopregnanolone synthesis. Allopregnanolone, a derivative of progesterone, induces anxiolytic, hypnotic, and anticonvulsant effects upon systemic administration. Such neurosteroids have been linked to modification of neuronal excitability, mood, and adaption to stress. Levels of allopregnanolone are significantly decreased in women with maternity 'blues', the mildest form of depression, postnatal depression being more serious [54]. The 40-70% of postpartum women who develop maternal blues, are four times more likely to develop postnatal depression. Allopregnanolone levels are also significantly reduced in women with premenstrual syndrome.

PREMATURE LABOUR AND CRH

Cross-sectional studies have revealed that the concentrations of placental CRH in maternal plasma are elevated in women who have delivered preterm, Fig. (**6**) [18].

This regulation of the length of pregnancy by CRH has been termed a 'placental clock' [18]. Differences in maternal CRH concentrations are significant at approximately 20 weeks of gestation [18], with the most significant elevation of CRH seen in high risk populations that deliver before 34 weeks [55]. The significance of this finding has not gone unnoticed, with much interest in the use of maternal CRH concentrations as a biological marker.

Interest has also been directed towards the use of CRH as a drug target. The presence of CRH and its receptors in reproductive tissues supports a functional role for CRH in gestation, as does the elevated levels of maternal plasma CRH during this period. It is believed that antagonism of CRH would appropriately delay labour in women destined to deliver prematurely. Antagonism of CRH_1 receptor in sheep has been shown to delay the onset of parturition [56].

CRH_1 RECEPTOR ANTAGONISM

Structure-activity relationship (SAR) studies of the corticotropin-releasing hormone peptide revealed that residues 1-4 are not necessary for receptor binding or transduction, residues 4-8 are important for activation, and residues 12-41 are mainly responsible for binding [20]. A further SAR study, utilising an alanine replacement series of ovine CRH, found that the side chains of residues 5-19, in the N-terminal region, are important for receptor binding and

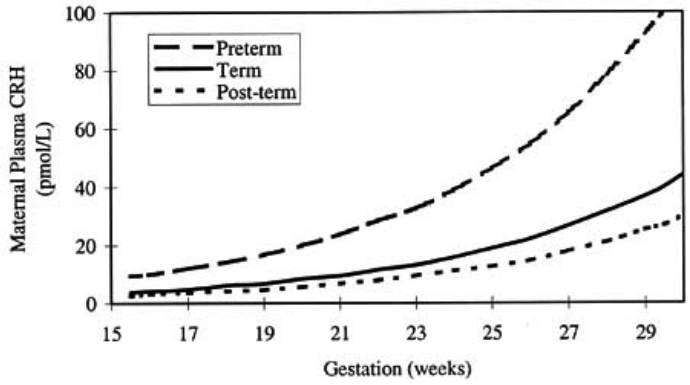

Fig. (6). Non-logarithmic representation of the three regression curves demonstrating the exponential increase of plasma CRH with advancing gestation [17].

activation [22]. C-Terminal amino acid residues are more responsible for structural conservation than for functional expression. The α-helix formed by CRH upon binding is highly amphiphilic in nature with hydrophobic and hydrophilic regions segregated on opposite faces of the helix [22].

Cleavage of the first eight to fourteen residues of CRH produces antagonists of varying potency to CRH, such as α-helical CRH_{9-41} [57]. Structural stabilisation of these antagonists, using D-amino acid residues to stabilise β turns, *i*-(*i*+3) Glu-Lys and Lys-Glu salt bridges or β-lactam rings to stabilise the α-helical structure, has also been shown to increase the potency of some of these agents [58,59]. Astressin {cyclo(30-33)[D-Phe [12], Nle [21,38], Glu [30], Lys [33]] rat/human CRH(12-41)}, a potent CRH antagonist has a tertiary structure stabilised by the presence of D-isomers and a *i*-(*i*+3) salt bridge [60]. The introduction of this salt bridge to astressins parent compound, [D-Phe [12], Nle [21,38]] r/hCRH(12-41), increases the potency of this molecule 30 fold. Astressin has a K_i of 2 nM and high affinity for CRH pituitary receptors [58].

These peptide antagonists have been shown to suffer from limited solubility, persistence of intrinsic activity and weak potency at the hypophyseal site of action [58]. The low bioavailability of peptide agents makes these antagonists of little therapeutic use, although they have been important in the investigation of CRH function and binding.

Recently, there have been large numbers of non-peptide low-molecular weight ligands emerging as CRH_1 antagonists. These antagonists have significant advantage over peptide molecules as they may be orally administered, have a longer duration of action and have activity within the CNS [61].

The current CRH_1 receptor antagonists have been designed for use in depression and anxiety related disorders. These all have a similar core structure consisting of a nitrogen containing, aromatic unit (for example, a pyrimidine, triazine or purine group), linked to a substituted anilino moiety [28]. Examples of these types of compounds are shown in Fig. (**7**). Numerous high affinity ligands have been developed in the pyrrolo-pyrimidine series of compounds, but attempts to increase the hydrophilicity of these compounds has resulted in a reduction in binding affinity [62].

Various derivatives of these types of structures have been made with the major variations being of the aliphatic substituents on the aryl ring, central heterocycle and the tertiary amino group [12]. Structure activity relationship (SAR) analysis of these derivatives has revealed that the

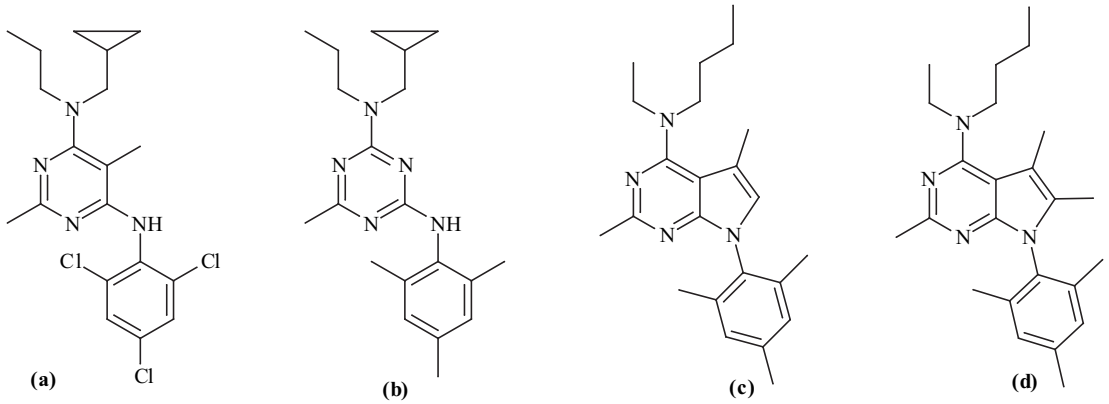

Fig. (7). CRH_1 antagonists. **(a)** Pyrimidine structure antagonist with a binding affinity of 2.3 nM (K_i). **(b)** Triazine analogue with a K_i of 57 nM. **(c)** Conformationally restricted purine based CRH_1 antagonist CP-154,256 K_i of 2.7 nM. **(d)** Antalarmin K_i of 1.7 nM [38].

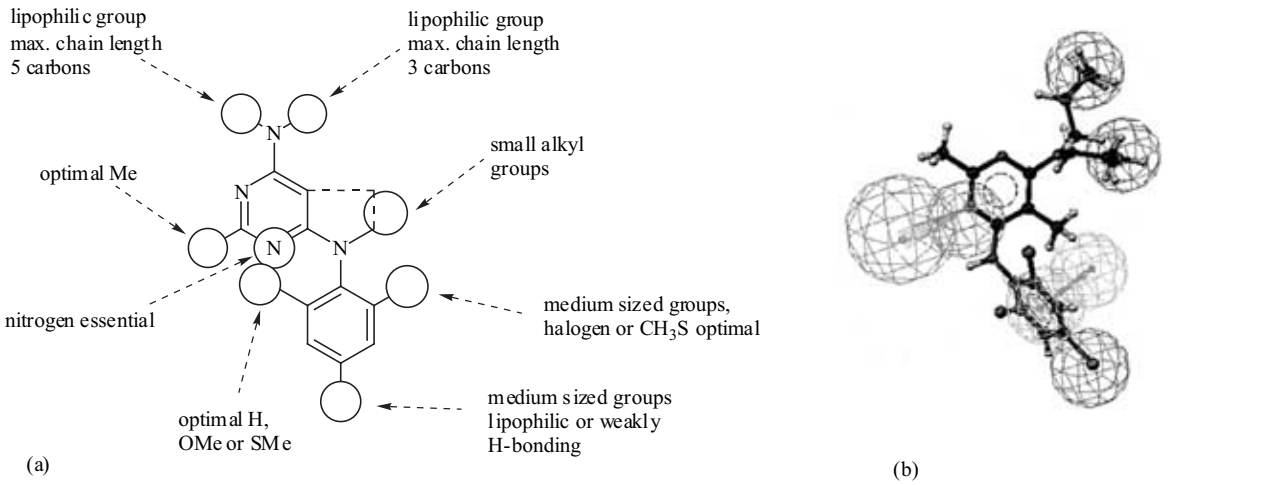

Fig. (8). (a) Structure activity relationship (SAR) study of CRH_1 antagonists. **(b)** Pharmacophore generated using a range of CRH_1 antagonists shown with the compound shown in Fig. (**7a**) overlayed. Hydrophobic regions are highlighted by spheres (bottom and top right); spheres (centre right) represent π-stacking interactions; hydrogen bond acceptors are represented by spheres (left) with the projected interactions illustrated by a cone [28].

anilino substituent should be either 2,4 or 2,4,6 substituted with medium sized lipophilic or weakly H-bonding groups, generally these are halogens or methyl substituents [12]. The nitrogen *ortho* to the anilino substituent is essential for activity and optimal activity is incurred with a teriary amine on the heterocyclic ring attached to two lipophilic chains with chain lengths no longer than 3 and 5 carbons respectively, Fig. (**8**). Recent SAR studies have also suggested that the tertiary amine can be replaced with alkyl ether substituents whilst retaining activity [62].

A pharmacophore model was generated [28] from a range of CRH_1 antagonists, Fig. (**8b**). From this model it can be seen that the heterocyclic nitrogen *ortho* to the anilino ring is involved in a hydrogen bonding interaction, the anilino ring itself is involved in a π-stacking interaction. There are also a number of hydrophobic interactions, notably involving one of the tertiary amino substituents and the 2 and 4 substituents on the anilino ring. This shows high correlation to the SAR model, lacking only the additional tertiary amino substituent and the methyl group on the heterocyclic ring Fig. (**8a**).

Two of the most active CRH_1 antagonists to date are CP-154,256, Fig. (**7c**), and antalarmin Fig. (**7d**) which has been shown to delay the onset of labour for one week in sheep [56]. To achieve this inhibition, the antalarmin was administered via infusion directly into the foetus over a 10 day period, using a 1:1 mixture of ethanol and polyethoxylated castor oil as a vehicle [56]. This inhibition supported the hypothesis of the potential use of CRH_1 antagonists at potential therapies for the prevention of preterm labour.

CONCLUSION

The design and synthesis of antagonists of the CRH family of receptors still requires significant improvement before a suitable therapeutic can be developed. Intimate knowledge of the receptor sites and potential modes of actions of different ligands provides valuable insight into this development, in particular with respect to therapeutics for the prevention of premature birth. While much about these receptors is still unknown, the emergence of new information will greatly benefit the development of new CRH receptor antagonists.

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