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The role of CRH receptors and their agonists in myometrial contractility and quiescence during pregnancy and labour

Dimitris K Grammatopoulos

Endocrinology and Metabolism, Division of Clinical Sciences, Warwick Medical School, University of Warwick, Coventry, CV4 7AL, United Kingdom

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1. ABSTRACT

The mechanism of human labor remains a scientific enigma. Corticotropin-releasing hormone (CRH), a hypothalamic peptide that controls the response of the body to stress and which is also produced by the placenta and intrauterine tissues during pregnancy is potentially involved in the onset of labor. CRH is part of a family of mammalian peptides that includes the urocortins (UCNs), which are also expressed by the placenta and intrauterine tissues. During human pregnancy, CRH appears to target multiple feto-maternal tissues, including the myometrium, implicating CRH in the regulation of the transition from relaxation to active uterine contractions. The myometrial actions of CRH are mediated via a wide network of specific G-protein coupled membrane-bound receptors. These receptors have various functional properties, depending on the receptor subtype, the ability of agonists to activate specific signalling cascades and the stage of pregnancy. In addition, their function is dependant upon other intracellular signals via communication between signalling cascades, suggesting potential multiple roles of CRH and other CRH-like peptides during pregnancy and labor. This review will provide the current concepts about the role of CRH and UCNs and their myometrial receptors during pregnancy, labor and delivery.

2. INTRODUCTION

The characterisation of mechanism(s) controlling human gestation and the onset of labour is an intriguing scientific problem. During gestation, the uterus remains in a state of quiescence to safeguard a secure intrauterine environment for the development of the foetus. At term and during labour, the cervix dilates and the uterus switches from quiescence to a state of co-ordinated contractility for securing a successful and safe outcome both for the mother and the newborn. Despite 50 years of intensive research, the molecular mechanisms underlying these complex physiological processes remain elusive. In up to 10% of human pregnancies, the mechanisms controlling these processes are altered in such a way that pre-term birth takes place.

Across mammals, the process of parturition appears to be unique in humans, although there are some features that are preserved in many species. One such feature is the activation of the maternal and fetal hypothalamo-pituitary adrenal axes, the primary function of which is to control the body's response to stress. This axis is under the control of corticotrophin releasing hormone (CRH) (1), a 41 amino-acid hypothalamic peptide that helps to co-ordinate the endocrine, autonomic, behavioural,

and immune responses to stress. CRH shares considerable sequence homology with a family of related mammalian peptides, the urocortins (2-4), that mediate diverse physiological responses.

3. CRH AND UCNs IN PREGNANCY AND LABOUR

CRH and CRH-related peptides are expressed in placenta and intrauterine tissues during pregnancy and labour (5-7). The circulating levels of placental-derived CRH, but not UCN I, increase exponentially during human pregnancy leading to the suggestion that CRH may act as a "placental clock" determining the length of pregnancy (8, 9). Women destined to have premature delivery have higher midpregnancy levels that those who deliver at term, and this may be used as a marker for identifying women at risk of prematurity (10). The precise biological functions of CRH during human pregnancy, however, are still unknown. The maternal pituitary does not appear to be regulated by placental CRH since its biological activity is attenuated by the presence of a circulating CRH binding protein (CRH-BP), of placental and hepatic origin (11-13) that blocks the ability of circulating CRH to induce ACTH release from pituitary corticotrophs. The evidence available suggests that increased levels of placental CRH are readily available to stimulate the fetal pituitary-adrenal axis which appears to be mature during the second trimester (14-15). This results in increased output of fetal adrenal steroids and would lead to fetal lung maturation in a synchronised mode of action with forces that induce the onset of labour. Therefore, it is possible that maternal stress could play an important role in initiating a cascade of intracellular signals leading to pre-term labour (16).

Thus, a series of endocrine events mediated via autocrine and paracrine actions of CRH (and possibly CRH-related peptides) appear to be activated during the different stages of pregnancy in order to prepare the fetus and uterus for parturition. Over the past decade a number of tissues have been identified as targets of CRH actions: the myometrium, the placenta and fetal membranes, the fetal adrenal cortex and the vasculature (17-20). In these tissues CRH and CRH-related peptides might play a role in the control of myometrial contractility, placental control of vascular tone, peptide and prostaglandin production and adrenal steroidogenesis and probably many more, yet unidentified processes. The actions of CRH and its related peptides in these tissues are mediated via an extensive network of specific G-protein coupled membrane-bound receptors that exhibit distinct functional characteristics, and are differentially regulated during pregnancy.

4. CRH RECEPTORS AND CONTROL OF MYOMETRIAL CONTRACTILITY/QUIESCENCE

4.1. Biology of CRH receptors

The CRH receptors (CRH-R) belong to the class II receptor superfamily. These receptors are membrane-bound proteins that belong to the family of seven transmembrane (7 TMD) G-protein coupled receptors (GPCRs) which, upon agonist binding, transduce signals across cells mainly through activation of heterotrimeric G-proteins, which regulate a diverse network of effector systems. In human tissues, including the myometrial smooth muscle cells, two types of CRH-R are expressed, named R1 and R2, encoded by 2 separate genes (21-22) and the primary RNA transcripts of each gene are subjected to significant alternative splicing to generate a family of spliced variant mRNAs producing in turn a family of related receptor proteins. The fully active CRH-R1 subtype, termed CRH-R1alpha, is a 415 amino acid protein comprising seven putative hydrophobic membrane-spanning domains with the hydrophilic domain at the N-terminus that may encode a signal peptide. A growing number of CRH-R1 mRNA splice variants have been described in humans and other species (23-26). These variants, termed R1beta, R1c-n are generated by various partial or complete exon(s) insertions or deletions, some of which are associated with a frameshift in the open reading frame (ORF). For example, the CRH-R1beta, which can be regarded as a "pro-CRH-R1" receptor variant, is the only R1 variant that contains an extra 29-aminocid insert (exon 6) in the 1st intracellular loop, whereas CRH-R1c is missing exon 3 which encodes 40 amino acids of the N-terminus. The CRH-R1d (24) has exon 13 deleted, which leads to the loss of 14 amino acids from the carboxyl-terminal end of the putative 7th transmembrane domain (TMD), potentially resulting in either a short 7th TMD where the proximal residues of the C-terminus are drawn into the membrane or a 6-TMD receptor variant containing a protein segment that fails to segregate into the membrane lipid bilayer leading to an extracellular C-terminus, similar to the CT-R variant Delta/e13 (27). The CRH-R2 gene exhibits a completely different splicing pattern compared to CRH-R1, possibly relevant to its distinct role in mammalian physiology. Three CRH-R2 variants have been identified in humans: CRH-R2alpha, R2beta and R2gamma. All three variant mRNAs are produced by the use of an alternate 5' exon 1 that splice onto a common set of downstream exons (exons 2-12), resulting in R2 variants that differ only in their N-terminal extracellular domains (28-30). However, the different N-termini do not significantly alter agonist binding and signalling properties of the various CRH-related peptides. The arrangement of the CRH-R2 gene provides the potential for generating multiple alternate splice forms of mRNA (31). In support of this, a number of different types of aberrant CRH-R2 mRNA splice variants have been reported so far (32, 33).

Interestingly, gene transcription of the two types of human CRH-Rs is differentially regulated; the R1 gene expression is driven by a single TATA-less promoter, whereas three distinct promoters, each controlling transcription of the three CRH-R2 isoforms (31, 34). Pharmacological studies on the CRH-R1 receptor have shown that CRH and UCN I have similar binding affinities and potencies in generating cAMP (2). In contrast, the two other members of the CRH-related family of peptides, UCN II and UCN III exhibit insignificant binding affinity for the CRH-R1 (3-4). The CRH-R2 (shares 70% homology at the amino acid level with the CRH-R1 receptor. Agonist binding and second messenger studies have shown that UCN I, UCNII and UCN III are significantly more potent than CRH at binding and

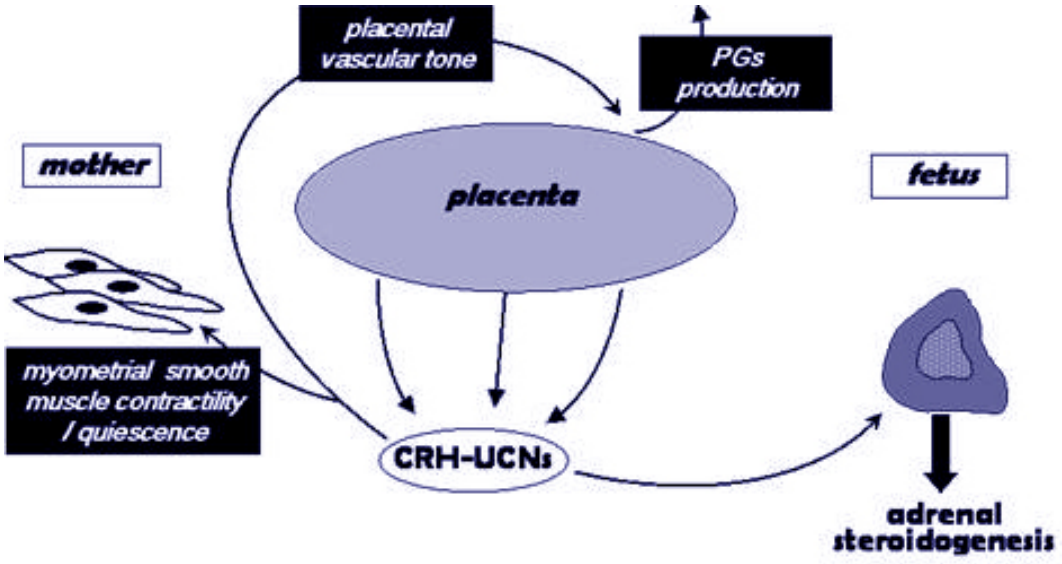

Figure 1. Potential actions of CRH and UCNs in the feto-maternal tissues. During pregnancy a number of tissues have been identified as targets of CRH actions: the myometrium, the placenta/ fetal membranes and the fetal adrenal cortex. In these tissues CRH and UCNs appear to be involved the control of myometrial contractility, placental control of vascular tone, peptide and prostaglandin production and adrenal steroidogenesis and probably many more, yet unidentified processes. The actions of CRH and its related peptides in these tissues are mediated via an extensive network of specific G-protein coupled membrane-bound receptors that exhibit distinct functional characteristics, and are differentially regulated during pregnancy.

activating CRH-R2 receptors (2-4) suggesting that the peptides may be the native agonists for the CRH-R2 receptor.

4.2. Myometrial expression of CRH-R during pregnancy and labour

During pregnancy the circulating CRH is predominantly of placental origin. In addition, myometrial smooth muscle cells produce both CRH and UCNs (5-7, 35) and therefore, functional CRH-R could be activated via paracrine/autocrine as well as endocrine mechanisms. Messenger RNA and protein studies have demonstrated CRH-R heterogeneity in human myometrium (36, 37); two proteins with apparent *Mw* of 70 and 46 kDa respectively were present when myometrial membrane homogenates were resolved by SDS-PAGE and ^{125}I-CRH was covalently attached to the CRH-R by chemical affinity cross-linking, whereas, use of isoelectric focusing (IEF) identified five CRH-R isoforms with pIs ranging from 4.65 to 5.2. Molecular studies demonstrated the presence of both R1 and R2 receptor mRNA in human myometrium and identified multiple receptor variants (37). Interestingly, the profile of CRH-R subtypes expression is dynamically regulated during pregnancy by, as yet, unidentified mechanisms; as pregnancy progresses towards labour there is differential receptor variant expression and at least four CRH-R1 variants, R1alpha, R1beta, R1c, R1d, and two CRH-R2 variants, R2alpha and R2beta, are expressed in human pregnant myometrium at term whereas only three receptor subtypes, CRH-R1alpha, R1beta, and R2beta are present in the non-pregnant myometrium. The splicing mechanisms involved in the transcription of the CRH-R1 mature mRNA are unknown, however it is conceivable that these mechanisms are activated during pregnancy and/or labour and result in the differential expression of each CRH-R variant. Both R1c and R1d receptor variants, which have reduced agonist binding and/or signalling properties, are expressed only in term (>37 weeks of gestation), but not pre-term myometrium (24), suggesting a potentially important functional role for these variants towards the end of pregnancy with important consequences for the effectiveness of CRH and UCN I actions. There are also reports suggesting that the levels of CRH-R1 expressed in myometrium are increased in patients in labour whether at term or pre-term (38); however, it is possible that increased expression of signalling-deficient R1 variants would result in overall diminished myometrial responsiveness to CRH actions (Figure .2). Some evidence also, suggests that the anatomical localisation and levels of expression of the myometrial CRH receptor subtypes varies, and that the levels of CRH-R1 mRNA in lower segment myometrium are much higher than those found in the fundal region (38).

These data suggest that CRH, acting via different receptor variants, is able to exert different actions on the myometrium in the pregnant state compared to the non-pregnant state, and activation of different CRH-R subtypes might have distinct effects on myometrial contractility/quiescence. The molecular basis of these changes remains unresolved.

4.3. Functional characteristics of myometrial CRH-R during pregnancy and labour

The identification of functional CRH-R in the human myometrium (39, 40), led us to hypothesize that CRH and CRH-related agonists might be involved in the control of myometrial contractility/quiescence during

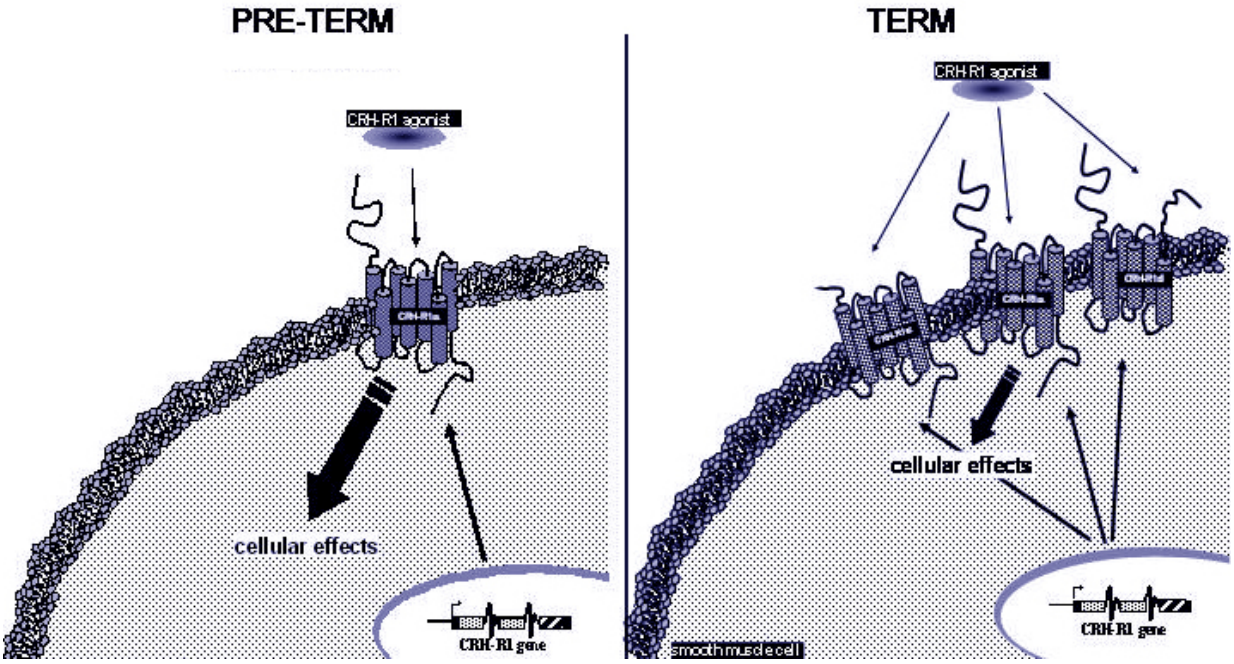

Figure 2. Differences of myometrial CRH-R1 expression during pregnancy. As pregnancy progresses towards term and delivery, the profile of CRH-R subtypes expressed in myometrial smooth muscle cells changes and there is differential CRH receptor variant expression. Despite increases in circulating CRH levels in maternal plasma, term myometrium is characterised by expression of signalling impaired CRH-R variants such as CRH-R1c and CRH-R1d. This would have significant effects on myometrial tissue responsiveness to the "protective" actions of CRH and might contribute to the transition of the uterus towards active contractions and labour.

pregnancy and labour. As mentioned previously, the intracellular mechanisms controlling the transition of the quiescent myometrial smooth muscle of pregnancy towards a state of co-ordinated contractility during the onset of labour, are not fully understood. A key step in this mechanism, is the accelerated interaction between actin and myosin (41) that leads to increased smooth muscle contractility and successful parturition. Interestingly, unlike other mammals, the onset of labour in humans, occurs without apparent changes in circulating steroid levels, although a functional "progesterone withdrawal" has been observed in human myometrium at term mediated through altered expression of progesterone receptors (42). This process is multifactorial and controlled by a complex interplay of hormonal signals; raised levels of intracellular calcium lead to increased activity of Ca^{2+}–calmodulin-dependent myosin light chain kinase (MLCK) (43). Myometrial contractility is also promoted by calcium-independent pathways involving activation of Rho kinase and inhibition of myosin phosphatase (44-45). These intracellular pathways are under the functional regulation of many GPCRs. For example, receptors coupled to $Galpha_q$, activated by uterotonins, stimulate contractility by activating the phospholipase C/Ca^{2+} pathway; receptors coupled to $Galpha_s$ (such as $beta_2$-adrenergic receptors, prostanoid EP2,) relax the uterus by activating the key enzyme adenylyl cyclase and increasing myometrial cAMP levels and receptors coupled to $Galpha_i$ (i.e $alpha_2$-adrenergic receptors) potentiate contractility, probably by inhibiting cAMP production.

In this mechanism, CRH and CRH-Rs are thought to play key roles. Studies on the intracellular signals generated downstream of CRH-R/G-protein activation demonstrated that during human pregnancy, the myometrial CRH-R are primarily linked to the adenylyl cyclase/ cAMP signalling pathway (40). Activation of this signalling pathway would favour myometrial quiescence, through inhibition of myosin light chain phosphorylation, required during pregnancy and before the onset of labour. Despite early preliminary studies suggesting that CRH may augment uterotonin-induced contractility (46-48), recent isometric contractility studies in myometrial tissue strips utilizing highly sophisticated mathematical models have confirmed CRH ability to relax the human pregnant myometrium (49). The effects of cAMP/PKA pathway can be further amplified through "cross-talk" with multiple signalling molecules involved in the relaxation of myometrium during pregnancy; a protein kinase A-dependent mechanism leads to transactivation of the membrane-bound form of guanylyl cyclase and increased production of cGMP (50). In addition, the cAMP/PKA pathway limits the ability of specific CRH-R1 agonists such as UCN I to activate the extracellular signal regulated kinase (ERK) cascade which stimulates myometrial contractility. This is achieved via a "self-regulatory" mechanism in which PKA-induced phosphorylation of Ser^{301} in the 3^{rd} intracellular loop of CRH-R1alpha attenuates Gaq-protein activation and ERK phosphorylation (51). Independently of receptor/Gaq-protein interaction,

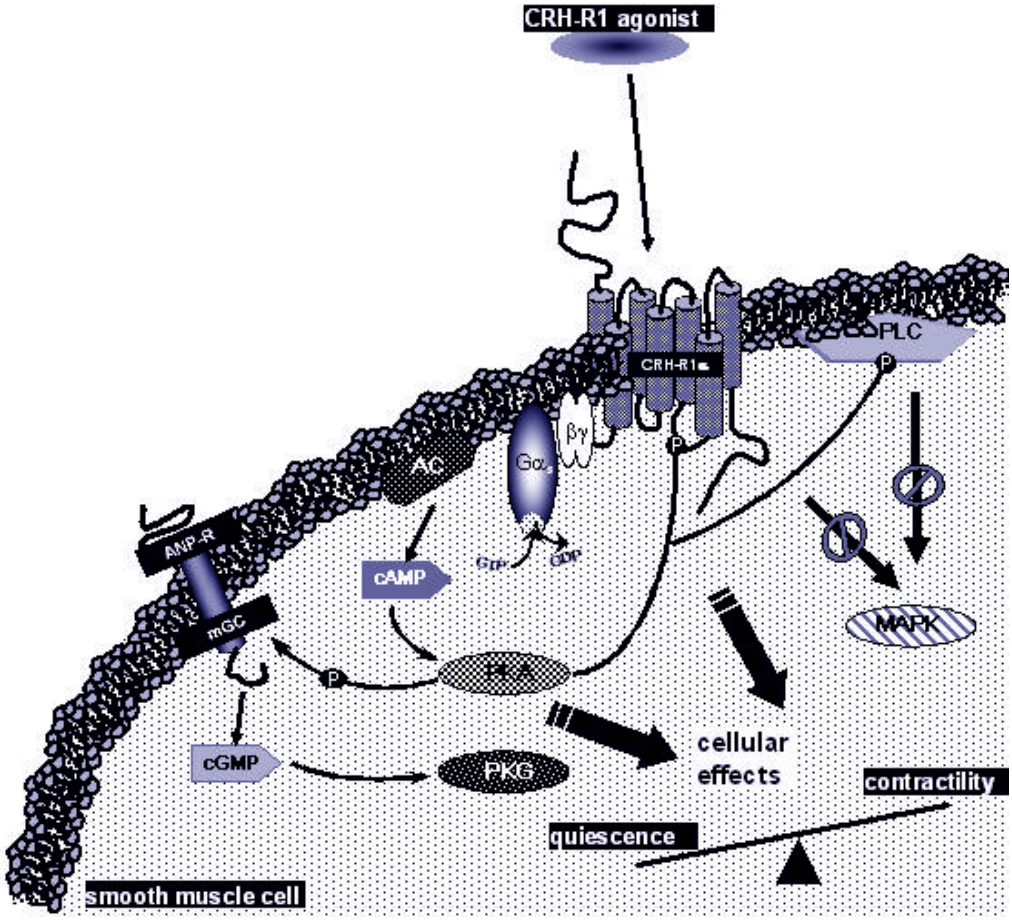

Figure 3. Signalling characteristics of myometrial CRH-R1 receptors: the critical role of AC/cAMP/PKA pathway. The myometrial CRH-R is primarily linked to the adenylyl cyclase/cAMP signalling pathway. Activation of this signalling pathway would favour myometrial quiescence, through inhibition of myosin light chain phosphorylation, required during pregnancy and before the onset of labour. The effects of cAMP/PKA pathway can be further amplified through "cross-talk" with multiple signalling pathways, such as transactivation of the membrane-bound form of guanylyl cyclase and increased production of cGMP and inhibition of PLC□□ activation and contractile signaling pathways . In addition, the cAMP/PKA pathway limits the ability of specific CRH-R1 agonists like UCN I to activate the extracellular signal regulated kinase (ERK) cascade via a "self-regulatory" mechanism in which PKA-induced phosphorylation of CRH-R1 alpha attenuates Gaq-protein activation and ERK phosphorylation.

PKA can inhibit stimulation of PLCbeta$_3$ and contractile signaling pathways by phosphorylation of Ser^{1105} of PLCbeta$_3$ (52) (Figure 3).

Although the CRH-Rs are primarily coupled to Galpha$_s$-activation (21), the myometrial CRH-Rs can couple to and activate multiple G-proteins, namely Galpha$_i$, Galpha$_o$, Galpha$_q$, Galpha$_z$, similar to endogenous CRH-Rs in other tissues (53). This allows CRH and CRH-related agonists to regulate multiple intracellular signalling pathways and generate diverse biological responses. In addition, as pregnancy progresses towards labour the pattern of G-protein activation appears to be altered under the influence of unknown mechanisms. In pre-term myometrial biopsies (<35 weeks of gestation), CRH can activate both the short (45 kDa) and the long (52 kDa) forms of the Galpha$_s$-protein but it has no effect on Galpha$_q$-protein activation, whereas, in term (>37 weeks of gestation) myometrium, CRH can activate only the short (45 kDa) form of the Galpha$_s$-protein as well as the Galpha$_q$-protein (54). In this mechanism, changes in the expression levels of signalling molecules are also playing an important role; for example the well-documented decreased expression and down-regulation of Galpha$_s$ (especially the long form) towards term (55). Although the functional role of the short and long forms of the Galpha$_s$-proteins is unknown and therefore the physiological significance of these events remains to be determined, this finding might explain the reduced ability of CRH to activate the Galpha$_s$-protein, mentioned previously, and the adenylyl cyclase/cAMP pathway (56).

Not all CRH actions are mediated through the adenylyl cyclase/cAMP/PKA pathway, in agreement with the multiple G-protein and signalling model described above; CRH can up-regulate the constitutive but not the

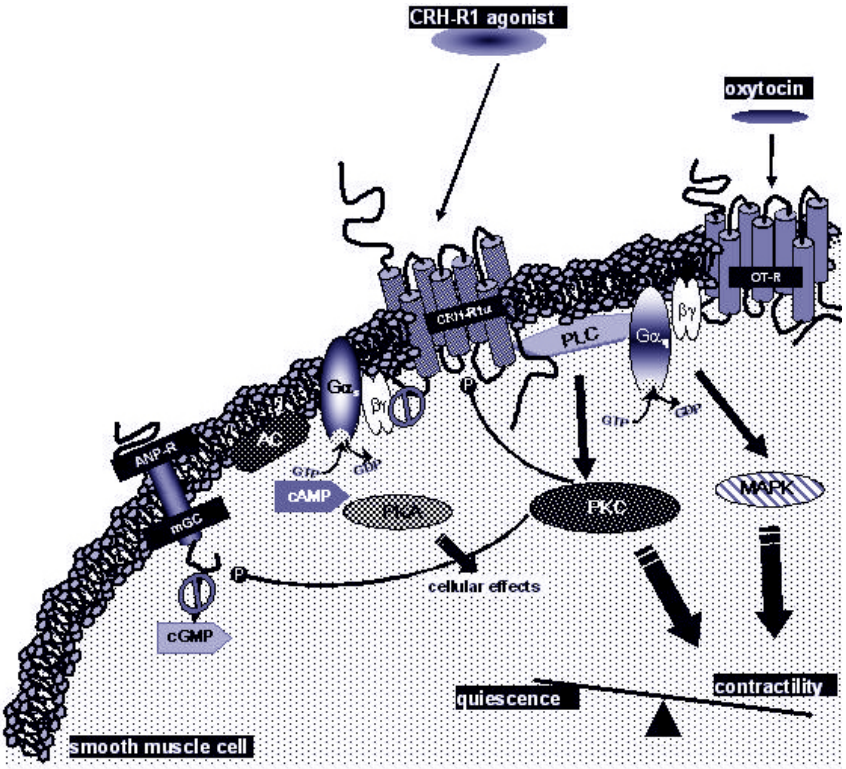

Figure 4. Modulation of myometrial CRH-R signalling pathways by uterotonin-activated PKC at term of pregnancy. As the myometrium is prepared for active labour, myometrial CRH-Rs activity appears to be modulated by the action of uterotonins such as oxytocin. Oxytocin's actions target CRH-R-effector coupling efficiency via activation of protein kinase C (PKC) and lead to CRH-R phosphorylation, and reduction in CRH-Rs signalling potency Furthermore, PKC can also decrease membrane bound guanylyl cyclase activity leading to reduced GMP production and therefore it can neutralize relaxation pathways at multiple levels. This decrease in the biological activity of the CRH receptor can potentially shift the intracellular microenvironment balance towards myometrial contractility.

inducible forms of nitric oxide synthase leading to increased NO and cGMP production (50) as well as stimulate the soluble form of guanylyl cyclase via a complex mechanism, not fully elucidated, which is PKA-independent. Collectively this data, supported by myometrial contractility studies, suggest that CRH plays a "protective" role for the myometrium by generating intracellular signals that help to maintain the pregnant myometrium in a state of relaxation and preventing uterine contractions. Paradoxically, this hypothesis appears to contradict the proposed physiological role of CRH based on the observed elevations in maternal peripheral plasma CRH concentrations which peak around labour and are suggested to predict women at risk of increased uterine activity and pre-term labour (10). The two hypotheses are not mutually exclusive since it is possible that: a) CRH and myometrial CRH-Rs play distinct and sometimes opposing roles during the different stages of pregnancy and b) the significantly higher levels of maternal circulating CRH in abnormal pregnancy states (57) might be produced in response to an abnormal or premature labouring process, in order to prevent inappropriate contractions and protect the fetus from expulsion. In support of the latter, CRH can inhibit cytokine-induced stimulation of myometrial prostaglandin production, in particular those acting as uterotonins such as PGE_2 (58). Activation of myometrial prostaglandins by cytokines have been proposed as a mechanism for infection-induced pre-term labour (59).

At term of pregnancy, myometrial CRH-Rs activity appears to be modulated by the action of uterotonins such as oxytocin (60). As the myometrium is prepared for active labour, there is a dramatic up-regulation of oxytocin receptors by as much as 200-fold (61) resulting in increased responsiveness of the myometrium to oxytocin, one of the main activators of uterine activity. Oxytocin's actions target CRH-R-effector coupling efficiency via activation of the inositol triphosphate pathway with subsequent activation of protein kinase C (PKC) and lead to CRH-R phosphorylation at one or more Ser or Thr residues, receptor desensitization and a reduction in CRH-Rs signalling potency (60). Both type 1 and type 2 CRH-Rs have multiple potential PKC phosphorylation sites which are identical in both receptor families. Furthermore, PKC has multiple actions; it can also decrease membrane bound guanylyl cyclase activity leading to reduced GMP production (50) and therefore it can neutralize relaxation pathways at multiple levels. This decrease in the biological activity of the CRH-R shifts the intracellular microenvironment balance towards myometrial contractility (Figure .4). Comparative studies between term and pre-term human myometrial tissue

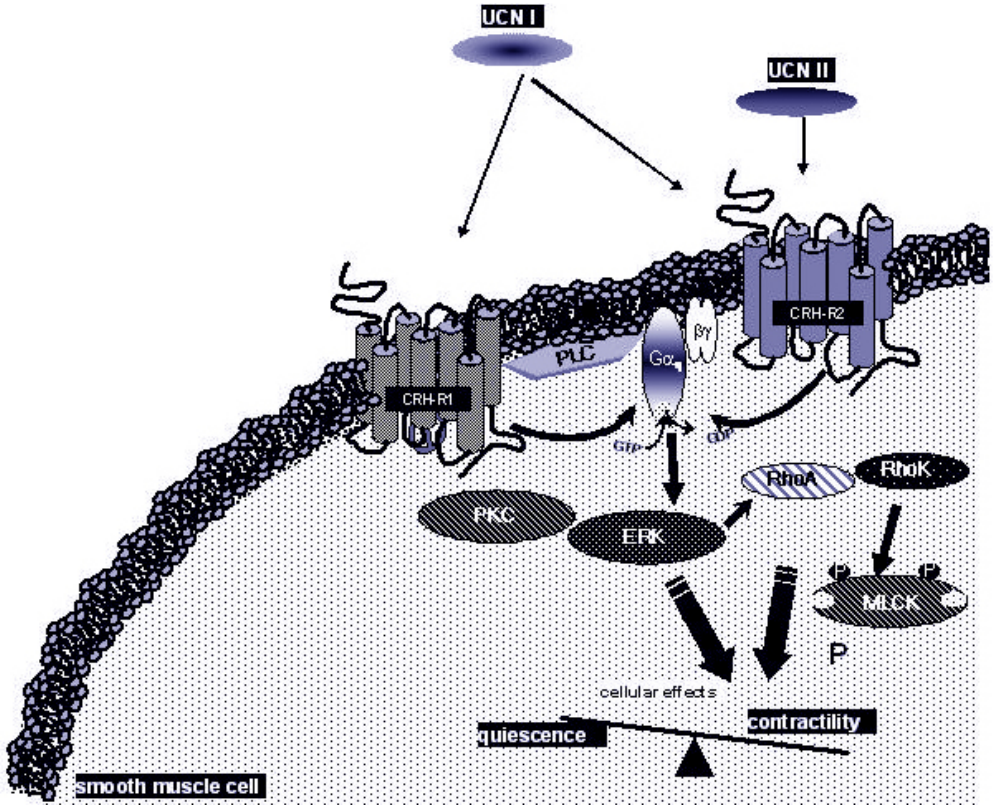

Figure 5. Signalling characteristics of UCNs in human myometrium during pregnancy. CRH-R agonists such as UCNs are now emerging as important regulators of myometrial contractility. UCN I acting via both R1 and R2 types of CRH-R can potentially promote myometrial contractility via activation of the ERK signalling pathway whereas UCN II (a specific CRH-R2 agonist) activates myosin light chain (MLC20) phosphorylation via a pathway involving sequential activation of PKC, MAPK kinase 1, ERK1/2, RhoA, and RhoA-associated kinase. These signalling pathways would enhance the contractile response of the myometrium.

demonstrated that the PKC activation occurs only in term myometrium, possibly due to lack of oxytocin receptors in preterm myometrial tissues.

Interestingly, not all CRH-R subtypes appear to be sensitive to the action of OT/PKC. Isoelectric focusing studies have shown that CRH binding to some but not all subtypes, can be reduced by oxytocin's action (60). Preliminary studies investigating the characteristics of the PKC-induced phosphorylation of the CRH receptors using individually expressed receptor subtypes, showed that activation of PKC had distinct effects on specific receptor subtype function. In particular, CRH-R1beta but not the CRH-R1alpha was susceptible to desensitization (62) which raises the possibility of a distinct and potentially important role for the type R1beta receptor subtype in myometrial physiology. It is possible that PKC-sensitive CRH-Rs subtypes are responsible mainly for the generation of cAMP and myometrial relaxation. Thus, inhibition of their biological activity by oxytocin might neutralise the impact of these receptor subtypes on myometrial contractility and enable CRH and/or CRH-related agonists to exert distinct actions by activating other subtypes. For example there is evidence that at term, CRH and UCN I can augment the myometrial contractile response to PGF_{2alpha} and oxytocin (46, 47), although they have no direct stimulatory action on their own.

Other CRH-R agonists such as UCNs are now emerging as important regulators of myometrial contractility. UCN I acting via both R1 and R2 types of CRH-Rs can potentially promote myometrial contractility via activation of the ERK signalling pathway (63) and UCN II (a specific CRH-R2 agonist) activates myosin light chain (MLC20) phosphorylation via a pathway involving sequential activation of PKC, MAPK kinase 1, ERK1/2, RhoA, and RhoA-associated kinase (35). These observations point towards a dual role for CRH and/or UCNs in the control of myometrial contractility. The existence of such mechanisms would enable CRH-like agonists to switch their actions at term and enhance the contractile response of the myometrium (Figure .5). This model would place CRH and UCNs in a central role in co-ordinating the smooth transition of myometrial tissue from a state of relaxation to one of contraction at term.

5. CONCLUSIONS

The actions of placental-derived CRH and CRH-related agonists during human pregnancy are exceedingly

complex. The suggestion that CRH may act as a "placental clock" determining the length of pregnancy is extremely attractive and in certain cases of threatened pregnancy, such as pre-term labour, might warrant the use of preventative measures to adjust the function of this "clock" (64, 65). Specific CRH-R antagonists for potential pharmacological interventions are available; however, characterization of their biological and physiological roles is not yet, complete. Furthermore, the contrasting effects of these CRH-R antagonists in different species (66, 67), highlights the diversity across mammals of the mechanisms regulating the onset of labour and the need for better understanding of these mechanisms.

There is little doubt that during pregnancy CRH and UCNs target the human myometrium to potentially modulate smooth muscle contractility and quiescence. A complex network of functional CRH-Rs mediates the actions of CRH; these receptors appear to be extremely versatile in their ability to transduce signals across smooth muscle cells. The myometrial tissue sensitivity to CRH and CRH changes, in order to adapt to the different biological requirements as pregnancy progresses towards term and labour, through an interplay of signals and mechanisms that alter CRH-R expression and functional activity, and allow these peptides to exert distinct actions. At present our knowledge is incomplete and we have only fragmented data regarding the exact roles of these agonists and their respective receptors. There are still many unanswered questions; without any doubt, many more studies are required to elucidate the signalling pathways controlled by CRH, UCNs and their receptors.

6. ACKNOWLEDGEMENTS

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Send correspondence to: Dr D.Grammatopoulos, Sir Quinton Hazell Molecular Medicine Research Centre, Department of Biological Sciences, The University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK, Tel: 44-2476-524206, Fax: 44-2476-574637, E-mail: d.grammatopoulos@warwick.ac.uk