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Corticotropin-releasing hormone acts on CRH-R1 to inhibit the spontaneous contractility of non-labouring human myometrium at term

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ABSTRACT

Aims: Corticotropin-releasing hormone (CRH) has been implicated in the mechanisms controlling human parturition. The aims of the present study were to explore effects of CRH on contractility of human term myometrium and compare these effects in labouring and non-labouring myometrial strips.
Main methods: The cumulative effects of CRH (10^{-10} to 10^{-7} mol/l) on the spontaneous contractility of labouring and non-labouring myometrial samples were evaluated using isometric tension recordings.
Key findings: CRH exhibited a concentration-dependent relaxant effect on spontaneous contractions in non-labouring term myometrium. This effect was mediated principally via a reduction in the amplitude rather than any changes in the frequency of contractions. The CRH-induced inhibitory effect on contractility could be blocked by pre-treatment with a CRH-R1 antagonist antalarmin, but not by pre-treatment with the CRH-R2 antagonist astressin 2B. CRH had no effect on spontaneous contractions in the labouring myometrium, as no change in either the amplitude or the frequency was observed.
Significance: Our findings indicate that CRH acts on CRH-R1 to inhibit spontaneous contractions in term myometrium from women who were not undergoing labour, but not those who were undergoing labour, supporting the hypothesis that CRH exerts dual effect on myometrium during pregnancy.

Introduction

Corticotropin-releasing hormone (CRH), a 41 amino acid neuropeptide, has been identified in human gestational tissues. During human pregnancy, the placenta and fetal membrane produce large amounts of CRH and release it into maternal and fetal circulation (Sasaki et al., 1987; Riley and Challis, 1991; Riley et al., 1991). CRH concentrations in maternal plasma are exponentially increased with advancing gestation and reach a peak at the time of labour (Sasaki et al., 1987; McLean et al., 1995; Makrigiannakis et al., 2007). Abnormal rates of increase or excessive levels of placental CRH are significant risk factors for an earlier onset of spontaneous birth (Warren et al., 1992; McLean et al., 1995; Korebrits et al., 1998; Makrigiannakis et al., 2007). Thus, CRH is proposed to regulate a placental clock that controls a cascade of physiological events leading to parturition (McLean et al., 1995; Sandman et al., 2006). Despite general agreement concerning the significance of CRH for the timing of birth, the possible biological effects of CRH on parturition remain to be elucidated.

An increasing body of evidence suggests that CRH is involved in the mechanisms controlling myometrial contractility. Two types of CRH receptors, termed CRH-R1 and CRH-R2, have been identified in the human myometrium (Grammatopoulos et al., 1995; Rodriguez-Linares et al., 1998; Wetzka et al., 2003; Jin et al., 2007). Current data from cultured myometrial cells suggests that CRH-R1 and -R2 mediate distinct actions of CRH on the pregnant myometrium (Grammatopoulos et al., 2000; Hillhouse and Grammatopoulos, 2001; Aggelidou et al., 2002; Karteris et al., 2004). For example, CRH-R1 activation up-regulates the expression of a constitutive form of nitric oxide synthase (Aggelidou et al., 2002), thereby promoting myometrial quiescence. In contrast, CRH-R2 activation can activate extracellular regulated-signalling kinase (ERK) 1/2 and RhoA pathways that actively promote myometrial contractility (Karteris et al., 2004). However, other studies investigating the effects of CRH on contractions in human pregnant myometrium strips have provided contradictory data. Studies by Quartero and Fry (1989) and Benedetto et al. (1994) showed that CRH potentiated contractile effects of oxytocin and prostaglandin (PG) $F2_{\alpha}$ in the pregnant myometrium. In contrast, Simpkin et al. (1999) found that the contractile responses to oxytocin and $PGF_{2\alpha}$ were not modified by CRH. More recently, Mignot et al. (2005) reported that CRH is able to relax the pregnant human myometrium prior to the onset of labour.

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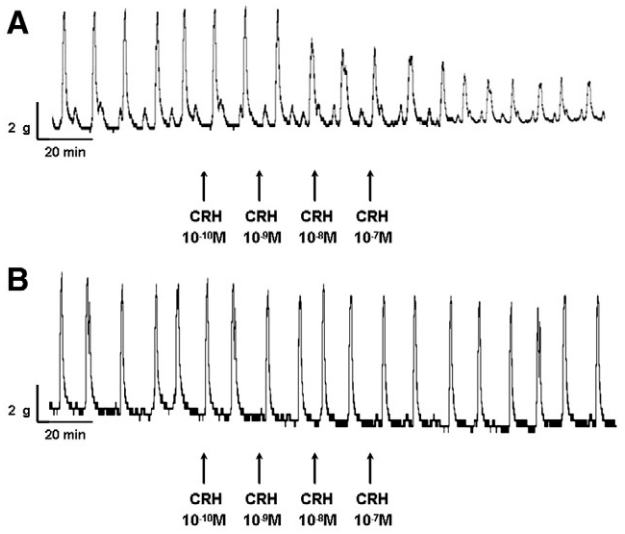

Fig. 1. A representative recording of spontaneous contraction of term myometrial strips demonstrates a cumulative effect of CRH on non-labouring myometrium (A) and labouring myometrium (B).

In the present study, we compared pregnant women undergoing labour and those not undergoing labour for in vitro CRH myometrial responsiveness and explored the specific CRH receptor involved. We found that CRH significantly inhibited the spontaneous contractility of non-labouring myometrium, but failed to inhibit the spontaneous contractility of the labouring myometrium. These effects were mediated by CRH-R1, and not by CRH-R2.

Materials and methods

Tissue collection and preparation

This study was approved by the human ethics committee of Changhai Hospital, Second Military Medical University, and informed consent was obtained from all patients. Human myometrial tissues were obtained from ten women undergoing elective caesarean section (non-labour) and eight patients undergoing emergency caesarean section (labour) at term (37–42 weeks). Indications for caesarean section included breech presentation, placenta previa, previous caesarean section, cephalopelvic disproportion, failure of labour to progress, fetal distress, or maternal request. None of the women included in this study showed evidence of underlying disease (e.g. hypertension, diabetes, pre-eclampsia, intrauterine growth restriction, etc.). Tissue samples were obtained from the upper edge of the incision line in the lower uterine segment at the caesarean section. All myometrial samples were taken from the external longitudinal layer. Samples were dissected free of serosa and immediately placed at 4 °C in Kreb's solution: NaCl (118.0 mmol/l), KCl (4.7 mmol/l), $CaCl_2$ (2.5 mmol/l), $MgSO_4$ (1.0 mmol/l), KH_2PO_4 (1.0 mmol/l), glucose (11.0 mmol/l) and $NaHCO_3$ (25.0 mmol/l). Samples were then transported to the laboratory where they were stored at 4 °C and used within 6 h.

Measurement of isometric tension in human uterine strips

The myometrial tissues were cut into 3×3×10 mm pieces, mounted on parallel wires and placed in a 10 ml organ bath filled with Kreb's solution maintained at 37 °C, and bubbled with a gas mixture (95% O_2, 5% CO_2). The contractile activity was measured isometrically by a tension transducer, followed by computerized recording and processing (MedLab, Nanjing, China). Each strip of myometrium was set up under an initial tension of 2 g and allowed to equilibrate for 2 h, and the Kreb's solution was changed every 20 min. Any muscle strip that did not develop spontaneous contraction, regular in frequency and strength at the end of the equilibration period, was discarded. After equilibration, spontaneously active tissue was exposed to a cumulative increase in concentrations of human/rat CRH (Sigma-Aldrich, St. Louis, MO) or urocortin II (Bachem California Inc., Torrance, CA) every 20 min. The CRH receptor type 1 antagonist antalarmin (Sigma-Aldrich) or type 2 antagonist astressin 2B (Sigma-Aldrich) was added at 10^{-6} mol/l 20 min before the strips were incubated with 10^{-8} mol/l CRH.

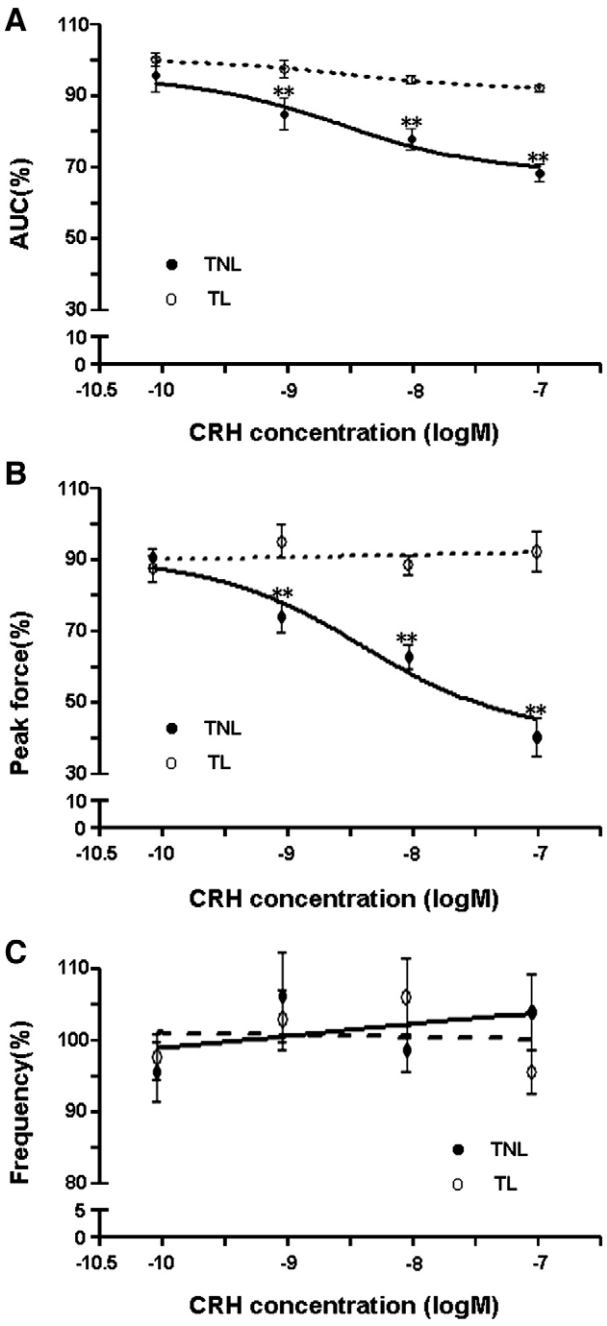

Fig. 2. Cumulative data of AUC (A), amplitude (B), and frequency (C), showing the effects of CRH on the spontaneous contractility of term pregnant myometrium. Cumulative increases in CRH (10^{-10} to 10^{-7} M) were applied to pregnant myometrial strips. Data are presented as the mean (SEM) percentage of the results obtained before drug application for each individual strip (control). $^{**}P<0.01$ vs. control. TNL: term no labour (from 10 patients, $n=10$); TL: term labour (from eight patients, $n=8$).

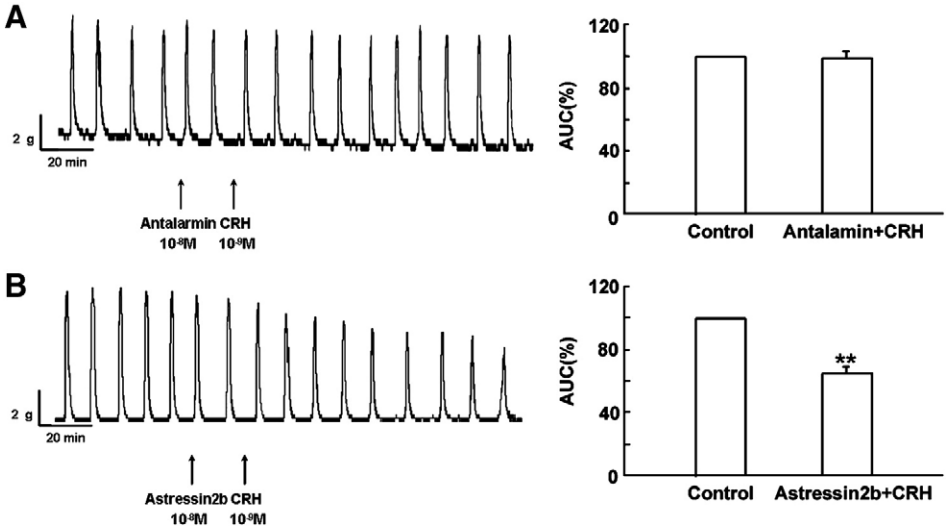

Fig. 3. Effects of the CRH-R1 antagonist antalarmin (A) and the CRH-R2 antagonist astressin 2B (B) on CRH-induced relaxation of non-labouring myometrium. Antalarmin (10^{-6} M) or astressin 2B (10^{-6} M) was applied to non-labouring myometrial strips prior to CRH exposure (10^{-8} M). Data are presented as mean ± SEM percentage of the results obtained before any drug application for each individual strip (control). Data were obtained from 6 patients (n=6). **P<0.01 vs. control.

Appropriate controls (incubation with solvents) were run under similar experimental conditions in rings of uterus obtained from the same woman. Only one concentration–response curve was performed for each uterine strip.

The responses were quantified by the amplitude and frequency of the contractions as well as integration of the area under each contractile record (AUC) using software written specifically for this purpose. The AUC was measured from the basal tension over a 10-min period after each stimulus. The effects were evaluated by comparing the experimental responses with the controls, which were set at 100%.

Statistical analysis

Contractile responses to CRH were expressed as a percentage of the spontaneous contraction at basal tension (control). The amplitude, frequency and AUC of contractions were evaluated. Data are expressed as mean ± SEM for the specified number of samples tested. Analysis of variance (ANOVA) for repeated measures was performed, followed by the Bonferroni corrected t-test. Individual comparisons were made by Student's t-test for paired data. A p-value of <0.05 was considered to be statistically significant.

Results

Effects of CRH on the spontaneous contractility of non-labouring and labouring myometrium

Human myometrium strips from all pregnant women participating in this study developed spontaneous phasic contraction although not all of the pieces developed spontaneous contractions.

Myometrium strips from patients who underwent elective caesarean section were treated with increasing concentrations of CRH (10^{-10} to 10^{-7} mol/l), which caused a concentration-dependent partial inhibition of the phasic contractile activity (see Fig. 1A). The mean AUC calculated for the highest concentration of CRH was 68.32 ± 4.56% of control. CRH treatment significantly decreased the amplitude of contraction, but no significant change in the frequency of contractions was seen (Fig. 2).

Administration of increasing concentrations of CRH (10^{-10} to 10^{-7} mol/l) had no effect on the spontaneous contractility of myometrial strips from patients who were undergoing labour (Figs. 1B and 2).

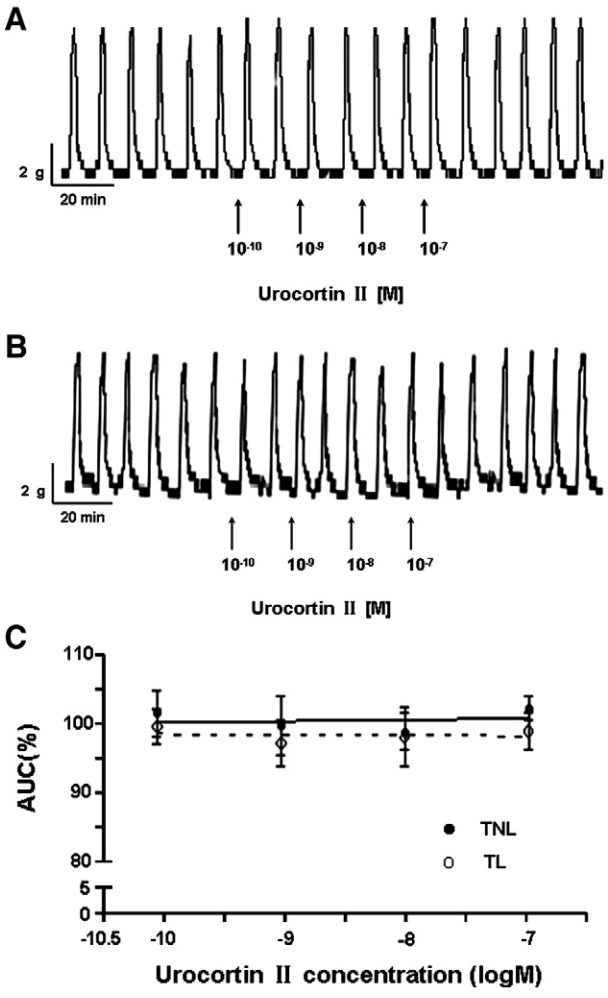

Fig. 4. Effect of urocortin II on spontaneous contractions in term myometrial strips. Cumulative increases in urocortin II (10^{-10} to 10^{-7} M) were applied to pregnant myometrial strips. Representative recordings of spontaneous contraction of non-labouring strips (A) and labouring myometrium (B) in the presence of urocortin II. (C) Cumulative data of AUC showing the effect of urocortin II on non-labouring and labouring myometrium. Data are presented as mean ± SEM percentage of the results obtained before any drug application for each individual strip (control). TNL: term no labour (n=7); TL: term labour (n=7).

CRH-R1, but not CRH-R2 mediates the effect of CRH on spontaneous contractility

Pre-treatment with the CRH-R1 antagonist antalarmin (10^{-6} mol/l), which by itself did not change uterine contractility, blocked CRH (10^{-8} mol/l) induced inhibition of spontaneous contraction (Fig. 3A). However, pre-treatment with astressin 2B induced no significant change in CRH (10^{-8} mol/l) induced relaxation of uterine strip from patients not undergoing labour (Fig. 3B).

In order to further elucidate the role of CRH-R2 in the regulation of myometrial contractility, we used the exclusive CRH-R2 agonist urocortin II. It was found that cumulative administration of urocortin II (10^{-10} to 10^{-7} mol/l) had no significant effect on the spontaneous contractility of non-labouring as well as labouring myometrium strips (Fig. 4).

Discussion

In this study we have demonstrated for the first time that CRH exhibits differential effects on the contractility of myometria from pregnant women who were undergoing labour and those who were not undergoing labour. CRH dose-dependently inhibited the spontaneous contractility of non-labouring myometrium, although it did not have a significant effect on spontaneous contractions in labouring myometrium strips. The relaxant effect of CRH on non-labouring myometrium appeared to be mediated exclusively via CRH-R1, as shown by the use of specific CRH receptor antagonists.

Our finding that CRH acted on CRH-R1 to inhibit spontaneous contractions in non-labouring myometrium, while having no effect on the contractions in labouring myometrium, suggests that CRH has dual effects on myometrium during pregnancy. Previous studies by Makrigiannakis et al. (2001) suggested dual roles for CRH via CRH-R1 in the regulation of implantation and maintenance of early pregnancy. CRH, when expressed in normal quantities in the placenta, facilitates the immune tolerance of the invading trophoblast acting through CRH-R1. However, when CRH is expressed in high quantities, apoptosis of trophoblasts via CRH-R1 is induced (Minas et al., 2007). It is known that maternal CRH levels increase after labour onset compared with the level of CRH before labour. However, in this study, CRH in a concentration range from 10^{-10} to 10^{-7} mol/l did not exhibit opposing effects on contractility in vitro.

Our previous study demonstrated no significant difference in protein level of either CRH-R1 or CRH-R2 between labouring and non-labouring myometrial tissues (Jin et al., 2007), which cannot explain the differential effects of CRH on labouring and non-labouring myometrium. Notably, both CRH-R1 and CRH-R2 have multiple mRNA splice variants (Liaw et al., 1996, Valdenaire et al., 1997, Kostich et al., 1998, Pisarchik and Slominski, 2001). Currently, eight variants of mRNA for CRH-R1, termed R1α-h, have been described, although their encoding proteins have not been characterized (Pisarchik and Slominski, 2001, 2004). Of those, CRH-R1α is believed to be the main functional CRH-R1 variant, while -R1β can be considered as "pro-CRH-R1" receptor variant (Grammatopoulos et al., 2000, Karteris et al., 2003, Sirianni et al., 2005, Hillhouse and Grammatopoulos, 2006). Other CRH-R1 variants have unique binding and signalling properties. For example, CRH-R1c has a decreased binding capacity (Wille et al., 1999; Hillhouse and Grammatopoulos, 2006), while CRH-R1e appears to attenuate CRH-R1α signalling in coexpression experiments (Wille et al., 1999; Pisarchik and Slominski, 2004; Hillhouse and Grammatopoulos, 2006). CRH-R1d and -R1f variants have an impaired signal transduction (Grammatopoulos et al., 1999; Hillhouse and Grammatopoulos, 2006). Markovic et al. (2007) demonstrated that expression of CRH-R1α and -R1d was increased while CRH-R1β expression was reduced in human term myometrium with parturition. However, our previous study showed that the CRH-R1α, -R1β, -R1c, -R1e and -R1f variants were all expressed in the term myometrium although not all of them were identified in each sample. It seemed that the detectable rates of CRH-R1β, -R1e and -R1f were higher in labouring myometrium than in non-labouring myometrium (Jin et al., 2007). In addition, studies by Hillhouse and Grammatopoulos demonstrated changes in signalling pathways activated by CRH toward the end of pregnancy (Hillhouse and Grammatopoulos, 2001, 2002). During pregnancy, CRH can activate Gsα protein and adenylate cyclase, leading to production of cAMP, which promotes myometrial quiescence. At term, CRH can activate the Gqα protein with subsequent stimulation of the phospholipase C/inositol triphosphate pathway, which favours myometrial contraction. Taken together, changes in CRH receptor variant and their signalling pathway systems in human myometrium may account for a shift in the roles of CRH in myometrium towards the end of pregnancy. Nevertheless, the mechanisms by which CRH controls myometrial contractility during pregnancy need to be further characterized.

Conclusion

CRH acts on CRH-R1 to inhibit spontaneous contractions in term non-labouring myometrium, but does not affect those in term labouring myometrium. This suggests that CRH exerts dual effects on myometrium during pregnancy.

Acknowledgements

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