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Distribution and function of 3′,5′-Cyclic-AMP phosphodiesterases in the human ovary

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ABSTRACT

The concentration of the important second messenger cAMP is regulated by phosphodiesterases (PDEs) and hence an attractive drug target. However, limited human data are available about the PDEs in the ovary. The aim of the present study was to describe and characterise the PDEs in the human ovary. Results were obtained by analysis of mRNA microarray data from follicles and granulosa cells (GCs), combined RT-PCR and enzymatic activity analysis in GCs, immunohistochemical analysis of ovarian sections and by studying the effect of PDE inhibitors on progesterone production from cultured GCs. We found that PDE3, PDE4, PDE7 and PDE8 are the major families present while PDE11A was not detected. *PDE8B* was differentially expressed during folliculogenesis. In cultured GCs, inhibition of PDE7 and PDE8 increased basal progesterone secretion while PDE4 inhibition increased forskolin-stimulated progesterone secretion. In conclusion, we identified PDE3, PDE4, PDE7 and PDE8 as the major PDEs in the human ovary.

1. Introduction

Cyclic adenosine monophosphate (cAMP) is an important intracellular second messenger produced by adenylate cyclase in response to activation of mainly G_s coupled cell surface attached receptors (Sassone-Corsi, 2012). In connection with the ovaries, the follicle stimulating hormone (FSH) receptor (FSHR) and luteinising hormone (LH) receptor (LHR) represent important G_s coupled receptors (Menon and Menon, 2012).

The production of cAMP leads to activation of protein kinase A, which in turn phosphorylates a number of proteins including the cAMP-response element-binding protein (CREB) that acts as a transcription factor to many genes (Escamilla-Hernandez et al., 2008; Lavoie and King, 2009; Priyanka and Medhamurthy, 2007). Furthermore cAMP binds to the exchange protein directly activated by cAMP (EPAC), which is a guanine nucleotide exchange factor (GEF) leading to activation of proteins in the Ras superfamily that again activates various signalling pathways (Schmidt et al., 2013).

Inactivation of cAMP is solely accomplished by enzymatic degradation by phosphodiesterases (PDEs) (Azevedo et al., 2014). Hence, the PDEs are important regulators of ovarian follicle growth, maturation and ovulation (Conti, 2002; Menon and Menon, 2012). However, while the function of PDEs in the rodent ovary is well described there is only limited information available in humans. In addition to hydrolysing cAMP, PDEs may also inactivate cyclic guanidine monophosphate (cGMP) that similar to cAMP acts as an important intracellular second messenger. The PDEs consist of several different enzymes, which are currently grouped into 11 families (i.e., PDE1–PDE11) each differing in tissue distribution, in substrate specificity for cAMP and cGMP and in the characteristics of the enzyme (Table S1). Each family is composed of 1–4 different subtypes (e.g., PDE8A) encoded by different genes each with several splice forms. Enzymes in three (PDE4, PDE7 and PDE8) of the families are capable of hydrolysing only cAMP, while PDE1, PDE2, PDE3, PDE10 and PDE11 are dual-substrate PDEs. The remaining three families (PDE5, PDE6 and PDE9) can only hydrolyse cGMP (Azevedo et al., 2014).

Inhibitors of PDEs have received much attention from the pharmaceutical industry due their key role in regulating cAMP levels. PDE inhibitors have been developed for treatment of inflammatory diseases, such as chronic obstructive pulmonary disease, asthma, inflammatory bowel disease and psoriasis, as well as neurological and psychiatric disorders, such as depression, schizophrenia,

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Parkinson's disease and chronic heart failure (Azevedo et al., 2014; Bender and Beavo, 2006). In reproductive medicine PDE4 inhibition has been suggested as a mechanism to be used for ovulation induction and as a tocolytic drug (Franova et al., 2009; McKenna et al., 2005). During preclinical development several PDE4 inhibitors have shown effects on the reproductive system in rodents (Losco et al., 2010; Nishiyama et al., 2006; U.S. Food and Drug Administration, 2011), but no reproductive effects have been reported in humans.

PDE3, PDE4 and PDE8 are known to be present in the ovary of various species. PDE3A is localised to the oocyte where it exerts an important function in controlling resumption of meiosis in connection with ovulation (Andersen et al., 1999). Prior to the mid-cycle surge of gonadotropins, PDE3A activity in the oocyte is inhibited by cGMP originating from the cumulus cells. This ensures a high intracellular cAMP concentration deriving from the constitutive active GPR3 (G protein-coupled receptor 3) which maintains meiotic arrest (DiLuigi et al., 2008; Vaccari et al., 2008). In connection with the mid-cycle surge of gonadotropins the cGMP concentration drops rapidly in the cumulus cells. This removes the inhibition of PDE3A leading to a reduction in cAMP levels and meiotic progression (Norris et al., 2009; Tsuji et al., 2012; Vaccari et al., 2009).

Further, PDE4D is critical in controlling ovulation in rodents (Park et al., 2003). PDE4D knock-out mice have reduced fertility due to disrupted ovulation with entrapped oocytes and premature luteinisation of the follicles and blunted cAMP response to human chorionic gonadotropin (hCG) stimulation, most likely due to diminished activity of the LHR (Jin et al., 1999; Park et al., 2003). In contrast, acute treatment with PDE4 inhibitors in FSH primed immature rats is sufficient to induce ovulation, either alone or with a suboptimal dose of hCG (McKenna et al., 2005). Continuous treatment with PDE4 inhibitors in mice and rats leads to disturbance of the oestrus cycle and signs of increased progesterone production with persistent corpora lutea (Losco et al., 2010; Nishiyama et al., 2006).

A single bovine study found that PDE8A was a major contributor to the total PDE activity in antral follicles, and that inhibition of PDE8 with dipyridamol delayed the resumption of meiosis (Sasseville et al., 2009).

In contrast, no reduction of litter size was reported in PDE11A knock-out mice suggesting that PDE11A is not critical in supporting fertility (Kelly et al., 2010).

Taken together, PDEs exert a number of important functions in the ovaries including a central role in regulating oocyte resumption of meiosis and final oocyte maturation. The vast majority of data generated on PDEs in the ovaries is based on rodent studies and since differences in PDE distribution between species in other tissue have been reported (Bian et al., 2004; Johnson et al., 2012), it is important to increase our knowledge about PDEs in humans to properly interpret the rodent data in a human context. The aim of the present study is to characterise the distribution and activity of cAMP hydrolysing PDEs in the human ovary. This was achieved by examining the gene expression and potential differential splicing throughout the folliculogenesis using microarray data and qPCR, evaluating the enzymatic activity in GCs after ovulation induction, by performing immunohistochemical analysis of ovarian sections and measuring the effect of PDE inhibitors on progesterone output from cultured GCs.

2. Methods and materials

2.1. Human tissue

Ovarian tissue for immunostaining and mRNA microarray analysis was isolated from patients undergoing fertility preservation by having ovarian tissue cryopreserved (Rosendahl et al., 2011).

Mature GCs were used for qPCR, protein and enzyme activity analysis, and cell culture experiments were obtained from women undergoing IVF treatment at oocyte aspiration 36 hours after administration of hCG (10.000 IU). The women received controlled ovarian stimulation following the standard long agonist protocol and received 110–250 IU rFSH daily until the leading follicle was 17 mm in diameter when ovulation was triggered. None of the women had any endocrine abnormalities (e.g. PCOS, endometriosis) and were all under the age of 38 years. Follicles were aspirated individually by manual suction and the GC were isolated from follicles exceeding a volume of 1.5–2.0 mL.

The follicular aspirate was collected by centrifugation at 300 × *g* for 5 minutes, the supernatant was discharged and the cell pellet was resuspended in phosphate-buffered saline (PBS) (Life Technologies, Nærum, Denmark) and purified by centrifugation on a Lymphoprep (Axis-Shield PoC, Oslo, Norway) gradient for RNA isolation followed by lysis of potential residual red blood cells in Red Blood Cell Lysis Buffer (Roche A/S, Hvidovre, Denmark) and snap-frozen in liquid nitrogen and stored at –80 °C until RNA isolation. For cell culture experiments the cells were run on Lymphoprep gradient twice using an intermediate 5 minutes incubation in trypsin (TrypLE, Life technology).

The use of surplus ovarian tissue and GCs were approved by the ethical committee of the Capital Region nos: H-4-2011-102 and H-2-2011-044 and participating women gave informed consent.

2.2. Microarray

Microarray data from three previously published studies were analysed (Borgbo et al., 2013; Kristensen et al., 2014; Wissing et al., 2014). None of the studies have specifically examined the expression of PDEs but instead focused on the gene expression patterns in preantral follicles, pre- and post-ovulatory follicles, and the effect of GnRH agonists versus hCG on cumulus cells and GCs for ovulation induction. In addition, we used data from two unpublished studies with GCs isolated from 4–6 mm antral follicles and preantral follicles. The included studies covered the folliculogenesis from the preantral stage to after induction of ovulation. All arrays used the Affymetrix Human Gene ST v1.0 GeneChip array (Affymetrix, Santa Clara, California, USA) and were processed at the same facility (microarray core facility at Rigshospitalet Copenhagen). The amplification, labelling, hybridisation and scanning for all arrays were performed as described previously (Kristensen et al., 2014). An overview of the included studies is given in Table 1. The previous unpublished study in preantral follicles was conducted in the same manner as Kristensen et al. (2014). In the second unpublished study RNA were isolated from GCs aspirated from antral follicles with a diameter of 4–6 mm as previously described (Jeppesen et al., 2013). The total RNA quality and level of degradation were evaluated on an Agilent 2100 Bioanalyzer using the Agilent RNA 6000 Pico LabChip (Agilent Technologies, Waldbronn, Germany). The study was MIAME compliant (ArrayExpress accession number E-MTAB-2862).

For comparison, dataset from heart (Pilbrow et al., 2012; Synnergren et al., 2011), parietal cortex (Chen et al., 2013), cerebellum (Chen et al., 2013), lung (Kabbout et al., 2013), and peripheral blood mononucleated cells (Zhang et al., 2011) were included in addition to various tissues from Affymetrix's sample data set.

2.3. qPCR

All reagents used were purchased from Sigma-Aldrich, Copenhagen, Denmark unless otherwise stated. Simultaneous isolation of mRNA and native protein, first strand cDNA synthesis and real-time quantitative PCR was performed as described previously (Petersen and Andersen, 2014). TaqMan® Gene Expression Assays (pre-designed) for qPCR were purchased from Life Technologies