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Cyclic AMP enhances progesterone action in human myometrial cells

Li Chen [a,b], Kaiyu Lei [a], Johann Malawana [a], Angela Yulia [a], Suren R. Sooranna [a], Phillip R. Bennett [a], Zhiqing Liang [b], Dimitri Grammatopoulos [c], Mark R. Johnson [a,*]

[a] Imperial College Parturition Research Group, Academic Department of Obstetrics & Gynaecology, Imperial College School of Medicine, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH, UK
[b] Department of Obstetrics and Gynecology, Third Military Medical University Southwest Hospital, Chongqing 400038, PR China
[c] Division of Metabolic and Vascular Health, Warwick Medical School, The University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK

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ABSTRACT

Cyclic AMP (cAMP) has been shown to promote progesterone and glucocorticoid action in a variety of cellular settings. In this study, we have used human myometrial cells to investigate whether cAMP potentiates the ability of progesterone to repress IL-1β-driven COX-2 expression. We found that forskolin enhanced progesterone-repression of IL-1β-driven COX-2 expression in association with delayed IL-1β-induced nuclear phospho-p65 entry and reduced NF-κB binding to the COX-2 promoter. Further, forskolin enhanced the progesterone-induced expression of FKBP5 and 11βHSD1, progesterone-driven activity of a progesterone response element (PRE) and progesterone receptor (PR)-B binding to a transfected PRE. In addition, forskolin treatment increased PR-B levels and reduced the PR-A:PR-B ratio while acutely decreasing the association between PR and nuclear receptor co-repressor (NCoR) and reducing NCoR levels after 6 h. These findings are of importance in situations where enhancing progesterone activity is desirable, for example in the management of endometrial cancer, the promotion of endometrial receptivity or the maintenance of myometrial quiescence during pregnancy.

1. Introduction

Several studies have shown that cyclic AMP (cAMP), a common second messenger for a number of hormones, cannot only enhance agonist bound PR activity (Beck et al., 1993; Denner et al., 1990; Sartorius et al., 1993) but also convert the PR antagonist, RU486, into a partial agonist (Beck et al., 1993; Sartorius et al., 1993). Initially, it was thought that cAMP-induced PKA activation might enhance PR activity via increasing its phosphorylation, but PR phosphorylation status was not changed by cAMP analogs (Bai et al., 1997; Beck et al., 1992; Sartorius et al., 1993). Subsequent studies, determined that a reduction in the association between PR and the co-repressors nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid hormone receptor (SMRT) underpinned the ability of cAMP to potentiate PR transcriptional (Wagner et al., 1998). This may be mediated via a cAMP-induced reduction in co-repressor affinity or increase in co-activator affinity leading to displacement of the co-repressor. The data are limited on this subject, however, cAMP induced increase in SRC-1 (PR co-activator) phosphorylation has been shown to enhance ligand-driven PR activity (Rowan et al., 2000b), supporting the second possibility. Further, cAMP also enhances the transcriptional activities of other nuclear receptors such as oestrogen receptor, glucocorticoid receptor and retinoic acid receptor (Coleman et al., 2003; Huggenvik et al., 1993; Zhang and Danielsen, 1995) and these effects may also be mediated through changes in their interaction with either NCoR or SMRT, (Chen and Evans, 1995; Horlein et al., 1995). Clinically, the interaction between cAMP and the glucorticoid receptor is of critical importance in the management of asthma, where β-agonists have been able to enhance steroid action and to reverse steroid resistance (Kaur et al., 2008; Rider et al., 2011). Similarly, in the case of the oestrogen receptor where increased cAMP levels may play a role in tamoxifen resistance observed in breast cancer (Dudek and Picard, 2008).

Progesterone has been implicated in all aspects of female reproductive function from sexual behaviour, mammary gland development, ovulation and implantation to the maintenance of pregnancy (Spencer and Bazer, 2002; Wetendorf and DeMayo, 2012). Further, therapeutically progesterone is used to promote fertility, maintain pregnancy and to correct abnormalities of endometrial function, such as endometrial cancer, hyperplasia and endometriosis (Kim et al., 2013). Consequently, the ability of cAMP to enhance

Abbreviations: cAMP, cyclic AMP; NCoR, nuclear receptor co-repressor; PR, progesterone receptor; PRE, progesterone response element; SMRT, silencing mediator for retinoid and thyroid hormone receptor.

* Corresponding author. Tel.: +44 20 8846 7887; fax: +44 20 8846 7796.
E-mail address: mark.johnson@imperial.ac.uk (M.R. Johnson).

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progesterone action may be important both physiologically and therapeutically. However, how cAMP potentiates progesterone driven transactivation is still not clear, further, whether cAMP can enhance progesterone repression, for example in the context of IL-1β-induced COX-2 expression, is also unknown. In this study, as the myometrium is a potentially important site of cAMP/progesterone interaction, we have used human myometrial cells to explore whether cAMP enhances progesterone transactivation and transrepression and to investigate the mechanisms involved.

2. Material and methods

2.1. Tissue collection and myometrial cell culture

The local ethics committee approved the study and women donating tissue gave informed written consent. Myometrial biopsies ($0.5 \times 0.5 \times 0.5\ cm^3$) of term human myometrium were collected at the time of elective caesarean section from the upper margin of the incision made in the lower segment of the uterus from women not in labour. Myometrial cells were cultured as previously described (Lei et al., 2011) and were incubated with progesterone or MPA and the cAMP agonist forskolin either alone or in combination and then the cells were exposed to IL-1β for up to 48 h.

2.2. rtPCR

The rtPCR was performed as previously described (Lei et al., 2011), Primer pair sequences are in Table 1).

2.3. Western-blotting

Western blotting was performed as previously described (Lei et al., 2011). The membranes were incubated with antibodies for COX-2 (sc-1745: Santa Cruz Biotechnology Inc., Santa Cruz, California, USA), progesterone receptor (ChIP grade, ab68195, Abcam Ltd., Cambridge, UK), NCoR (ChIP grade, ab24551, Abcam Ltd.), SRC1 (ChIP grade, ab84 Abcam Ltd.), SMRT (Sigma–Aldrich Co. Ltd.), IκBα (New England Biolabs (UK) Ltd., Hitchin, Hertfordshire, UK) at a dilution of 1:1000, and incubated overnight at 4 °C.

2.4. Luciferase assay

The promoter assays were performed as previously described (Chen et al., 2012). The pSG5 was the kind gift from Dr. John White, Imperial College London, UK; and pSG5-PRB was a kind gift from Dr. Pierre Chambon, Institute de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France and reporter vector progesterone response element (PRE) was kindly provided by Dr. Birgit Gellersen, Hamburg, Germany.

Table 1
Primer pair sequences with gene accession numbers.

Name	Primer sequence(5′–3′)	GeneBank/EMBL accession No.
COX-2	F: TGTGCAACACTTGAGTGGCT R: ACTTTCTGTACTGCGGGTGG	AY151286
FKBP5	F: TCCCTCGAATGCAACTCTCT R: GCCACATCTCTGCAGTCAAA	NM_001145775
GAPDH	F: TGATGACATCAAGAAGGTGGTGAAG R: TCCTTGGAGGCCATGTAGGCCAT	BC014085
HSD11β1	F: ACCTTCGCAGAGCAATTTGT R: GCCAGAGAGGAGACGACAAC	NM_005525
PR-B	F: AACTCAGCGAGGGACTGAGA R: GAGGACTGGAGACGCAGAGT	X51730
PR-total	F: AGCCCACAATACAGCTTCGAG R: TTTCGACCTCCAAGGACCAT	NM_000926

F: forwards; R: reverse.

2.5. Co-immunoprecipitation studies

When the cells were 80–90% confluent, growth medium was aspirated and the cells washed with ice-cold PBS or TBS; the PBS was drained and ice-cold lysis buffer was added (1% NP40 buffer, 1% Triton X100, 150 mM NaCl, 50 mM Tris–HCl pH 7.4, 1 mM EDTA, 10% Glycerol). The cells were lysed for 3–4 min and then gently transferred into a pre-cooled tube. The lysates were centrifuged at maximum speed (~13,000 rpm) in a chilled centrifuge for 10 min. The tubes were gently removed from the centrifuge and placed on ice, the supernatant was then aspirated and transferred into a fresh tube and kept on ice and the pellet was discarded. The lysate was pre-cleared by incubating for 10–30 min with 40 μL Protein G agarose bead slurry (previously washed in IP buffer) per mL of lysate with end-over-end mixing at 4 °C. The samples were centrifuged at 3000 rpm for 2 min at 4 °C and the supernatants were transferred to fresh tubes. These were centrifuged again at 13,000 rpm for 5 min at 4 °C and the supernatant transferred to fresh tubes. 600 μL of each lysate was transferred to a cold tube containing the 8 μL of different antibodies. 5% of the lysate was kept as input. 60 μL of the beads (Thermo Fisher Scientific) were taken per sample and washed twice with cold IP buffer, and diluted back to 50% slurry with fresh lysis buffer. The immunoprecipitates were incubated overnight at 4 °C with gentle end-over-end mixing. After the last wash, the beads were resuspended in 20 μL IP buffer (with an additional 2 μL of 40 mM of dithiothreitol added) and 20 μL of 4× SDS PAGE sample buffer at 70 °C for 10 min. Then the samples were centrifuged and the pellets and supernatants stored separately until electrophoresed on SDS gels.

2.6. Chromatin immunoprecipitation studies

When cells were approximately 80% confluent, the medium was replaced with 10 mL of fresh medium. Then, 625 μL of 16% formaldehyde was added (final concentration = 1%) and incubated at room temperature for 10–20 min with continuous shaking. 1 mL of 1.25 M glycine was then added to the sample and incubated for 5–10 min at room temperature in order to stop the reaction. The cells were carefully washed twice with 5 mL of cold PBS at 4 °C. All tubes were kept on ice during this procedure. A cell scraper was used to transfer the cells into a 1.5 mL tube. The cells were centrifuged at approximately 200*g* for 10 min at 4 °C and the pelleted cells resuspended in Szak RIPA buffer. The lysates were sonicated for 15 min in 30-s pulses using a Soniprep 150 ultrasonic processor to shear the DNA into 200–1000 bp pieces and the samples were then spun at maximum speed for 10 min at 4 °C. While these cell lysates were sonicated, the antibody-coupling dynabeads were prepared. 4 μg of antibody against the protein of interest and preimmune rabbit polyclonal IgG were incubated with dynabeads for 2 h at 4 °C, respectively. The sonicated cell supernatant was diluted to 1 mL aliquots and incubated with dynabeads for 1 h at 4 °C to pre-clear. After pre-clearing, 10% of cell lysates were kept as "input" positive control for subsequent qPCR analysis. The rest of pre-cleared cell lysates were incubated with antibody-coupling dynabeads for 2 h at 4 °C with rotation. The beads were collected in the tube using the DynaMag-PCR magnet and the supernatant was discarded. The beads were washed with different buffers in the following order: 1× in Szak RIPA buffer, 2× in Low-salt buffer, 2× in High-salt buffer and 2× in TE buffer. Each wash step was carried out with 1 mL buffer for 5 min at 4 °C with rotation.