mRNA expression profiles for corticotrophin-releasing hormone, urocortin, CRH-binding protein and CRH receptors in human term gestational tissues determined by real-time quantitative RT-PCR

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Abstract

Increasing maternal plasma levels of corticotrophin-releasing hormone (CRH) during the last weeks of pregnancy suggest that this stress hormone plays an important role in the control of human parturition. Little is known about the quantitative contribution of gestational tissues (other than placenta) to intrauterine formation of CRH, urocortin and CRH-binding protein (CRH-BP), or about the distribution of CRH receptors within the uterus.

We have investigated the mRNA expression of CRH, urocortin, CRH-BP and CRH receptors 1 and 2 (CRH-R1 and -R2) in gestational tissues by real-time RT-PCR. Placenta, myometrium and choriodecidua were collected after uncomplicated pregnancies at term, before the onset of labour. Distribution of CRH-R1 and CRH-R2 protein was also investigated by immunostaining with receptor subtype-specific antibodies.

The placenta was identified as the main site of CRH and CRH-BP mRNA expression, displaying mRNA levels >1000 and >20 times higher than those found in the myometrium and choriodecidua respectively ($P < 0.05$ in each case). mRNA expression of urocortin was low in all tissues investigated. Myometrium and choriodecidua expressed relevant amounts of both receptor subtypes, whereas the CRH receptor population in placenta consisted mainly of CRH-R2.

The high expression of CRH in placenta and the substantial expression of CRH receptors in choriodecidua and myometrium suggested that CRH derived from placenta exerts direct or indirect actions on these tissues. Neither CRH produced by myometrium or choriodecidua nor urocortin from other intrauterine sources seem to play a major role in the control of labour.

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Introduction

The hypothalamic 41 amino acid neuropeptide corticotrophin-releasing hormone (CRH) is the key regulator of the stress response in the hypothalamic–pituitary–adrenal (HPA) axis. It exerts its actions via specific receptors belonging to a family of neuropeptide receptors with seven transmembrane domains. Two different isoforms of CRH receptors (CRH-R1 and CRH-R2) are known; these share 70% identity at the amino acid level and exist in several variants resulting from differential RNA splicing (Dieterich *et al.* 1997,

Hillhouse *et al.* 2002). The biological activity of CRH is attenuated by a circulating CRH-binding protein (CRH-BP).

Apart from the HPA axis, the formation of CRH or related peptides (e.g. urocortin) and the expression of CRH receptors have been described in numerous peripheral tissues, e.g. placenta (Sasaki *et al.* 1988, Florio *et al.* 2000), endometrium (Gravanis *et al.* 2001), lymphatic organs or immune cells (Baigent 2001) and skin (Slominski *et al.* 2001). In the central nervous system, CRH attenuates inflammatory reactions via glucocorticoid action. In contrast, CRH produced by peripheral

tissues has predominantly proinflammatory effects and may constitute an important link between exogenous stress and local immunological or inflammatory reactions (Webster *et al.* 1998, Baigent 2001).

Parturition is a complex, highly co-ordinated process comprising numerous inflammatory-like processes (Kelly 1996). It has been suggested that placental CRH plays a key role in the timing of birth, as there is a sharp increase in its bioavailability prior to the onset of labour (Mc Lean *et al.* 1995). Currently available data suggest multiple actions of CRH during the birth process (Challis *et al.* 2000, Hillhouse & Grammatopoulos 2002). In the foetal adrenals it directly stimulates dehydroepiandrosterone sulphate and cortisol formation, which subsequently trigger placental oestrogen synthesis and foetal lung maturation respectively (Smith *et al.* 1998). In a placental perfusion model, CRH was described as a potent vasodilator acting via a nitric oxide/cGMP-dependent pathway (Clifton *et al.* 1995). Other possible biological effects of CRH in parturition remain to be elucidated. To understand the involvement of peripheral CRH in the intrauterine neuroendocrine-immune network, it is of major interest to investigate the contribution of different gestational tissues to intrauterine CRH formation and the quantified distribution of CRH receptors within these tissues.

The presence of components of the CRH system has been described previously at both the mRNA and the protein level in many gestational tissues (Riley *et al.* 1991, Petraglia *et al.* 1993, 1996, Clifton *et al.* 1998, Grammatopoulos *et al.* 1998, Karteris *et al.* 1998, Rodriguez-Linares *et al.* 1998, Stevens *et al.* 1998, Florio *et al.* 2000, Wetzka *et al.* 2003). However, these findings require further differentiation with regard to quantification of relative expression patterns of CRH, urocortin, CRH-BP, CRH-R1 and CRH-R2. No experimental data are available as to whether CRH from intrauterine sources other than the placenta, or urocortin, are involved in the control of labour, or whether their activity might be outbalanced by locally produced CRH-BP (Linton *et al.* 2001). In this study, data obtained by real-time quantitative RT-PCR on the mRNA expression of these components of the CRH system in placenta, choriodecidua and myometrium at term are presented.

Materials and methods

Tissue collection

Tissue samples were collected from nine patients (aged 28–42 years) undergoing Caesarean section between the 38th and 40th week of gestation, before the onset of labour, after uncomplicated pregnancies. The indications for Caesarean section were cephalopelvic disproportion ($n=5$), breech presentation ($n=3$) and placenta previa ($n=1$). In order to compare placental CRH and urocortin mRNA expression in labouring and non-labouring women, another set of samples of placenta obtained from three different groups were investigated. Each group consisted of six women delivering between the 38th and 40th week of gestation, either by primary Caesarean section (cephalopelvic disproportion, breech presentation), secondary Caesarean section (due to failure to progress in labour) or spontaneous birth. In addition, a single biopsy from the myometrial fundus was taken from a 28-year-old woman after a hysterectomy for cervical cancer at 35 weeks of gestation.

The placenta samples were excised from cotyledons after ablation of the choriodecidual layer. Samples with an area of 100–200 cm² were dissected from foetal membranes. Small strips of myometrium (1–2 g) were cut from the upper incision of the uterotomy. The biopsies were transferred immediately to the laboratory and rinsed thoroughly with phosphate-buffered saline. For preparation of the choriodecidua, the amnion was peeled off the foetal membranes and the choriodecidua was carefully scraped off the chorion layer. As term decidua and chorion are entangled, it is not possible to obtain pure decidua samples. Tissue samples for analysis of mRNA expression were snap-frozen within 30–60 min in liquid nitrogen and stored at -80°C . The biopsies were fixed for immunostaining in buffered formalin prior to processing the paraffin sections. The study was approved by the Ethics Committee of the University of Freiburg and patients gave their written informed consent.

RNA extraction and cDNA preparation

Total RNA was extracted from frozen tissue samples using a commercially available kit (Trizol; Invitrogen, Karlsruhe, Germany). Approximately 100 µg total RNA was subsequently treated with

DNAse (DNAse I Set; Quiagen, Hilden, Germany) on RNeasy mini-columns (Quiagen). Quantification of total RNA was performed by measuring absorbance at OD₂₆₀. Concentrations of all RNA templates were >100 ng/μl. The quality of total RNA was controlled by running 1.5% agarose gels buffered in 89 mM Tris, 89 mM boric acid and 2 mM ethylene diamine-tetra acetic acid (pH 8.3) and assessed as acceptable if strong and intact 28S rRNA and 18S rRNA bands were visible under ultraviolet light after staining with ethidium bromide. No bands of genomic DNA were observed in agarose gels after DNAse treatment.

cDNA synthesis from total RNA (2 μg) was carried out in a reaction volume of 20 μl containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 5 μM random hexamer primer, 2.7 mM deoxynucleoside triphosphate and 10 units/μl Superscript II reverse transcriptase (all reagents obtained from Invitrogen). RNA was initially denatured at 85 °C for 5 min. The reaction mixture was then added and reverse transcription was performed at 42 °C for 90 min. The reaction was stopped by denaturing the enzyme at 85 °C for 15 min. The cDNA was stored at -20 °C. For each RNA sample, a parallel reaction tube was prepared as described above, but without reverse transcriptase (RT-negative control).

Real-time quantitative RT-PCR analysis

To quantify mRNA expression of CRH, urocortin, CRH-BP, CRH-R1 and CRH-R2, real-time quantitative RT-PCR (TaqMan PCR; Applied Biosystems, Weiterstadt, Germany) using an ABI Prism 7700 sequence detector (Applied Biosystems) was performed. All samples were run in duplicates on 96-well optical PCR plates (Applied Biosystems) with a TaqMan Universal PCR Master Mix (Applied Biosystems). Standard RNA preparations were included in every RT-PCR run. Primers and probes were designed using the primer design software Primer Express 1.0 (Applied Biosystems) or taken from the commercially available 'Assays on Demand' (AoD; Applied Biosystems). Details of the primer and probe sequences, accession numbers and concentrations are given in Table 1. Self-designed primers were synthesised by TIB Molbiol (Berlin, Germany) and self-designed probes were produced by Applied Biosystems. The

latter were labelled with the reporter dye 6-carboxyfluorescein at the 5'-terminus and with the downstream quencher dye 6-carboxy-tetramethyl-rhodamin at the 3'-terminus. Cyclophilin A was quantified by using 5'-VICTM-labelled predeveloped assay reagents from Applied Biosystems. All assays for the target sequences investigated were optimised to the universal PCR protocol of the manufacturer in order to investigate different target mRNAs on one plate. After initial denaturation for 10 min at 95 °C, denaturation at the subsequent 40–50 cycles was performed for 15 s at 95 °C followed by primer annealing and elongation at 60 °C for 1 min. Prior to the PCR reaction, incubation with AmpErase UNG (Applied Biosystems) was performed for 2 min at 50 °C to destroy contaminating amplicons of preceding TaqMan reactions. The relative expression of the genes of interest was determined by using relative standard dilutions of specific cDNAs and normalising against total RNA. Cyclophilin was inappropriate as the housekeeping gene, as its expression in placenta was significantly lower than in myometrium and choriondecidua ($P < 0.05$). The $\Delta\Delta C_T$ method (Livak & Schmittgen 2001) was applied as a second, comparative method of quantification. However, the data presented for CRH, CRH-BP and CRH receptors were not significantly different when calculated by the $\Delta\Delta C_T$ method (data not shown).

Immunohistochemistry

Paraffin sections (5 μm) were cut, rehydrated and incubated with pronase (0.1%; Linares, Wertheim-Bettingen, Germany) to expose antigens. After inhibition of endogenous peroxidases with 3% H₂O₂, unspecific antibody binding was blocked with 10% rabbit serum for 30 min. Serial sections were then incubated with specific antibodies against human CRH-R1 (sc-12381) and murine CRH-R2β (sc-1826, both obtained from Santa Cruz Biotechnology, Heidelberg, Germany) (1:200) overnight at 4 °C. The CRH-R1 antibody is directed against an epitope between amino acid positions 81 and 109, where no sequence homology exists to CRH-R2, and all CRH-R1 subtypes with the exception of CRH-R1g are detected. The antibody against CRH-R2 was raised against an epitope between amino acids 47 and 66 of the

Table 1 Primers and TaqMan probes used for real-time quantitative RT-PCR

Name	Sequence	Concentration used (nM)	Position
CRH (NM_00756) ¹			
CRH-bs1			
s-Primer	5'-TCCCATCTCCCTGGATCTCAC-3'	600	656–740
as-Primer	5'-GTGAGCTTGCTGTGCTAACTGCT-3'	300	
Probe	5'-TGGCCATTTCCAAGACTTCCCGGA-3'	200	
Urocortin (NM_003553) ¹			
UCN-bs1			
s-Primer	5'-CGAGCAGAACCGCATCATATT-3'	900	452–575
as-Primer	5'-ACAGTGCCCTGGTGGCTCT-3'	600	
Probe	5'-GGCCATCACTTGCCCACCGAGT-3'	200	
CRH-BP (X58022) ¹			
BP-AoD (Hs00181810_ml) ²	5'-CCTTTCATGGCCCGGCCAGATGAA-3'		843–867 ³
CRH-R1 (L23332) ¹			
R1-nh ⁴			
s-Primer	5'-CGCATCCTCATGACCAAGCT-3'	300	911–977
as-Primer	5'-TCACAGCCTTCCTGTAATG-3'	300	
Probe	5'-CGGGCATCCACCACGTCTGAGA-3'	200	
CRH-R2 (U34587) ¹			
R2-bs1 ⁵			
s-Primer	5'-TGCGGAGCATTGCTGT-3'	600	422–488
as-Primer	5'-TTTCGCAGGATAAAGGTGGTG-3'	900	
Probe	5'-TGAGGTTCCAGTGAATCACATTCCGCA-3'	200	

¹GenBank Accession no.; ²Article no. of the manufacturer (Applied Biosystems); ³25 bp partial sequence (exact position not given by the manufacturer); ⁴Primer sequences after Hiroi *et al.* 2001, CRH-R1-subtypes α , β , γ , δ , ϵ , ζ , η , θ (but not CRH-R1g) were detected; ⁵Self-designed primers, which detect CRH-R2 α , CRH-R2 β and CRH-R2 γ .
s, sense; as, antisense.

murine CRH-R2 and detects CRH-R2 α , CRH-R2 β and CRH-R2 γ . It cross-reacts with human CRH-R2. The bound antibodies were detected with the biotin–streptavidin–peroxidase system (Vectastain-ABC-kit; Serva, Heidelberg, Germany) using diaminobenzidine (Sigma-Aldrich, Deisenhofen, Germany) as chromogen. Counter-staining was performed with hemalum (Merck Eurolab, Darmstadt, Germany) and the sections were mounted in entellan (Merck Eurolab).

Negative controls were performed by omitting the primary antibody or after preabsorption of the primary antibody with a tenfold excess of the blocking peptides sc-12381P or sc-1826P (both obtained from Santa Cruz). As positive controls, the mouse pituitary cell line AtT20 (ECACC, Salisbury, Hants, UK) for CRH-R1 and murine heart sections (kindly provided by T Guenther, Centre for Clinical Research, University of Freiburg) for CRH-R2 were used.

The sections were examined by conventional light microscopy.

Statistics

Results are expressed as the means $\pm$ s.d. Comparison between two group means was performed by the Student's *t*-test (normal distribution) or the Mann–Whitney rank sum test (skewed distribution). If more than two groups were compared, a one-way ANOVA was applied, followed by a Tukey test for identification of the statistically distinct groups. Significance was accepted at a *P* value < 0.05 .

Results

CRH and urocortin

The expression of CRH in placenta was > 1000 times higher than in myometrium and chorio-decidua ($P < 0.05$; Fig. 1A). The number of

Figure 1 Distribution of CRH, CRH-BP, CRH-R1 and CRH-R2 mRNA in term gestational tissues. Results of real-time quantitative RT-PCR demonstrate that the placenta is the main site of (A) CRH and (B) CRH-BP mRNA expression. Relevant quantities of (C) CRH-R1 and (D) CRH-R2 mRNA are expressed in myometrium and choriodecidua. TaqMan runs were performed with cDNA preparations reversibly transcribed in the same RT reaction from 100 ng total RNA in each case. Results are expressed as mean values $\pm$ S.D. of relative mRNA expression levels normalised against total RNA. * $P < 0.05$. The mean results for myometrium were arbitrarily assigned the value 1.

threshold cycles (C_T) of CRH in placenta was low (21.0–23.5) indicating high levels of mRNA. In contrast, in myometrium and choriodecidua, CRH mRNA expression was low ($C_T \approx 35$) or at the detection limit ($C_T > 38$). Preliminary experiments revealed no significant differences in CRH mRNA levels in placenta tissues obtained after Caesarean section, with or without labour, or after vaginal delivery (data not shown).

The mRNA expression of urocortin was low in all tissues. No significant differences in urocortin mRNA expression were observed between placenta, myometrium and choriodecidua (Fig. 2). Despite the low levels, expression of urocortin mRNA was unequivocally detectable in the patient samples compared with the RT-negative controls. In preliminary experiments, no significant elevation in urocortin mRNA expression was observed after labour (Caesarean section or spontaneous delivery) compared with primary Caesarean section without labour (data not shown).

CRH-BP

The mRNA expression of CRH-BP in placenta, myometrium and choriodecidua was investigated in the same set of samples used for CRH mRNA. In placenta, the mRNA levels were >20 times higher than those detected in myometrium and choriodecidua ($P < 0.05$; Fig. 1B).

CRH receptors

Expression of CRH-R1 mRNA was moderate in myometrium and choriodecidua and very low in placenta (Fig. 1C). CRH-R2 mRNA was more evenly distributed (Fig. 1D). The relative expression of both CRH-R1 and CRH-R2 mRNA was highest in myometrium. In one patient undergoing hysterectomy immediately after birth, the level of CRH-R2 mRNA (but not of CRH-R1 mRNA) was higher in the fundus than in the lower uterine segment (data not shown).

Figure 2 Expression of urocortin mRNA in gestational tissues. The TaqMan data shown illustrate that expression of urocortin mRNA was low, but could be clearly discriminated from RT-negative controls in all the gestational tissues investigated. The number of C_T of samples (solid bars) and RT-negative controls (shaded bars) of one representative patient are presented as means $\pm$ s.d. TaqMan runs were performed in duplicate with cDNA preparations derived from 100 ng total RNA in each case.

Immunostaining of CRH-R1 and CRH-R2

In placenta, there was strong staining for CRH-R2, mainly on the syncytiotrophoblast, whereas only weak staining was observed for CRH-R1 (Fig. 3C and D). In myometrium, staining of both receptor isotypes was confined to nests of few leiomyocytes (Fig. 3G and H). Choriodecidua was positively stained for both receptor subtypes (Fig. 3K and L). The positive controls for CRH-R1 (AtT20 cells) and CRH-R2 (murine heart) were strongly stained (not shown).

Discussion

This study presents for the first time quantitative data on differential mRNA expression profiles of CRH, urocortin, CRH-BP and CRH-R1 and -R2 in placenta, myometrium and choriodecidua as well as providing data on the distribution of CRH-R1 and CRH-R2 protein in these tissues. Whereas in previous studies non-quantitative or semi-quantitative methods were applied (e.g. Grino *et al.* 1987, Petraglia *et al.* 1993, 1996, Clifton *et al.* 1998, 2000, Grammatopoulos *et al.* 1998, Karteris *et al.* 1998, Watanabe *et al.* 1999, Florio *et al.* 2000, Wetzka *et al.* 2003) we investigated differential mRNA expression patterns by using real-time quantitative RT-PCR. CRH and CRH-BP mRNA was mainly expressed by placenta. CRH-R1 and CRH-R2 mRNA and protein were predominantly

expressed in myometrium and choriodecidua. Urocortin mRNA expression was low in all tissues investigated.

The high expression of the CRH gene observed in placenta is in agreement with earlier findings, which describe this organ as the main source of intrauterine CRH protein production (Shibasaki *et al.* 1982, Goland *et al.* 1988, Sasaki *et al.* 1988, Glynn *et al.* 1998). To date, the possible quantitative contribution of other gestational tissues to intrauterine CRH synthesis has hardly been addressed. Our quantitative RT-PCR results demonstrated that the contribution of myometrium and choriodecidua to intrauterine CRH mRNA expression is negligible. As recently recommended (Bustin 2002), the data shown were normalised to total RNA.

In the present study, the expression of urocortin was low (but clearly higher than zero) in all gestational tissues including pregnant myometrium. The expression of urocortin mRNA in placenta, foetal membranes and choriodecidua has previously been demonstrated by non-quantitative methods such as conventional RT-PCR (Petraglia *et al.* 1996) and *in situ* hybridisation (Watanabe *et al.* 1999). Urocortin protein concentrations in maternal plasma and pregnancy tissues, however, were reported to be low (Glynn *et al.* 1998, Clifton *et al.* 2000). Considered together, our data and the previous findings suggest that urocortin plays, at most, a local role in the control of human labour.

We further demonstrated that expression of CRH-BP mRNA was more than 20 times higher in placenta than in myometrium and choriodecidua. The expression pattern of CRH-BP thus resembles that of CRH. To date, the role of myometrial and decidual CRH and CRH-BP has been unclear (Linton *et al.* 2001). Our present and earlier findings (Wetzka *et al.* 2003) demonstrate that both CRH and CRH-BP are expressed in myometrium and choriodecidua in low quantities only, suggesting only a minor significance for paracrine or autocrine CRH action in these tissues during the birth process.

As far as CRH receptors are concerned, we have elaborated the quantitative data on mRNA distribution of CRH-R1 and -R2 in term gestational tissues. In addition, we have investigated the tissue-specific protein distribution of CRH receptors by immunostaining with CRH-R1 and CRH-R2 subtype-specific antibodies.

Figure 3 Immunolocalisation of CRH-R1 and CRH-R2 in placenta, myometrium and choriodecidua. The figure shows representative sections of (A–D) placenta ($n=2$), (E–H) myometrium ($n=3$) and (I–L) choriodecidua ($n=4$) immunostained with subtype-specific antibodies against (C, G and K) CRH-R1 and (D, H and L) CRH-R2. (B) Negative control without primary antibody, (A, E and I) negative controls (preabsorption) for CRH-R1 and (F and J) negative controls (preabsorption) for CRH-R2.

Myometrium and choriodecidua expressed substantial amounts of both receptor subtypes, whereas the CRH receptor population in placenta mainly consisted of CRH-R2. When interpreting the findings in myometrium, one should bear in mind the potential topographic distinct expression patterns of receptors. The biopsies investigated were taken from the lower uterine segment, where the CRH receptor distribution is probably different from that in the fundus (Stevens *et al.* 1998). This has also been demonstrated for prostaglandin and oxytocin receptors (Fuchs *et al.* 1984). Our preliminary TaqMan PCR findings suggest that CRH-R2 expression in the fundus is more pronounced than in the lower uterine segment. On the basis of these observations, we present a more accurate description of quantitative CRH receptor expression in gestational tissues than previous contributions based on conventional PCR methods (Glynn *et al.* 1998, Grammatopoulos *et al.* 1998, Karteris *et al.* 1998, Stevens *et al.* 1998, Florio *et al.* 2000).

The specific contribution of CRH receptor isoforms to parturition is currently a subject of intense discussion (Linton *et al.* 2001, Hillhouse & Grammatopoulos 2002). Evaluation of the biological relevance of CRH receptor expression in gestational tissues is complex, as several variants of the CRH-R1 and -R2 subtypes have been identified, at least in human myometrium (Grammatopoulos *et al.* 1998, Linton *et al.* 2001). These different CRH receptor isoforms are believed to be coupled to different signal transduction pathways. Primarily, CRH receptor activation is linked to the generation of cAMP which mediates myometrial relaxation. In certain CRH receptor isoforms (e.g. CRH-R1 β , CRH-R1c, CRH-R1d, CRH-R2 α truncated), the potential for activation of adenylate cyclase is reduced or lost (Linton *et al.* 2001). As far as the potential role of CRH in human myometrium is concerned, it has been speculated that spatial and temporal differential expression of CRH receptor variants permits CRH to act in a pleiotropic manner, in particular, to promote uterine quiescence during the majority of pregnancy and to contribute to the enhancement of contractility at term (Hillhouse & Grammatopoulos 2002).

Overall, the biological action of CRH in the pregnant human uterus at term is, as yet, only partially understood. It is generally accepted that

CRH exerts a potent vasodilative effect on the human foetoplacental circulation via nitric oxide (Clifton *et al.* 1995) and stimulates biosynthesis of dehydroepiandrosterone sulphate and cortisol in the foetal adrenals (Smith *et al.* 1998). In contrast, early reports of enhancing effects of CRH on myometrial contractility in combination with oxytocin or prostaglandin F_{2 α} could not be confirmed (Simpkin *et al.* 1999). No direct CRH effects on the secretion of inflammatory cytokines in human myometrial tissues have been observed (Schringer *et al.* 2000), but, in a recent study, it was demonstrated that CRH increases the lipopolysaccharide-triggered formation of interleukin (IL)-1 β , IL-6 and tumour necrosis factor α in macrophages (Agelaki *et al.* 2002), which indicates that CRH plays an amplifying role in neuroendocrine-immune interactions.

CRH receptors are widely distributed in human organs and tissues, particularly in the brain, heart, lung, skeletal muscle and adrenals (Hiroi *et al.* 2001). It has been suggested that peripheral CRH systems act as a link between stress stimuli and pathophysiological somatic responses, e.g. activation of the immune system and inflammatory reactions (Webster *et al.* 1998, Baigent 2001). Interestingly, the last trimester of pregnancy is characterised by increasing stress (induced for example by the growing foetus) and parturition resembles in many aspects an inflammatory process (Kelly 1996).

In summary, the detection of substantial quantities of CRH receptor mRNA and protein in placenta, myometrium and choriodecidua suggests that these tissues are relevant targets for locally produced CRH. The placenta has been identified as the main source of CRH in gestational tissues. The expression of urocortin was low in all the tissues investigated. Potentially different expression patterns of the CRH system in preterm deliveries, or other pathological pregnancies, remain to be determined.

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