SHORT COMMUNICATION

HONG KONG, SAR CHINA

Ratio of mRNA expression of progesterone receptor isoforms AB is to b in human oviduct mucosal cells during the ovulatory cycle*

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The ratio of the active progesterone receptor B isoform is higher in the ampullary region of the oviduct.

Purpose: To examine mRNA expression of progesterone receptor isoforms AB and B in oviduct mucosal tissue during the ovulatory cycle and in the different functional regions of the human oviduct.

Methods: The mucosal layer was isolated from human oviduct tissue and semi-quantitative RT-PCR for progesterone isoforms AB and B was performed. The RT-PCR results were verified by immunohistochemistry.

Results: The isthmus region showed no mRNA expression of either progesterone receptor isoform while the relative ratio of the B isoform was significantly higher in the ampullary region compared to the fimbrial region. There was a significant increase in the ratio of PRB to PRAB in the ampullary region compared to the fimbrial region in all samples.

Conclusions: We found an increase in the relative abundance of the progesterone receptor B isoform in the ampullary region which is the site of fertilization and early embryo cleavage. Our results indicate that progesterone responsive genes are more likely to be activated in the ampullary region of the oviduct due to the difference in PRAB to PRB ratio. Providing support for the hypothesis that progesterone may play a specific role in providing an appropriate environment for sperm capacitation, fertilization and early embryo cleavage.

KEY WORDS: Human oviduct; immunohistochemistry; mRNA; progesterone receptor isoforms AB and B.

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INTRODUCTION

One of the functions of the oviduct is to provide a suitable environment for oocyte fertilization and early embryo development. However, while P receptors have been identified in the human oviduct, little is known about P receptor regulation. In other P target tissues, two isoforms of P receptors have been identified, isoform AB (PRAB) and B (PRB). Sun *et al.* (1) demonstrated the presence of the PRB isoform in the human oviduct as well as the endometrium during the peri-ovulatory phase. There are no data on the regulation of PRAB and PRB during the ovulatory cycle.

The progesterone receptor is a nuclear receptor that regulates a number of physiological and morphological processes in response to ligand binding. A single gene codes for the PRAB and PRB isoforms that are translated from individual messenger RNA (mRNA) species. These are under the control of different promoters (2). The transcription of both isoforms is indirectly stimulated by estradiol via the estrogen receptor. The B isoform contains an additional 164 amino acids at the N-terminal that are absent in the A isoform (3). Therefore, while it is not possible to distinguish the A isoform, it is possible to do this for the B isoform.

The relative expression of PRAB and PRB may determine the tissue response to circulating P (4). The PRAB and PRB isoforms lead to differential gene regulation (5) and interact with each other following receptor binding.

This study examines the mRNA expression of the two P receptor isoforms in oviduct mucosal cells during the ovulatory cycle. The expression will also be assessed in samples taken in early pregnancy, the postpartum period and from postmenopausal subjects. This study also examines the mRNA expression of the two P receptor isoforms in oviduct mucosal cells from the three different functional regions of the oviduct.

MATERIALS AND METHODS

Collection of Oviduct Tissue

Oviduct tissue ($N = 20$) was obtained from women who were undergoing either a laparoscopy for tubal sterilization or a hysterectomy for a benign uterine condition. The stage of the cycle was determined by the date of commencement of the last menstrual period and was confirmed by appropriate serum

concentrations of luteinizing hormone (LH), progesterone (P) and estradiol (E₂).

Excised oviduct tissue was immediately placed in 20 mL of Hepes buffered Quinn's Human Tubal Fluid (Irvine Scientific, Santa Ana, CA, USA). This medium was used for all tissue manipulation. The oviduct was rinsed to minimize blood cell contamination. The lumen of the oviduct was exposed by incising along the anti-mesenteric border and the mucosal folds were dissected off macroscopically. The samples were taken from the fimbrial, ampullary and isthmus regions of the oviduct. Only two of the investigators performed the tissue dissection and the technique was closely monitored to ensure accurate replication.

Semi-quantitative RT-PCR Analysis

mRNA was extracted from the oviduct mucosal tissue using the Oligotex direct mRNA kit (Qiagen, Hilden, Germany). All mRNA samples were treated with 1 U of DNase (Boehringer Mannheim/Hoffman-La Roche, Basel, Switzerland). The DNase was inactivated by incubating for 5 min at 90°C. 100 ng of mRNA was used for cDNA synthesis with Multiscribe reverse transcriptase (PE Biosystems, Foster City CA, USA). All the resultant cDNA was then used for PCR with AmpliTaq Gold DNA polymerase (PE Biosystems, Foster City CA, USA). Each PCR cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. Thirty cycles were performed, followed by a final extension at 72°C for 4 min. The RT-PCR assay was validated by establishing that the coamplified product for the (experimental) PRAB or PRB and (internal standard) β -actin primer sets were linear and parallel with increasing amounts of start cDNA and the cycle number and primer concentrations were optimized in the exponentially increasing phase of the detectable product.

Forward and reverse primers specific to PRAB isoform were derived from Kumar *et al.* (6). The sequences of the primers were: forward-ACA GAA TTC ATG ACT GAG CTG AAG GCA AAG GGT reverse-ACA AGA TCT CAA ACA GGC ACC AAG AGC TGC TGA (Mwgag Biotech, Ebersberg, Germany). Forward and reverse primers specific to PRB isoform were derived from Kumar *et al.* (6). The sequences of the primers were: forward-ACA GAA TTC ATG ACT GAG CTG AAG GCA AAG GGT reverse-ACA AGA TCT CAA ACA GGC ACC

AAG AGC TGC TGA (Mwgag Biotech, Ebersberg, Germany). β -actin was co-amplified with PRAB or PRB to provide a semi-quantitative internal control for RNA quantity and PCR reaction efficiency. β -actin is commonly used as a standard when comparing samples under different hormonal conditions, as it is constitutively expressed (7). Forward and reverse primers specific to β -actin were derived from published primer sequences (7). The sequences of the β -actin primers were: forward-ATC GTG GGG CGC CCC AGG CAC and reverse-CTC CTT AAT GTC ACG CAC GAT TTC (Mwgag Biotech, Ebersberg, Germany).

Twenty percent of each PCR reaction was separated by gel electrophoresis on a 2% agarose gel with 0.5 μ g/mL ethidium bromide in TBE buffer. The separated PCR products were visualized under Ultra Violet illumination. A video camera sent the UV illuminated gel image to a computer where the software package Gel Doc enabled an image of the gel to be recorded. The integrated optical density (IOD) was determined for each PCR product by the image analyzer, Gel Doc System. The IOD ratio between the PCR amplified PRAB or PRB product with its simultaneously amplified control, β -actin was obtained for each sample.

Statistics

The nonparametric Wilcoxon sign test was used to determine whether significant differences were present between the ratio of and PRAB or PRB mRNA expression in different regions of the oviduct. A probability value was considered significant when $P < 0.05$.

Immunohistochemistry

Seventy tissue sections from the 20 oviducts consisting of fimbrial, ampullary and isthmus regions were analyzed for the presence of PRAB or PRB by specific immunohistochemical staining. Tissue sections fixed on glass slides were dewaxed in graded xylenes, rehydrated through graded alcohols and washed in phosphate buffered saline (PBS).

Sections were stained for PRAB or PRB with mouse monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The secondary antibody and chromogenic substrate addition was performed using the DAKO Catalyzed Signal Amplification System reagents and protocol (Dako,

Fig. 1. (A) RT-PCR products for the control gene β -actin (543 bp) and for PRAB (243 bp). (B) RT-PCR products for the control gene β -actin (543 bp) and for PRB (429 bp). MW: 50 bp molecular weight marker. a: mRNA from oviduct mucosal cells obtained during the early follicular phase with low circulating concentrations of E_2 , LH and P. b: mRNA from oviduct mucosal cells obtained during the mid follicular phase with high circulating E_2 and low circulating LH and P concentrations. c: mRNA from oviductal mucosal cells obtained during the late follicular phase with high circulating concentrations of E_2 and LH and low circulating concentrations of P. d: mRNA from oviductal mucosal cells obtained during the luteal phase with low circulating concentrations of E_2 and LH and high circulating concentrations of P. e: mRNA from oviductal mucosal cells obtained during early pregnancy. f: mRNA from oviduct mucosal cells obtained during the postpartum phase. g: mRNA from oviduct mucosal cells obtained from a menopausal subject.

Glostrup, Denmark). Negative controls were performed by omitting addition of primary antibody. Staining was detected under light microscopy.

RESULTS

Three samples were collected in the early follicular phase, three from the mid-follicular phase, three from the late follicular phase, three from the early luteal phase and two from the late luteal phase.

Two samples were collected during early pregnancy (tubal ligation following first trimester termination of pregnancy), two samples were collected in the postpartum period and two were collected from postmenopausal women.

A PCR product of the appropriate size was obtained from both the PRAB and PRB PCR primers in samples collected from the mid and late follicular phase but not in samples collected from the early follicular phase or from the luteal phase (Fig. 1a and b). One sample in the late luteal phase showed weak

Fig. 2. A bar graph showing the mean $\pm$ standard error for PRAB and PRB mRNA expression in the fimbrial and ampullary regions. * $P \leq 0.0005$.

expression of PRAB but all other luteal phase samples showed no expression. Samples collected during early pregnancy showed high expression of both PRAB and PRB while samples from the postpartum period and from menopausal women were negative for both.

Ratio of PRAB: PRB mRNA Expression in the Follicular Phase

There was no significant difference between the mRNA expression of PRAB in the fimbrial com-

pared to the ampullary region (Fig. 3b and c). However, there was significantly higher PRB expression in the ampullary compared to fimbrial region, where $P < 0.0005$ (Fig. 2).

Localization of PRAB and PRB in the Three Functional Regions of the Oviduct by Immunohistochemistry in Samples Obtained in the Follicular Phase

Strong and specific cytoplasmic and nuclear PRAB and PRB staining was observed in the

Fig. 3. Immunolocalization of PRAB and PRB in human ampullary region oviduct by mouse monoclonal antibody (*brown*) with counterstaining (*blue*). All samples shown are from the same late follicular phase subject. (A) Negative control with PBS and 1.5% fetal bovine serum substituted for the primary anti-PRAB or PRB antibodies. (B) PRAB staining in the fimbrial region. (C) PRAB staining in the ampullary region. (D) PRAB staining in the isthmic region. (E) PRB staining in the fimbrial region; PRB staining in the ampullary region. (F) PRB staining in the isthmic region. ep: epithelial cells; sm: smooth muscle cells.

mucosal epithelial cells and more intense staining for both was observed in the smooth muscle cells in samples from the fimbrial region (Fig. 3b and e) and in samples from the ampullary region (Fig. 3c and f). The two proteins co-localized in the same cell types. Despite no RT-PCR product being detectable in isthmic samples, faint staining of PRAB and PRB was observed in some mucosal epithelial cells, with a much stronger staining of both in the smooth muscle cells (Fig. 3d and g).

DISCUSSION

The results of this study demonstrate that peak expression of both PRAB and PRB occurs during the late follicular phase in mucosal epithelial cells. This temporal regulation may indicate a role in the early events of reproduction. This study focused on the regulation of PRAB and PRB in the mucosal cells as these are the cells that interact directly with gametes and early embryos. The results of the immunohistochemistry show that regulation of the P receptor isoforms in the smooth muscle layers of the oviduct may be under different control. This may reflect the different function of the muscle layer for gamete and embryo transport.

Both PRAB and PRB isoforms were strongly expressed in the fimbrial and ampullary regions but not in the isthmic region. This may reflect the function of the fimbrial and ampullary regions to maintain an environment suitable for supporting gamete and embryo survival and growth as the gametes and embryos traverse these regions slowly compared to the reported rapid descent of the embryo through the isthmic region (8).

Both PR isoforms were expressed in the fimbrial and ampullary regions. In these samples there was simultaneous expression of PRAB and PRB.

The limited sensitivity of our RT-PCR assay may explain the presence of PRAB and PRB protein in isthmic region samples that were negative for both isoforms in the RT-PCR assay. While the PCR method is very sensitive our agarose gel method of analyzing the PCR products is not. It is therefore possible that very low levels of expression may not have been identified using the RT-PCR method.

The immunohistochemistry results showed that the regulation of PRAB and PRB isoforms in the oviduct smooth muscle cells may be through a different mechanism than the mucosal cells and this may be due to the different functions of the spe-

cific cell types within the oviduct. The function of the smooth muscle cells in regulating the transport of gametes and embryos may require different timing to the function of the mucosal cells to facilitate fertilization and early embryo cleavage. The precise local control of the ratio of repressor PRAB and activator PRB expression in each tissue type could lead to different action in these tissues despite exposure to similar concentrations of progesterone.

The results of this study demonstrate that progesterone is likely to function in a different manner in the fimbrial compared to the ampullary region. Consistent among all samples was a relative increase in the proportion of the transcription activator PRB isoform to the transcription repressor PRAB isoform in the fimbrial region. The fimbrial region functions to capture and transport oocytes to the ampullary region where sperm capacitation, oocyte-sperm binding, fertilization and early embryo cleavage takes place. Progesterone responsive genes are more likely to be activated in the ampullary region of the oviduct than in the fimbrial region due to the change in PRAB to PRB ratio (9,10).

CONCLUSION

This study focused on the regulation of PRAB and PRB in mucosal cells as these are the cells that interact directly with gametes and early embryos. Our data provides further evidence to support the hypothesis that P may play a specific role in providing an appropriate environment for sperm capacitation, fertilization and early embryo cleavage in oviduct mucosal cells.

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REFERENCES

1. Sun X, Christow A, Marions L, Gemzell-Danielsson K: Progesterone receptor isoform B in the human Fallopian tube and endometrium following mifepristone. *Contraception* 2003;67:319–326
2. Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P: Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally

- different human progesterone receptor forms A and B. *EMBO J* 1990;9:1603–1614
3. Horowitz K, Alexander P: *In situ* photolinked nuclear progesterone receptors of human breast cancer cell lines: Subunit molecular weights after transformation and translocation. *Endocrinology* 1983;113:2195–2201
  4. Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP: Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor function. *Mol Endocrinol* 1993;7:1244–1255
  5. Meyer ME, Pornon A, Ji JW, Bocquel MT, Chambon P, Gronemeyer H: Agonistic and antagonistic activities of RU486 on the functions of the human progesterone receptor. *EMBO J* 1990;9:3923–3932
  6. Kumar NS, Richer J, Owen G, Litman E, Horowitz KB, Leslie KK: Selective down-regulation of progesterone receptor isoforms B in poorly differentiated human endometrial cancer cells: Implications for unopposed estrogen action. *Cancer Res* 1998;58:1860–1865
  7. Soutar RL, Dillon J, Ralston SH: Control genes for reverse-transcription-polymerase chain reaction: a comparison of beta-actin and glyceraldehyde phosphate dehydrogenase. *Br J Haematol* 1997;97:247–248
  8. Amso NN, Crow J, Shaw RW: Comparative immunohistochemical study of oestrogen and progesterone receptors in the Fallopian tube and uterus at different stages of the menstrual cycle and the menopause. *Hum Reprod* 1994;9:1027–1037
  9. Giangrande PH, Pollio G, McDonnell DP: Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. *J Biol Chem* 1997;272:32889–32900
  10. McDonnell DP, Shahbaz MM, Vegeto E, Goldman ME: The human progesterone receptor A-form functions as a transcriptional modulator of mineral corticoid receptor transcriptional activity. *J Steroid Biochem Mol Biol* 1994;48:425–432

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