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Human Chorionic Gonadotrophin Relaxation of Human Pregnant Myometrium and Activation of the BK$_{Ca}$ Channel

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The uterorelaxant effect of human chorionic gonadotropin (hCG) is regarded as an important mediator in maintenance of uterine quiescence during pregnancy with clinical potential for tocolysis, the mechanisms of which are unknown. The large conductance calcium-activated K$^+$ channel (BK$_{Ca}$) is ubiquitously encountered in human uterine tissue and plays a significant role in modulating myometrial cell membrane potential and excitability. The objective of this study was to investigate the involvement of BK$_{Ca}$ channel function in the response of human myometrial cells to hCG. Single electrophysiological BK$_{Ca}$ channel recordings from freshly dispersed myocytes were obtained in the presence and absence of increasing hCG concentrations. Isometric tension studies, investigating the effects of hCG on isolated myometrial contrac- tions, in the presence and absence of the BK$_{Ca}$ channel blocker, iberiotoxin, were performed. The hCG significantly increased the open-state probability of these channels in a concentration-dependent manner [control 0.036 ± 0.01; 1 IU/ml hCG 0.065 ± 0.014 ($P = 0.262$); 10 IU/ml hCG 0.111 ± 0.009 ($P = 0.001$); and 100 IU/ml hCG 0.098 ± 0.004 ($P = 0.007$)]. *In vitro* functional studies demonstrated that hCG exerted a significant concentration-dependent relaxant effect on human myometrial tissue. This effect was significantly attenuated by preincubation with iberiotoxin ($P < 0.05$). These findings outline that activation of BK$_{Ca}$ channel activity may explain the potent uterorelaxant effect of hCG. (*J Clin Endocrinol Metab* 88: 4310–4315, 2003)

THE FACTORS REGULATING myometrial quiescence during human pregnancy, and the subsequent transformation to a state of maximal excitability during labor, are poorly understood (1–3). Knowledge pertaining to such factors is essential to both an understanding of the physiology of human parturition and the clinical management of human labor and disorders thereof. The major clinical problem associated with human labor is that of preterm labor, which is the leading cause of perinatal mortality and morbidity in the developed world (1, 4, 5).

Human chorionic gonadotropin (hCG) is a heterodimeric glycoprotein produced primarily in the placenta (6). Although the importance of hCG in maintenance of early pregnancy has been widely accepted, recent reports have highlighted a potential role of hCG in maintaining uterine quiescence in the third trimester. The hCG exerts a potent concentration dependent inhibitory effect on human myometrial contractions *in vitro* (7). The hCG receptors in human myometrium are down-regulated following the onset of labor, in both term and preterm deliveries (7, 8). Although possible explanations for the inhibitory effect of hCG in human myometrial smooth muscle have included a direct reduction of intracellular calcium availability (9, 10), downregulation of myometrial gap junctions (11) and inhibition of uterotonic agents such as eicosanoids (8), little is known of the endogenous mechanisms of hCG regulation of uterine contractility during pregnancy.

The state of contractility of the myometrium is ultimately dependent on both the concentration of and sensitivity to intracellular calcium (2, 12). Cell membrane ion channels play a major role in regulation of smooth muscle activity by controlling cell membrane potential and in the regulation of intracellular calcium concentration. For myometrium, Ca^{2+}, Na$^+$, K$^+$, and Cl$^-$ channels all contribute to the regulation of the resting membrane potential and the action potential, and both directly and indirectly contribute to the regulation of intracellular free Ca^{2+} concentrations (12). The K$^+$ channels are the largest category of ion channels in the cell and hence are of primary importance in the regulation of membrane potential and cell excitability in the myometrium (13–16). The large conductance calcium-activated potassium channel (BK$_{Ca}$ or maxi-K channel) is the predominant K$^+$ channel type encountered in nonpregnant and pregnant human myometrium (14, 15) and has been reported as playing a significant role in modulating myometrial function in both animal models and in humans (12, 16). Although it is established that BK$_{Ca}$ channel function in smooth muscle is closely linked to membrane conductance and cellular excitability, little is known about the actual mechanisms or role of BK$_{Ca}$ modulation by endogenous substances. This study was undertaken to investigate whether hCG exerted an effect on open-state probability of BK$_{Ca}$ in single channel electrophysiological recordings of human uterine myocytes and, second, to evaluate whether the relaxant effect of hCG on myometrial

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Abbreviations: BK$_{Ca}$, Calcium-activated K$^+$ channel; hCG, human chorionic gonadotropin; IbTX, iberiotoxin; NP$_o$, mean open probability; PSS, physiologic saline solution; TEA, tetraethylammonium.

contractions was altered by BK$_{Ca}$ blockade in functional studies using iberiotoxin (IbTX).

Materials and Methods

Tissue collection

Biopsies of human myometrium were obtained from women undergoing elective cesarean section in the third trimester of pregnancy in the Department of Obstetrics and Gynecology, University College Hospital, Galway, Ireland. The biopsies were excised from the midline portion of the upper lip of the incision in the lower uterine segment. Women who had received exogenous prostaglandins, oxytocin, or corticosteroids were excluded from the study. Recruitment was by written informed consent. Ethics committee approval for the study was obtained from the Research Ethics Committee, University College Hospital, Galway. For electrophysiological studies, tissue samples were immediately placed in sterile Ham's F-12 medium (Sigma-Aldrich, Dublin, Ireland) supplemented with 100U/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich). Tissue samples for isometric recordings were placed in fresh Krebs-Henseleit physiologic saline solution (PSS) of the following composition (mmol/liter): 4.7 KCl, 118 NaCl; 1.2 MgSO$_4$, 1.2 CaCl$_2$, 1.2 KH$_2$PO$_4$, 25 NaHCO$_3$, and 11 glucose (Sigma-Aldrich). Tissue was stored at 4 C and used within 6 h of collection.

Tissue dispersion/primary human myometrial cells

The preparation of single myometrial cells for electrophysiological recordings was performed using methodology previously described (17, 18). Freshly isolated myometrial cells were obtained by enzymatic digestion of finely minced myometrium with 2 mg/ml collagenase (type IA, 300–400 U/mg) (Sigma-Aldrich) in Hanks' buffered salt solution. The incubation with enzyme was performed at 37 C for 2 h followed by centrifugation (1000 revolutions per minute) in 50% Percoll (Sigma-Aldrich) for 10 min. The cell layer was removed, washed, and spun in physiological solution to remove excess red blood cells. The cell suspension was then triturated and filtered through an 80-μm nylon mesh filter. Single cells were placed in a recording chamber (Warner Instrument Corp., Hamden, CT) and electrophysiological experiments begun immediately. Morphologically, freshly dissociated uterine myocytes were characterized by a long, slender fusiform shape. All myocytes used for this work were relaxed and adhered to the bottom of the recording chamber with no additional substrate.

Electrophysiological recordings of the BK$_{Ca}$ channel

Single-channel recordings using the cell-attached configuration of the patch clamp technique were performed. Several drops of cell suspension were placed in the recording chamber containing a solution of the following composition (mmol/liter): 140 KCl, 10 MgCl$_2$, 0.1 CaCl$_2$, 10 HEPES, and 30 glucose (pH 7.4, 22–25 C). Single potassium channel currents were measured in cell-attached patches by filling the patch pipette (2–5 MΩ) with Ringer solution composed of (mmol/liter): 140 NaCl, 5 KCl, 1 MgCl$_2$, 2 CaCl$_2$, and 10 HEPES, and making a gigaohm seal on a single myocyte. Voltage across the patch was controlled by setting the cellular membrane potential to 0 mV using a high-[K$^+$] extracellular solution. Average channel activity in patches was measured as mean open probability (NP$_o$). The effects of hCG (1, 10, and 100 IU/ml) on channel activity were measured by recording about 10 sec of continuous recording at +40 mV before and 15 min after drug treatment. Currents were filtered at 1 kHz and digitized at 10 kHz. In all experiments, voltage-clamp and voltage-pulse generation were controlled with an Axopatch 200-A patch-clamp amplifier (Axon Instruments, Inc., Union City, CA), and data were acquired and analyzed with pClamp 8. For statistical purposes and to ensure accuracy, NP$_o$ was calculated for single BK$_{Ca}$ channels at a depolarized potential in which channel openings can be clearly differentiated from other channel species. BK$_{Ca}$ channels are easily visualized at +40 mV because there is virtually no contamination with other channel species. In addition, at such depolarized voltages, there is no other ion channel expressed in these cells with an amplitude of 7–9 pA, making statistical analysis of BK$_{Ca}$ channel activity more accurate (19). Moreover, recording single-channel activity at more depolarized potentials helps compensate the artificial depression of

activity otherwise encountered from recording channel activity at room temperature (22–25 C) (19).

Isometric tension recordings

Longitudinal myometrial strips (measuring approximately 2 × 2 × 10 mm) were dissected free from uterine decidua and serosa and mounted isometrically in an organ tissue bath under 2 g tension for recordings, as previously described (20, 21). The tissue baths contained 20 ml of Krebs-Henseleit PSS maintained at 37 C, pH 7.4, and were gassed continuously with a mixture of 95% oxygen/5% carbon dioxide. During a period of equilibration of 1 h, the Krebs-Henseleit PSS in the tissue baths was changed every 15 min. The experiments evaluating effects on myometrial contractility were then designed in four groups as follows: group 1: oxytocin alone; group 2: oxytocin + hCG; group 3: oxytocin + IbTX; group 4: oxytocin + IbTX + hCG. Following equilibration, all myometrial strips were incubated with 0.5 nmol/liter oxytocin to elicit regular rhythmic contractions. After 30 min, strips from groups 3 and 4 were exposed to IbTX at a bath concentration of 100 nmol/liter for a further 30 min, and group 1 strips were exposed to Krebs-Henseleit PSS only. Contractile activity was measured for a 20-min period at which time bath addition of hCG to groups 2 and 4 strips took place. Highly purified hCG was added to the tissue bath in a cumulative manner at bath concentrations 0.1, 0.5, 1, 5, and 10 IU/ml at 20-min intervals, and the resultant contractile activity was measured for each period and expressed as a percentage of the integral obtained in the 20-min period before any hCG addition (*i.e.* percentage of contractility). Measurement of contractile activity was performed by calculation of the integral of the selected area with the PowerLab (AD Instruments, Hastings, UK) hardware unit and Chart version 3.6 software (AD Instruments).

Drugs and solutions

Highly purified hCG (CR-127 14,900 IU/mg) was obtained as a gift from Dr. A. G. Parlow, National Hormone and Pituitary Program, Torrance, CA. A stock solution (10,000 IU/ml) of hCG was prepared in sterile saline. A stock solution of oxytocin (1 mmol/liter; Sigma-Aldrich) was made in ethanol. Series of dilutions were made in deionized water on the day of experimentation and maintained on ice for the duration of the experiment. A stock solution of IbTX (Sigma-Aldrich) (1 × 10^{-5} mol/liter) was made in saline. Fresh Krebs-Henseleit PSS was prepared daily.

Statistical analysis

For single-channel activity data (NP$_o$ values) are expressed as mean ± SE of the mean. Comparisons between groups were made by one-way ANOVA, with a *post hoc* Tukey test to determine significant differences among data groups. For isometric recordings, multiple comparisons of measured integrals of contractility were performed using two-way ANOVA, followed by *post hoc* testing using Newman-Keuls test. The statistical packages (SPSS, version 11.0, SPSS Inc., Chicago, IL) and GB Stat (version 6.5; GB Stat, Silver Spring, MD) were used for statistical calculations. A *P* value <0.05 was accepted as statistically significant.

Results

Myometrial tissue samples

Myometrial biopsies were obtained from 15 women. The reasons for cesarean section included breech presentation (n = 3), previous cesarean section (n = 9), maternal spine abnormality (n = 1), and cephalopelvic disproportion (n = 2). All cesarean sections were performed before the onset of labor under regional anesthesia. The maternal demographic details were as follows: mean age 31.3 yr (range 23–42); median period of gestation 38 wk (range 37–39); and median parity 1 (range 0–2).

BK$_{Ca}$ channel activity

Single-channel recordings from freshly dispersed myocytes, using the cell attached configuration of the patch clamp technique, revealed ion channel activity with an amplitude average of 7–10 pA at +40 mV identifying the BK$_{Ca}$ channel (19). NS1619, a specific BK$_{Ca}$ channel opener, elicited potent channel activation at applied concentration of 20 μmol/liter (Fig. 1). Furthermore, application of 10 mmol/liter tetraethylammonium (TEA), which exhibits selectivity for BK$_{Ca}$ channels, reversed the NS1619 activation of this channel. These experiments identified the BK$_{Ca}$ channel as the predominant K$^+$ channel expressed in human myometrial myocytes, consistent with previous studies on these cells (13, 14, 16).

A representative recording, demonstrating minimum BK$_{Ca}$ channel activity under control conditions (NP$_o$ = 0.042), is shown in Fig. 2. Cumulative additions of hCG resulted in a potent activation of BK$_{Ca}$ channel activity in a concentration-dependent fashion, as illustrated. BK$_{Ca}$ channel activity for this recording was increased following the addition of 1 (NP$_o$ = 0.088), 10 (NP$_o$ = 0.121), and 100 (NP$_o$ = 0.107) IU/ml hCG, respectively. The average NP$_o$ (Fig. 3B) before and after addition of hCG was as follows: control 0.036 ± 0.01 (n = 5); 1 IU/ml hCG 0.065 ± 0.014 (n = 5; P = 0.262); 10 IU/ml hCG 0.111 ± 0.009 (n = 5; P = 0.001); and 100 IU/ml hCG 0.098 ± 0.004 (n = 4; P = 0.007). The hCG stimulated the BK$_{Ca}$ channel with a maximal activation effect observed at a concentration of 10 IU/ml, and the effect appeared to plateau at higher concentrations. Open-state channel activity was stimulated 2- and 3-fold by 1 and 10 IU/ml hCG, respectively; however, subsequent addition of 100 IU/ml hCG did not elicit significant further effect. In general, there was a 5- to 10-min latency period before observation of hCG-stimulated channel activity, and this effect appeared to be maximal within 15–20 min. In all patches this activity
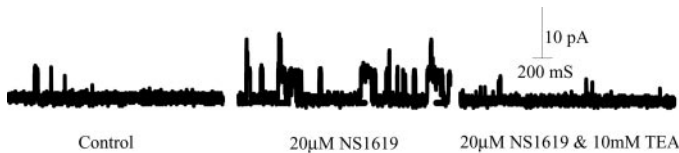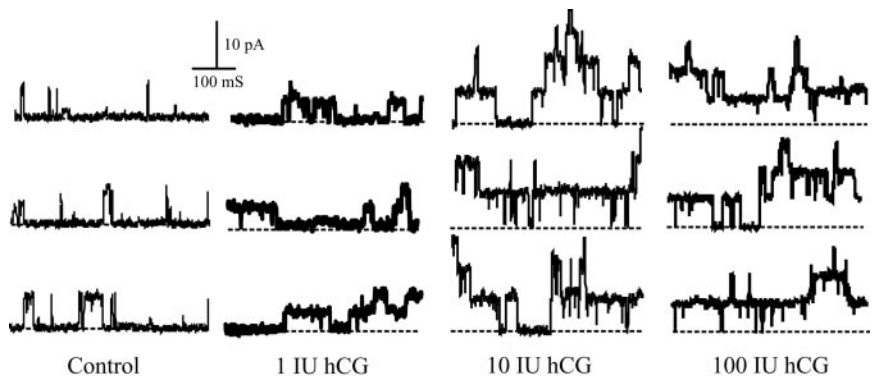
persisted until either seal integrity was lost, or the experiment was terminated.

In vitro contractility

The hCG exerted a cumulative inhibitory effect on contractility in isolated pregnant human myometrium (n = 6). Figure 4A demonstrates a representative recording of oxytocin (0.5 nmol/liter) elicited myometrial contractility, and in Fig. 4B the effects of cumulative additions of hCG (0.1, 0.5, 1, 5, and 10 IU/ml) to the tissue bath are shown. ANOVA
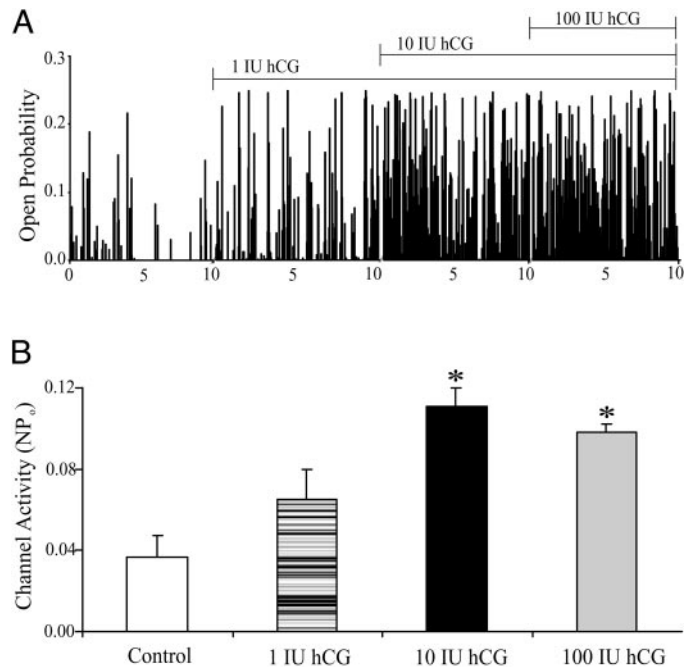
FIG. 3. Human CG opens BK$_{Ca}$ channel activity in human myometrial smooth muscle cells in a concentration-dependent manner. A, Activity plot of BK$_{Ca}$ channel open probability in a single cell-attached patch before and 15 min after exposure to cumulative doses of hCG (1, 10, and 100 IU/ml). *Vertical bars* are channel activity during a 100-msec test pulse to +40 mV. Recording time under each condition was 10 sec, as indicated on the time axis. Breaks in the time axis represent drug incubation periods when channel activity was not recorded. Period of drug exposure is indicated by *horizontal lines* above the histogram. B, Each bar represents the average channel open probability obtained from cell attached patches (+40 mV) before and 15 min after 1, and subsequent additions of 10 and 100 IU/ml hCG, respectively. *, Significant increase in channel activity, compared with control (n = 5; $P < 0.01$).

FIG. 1. Representative recordings from the same membrane patch (+40 mV) in the cell-attached configuration and after addition of 20 μmol/liter NS1619 and subsequent addition of 10 mmol/liter TEA. TEA reversed NS1619-stimulated channel activity.

FIG. 2. Human CG stimulates BK$_{Ca}$ channel activity in human myometrial smooth muscle cells. Representative continuous recordings were recorded from the same cell attached patch (+40 mV) before (control) and 15 min after cumulative exposure to 1, 10, and 100 IU/ml hCG, respectively. Channel openings are upward deflections from the baseline (closed state) (*dashed line*).

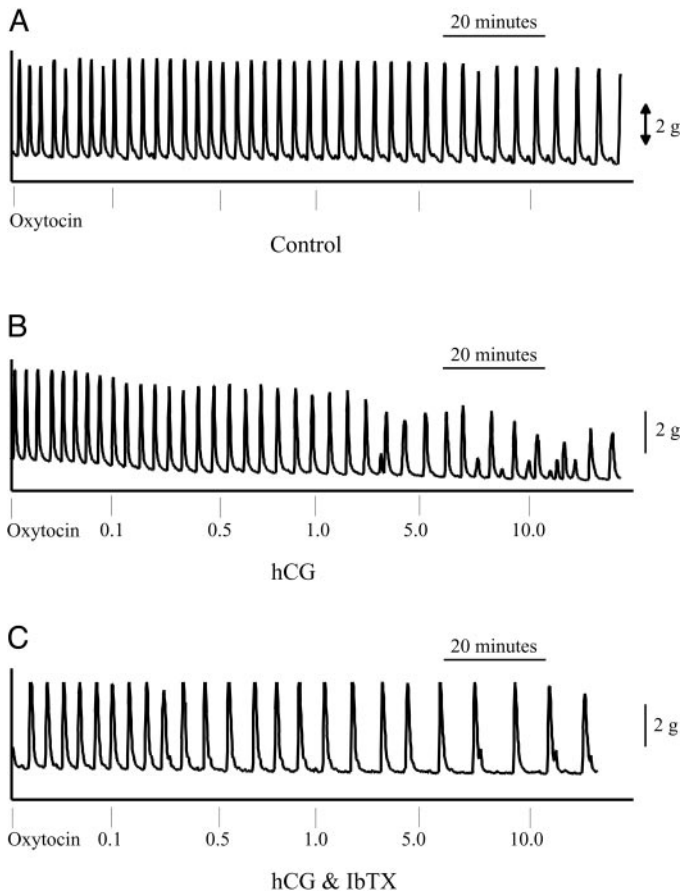Control   1 IU hCG   10 IU hCG   100 IU hCG

FIG. 4. Effects of hCG on oxytocin induced myometrial contractions. Representative recordings of oxytocin induced contractions of pregnant human myometrium (A) and the effects of cumulative additions of hCG (0.1, 0.5, 1, 5, and 10 IU) at 20-min intervals are shown (B). The uterorelaxant effect was significantly attenuated by preincubation with the BK$_{Ca}$ channel antagonist IbTX (C).

revealed a significant effect on contractility of both addition of hCG (group 1 *vs.* group 2; $P < 0.05$) and increasing hCG concentrations. *Post hoc* analysis showed a statistically significant relaxant effect of hCG at bath concentrations of 1 (n = 6; $P < 0.01$), 5 (n = 6; $P < 0.01$), and 10 (n = 6; $P < 0.01$) IU/ml when compared with respective control values measured from strips exposed to oxytocin alone.

Incubation of strips with IbTX, in addition to oxytocin, did not significantly alter the integrals of contractile activity measured (group 1 *vs.* group 3; n = 5; $P = 0.83$). However, addition of IbTX, before bath addition of hCG, significantly attenuated the relaxant effect exerted by hCG. In Fig. 4C a representative recording demonstrating the effects of preincubation with the BK$_{Ca}$ channel antagonist IbTX, on the uterorelaxant effects of hCG, is shown. Comparison of the inhibitory effects of hCG on the oxytocin-induced contractility of myometrial strips, in the presence or absence of IbTX, revealed a significant difference across the groups (group 2 *vs.* group 4; n = 6; $P < 0.05$). *Post hoc* analysis revealed that preincubation with IbTX (100 nmol/liter) significantly attenuated the uterorelaxant effect of hCG at all test concentrations, resulting in no significant difference when compared with control values [group 3 (n = 5) *vs.* group 4 (n =

6); $P > 0.05$] at all hCG bath concentrations. In Fig. 5 the effects of cumulative additions of hCG (0.1, 0.5, 1, 5, and 10 IU/ml) on contractions of myometrial strips, in the absence and presence of IbTX, are shown in graphical form alongside the integrals observed in control strips.

Discussion

The concept that hCG plays a role in maintenance of human uterine quiescence throughout pregnancy, and particularly in the third trimester, has been recently highlighted (7, 8). Based on hCG receptor quantification data, using mRNA and protein studies (8), it has been suggested that this relaxant effect of hCG is down-regulated at the time of labor. The exact mechanism, or mechanisms, of the inhibitory effect of hCG in myometrial smooth muscle are unknown, but direct reduction in intracellular calcium availability (10, 22) and down-regulation of gap junctions have previously been suggested (11). The potential inhibition of unknown uterotonic agents by hCG has also been speculated (8). Our data demonstrate a clear and potent activation of the BK$_{Ca}$ channel, directly by hCG, in human myometrium during pregnancy, at both the single ion channel level and in functional studies. Activation of the BK$_{Ca}$ channel results in an efflux of K$^+$ ions from the intracellular compartment, drawing the cell membrane potential closer to the potassium equilibrium potential and thereby reducing cellular excitability and contractility. These results provide a clear explanation of mechanism of action of the uterorelaxant effect of hCG; however, inhibition via other pathways is also possible. Our findings also account for the direct reduction in intracellular calcium elicited by hCG in myometrial tissue (10) by inactivation of
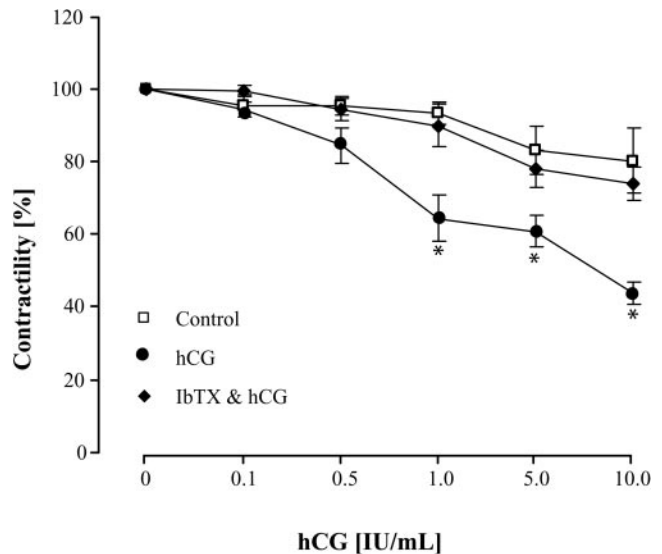

FIG. 5. Dose response curves for hCG- and oxytocin-induced myometrial contractions. Graphical representation of the effects of cumulatively increasing tissue bath concentrations of hCG (0.1, 0.5, 1, 5, and 10 IU/ml) at 20-min intervals on oxytocin-induced contracting pregnant myometrium in the presence (*shaded diamonds*) and absence (*shaded circle*) of IbTX. A control trace showing uninterrupted oxytocin induced contractions in pregnant myometrium are shown for comparison (*open squares*). The *symbols* used represent the mean values within each group. *Vertical error bars* represent the SE of the mean.

voltage-gated calcium channels because of hyperpolarization of the cell membrane via BK_{Ca} channel activation.

In our experiments, single-channel patch clamp studies clearly identified BK_{Ca} channels in the isolated myocytes and demonstrated that hCG significantly increases their open state probability. These studies are consistent with previous reports indicating that the BK_{Ca} channel is the predominant potassium channel in primary myocytes from human pregnant myometrium (13–15). Because of its large conductance, and high density of expression, this channel plays an important role in the regulation of membrane potential and cellular excitability of the myometrium and hence has been reported as playing a significant role in modulating myometrial function in both animal models and humans. Initial links between the myometrial BK_{Ca} channel and parturition were evident with the report outlining that it apparently loses its Ca^{2+} and voltage sensitivity in human myometrium with the onset of labor (13). In addition, NS1619, the specific BK_{Ca} channel activator, exerts a potent relaxant effect on pregnant human myometrium, in *in vitro* experiments (16). Using both inside-out and outside-out configurations of the patch clamp technique, NS1619 appears to act directly on myometrial BK_{Ca} channels and stimulates channel activity by increasing their time spent in the open state (16). The findings from this study indicate that hCG exerts its uterorelaxant effect in a manner similar to that of NS1619.

The results from single BK_{Ca} channel recordings concur with the data obtained from isometric tension recordings with human myometrial strips. After preincubation with IbTX, the contractility measured following hCG exposure, was not significantly different to that of control strips (*i.e.* strips exposed to oxytocin alone or oxytocin and IbTX). This confirms the fact that BK_{Ca} channel function is intrinsic to myometrial contractions and also providing evidence that this channel plays a central role in hCG-mediated relaxation. In our study, after the equilibration period, the strips were exposed to oxytocin to achieve regular rhythmic contractions, which are reproducible over a period of hours and hence serve as a reliable control, unlike spontaneous contractions *in vitro* (23). In our experiments, there was no significant difference observed in integrals of contractility in strips exposed to oxytocin alone *vs.* those exposed to oxytocin and IbTX, before bath addition of hCG.

Although the physiological and clinical importance of endogenous factors regulating myometrial quiescence during pregnancy are obvious, the potential use of hCG, administered exogenously, as a therapeutic agent for preterm labor, has been recently debated (7, 24, 25). Whole hCG exhibited potent inhibition of $PGF_{2\alpha}$-induced preterm delivery in mice, and the effect was dose dependent (25). There are no reliable data in relation to clinical studies, to our knowledge. Our findings support the hypothesis that clinical tocolysis using hCG remains a possibility, with a predominant mechanism of action via BK_{Ca} channel opening in myometrial cells.

We did not address the possibility that the effects outlined in our results may be altered by labor onset. This remains an exciting study for further evaluation of both hCG effector mechanisms, and BK_{Ca} channel activity, in myometrial tissue obtained during spontaneous labor, with similar clinical criteria as outlined for this study.

In conclusion, we report the first direct evidence that hCG exerts a potent uterorelaxant effect in human pregnancy via BK_{Ca} channel activation. These novel data raise further questions about a central role of hCG, and BK_{Ca} channel function, in association with uterine quiescence and parturition. These findings support the growing hypothesis that hCG may confer therapeutic benefit in preterm labor management.

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