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EXPRESSION OF LHCG RECEPTOR IN THE MOUSE VULVA

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ABSTRACT

The elevated serum LHCG concentrations have been detected between 40 and 70 years both in male and female. In previous studies LHCG receptor was detected in the mouse and human penis both in corpus cavernosum penis and corpus spongiosum penis. There is no information about the expression of LHCG receptor in the vulva up to now. The aim of present study is to investigate the expression of the LHCG receptor in the mouse vulva tissue to see, if the LH effects can be possible in vulva.

12 Balb/c mice (7–8 weeks old) were used as donors of normal vulva tissue. Immunohistochemistry was used for the detection of the LHCG receptor.

Positive immunoreaction for LHCG receptors was detected in the epithelial cells in all layers of the mouse vulva epithelium, in the epithelial cells of the gland ducts and in the interstitial cells of subepithelial connective tissue.

The high postmenopausal gonadotrophin concentrations could have direct effects on the vulvar tissue, if there are receptors for them. LHCG receptors were detected in the different cell types in the mouse vulva. It could be possible that the higher serum LH concentrations both in aging male and female could have direct effects on the spongious tissue both in the individual and his/her partner. Further studies are indicated.

Keywords: *luteinizing hormone; luteinizing hormone receptor; vulva*

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INTRODUCTION

The dominant role of luteinizing hormone (LH) is to regulate gonadal function both in male and female. The luteinizing hormone/choriogonadotropin (LHCG) receptors have been found in Leydig cells, where they mediate the stimulating effects of LH on steroidogenesis [3]. Human ejaculated spermatozoa also contains LHCG receptors [4]. In the ovary LHCG receptors are expressed in the luteal cells, where they regulate progesterone production and in the theca interna cells, where they regulate androgen production [5].

Besides gonadal location numerous publications have indicated the LHCG receptors presence and function in a variety of extragonadal organs and tissues- in uterus [14], umbilical arteries and vein [16], embryonic stem cells [6], some of the brain cells, prostate, epididymis [15]. However, the physiological significance of such possible effects on these tissues has remaind unclear [12].

LH exerts its effects through binding to its cognate receptor, the LHCG receptor, which belongs to the group of glycoprotein hormone receptors (GpHRs) [1]. The placental analogue of LH, human chorionic gonadotropin (hCG) interacts with the same receptor [10].

In previous studies LHCG receptor was detected in the mouse penis [11]. Using immunohistochemistry, LHCG receptor was found in urethral epithelium, also in the endothelial cells of cavernous spaces both in corpus cavernosum and corpus spongiosum penis. Western blotting analyses and the quantitative RT–PCRs confirmed the presence of LHCG receptor in the mouse penis [11]. LHCG receptor was also found in the human penis in the endothelial cells of cavernous spaces both in the corpus cavernosum penis and corpus spongiosum penis and in the endothelial cells of capillary walls [18].

It is known that LHCG regulates cellular function mostly through the adenylate cyclase/cyclic AMP (cAMP) signal transduction pathway. The transcription factor cAMP response element binding protein (CREB) also is involved into that pathway. By binding to its receptor, LH leads to an increase in the intracellular cAMP level [12]. Both cAMP and CREB were found in the human penis [17]. cAMP was detected in the most cells of all layers of urethral epithelium, in fibroblast-like cells of interstitial tissue and in endothelial cells of cavernous spaces in corpus spongiosum penis and corpus cavernosum penis. CREB was found in superficial and intermedial layer of urethral epithelium and in endothelial cells of cavernous spaces and in fibroblast-like cells of interstitial tissue in corpus spongiosum penis and corpus cavernosum penis [17].

It is not known yet what functions LHCG receptors may have in the penis, but it's possible that age–associated increased LHCG levels may directly affect

penile tissue and thereby play an important role in the development of erectile disturbances [18]. The studies in the mice, expressing a constitutively activating mutation in LHCG receptors, demonstrate that this mutation can cause erectile dysfunction due to impairment of the NO–mediated signaling pathway in the penile smooth muscle [8].

The higher serum LH concentrations both in aging males and females could have direct effects on the spongious tissue both in the individual and his/her partner.

There is no information about the expression of LHCG receptor in the vulva up to now. The aim of present study is to investigate the expression of the LHCG receptor in the mouse vulva to see if LH effects can be possible in vulva.

MATERIALS AND METHODS

Animals and housing

Mice used in this study were bred and cared at the Laboratory Animal Centre of the University of Tartu, Estonia. According to the national guidelines, no permission was required by authorities to collect tissue specimens from sacrificed mice. The animals had free access to food and water and they were maintained in a normal dark/light cycle.

12 Balb/c mice (7–8 weeks old) were used as donors of normal vulva tissue and and 2 Balb/c mice (7–8 weeks old) were used as donors of normal ovarium tissue (positive control). The mice vulvas and ovaries were removed after sacrificing the mice with cervical dislocation.

Samples were fixed in 4% formalin overnight at 4 °C. After fixation, the samples were stored in 70% ethanol until embedding in paraffin.

Immunohistochemistry

Paraffin sections of 5 μm in thickness were cut and mounted on slides. After deparaffinization, the sections were treated with 0.9% H_2O_2 for 15 minutes to inactivate endogenous peroxidase. The sections were treated with Dako REAL Antibody Diluent (S2022; Dako Denmark A/S, Glostrup, Denmark) to block non-specific binding. After blocking, the sections were incubated with the rabbit polyclonal antibody to LHCG receptor (TA340817, Origene) or control serum overnight at 4 °C. Primary antibody dilution was 1:200. Visualization of the primary antibody was performed using the commercial kit "Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, Rabbit/Mouse" (K5007; Dako

Denmark A/S, Glostrup, Denmark). Washing steps in-between were done in phosphate buffered saline (PBS) which contained 0.07% of Tween 20 as the detergent. Toluidine blue (Applichem, Darmstadt, Germany) was used for background staining. No immunohistochemical staining was noted in negative controls where the primary antibody was omitted.

RESULTS

Positive immunoreaction for LHCG receptors was present in the epithelial cells in all layers (superficial, intermedial and basal layer) of the mouse vulva epithelium (Figure 1a). Positive immunoreaction was also detected in the interstitial cells of subepithelial connective tissue (Figures 1a, b), included macrophages (Figure 1c). Positive immunoreaction was found in glandular cells of the mouse vulva (Figure 1d).

Positive immunoreaction was found in tissue samples of all 12 mice.

No positive cells were visible in negative controls of the mouse vulva. (Figure 1e).

Positive cortical parenchymal cells were detected in the mouse ovarium tissue used as positive controls (Figure 1f).

DISCUSSION

We showed for the first time the presence of LHCG receptor in the mouse vulva.

The role of LH in ovaries is well-known and LHCG receptors also were found outside of the gonads in the female reproductive system–including uterus. By increasing LHCG receptors levels in the human myometrium during pregnancy the uterine relaxation during fetal maturation is possible [14].

The effects of different hormones on sexual function and the quality of life of postmenopausal women have been investigated in many studies. With regard to sexual hormones and sexual function, a relationship was found between orgasm and luteinizing hormone ($r = 0.37$) [16].

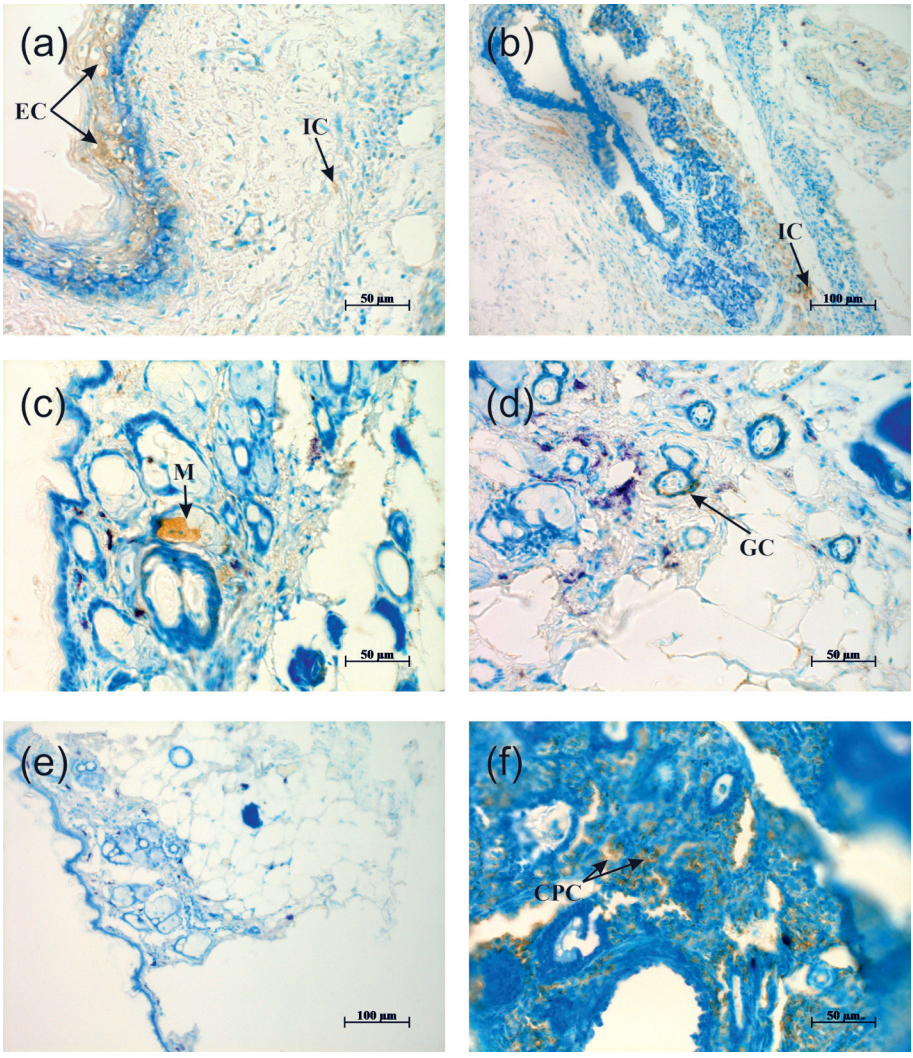

Figure 1. Expression of LHCG receptors in the mouse vulva. Positive cells are pointed by arrows. Note (a) the presence of LHCG receptors in the epithelial cells (EC) of the mouse vulva epithelium and (a, b) in the interstitial cells (IC) of subepithelial connective tissue. (c) Positive immunoreaction is also present in macrophages (M) and (d) glandular cells (GC). (e) No positive cells were present in negative controls. (f) Positive control – mouse ovarium. Cortical parenchymal cells (CPC). The scalebar is on the lower right corner of the figures.

It is not clear at the present, what functions LHCG receptors may have in different cells of vulva. LHCG receptors were also detected in the mouse and human penis – in the endothelial cells of the cavernous spaces both in corpus cavernosum and corpus spongiosum penis and in the endothelial cells of capillary walls, also in urethral epithelium [11; 18]. It is possible, that the higher serum LH concentrations in the aging males and females [9] could have direct effects on the spongious tissue of penis and vulva. The experiments with the mouse model of familial male-limited precocious puberty mice demonstrate that activating mutations in the mouse LHCGR cause erectile dysfunction due to impairment of the NO–mediated signaling pathway in the penile smooth muscle [8]. This study could support our hypothesis that continuously elevated LH levels (LH receptor activity) could affect the mechanism of erection.

It is often thought that the steroids have their effects on these tissues, but as there are high concentrations of gonadotrophins in the aging males and females and receptors for them at least in the penis, the high concentrations themselves could as well have direct effects on the spongious tissue in the penis. The high gonadotrophin concentrations are a signal, information that the receptors in the penile or vulvar tissue could receive, if there are receptors for them present as the present and earlier results suggest. It should also be taken into account that possibly even the penile tissue could be affected by the high gonadotrophin concentrations of the female during intercourse, if there is LH in high concentrations in the vaginal secretions. It should remembered that the postmenopausal women as well as women approaching ovulation have high serum LH levels and the pregnant women have high levels of serum hCG, suggesting that high female LH or hCG concentrations in the vaginal excretions might bind to the penile LHCG receptors, thus possibly affecting the penile function. However, the LHCG receptors are expressed by cells deep inside the penile tissue, suggesting that LH or hCG in the vaginal excretions may not reach these cells without active transport at some point between the penile surface and the spongious tissue [7]. Further studies are indicated.

CONCLUSION

The present study clearly shows the presence of the LHCG receptor in the different cell types in the mouse vulva. These findings support the view that the high postmenopausal gonadotrophin concentrations could have direct effects on the vulvar tissue.

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