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Department of Anatomy, University of Tartu, Estonia
Institute of Anatomy, University of Tampere, Finland

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Supervisors: Associate Professor Kersti Kokk, MD, dr.med
Department of Anatomy
Institute of Biomedicine and Translational Medicine
Faculty of Medicine
University of Tartu, Estonia

Professor of practice Pasi Pöllänen, MD, Dr.med.sci., PhD
Specialist in Obstetrics and Gynecology
Faculty of Medicine
University of Helsinki, Finland

Reviewers: Professor Helle Karro, MD, dr.med
Department of Obstetrics and Gynecology
Institute of Clinical Medicine
Faculty of Medicine
University of Tartu, Estonia

Professor Marika Väli, MD, PhD
Department of Pathological Anatomy and Forensic Medicine
Institute of Biomedicine and Translational Medicine
Faculty of Medicine
University of Tartu, Estonia

Opponent: Adjunct Professor (Docent) Esko Veräjänkorva, MD, PhD
Department of Clinical Medicine
School of Surgery, Division of Plastic and General Surgery
Faculty of Medicine
University of Turku and University Hospital of Turku, Finland

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II. The author took part in histological and immunohistological experiments, analysed the data and was the main person responsible for writing the manuscript.

III. The author took part in Western blotting, quantitative RT-PCR reaction, conducted immunohistochemical experiments and participated in writing the manuscript.

ABBREVIATIONS

ATF-1	activating transcription factor-1
ATP	adenosine trisphosphate
AR	androgen receptor
cAMP	adenosine 3',5'-cyclic monophosphate
cGMP	cyclic guanosine monophosphate
CREB	cAMP-response element-binding protein
CREM	cAMP response element modulator
DAB	3,3-diaminobenzidine
DM1	myotonic dystrophy type 1
DMPK	DM1 protein kinase
ED	erectile dysfunction
Epacs	exchange proteins activated by cAMP
FSH	follicle stimulating hormone
FSHR	follicle-stimulating hormone receptor
GnRH	gonadotropin-releasing hormone
GPCR	G-protein-coupled receptor
GPHR	glycoprotein hormone receptor
hCG	human choriogonadotropin
LHR	luteinizing hormone receptor
LH	luteinizing hormone
LHCGR	luteinizing hormone/choriogonadotropin receptor
NGS	normal goat serum
NO	nitric oxide
PBS	phosphate buffered saline
PKA	protein kinase A
SDS	sodium dodecyl sulfate
STAR	steroidogenic acute regulatory protein
T	testosterone
T3	tri-iodothyronine
T4	thyroxine
TBS	Tris-buffered saline
TSH	thyroid-stimulating hormone
TSHR	thyroid-stimulating hormone receptor

1. INTRODUCTION

In the context of aging populations in developed countries, researches related to erectile dysfunction (ED) are an important issue in modern science.

According to Massachusetts Male Aging Study, the mean probability of some degree of ED was 52% in the whole group of 1,290 men aged from 40 to 70 years (Feldman *et al*., 1994). In the USA, 6 million prescriptions for sildenafil were prescribed in the first 8 months after it became available (Morgentaler, 1999). According to the European Male Ageing Study, erectile dysfunction was reported in 30% of the entire study. It was higher in the older age groups, especially in men 70 years and older (64%) (Vasan, 2010). Until the early 1990s, ED was thought to be related with the natural aging process and was tolerated together with other age-related diseases. Extensive studies in this area have shown that ED is not just an age-related phenomenon (Solomon *et al*., 2005).

A large number of older men experience changes in the level of different sex hormones. The proportion of men with serum luteinizing hormone (LH) > 6.0 IU/L with normal serum testosterone > 9.8 nmol/L increases with age (Härkönen *et al*., 2003). It is possible that high LH levels in aging men may have an impact on the penile tissue and are thereby related to the development of erectile dysfunction. The prerequisite for such hypothesis would be the expression of LHCG receptors in the penis.

Luteinizing hormone belongs to a family of glycoprotein hormones and has an important role in reproduction, having its receptor in the Leydig cells in the testis but also in ovarian theca, granulosa, luteal and interstitial cells (McFarland *et al*., 1989).

The luteinizing hormone receptor (LHR), the follicle-stimulating hormone receptor (FSHR) and the structurally homologous thyroid stimulating hormone receptor (TSHR) form a glycoprotein hormone receptor subfamily that belongs to a large group of G protein-coupled receptors (GPCRs). Pituitary LH and placental choriogonadotropin both bind to LHR (Huhtaniemi and Alevizaki, 2006). Besides male and female gonadal cells, LHCGR has been found to be present in many nongonadal tissues described below, but its expression in the penis has never been studied before the present project.

Studies on nongonadal LH/hCG actions may increase the possibilities of using these hormones in new clinical indications in the future (Rahman and Rao, 2009).

In the present thesis, the expression of LHCG receptors in the mouse and the human penis is studied to analyse the possible involvement of LH in the development of erectile dysfunction. As it is known that LH mediates its steroidogenic actions through adenylate cyclase signaling pathway (Dufau *et al*., 1984), the presence of adenosine 3',5'-cyclic monophosphate (cAMP) and cAMP-response element-binding protein (CREB), two components of this pathway, is also investigated in the penile tissue.

2. REVIEW OF LITERATURE

2.1 Structure of the penis and the erectile mechanism

2.1.1 Structure of the penis

Penis is the male copulatory organ, but also the main part of the male urethra courses through it.

The weight of the penis is supported by the fundiform ligament from the inferior part of the linea alba and the suspensory ligament from the pubic symphysis. The penis is cylindrical in shape and has three main parts: body, root and glans. The body also consists of three cylindrical masses, the paired dorsolateral masses are corpora cavernosa and the smaller midventral mass, the corpus spongiosum penis, includes the spongy urethra and keeps it open during ejaculation (Tortora, 1995). The bodies are encased by fascial layers of the tunica albuginea – Buck's fascia and Colles' fascia – which separate the corporeal sinusoids from the dermis and skin layer (Hsieh *et al*., 2012).

The tunica albuginea of the corpora cavernosa is a bilayered structure, which has multiple sublayers. The collagen bundles of the inner layer are oriented circularly, supporting and containing the cavernous tissue. Bundles of the outer layer are directed longitudinally (Hsu *et al.,* 1992). Corpus spongiosum has a much thinner tunica albuginea and contains more elastic fibers (Andersson and Wagner, 1995).

The proximal ends of the corpora cavernosa, known as the crura, are anchored to the descending rami of the pubic arch. Cruras are covered by the ischio-cavernous muscle, which can direct blood from the crus into the penis shaft. Beneath the symphysis, the two crura unite into one unpaired body. An incomplete fibrous partition, the septum penis, runs in the midline along the entire length of the corpus cavernosum penis.

Corpus spongiosum penis is embedded in a longitudinal groove, which runs along the undersurface of the corpus cavernosum penis. It begins as bulbus penis, covered by the bulbospongiosus muscle. Bulbospongiosus muscle helps to squeeze out the contents from the urethra (Kahle *et al*., 1993).

The erectile tissue of the penis has many endothelially lined spaces which are variably shaped and separated from one another by trabeculae of connective tissue and smooth muscle cells (Gartner, 2016).

The corpora cavernosa contain numerous wide, irregularly shaped vascular spaces lined with vascular endothelium (Ross and Pawlina, 2011). The vascular spaces are smaller peripherally, near the tunica albuginea, and larger centrally (Gartner, 2016).

A thin layer of smooth muscle surrounds the spaces and forms trabeculae within the tunica albuginea interconnecting and crisscrossing the corpus cavernosum. Many nerve endings and lymphatic vessels are in the interstitial connective tissue (Ross and Pawlina, 2011).

In corpus spongiosum, the vascular spaces are similar in size throughout its extent. Compared to corpora cavernosa, the trabeculae of the corpus spongiosum contain more elastic fibers and fewer smooth muscle cells (Gartner, 2016).

Branches of the deep and dorsal arteries of the penis give blood to the erectile tissues of the corpora cavernosa. Penetrating the walls of the trabeculae of the erectile tissue, these branches form either capillary plexuses, which supply some blood flow into the vascular spaces, or helical arteries, which are important blood sources to the vascular spaces during erection of the penis (Gartner, 2016).

Venous drainage of the corpora cavernosa is mainly via the cavernous veins, with additional drainage through the crural, circumflex and deep dorsal veins (Aboseif *et al*., 1989).

2.1.2 Erectile mechanism

Penile erection as a vascular event is initiated by the central nervous system and maintained by complex interactions between vascular and neurologic events. The central nervous system responds to external or internal stimuli involving the sympathetic and parasympathetic innervation of the penis. Parasympathetic stimulation initiates erection by relaxation of the trabecular smooth muscle cells and dilation of the helicine arteries. Sympathetic stimulation terminates penile erection by causing contraction of the trabecular smooth muscle cells of the helicine arteries (Ross and Pawlina, 2011).

Many central neurotransmitters and neuropeptides also have a role in the erectile control. Serotonin, dopamine, oxytocin, nitric oxide (NO), adrenocorticotropin/α-melanocyte stimulating hormone, excitatory amino acids and opioid peptides are some best-known central mediators which can facilitate or inhibit penile erection by acting in several brain areas (Andersson and Martin, 2011). NO has a crucial role in the physiology of the penis and is an important factor for relaxation of vascular smooth muscles and smooth muscles of the corpus cavernosum (Burnett, 1995, 1997).

The erection of the penis requires adequate filling of the corpora cavernosa with blood at systolic pressure or slightly above (Kirby, 1994). The cavernous smooth musculature and the smooth muscles of the arteriolar and arterial walls have an important role in the erectile mechanism. In a relaxed state, these muscles are in a state of contraction by the sympathetic discharge. Vasoconstrictors secreted by endothelium allow only a small amount of arterial flow for nutritional purposes (El-Sakka and Lue, 2004). Relaxation of these muscles increases blood inflow into the lacunar spaces of the corpora cavernosa. The relaxed trabecular walls expand because of arterial pressure, thus expanding the tunica albuginea with elongation and compression of the draining venules, which restricts the outflow of blood. After ejaculation, the smooth muscle surrounding the arteries and the lacunar spaces contracts and the inflow of blood reduces. The penis returns then to a relaxed state because the venous

drainage of the corporeal spaces is opened again (Wagner and Saenz de Tejada, 1998).

The corpus spongiosum and the glans penis have somewhat different hemodynamics compared to corpora cavernosa. Although the arterial flow increases in a similar manner during erection, the pressure in the corpus spongiosum and the glans is only one-third to one-half of that in the corpora cavernosa because of the tunical covering which ensures minimal venous occlusion (El-Sakka and Lue, 2004).

2.2 Erectile dysfunction etiology

Erectile dysfunction (ED) is an increasing health problem, which shows age-dependent prevalence ranging from 2% in men younger than 40 years to 86% in men aged 80 years and older (Prins *et al*., 2002). It is predicted that by 2025 over 322 million men are affected by erectile dysfunction world-wide (Ayta *et al*., 1999).

By definition, as given in the DSM-5 by the American Psychiatry Association (2013) erectile dysfunction is the inability to obtain and/or to maintain an adequate erection to enable satisfactory sexual activities. ED can be the result of many health conditions, including neurologic, psychogenic, vascular, urogenital and hormonal abnormalities but can also develop post-traumatically and after surgery (Celik *et al*., 2014). Potency disturbances possibly also have a genetic dimension, as e.g. expression of the DM1 protein kinase (DMPK) gene may be associated with many age-related signs, including hypogonadism and erectile disorders and there can be CTG repeat expansions in this gene affecting its function (Brisson *et al*., 2002).

The problems with potency are usually multifactorial, but, generally, the disorders resulting in erectile dysfunction can be subdivided into four groups: psychogenic, endocrinological, neurogenic and vascular impotence (Kirby, 1994).

Predisposing factors related to the development of psychogenic erectile dysfunction are physical and mental health problems, traumatic past experiences, strict upbringing and inadequate sex education. Acute relationship problems, family or social pressures and major life events are also factors that may cause psychogenic erectile disorders (Shamloul and Ghanem, 2013).

Although most cases of ED in men under 40 are thought to have psychogenic etiology, several studies have identified organic etiologies in men with ED in this age group, including vascular, neurogenic, endocrine disorders and also Peyronie's disease or medication side effects (Ludwig and Phillips, 2014).

Many neurological disorders may lead to erectile dysfunction, including cerebral insult, multiple sclerosis, spinal cord injury, Parkinson's disease and Alzheimer's disease. Radical pelvic surgeries are also risk factors for development of erectile dysfunction. Endocrinological causes of erectile disorders are diabetes mellitus, hypogonadism and hyperprolactinaemia. Risk factors associated with penile arterial insufficiency are atherosclerosis, hypertension, hyper-

lipidaemia, diabetes mellitus, pelvic irradiation and cigarette smoking. Age, poor physical and psychological health, obesity, alcohol misuse, recreational drug use, sleep disorders, metabolic syndromes and also persistent medical disordes (chronic kidney, liver and pulmonary diseases) are all related to erectile dysfunction (Shamloul and Ghanem, 2013).

2.3 The family of glycoprotein hormones and their receptors

The first indication of the existence of gonadotropic substances in anterior pituitary extracts came from the pioneer work of Smith (1927) (Li and Pedersen, 1952).

Gonadotropins as glycoprotein hormones are large (up to 40 kDa) proteins which regulate sexual development, normal growth and reproduction (Mazina *et al*., 2017).

Luteinizing hormone (LH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and human choriogonadotropin (hCG) belong all to the family of glycoprotein hormones, the production of which occurs in the pituitary gland (LH, FSH, TSH) or in the placenta of a developing fetus (hCG) (Bousfield and Dias, 2011). As heterodimeric proteins, these hormones share a common α-subunit, and their hormonal specificity is determined by the β-chains (Tourkova *et al*., 2015). Both subunits are glycosylated in specific residues and are closely linked with disulfide bonds (Pierce and Parsons, 1981). A common α-subunit contains 92 amino acids, while β-subunits assign biological activity, displaying various degrees of homology, which, between hCG and LH, is approximately 80% (Stenman *et al*., 2006).

Within family A of the G-protein-coupled receptors (GPCR), the thyroid-stimulating hormone receptor (TSHR), follicle-stimulating hormone receptor (FSHR) and the luteinizing hormone/choriogonadotropin receptor (LHCGR) belong to the subfamily of glycoprotein-hormone receptors (GPHR) (Grzesik *et al*., 2014). There are mainly seven transmembrane helices, three extracellular and three intracellular loops and, in most, an intracellular C-terminal tail in the main structure of GPCRs (Puett *et al*., 2007). The interaction of the glycoprotein hormone with its receptor's ectodomain leads to the activation of the receptor's transmembrane domain for signal transduction (Dias *et al*., 2002). LH and hCG both bind to LHCGR (Puett *et al*., 2010). Polypeptides, which make hCG, form a group of three biologically important molecules with different physiological functions. These molecules are regular hCG, hyperglycosylated hCG and hyperglycosylated hCG free β-subunit. (Cole, 2009). The main role of hCG is to stimulate progesterone and oestrogen production in the corpus luteum (Hearn and Gomme, 2000). However, the understanding of the biological functions of hCG has developed significantly recently. For example, hCG maintains angiogenesis in the myometrial spiral arteries (Lei *et al*., 1992; Herr

et al., 2007), contributing to placental nutrient supply. The hyperglycosylated hCG regulates placental invasion, promotes cancer cell malignancy (Cole *et al*., 2006) and is a critical factor for implantation (Sasaki *et al*., 2008). Hyperglycosylated hCG free β, made by non-trophoblastic cancer cells, enhances cancer cell growth and malignancy (Butler and Iles, 2003; Cole *et al*., 2006).

FSH and LH regulate the spermatogenesis and steroidogenesis in the testes. While LH mainly controls the testosterone (T) secretion from Leydig cells, the role of FSH in the male gonads is to interact with Sertoli cells, resulting in the production of proteins which control spermatogenesis. In the female, LH and FSH control follicular growth, ovulation, development of the corpus luteum and steroid production in the follicle and corpus luteum. TSH regulates the thyroid hormone (tri-iodothyronine T3 and thyroxine T4) production and secretion from the thyroid gland (Hearn and Gomme, 2000).

2.4 Luteinizing hormone receptor and its role in Leydig cells

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are pituitary gonadotropic hormones, the pulsatile release of which is central in reproduction. These hormones regulate the gonadal function. In the testis, LH regulates the production and secretion of androgens by acting through plasma membrane receptors of the Leydig cells. In the testis, LHCGRs are expressed during the fetal life, postnatally, at puberty, and throughout adult life. LH also promotes the maturation of follicular cells in the ovary (Dufau, 1998).

The luteinizing hormone/choriogonadotropin receptor (LHCGR) is a member of glycoprotein hormone receptors (GPHR) (Ascoli *et al*., 2002; Caltabiano *et al*., 2008). LHCGR anchors within the cell membrane by seven transmembrane domains, and its gene is located on the short arm of chromosome 2 (2p21) (Rousseau-Merck *et al*., 1990, 1993). The human LHCGR gene contains 11 exons and 10 introns, complemented by the primate-specific exon 6A (Atger *et al*., 1995; Kossack *et al*., 2008). Inactivating mutations of the LHCGR gene are also known (Latronico and Arnhold, 2012).

LH receptor is expressed in many cell types, including the corpus luteal cells (Bukovsky *et al*., 2003), the decidual cells (Licht *et al.,* 2003), the myometrial smooth muscle cells (Phillips *et al*., 2005), the uterine vascular cells (Toth *et al*., 1994), the smooth muscle and endothelial cells of umbilical arteries and vein (Rao *et al*., 1993), the embryonic stem cells (Gallego *et al.*, 2010), the trophoblasts (Prast *et al*., 2008), some of the brain cells (Bukovsky *et al*., 2003; Cole, 2010), the testicular endothelial cells (Ghinea *et al*. 1994; Ghinea and Milgrom, 2001), the human ejaculated spermatozoa (Elben *et al*., 2001) and also certain cells in oviducts, spinal cord, neural retina, skin, breast, adrenals, urinary bladder, bone, cavernous sinus carotid rete vascular complex, prostates, seminal vesicles and epididymides (Rao, 2001).

Released from the anterior pituitary gland in a pulsatile manner, LH controls, among its other functions, the androgen production by the Leydig cells, thus maintaining testosterone production at the necessary level through interaction with LH receptors and activation of the steroidogenic signal transduction pathways of the Leydig cell. LH receptor activation leads to the stimulation of adenylate cyclase in the Leydig cell plasma membrane, causing phosphorylation of the key intermediate of its signal transduction pathway. After up-regulation of testosterone production in the Leydig cells, increased circulating testosterone concentration down-regulates LH production, which again leads to upregulation of the LH receptors and vice versa, when lowering serum testosterone levels induce increased LH production – like in aging men (Härkönen *et al.*, 2003) – or when hCG is given in high doses, LH receptor production may be down-regulated to a certain degree (Dufau *et al*., 1984).

2.5 cAMP and CREB and their role in signal transduction

Adenosine 3',5'-cyclic monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), nucleotides, lipids and other small molecules belong to the group of intracellular second messengers, which convert and magnify extracellular signals by activating protein kinases or acting on intracellular ligand-gated channels to alter membrane potential. One of the most important cyclic phosphate involved with signal transduction is cAMP (Yan *et al*., 2016).

In response to tropic hormone, activation of the adenylate cyclase enzyme causes intracellular increase in cAMP and PKA (protein kinase A) activation (Stocco and Clark, 1996; Stojkov *et al.*, 2014). Although cAMP mediates its intracellular effects mainly through PKA, it may also act via other target proteins, like Epacs (exchange proteins directly activated by cAMP) (de Rooij *et al.*, 1998).

After binding to its receptor, LH activates the adenylate cyclase enzyme, causing an increase in the production of cAMP from adenosine trisphosphate (ATP). The increased intracellular cAMP activates the cAMP-dependent protein kinase A (PKA). After translocation to the nucleus, the activated PKA participates in the regulation of gene expression via phosphorylating transcription factors like cAMP-response element binding protein (CREB), which initiates transcription of cAMP-response genes by binding to the cAMP response element in the promoter region of the cAMP-response genes (Zeleznik and Somers, 1999; Yan *et al.*, 2016).

The cAMP response element binding protein (CREB) belongs to a large family of DNA binding proteins (Priyanka and Medhamurthy, 2007), and it is the first transcription factor whose activity, together with its other family members, is known to be regulated by phosphorylation (Mayr and Montminy, 2001). CREB, CREM (cAMP response element modulator) and ATF-1 (activating transcription factor-1) are genes that share wide homology, form the CREB/CREM subfamily and mediate transcriptional activation. This subfamily has an

important role in the growth and development processes of many organs, is necessary for survival and mediates various biological functions (Manna *et al.,* 2002). CREB regulates numerous genes with various functions, like proliferation, survival, memory and learning (Kinjo *et al.,* 2005).

2.6 Hormonal changes in aging men

Although the gonadal function declines with age in both men and women, men have a more gradual decline in the gonadal function, which means there is no andropause equivalent of the menopause (Vermeulen, 2000).

Hypogonadism is a clinical condition caused by a testicular failure to produce physiological levels of testosterone and a normal number of spermatozoa due to hypothalamic-pituitary-testicular axis disorder. Primary hypogonadism, also known as hypergonadotropic hypogonadism, can be a result of several testicular injuries, tumour, infection (Dandona and Rosenberg, 2010) or renal failure, hepatic cirrhosis, drugs, autoimmune disease and irradiation (Gurung and Jialal, 2019). Despite high levels of LH and FSH, the gonads are unable to produce sufficient testosterone or spermatogenesis. It may also have congenital causes, such as androgen synthesis disorder, cryptorchidism or Klinefelter syndrome (Gurung and Jialal, 2019). The second form, which is more common and shows low T and low or inappropriately normal LH, is usually linked with overweight or chronic diseases (hypertension, diabetes, heart failure, metabolic syndrome, inflammatory arthritis, chronic obstructive lung disease etc.) (Huhtaniemi, 2014). Congenital isolated gonadotropin-releasing hormone (GnRH), LH or FSH deficiency may also be the cause of secondary hypogonadism (Gurung and Jialal, 2019). Dual defects, affecting both the pituitary and the testis, are also possible. Depending on which defect predominates, combined failure results in variable gonadotropin levels, impaired spermatogenesis and low testosterone levels (Bhasin *et al*., 2010). The study results of Delhez *et al*., 2003 on 153 men (aged 50–70 years) showed a decrease in the levels of free T, whereas FSH and LH increased. In the "Hypogonadism in Males" study (in men aged ≥45 years), patients with hypertension, hyperlipidaemia, diabetes, obesity, prostate disease and asthma or chronic obstructive pulmonary disease had significantly higher odds of hypogonadism compared to patients without these comorbidities (Mulligan *et al*., 2006). According to Rey *et al*., 2013, male hypogonadism can be classified based on the pathophysiology of the hypothalamic-pituitary-testicular axis in different life periods, considering the level of the hypothalamic-pituitary-testicular axis primarily affected, the testicular cell population initially impaired and the period of life when the gonadal function begins to fail.

2.7 Summary of the literature

Erectile dysfunction (ED) as an inability to achieve normal erection and also to maintain it sufficiently long, has a multifactorial etiology. It is an increasing health problem, especially in the context of an aging society. The general disorders resulting in ED can be subdivided into psychogenic, neurogenic, vascular and endocrinological subgroups, but also many other risk factors can affect the development of ED. Endocrinological causes of erectile dysfunction include diabetes mellitus, hyperprolactinaemia and hypogonadism. Although large number of older men experience changes in the level of different sex hormones and ED shows age-dependent prevalence, it is not only an age-related phenomenon. LH, TSH, FSH and hCG are all pituitary gonadotropic hormones, heterodimeric proteins, sharing a common α-subunit, while β-subunits determine hormonal specificity, assign biological activity and display various degrees of homology. LH mainly regulates gonadal function and exerts its actions through binding to its receptors and mediating its steroidogenic actions through adenylate cyclase signaling pathway. In the testis, LH regulates the production and secretion of androgens, mainly controlling the testosterone secretion from Leydig cells. LHCGR, together with FSHR and TSHR, belongs to a family of glycoprotein hormone receptors and besides gonadal cells it has found to be expressed in many extragonadal tissues. Until now there is no data about the expression of LH receptors in the penis. The study was started to find out if LH receptor is expressed in the penile tissue to analyse whether it could be involved in the pathogenesis of erectile dysfunction. Studies on the effects of nongonadal LH/hCG may increase the potential for these hormones to be used in new clinical indications in the future.

3. AIMS OF THE STUDY

The general aim of the present study is to evaluate the expression of the LH receptor and the components of its signal transduction pathway in the penis tissue.

Specific aims:

1. To investigate the expression of the LH receptor in the mouse penis
2. To evaluate the expression of the LH receptor in the human penis.
3. To find out if cAMP and CREB, the components of LH signal transduction pathway, are expressed in the human penis.
4. To analyse, based on the results of the study, if the elevated serum LH levels in aging men may have an impact on the corpus spongiosum and cavernosum penis and be related to the development of erectile dysfunction.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Animals (Paper III)

Balb/c mice (n=42) were used as donors of normal penis tissue and testis tissue (positive control). Fourteen mice (6–7 weeks old) were used for immunohistochemistry; 20 mice (10–12 weeks old), for Western blotting; and 8 mice (15 weeks old), for qRT-PCR (Table 1). The mice used in the present study were bred and cared at the Animal Care Center of the University of Tampere, Finland. According to the national guidelines, no permission from the authorities was required 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. The mice penises and testes were removed after sacrificing the mice with CO_2.

The penis was collected up to the body wall and the distal two-thirds of the body of the penis was used for each analysis. The penis was cut, not prepared free of the surrounding tissue, so the visible part of penis was collected. 14 penises and 2 testes (from 2 mice) were collected for immunohistochemistry, fixed in 4% formalin and embedded in paraffin according to standard methods. A total of 20 penises in one sample (0.62 g of penile tissue in 1.86 ml NaCl) and a total of 40 testes in one sample (4.59 g of testis tissue in 13.5 ml NaCl) were examined for Western blotting. Altogether 8 penises (1 penis/1 ml Eurozol, weight approximately 27–35 mg) and 6 testes (from 6 mice; 1 testicle/2.5 ml Eurozol, weight approximately 134–155 mg) were examined for qRT-PCR.

Table 1. Balb/c mice used as donors of penis tissue

Mice (n=42)	Age in weeks	Study method
14	6–7	immunohistochemistry
20	10–12	Western blotting
8	15	qRT-PCR

4.1.2 Human penis tissue (Papers I and II)

Penile tissue was obtained from three patients undergoing partial or total penectomy either due to rectal cancer or squamous cell carcinoma of the penis. The patients were being treated at the Tampere University Hospital.

Two patients (66-year-old and 64-year-old) were undergoing total penectomy due to rectal cancer with secondary penile metastasis, and one 83-year-old patient with squamous cell carcinoma of the penis was undergoing partial penectomy.

Samples from corpus cavernosum and corpus spongiosum penis were fixed in 4% formalin overnight at 4 °C. After fixation, the samples were stored in 70% ethanol until embedding in paraffin according to standard methods.

4.2 Methods

4.2.1 Immunohistochemistry

4.2.1.1 Avidin-Biotin Immunoperoxidase Method (Paper III)

Paraffin sections of 5 µm in thickness were cut and mounted on poly- L-lysine coated Super-Frost slides (Menzel-Gläser, Germany). After deparafinization, endogenous peroxidase blockade (0.5% H_2O_2 in methanol) was carried out for 30 min. Sections were washed in distilled water for 2 × 5 min and in phosphate buffered saline (PBS) 2 × 10 min. Slides were held in 0.01 M sodium citrate buffer (pH 6.0) in a microwave oven at 100 °C for 20 min. Sections were removed from heat and kept at room temperature in buffer for 20 min. After washing in Tris-buffered saline (TBS) for 5 min and in PBS for 5 min, non-specific binding sites were blocked by incubating the sections in 10% normal goat serum (NGS) for 30 min. The sections were then incubated with the primary antibody or the control serum overnight at +4 °C. Rabbit polyclonal anti-LH receptor antibody (Acris Antibodies GmbH, Germany) was used as the primary antibody diluted 1:750 in 1% NGS in PBS. The sections were washed for 3 × 10 min in PBS and incubated with goat anti-rabbit IgG (Acris Antibodies GmbH, Germany) for 30 min. The biotinylated secondary antibody was diluted 1:500 in PBS containing 1% NGS. After 3 × 10 min washing in PBS, the sections were incubated with the ABC reagent for 30 min (reagent A 90 µl; 10 ml PBS+ reagent B 90 µl; Vector Laboratories Inc, Burlingame, California). The sections were washed for 3 × 10 min in PBS followed by a demonstration of peroxidase activity, using 3,3'-diaminobenzidine (DAB; Sigma) as a substrate.

4.2.1.2 Peroxidase/DAB+ method with Dako REAL™ EnVision™ Detection System (Paper I and II)

Samples from corpus cavernosum and corpus spongiosum of the penis were embedded in paraffin after fixation in formalin. 5 µm sections were cut, deparaffinized and treated with 0.9% H_2O_2 to inactivate endogenous peroxidase. The sections were then 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 luteinizing hormone receptor (LHCGR) (C-term) (SP4594P, Acris Antibodies), the mouse monoclonal antibody to cAMP (ab24851, Abcam) or the rabbit monoclonal antibody to CREB (ab32096, Abcam) overnight at 4 °C. Primary antibody dilution was 1:500 for luteinizing hormone receptor and 1:200 for cAMP and

CREB. 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.

4.2.2 Western blotting (Paper III)

Penile and testis tissue homogenates were prepared in protease inhibitor suspension Complete Mini (Roche, # 11 836 153 001) in 0.9% NaCl. The tissue was placed in 6 ml of suspension buffer and homogenized. After incubation, the lysate was centrifuged at 250 x g for 15 min. The salts were removed from the supernatant, and the supernatant was centrifuged at 10000 x g for 30 min. The supernatant was collected, and the eluate was then freeze-dried at –70 °C. The protein was diluted to 1:2 in a solution of 10% sodium dodecyl sulfate (SDS), glycerol, 10% bromophenol blue and 5% β-mercaptoethanol in 0.5M TRIS buffer, pH 6.8. The samples were boiled for 5 min.

Denaturated 7.5% SDS polyacrylamide mini-gels were prepared, and 50-µl samples were loaded into the wells. High-molecular-weight markers were run parallel to the samples. Gels were run with 100-mA current and after electrophoresis, proteins were transferred to a nitro-cellulose filter for 60 min using 200-mA current. The nitro-cellulose filter was stained with Ponceau S and each separate line was cut off. The strips were blocked with TBS Blotto A (Santa Cruz, #SC-2333) for l hour at room temperature. Then the strips were incubated overnight at 4 °C with anti-LH receptor antibody (Acris Antibodies GmbH, Germany, #SP4594P) diluted 1:3000 in TBS Blotto A. Normal rabbit immunoglobulin G (IgG; Dako Cytomation, Denmark, #X 0903), diluted 1:3000 in TBS Blotto A, was used as negative control. After incubation, the strips were washed three times with TBS 0.05% Tween solution and then incubated for l hour in 1:30000 dilution of horseradish peroxidase-conjugated goat anti-rabbit Ig (Acris Antibodies GmbH) in TBS Blotto A per strip. Strips were washed three times with TBS 0.05% Tween solution and then allowed to react with Amersham ECL Western Blotting Detection Reagents RPN 2106. Strips were blotted dry before photographing.

4.2.3 Quantitative reverse transcriptase polymerase chain (qRT-PCR) reaction (Paper III)

The penis and testis tissues were homogenized using 1 ml of Eurozol (Euroclone, S.p.A. Milano, Cat. #EMR055100) per penis and 2.5 ml of Eurozol per testis. Chloroform (0.1 ml) was added to each sample. Three samples were run. Samples were mixed carefully, incubated on wet ice for 5 minutes and centrifuged at

12000 x g for 15 min. The upper aqueous phase containing RNA was collected in a new tube, 500 µl of cold isopropanol was added and the mixture was stored in wet ice for 15 min. After centrifugation at 12000 x g for 15 min, the samples were washed, adding 1 ml 75% ethanol. Samples were centrifuged at 8000 x g for 15 min and dried.

Relative quantification of LH in real-time RT-PCR was done using the Pfaffl model (Pfaffl, 2001). The cDNA was synthesized from the total RNA by RT-PCR using a High Capacity cDNA Archive kit (Applied Biosystems, Foster City, California). The reaction was performed at 37 °C for two hours. Samples were stored at –20 °C prior to the real-time PCR reaction. Expression of mRNA in the tissues was detected using real-time PCR. Target cDNA was amplified by PCR for 40 cycles (1 cycle: 95 °C for 15 seconds, 60 °C for 1 minute) in ABI PRISM® 7000 SDS using SYBR® Green solution (Applied Biosystems) and 20 ng of template cDNA. The PCR primer sequences were: forward 5'-TGTATGGAAATGGGTTTGAAGAAGT-3', reverse 5'-TTCTTTTAACTCCAGCGAGATTAGC-3' for the mouse LH receptor gene and forward 5'-GCTTCTTTGCAGCTCCTTCGT-3', reverse 5'-CCAGCGCAGCGATATCG-3' for the internal control gene mouse β-actin. Primers were designed by using Primer Express v2.0 software (Applied Biosystems). To avoid amplification of any genomic DNA, the forward and reverse primers for LH receptor gene were chosen from different exons. BLASTN searches were performed to confirm the specificity of the primer sequences. The final results, expressed as relative differences (ratio) in gene expression between samples, were calculated by using amplification efficiencies obtained from the standard curves and C_t values as described previously (Pfaffl, 2001). C_t is the cycle at which the threshold is crossed.

5. RESULTS

5.1 Expression of the LH receptor in the mouse penis (Paper III)

The results demonstrate the presence of the LH receptors in all investigated mice penises by using three different study methods. Results are presented by specific methods.

5.1.1 Immunohistochemistry

Positive immunoreaction for LH receptors was found in the mouse penis in the urethral epithelium. Positive immunoreaction was also detected in the endothelial cells of the cavernous spaces both in corpus spongiosum and corpus cavernosum penis. All these kinds of positive cells were found in all investigated penises (Fig. 1A). No positive cells were present in negative controls (Fig. 1B).

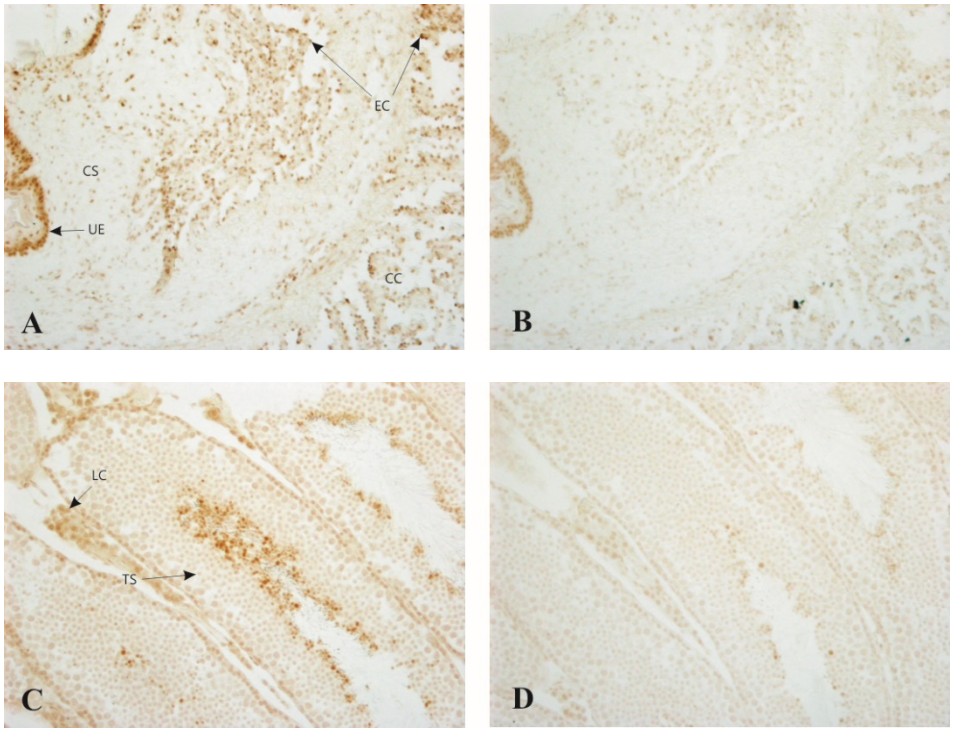

Figure 1. Expression of LHCG receptor in the mouse penis (A) and testis (C). Control sections of penis (B) and testis (D). UE – urethral epithelium; CS – corpus spongiosum penis; CC – corpus cavernosum penis; EC – endothelial cells of the cavernous spaces; LC – Leydig cells; TS – tubulus seminiferus. Staining: DAB. Magnification: × 2300.

Testis tissue was used as positive control for the detection of LH receptors in their target cells. Positive immunoreaction to the LH receptor was found in the Leydig cells and in the central part of seminiferous tubules next to the lumen, most likely in spermatozoas or in residual bodies (Fig. 1C).

No positive cells were present in negative controls (Fig. 1D).

5.1.2 Western blotting

The LH receptor antigen was repeatedly present in the mouse penises investigated in Western blotting (Fig. 2).

It was recognized at M_r = 97.4, occasionally, also at M_r = 78 kD. LH receptor antigen was present in the penile tissue.

Testis tissue was used as positive control. The LH receptor antigen was repeatedly recognized at M_r = 97.4 and 78 kD.

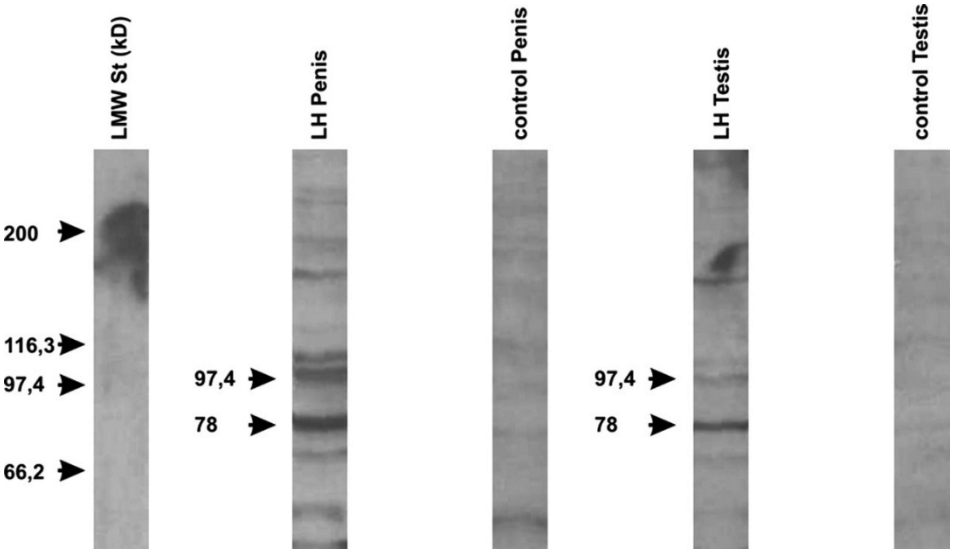

Figure 2. Western blot analyses of the mouse penis and testis tissue for luteinizing hormone (LH).

5.1.3 Quantitative reverse transcriptase polymerase chain (qRT-PCR) reaction

LHR was specifically detected in the penis in qRT-PCR (Table 2).

Table 2. Expression of the LHR in the penis in qRT-PCR

	C_t (LHR)	C_t (β-Actin)	Ratio
Penis	27.069 ± 0.049	17.921 ± 0.084	0.044 ± 0.005
Testis	23.836 ± 0.099	19.381 ± 0.138	1.000

Abbreviations: C_t, threshold cycle; LHR, luteinizing hormone receptor; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction.

5.2 Expression of the LH receptor in the human penis (Paper I)

Positive immunoreaction was found in all three investigated penises.

Positive immunoreaction for LHCG receptors was present in the endothelial cells of cavernous spaces in the corpus spongiosum (Fig. 3A) and cavernosum penis (Fig. 3B). Positive immunoreaction was present in fibroblast-like cells of interstitial tissue in the corpus spongiosum penis (Fig. 3A). Positive immunoreaction was also present in superficial, intermedial and basal layer of urethral epithelium and in fibroblast-like cells of interstitial tissue and endothelial cells of cavernous spaces in glans penis (Fig. 3C). Positive immunoreaction for LHCG receptors was detected in the endothelial cells of capillary walls (Fig. 3D).

No positive cells were visible in negative controls (Fig. 6A).

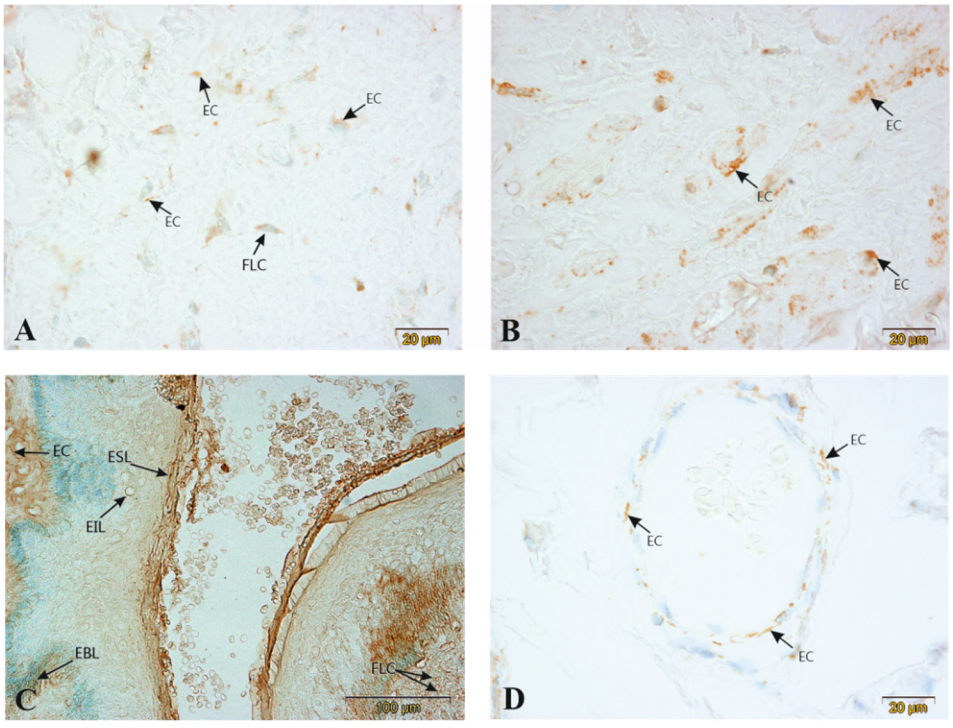

Figure 3. Expression of LHCG receptors in the human penis. Corpus spongiosum penis (A), corpus cavernosum penis (B), glans penis (C) and capillary wall (D). EC – endothelial cells; ESL – superficial layer of urethral epithelium; EIL – intermedial layer of urethral epithelium; EBL – basal layer of urethral epithelium; FLC – fibroblast-like cells of interstitial tissue. Staining: DAB + toluidine blue.

5.3 Expression of the cAMP and CREB in the human penis (Paper II)

Positive immunoreaction for cAMP was present in most cells of superficial, intermedial and basal layer of urethral epithelium and in fibroblast-like cells of interstitial tissue and endothelial cells of cavernous spaces in corpus spongiosum penis (Fig. 4A).

Positive staining for cAMP was also visible in endothelial cells of cavernous spaces and in fibroblast-like cells of interstitial tissue in corpus cavernosum penis (Fig. 4B).

Positive immunoreaction for CREB was present in superficial and intermedial layer of urethral epithelium and some positive staining was also noticed in endothelial cells of cavernous spaces and in fibroblast-like cells of interstitial tissue in corpus spongiosum penis (Fig. 5A).

Positive staining for CREB was also visible in endothelial cells of cavernous spaces and in fibroblast-like cells of interstitial tissue in corpus cavernosum penis (Fig. 5B). No positive cells were visible in negative controls (Fig. 6B).

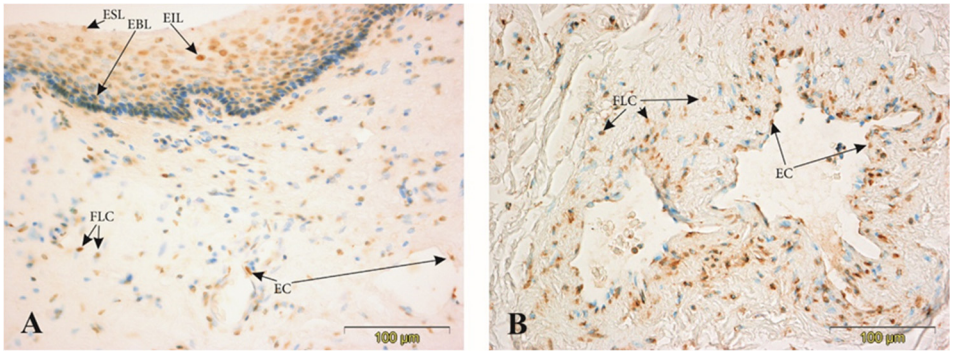

Figure 4. Expression of cAMP in the human penis. Corpus spongiosum penis (A), corpus cavernosum penis (B). EC – endothelial cells; ESL – superficial layer of urethral epithelium; EIL – intermedial layer of urethral epithelium; EBL – basal layer of urethral epithelium; FLC – fibroblast-like cells of interstitial tissue. Staining: DAB + toluidine blue.

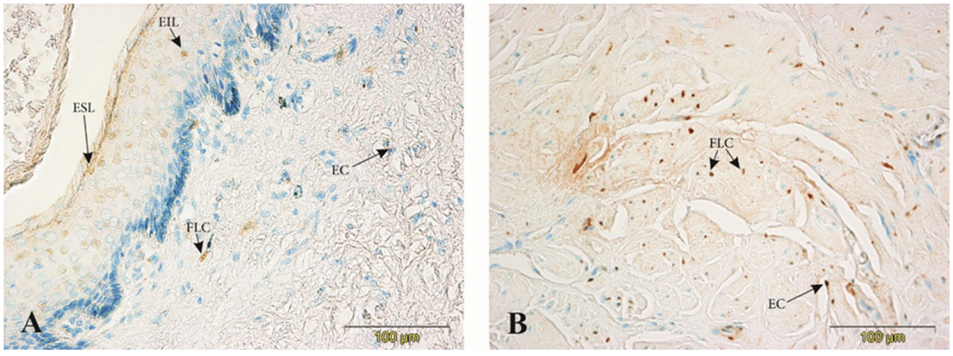

Figure 5. Expression of CREB in the human penis. Corpus spongiosum penis (A), corpus cavernosum penis (B). EC – endothelial cells; ESL – superficial layer of urethral epithelium; EIL – intermedial layer of urethral epithelium; FLC – fibroblast-like cells of interstitial tissue. Staining: DAB + toluidine blue.

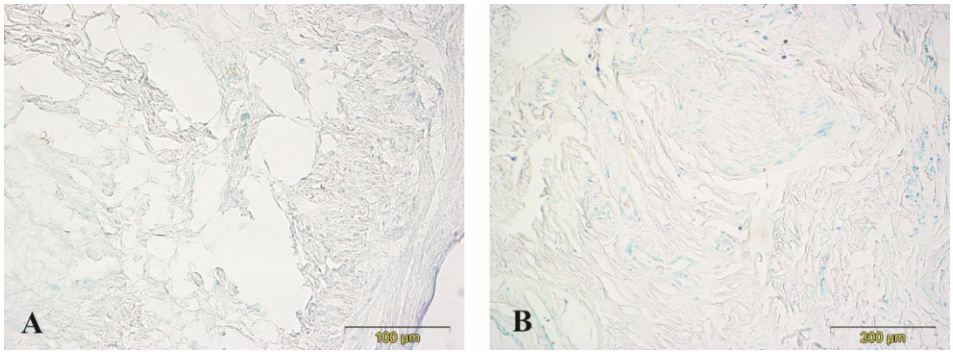

Figure 6. Negative controls for LHCG receptors (A) and cAMP and CREB (B) in the human penis. Staining: DAB + toluidine blue.

Table 3. Expression of the LHR, cAMP and CREB in the different types of cells in the human penis

	LHR	cAMP	CREB
Corpus spongiosum penis			
Superficial layer of urethral epithelium	+	+	+
Intermedial layer of urethral epithelium	+	+	+
Basal layer of urethral epithelium	+	+	N/A
Fibroblast-like cells of interstitial tissue	+	+	+
Endothelial cells of cavernous spaces	+	+	+
Corpus cavernosum penis			
Endothelial cells of cavernous spaces	+	+	+
Fibroblast-like cells of interstitial tissue	N/A	+	+

N/A not available

6. DISCUSSION

6.1 General discussion

The results of our studies show that the LH receptor exists in both mouse and human penile tissue (Kokk *et al*., 2011; Zirnask *et al*., 2018). We also succeeded in showing the presence of the two components, cAMP and CREB, related to the adenylate cyclase signal transduction pathway used by the LH receptor, in the penis (Zirnask *et al*., 2019). These findings support the view that LH has a previously unknown role in the penis, and its elevated levels could also affect the tissue of the corpus cavernosum and spongiosum penis, thereby having a role in the pathogenesis of erectile dysfunction.

It has been demonstrated that a significant proportion of aging men are in a subclinical hypogonadism with elevated serum LH concentrations (Härkonen *et al*., 2003; Foresta *et al*., 2015). As well the observations of Yu *et al*. (2017) confirmed the influence of increased age and LH on the aging male symptoms and on the 5-item version of the international index of erectile function scores. On the basis of the present results we may suggest that the chronically elevated LH in aging men might have an influence on the penile tissue through the penile LHCGR. This suggestion is supported by the previous results of the Turku Aging Male Study, as a statistically significant positive correlation was found between s-LH and the reported intensity of potency disturbances among aging men (Härkönen *et al*., 2003). Also according to the European Male Ageing Study, conducted on 3369 community-dwelling European men aged 40–79 years, men with incidentally high LH developed erectile dysfunction more often than men with persistently normal LH (Eendebak *et al*., 2018).

Although LH or LHCGR have not been reported to be associated with erectile dysfunction in genome-wide analyses this far (Kerns *et al*., 2010; Hotaling *et al*., 2012; Kerns *et al*., 2013), the FSH receptor sharing the α-subunit and having homology in its β-subunit with the β-subunit of LH receptor has been (Kerns *et al*., 2010). It may be of importance here that the cumulus cells have been reported to express LH receptor mRNA in response to FSH (Hattori *et al*., 2000; Nishida *et al*., 2000), suggesting that the effects of the elevated LH levels in aging men may lead to effects in the penis only if the FSH levels are elevated as well, and they often are in aging males (Lee *et al*., 2010). Based on the present and earlier data, it might be possible that the increased LH and FSH levels may cause erectile dysfunction together. However, further research is needed to see how LH receptor expression is regulated in the penis.

According to literature, the wild-type LHCG receptors and the wild-type FSH receptors can also form dimers (Segaloff, 2012), suggesting that the regulation of the penile tissue by the gonadotrophins is more complex and still needs more research.

The present findings also suggest that high female LH, hCG concentrations in the vaginal excretions might possibly affect the penile function if reaching

the LHCG receptors, as it is known that women have high serum LH levels after menopause (Edwards and Li, 2013) and high levels of hCG during pregnancy (Szczerba *et al*., 2016).

Our present data about the presence of cAMP and CREB in the human penis (Zirnask *et al*., 2019), two components of the adenylate cyclase signal transduction pathway used by the LH receptor, also support the possibility of potential actions of LH in the human penis, especially as the the expression of protein kinase A in the human penile tissue has been described before (Waldkirch *et al*., 2010).

As according to Cui *et al*., 2017, both the erectile function and the expression levels of cAMP were significantly lower in the aged than in the younger rats, it may be possible that elevated serum LH concentrations could act as a compensatory mechanism to increase the levels of cAMP in the penile endothelial cells of aging men.

Furthermore, it may be of interest that nitric oxide (NO), a substance known to affect the penile tissue, can affect FSH-induced LH receptor synthesis by granulosa cells (Nishida *et al*., 2000), and that LH has a significant stimulatory effect on NO synthase expression in vitro in a prostaglandin-dependent manner (Zamberlam *et al*., 2014). If the same mechanisms can be demonstrated in the penile LH receptor expressing cells at some point, we may understand much more about the regulation of the penile function and the cell biological background of erectile dysfunction.

It should also be taken into account that LH production is regulated by several factors, not only testosterone itself and that potency itself is regulated by several cell biological mechanisms, not only by LH action. Especially, it is interesting that although the proportion of men with serum LH in the uppermost quartile (> 6.0 IU/L) and serum testosterone above the lowest 10 % (> 9.8 nmol/L) was statistically significantly associated with age (11, 24 and 31 % among 41–50, 51–60 and 61–70-year-old men, respectively, Härkönen *et al*. 2003) and although LH and estradiol were positively correlated with the intensity of potency disturbances (Härkönen *et al*. 2003), there were fewer men with this hormonal condition (LH > 6.0 IU/L and T > 9.8 nmol/L) among those having > 23 androgen receptor (AR) CAG repeats compared with others (Härkönen *et al*. 2003). These same men with > 23 AR CAG repeats reported decreased potency less often than other men (Härkönen *et al*. 2003). Thus, it seems to be so that the potency is not regulated similarly in all men. There may be genetic subgroups among men in this respect. Regarding this, it is very interesting that the HAP4 AR haplotype has been reported to be associated with an increased risk for male infertility, although AR CAG repeat number was not different among infertile and control groups of men (Saare *et al*. 2008). Furthermore, it has been shown that in the females AR CAG repeat number is smaller among patients with premature ovarian failure (Laisk *et al*. 2010) and that AR CAG repeat number variations and X-chromosome inactivation pattern exert an effect on LH and FSH levels, supporting thus clearly the necessity of taking the genetic

variables into account in any analysis of the reproductive functions, including analysis of the potency disturbances.

Although it is clear by now that LHCG receptor together with cAMP and CREB, the components of the signal transduction pathway used by LH, are present in the penis, it is not clear yet what possible roles LH has in the penile tissue.

6.2 Strengths and limitations

To the knowledge of the author, the presence of the LH receptor in the penis has not been previously investigated. This study may provide a basis for a better understanding of penile tissue function in the future. Based on the results, it is clear that further research is needed in this area to investigate the role of the LH receptor in the penis.

One of the main limitations of the study, performed on human penis tissue, were the small number of patients the tissue samples were taken from. Only three penises were used for immunohistochemistry to detect the LH receptor and the components of its signal transduction pathway in the penis.

Another limitation may be the fact that penile tissue was taken from elderly cancer patients. This raises the question whether the treatment of cancer or the cancer itself may affect the outcome of the study. The use of human penile tissue in studies is limited due to difficult availability, not to mention the availability of healthy penis tissue. Furthermore, a strict legislation on using cadaver material for research purposes, the necessity of immediate removal of the tissues after death and the legal-ethical requirements, especially getting informed consent before death and sample-taking, and the limited possibility of getting material from sex assignment surgery, restrict studying the human penile tissue. For this reason, only one type of method was used to demonstrate LH in the human penile tissue, but this was considered sufficient, because the used antibody itself had already been tested using several methods in the mouse. On the other hand, we got the same results for LH receptor in the young healthy mouse penis tissue by using three different study methods and the same primary antibody for immunohistochemistry. The LH receptor was present in both the mouse and the human penises mostly in the same cell types.

6.3 Future perspectives

Further functional studies are indicated to determine the possible roles of LH in the penis and to evaluate whether it may be involved in the pathogenesis of erectile dysfunction. These functional studies could include e.g. electron microscopy on transfer of gold particle-labelled hCG to the penile interstitial tissue, as in the testis, hCG/LH seems to be actively transported through the endothelium of the testicular microvessels, i.e. in a receptor-mediated way (Ghinea *et al*.

1994; Ghinea and Milgrom 2001), and treatment with a low dose of ovine LH or inducing an endogenous LH peak by mating results in leucocyte accumulation and vascular leakage of carbon in the testis (Bergh *et al*. 1990), suggesting that more research on the vascular regulation of the erectile mechanisms by LH is necessary to understand the mechanisms of potency disturbances in aging men. If LH is transported actively – in a receptor-mediated way – to the penile interstitial tissue and high LH levels are involved in the generation of potency disturbances among the aging men, blocking such active transendothelial transport of LH to the penile tissue could lead to medical applications.

7. CONCLUSIONS

1. The present study clearly shows the presence of the LH receptor in the mouse penis.
2. LH receptor is present in the human penis.
3. The components of LH signal transduction pathway, cAMP and CREB are expressed in the human penis.
4. Based on the fact, that LH receptor and the components of its signal transduction pathway are present in the penis, it is possible, that LH regulates the function of corpus spongiosum and cavernosum penis and may thereby participate in the pathogenesis of erectile dysfunction.

REFERENCES

1. Aboseif SR, Breza J, Lue TF, Tanagho EA: Penile venous drainage in erectile dysfunction. Anatomical, radiological and functional considerations. Br J Urol 1989; 64(2):183–190.
2. American Psychiatric Association Task Force. Diagnostic and statistical manual of mental disorders. 5th ed. (DSM-5) Arlington, VA, Washington, D.C: American Psychiatric Association; 2013.
3. Andersson KE, Martin CM: Mechanisms of Penile Erection and Basis for Pharmacological Treatment of Erectile Dysfunction. Pharmacological Reviews 2011; 63(4):811–859.
4. Andersson KE, Wagner G: Physiology of penile erection. Physiol Rev 1995; 75(1):191–236.
5. Ascoli M, Fanelli F, Segaloff DL: The lutropin/choriogonadotropin receptor, a 2002 perspective. Endocr Rev 2002;23:141–174.
6. Atger M, Misrahi M, Sar S, Le Flem L, Dessen P, Milgrom E: Structure of the human luteinizing hormone-choriogonadotropin receptor gene: unusual promoter and 5' non-coding regions. Mol Cell Endocrinol 1995;111:113–123.
7. Ayta IA, McKinlay JB, Krane RJ: The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. BJU Int 1999;84:50–56.
8. Bergh A, Damber JE, Widmark A: A physiological increase in LH may influence vascular permeability in the rat testis. J Reprod Fertil 1990;89(1):23–31.
9. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS *et al*.: Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2010;95(6): 2536–2559.
10. Bousfield GR, Dias JA: Synthesis and secretion of gonadotropins including structure-function correlates. Rev Endocr Metab Disord 2011;12(4):289–302.
11. Brisson D, Houde G, St-Pierre J, Vohl MC, Mathieu J, Gaudet D: The pleiotropic expression of the myotonic dystrophy protein kinase gene illustrates the complex relationships between genetic, biological and clinical covariates of male aging. Aging Male 2002;5(4):223–32.
12. Bukovsky A, Indrapichate K, Fujiwara H, Cekanova M, Ayala ME, Dominguez R *et al*.: Multiple luteinizing hormone receptor (LHR) protein variants, interspecies reactivity of anti-LHR mAb clone 3B5, subcellular localization of LHR in human placenta, pelvic floor and brain, and possible role for LHR in the development of abnormal pregnancy, pelvic floor disorders and Alzheimer's disease. Reprod Biol Endocrinol 2003;1:46.
13. Burnett AL: Nitric oxide in the penis: physiology and pathology. J Urol 1997; 157(1):320–324.
14. Burnett AL: Role of nitric oxide in the physiology of erection. Biol Reprod 1995; 52(3):485–489.
15. Butler SA, Iles RK: Ectopic human chorionic gonadotrophin β secretion by epithelial tumors and human chorionic gonadotrophin β-induced apoptosis in Karposi's sarcoma Is there a connection? Clin Cancer Res 2003;9:4666–4673.
16. Caltabiano G, Campillo M, de Leener A, Smits G, Vassart G, Costagliola S *et al*.: The specificity of binding of glycoprotein hormones to their receptors. Cell Mol Life Sci 2008;65:2484–2492.

17. Celik O, Ipekci T, Akarken I, Ekin G, Koksal, T: To evaluate the etiology of erectile dysfunction: What should we know currently? Arch Ital Urol Androl 2014; 86:197–201.
18. Cole LA: Biological functions of hCG and hCG-related molecules. Reprod Biol Endocrinol 2010;8:102.
19. Cole LA:New discoveries on the biology and detection of human chorionic gonadotropin. Reprod Biol Endocrinol 2009;7:8.
20. Cole LA, Khanlian SA, Riley JM, Butler SA: Hyperglycosylated hCG in gestational implantation and in choriocarcinoma and testicular germ cell malignancy tumorigenesis. J Reprod Med 2006;51:919–925.
21. Cui K, Luan Y, Tang Z, Rao K, Wang T, Chen Z *et al*.: Involvement of DDAH/ ADMA/NOS/cGMP and COX-2/PTGIS/cAMP Pathways in Human Tissue Kallikrein 1 Protecting Erectile Function in Aged Rats. PLoS One 2017;12(1), e0170427.
22. Dandona P, Rosenberg MT: A practical guide to male hypogonadism in the primary care setting. Int J Clin Pract 2010;64:682–96.
23. Delhez M, Hansenne M, Legros JJ: Andropause and psychopathology: minor symptoms rather than pathological ones. Psychoneuroendocrinology 2003 Oct; 28(7):863–74.
24. Dias JA, Cohen BD, Lindau-Shepard B, Nechamen CA, Peterson AJ, Schmidt A: Molecular, structural, and cellular biology of follitropin and follitropin receptor. Vitam Horm 2002;64:249–322.
25. Dufau ML: The luteinizing hormone receptor. Annual Review of Physiology 1998; 60:461–496.
26. Dufau ML, Winters CA, Hattori M, Aquilano D, Barañao JL, Nozu K *et al*.: Hormonal regulation of androgen production by the Leydig cell. J Steroid Biochem 1984;20:161–73.
27. Eendebak RJAH, Ahern T, Swiecicka A, Pye SR, O'Neill TW, Bartfai G *et al.:* Elevated luteinizing hormone despite normal testosterone levels in older men-natural history, risk factors and clinical features. Clin Endocrinol 2018;88(3):479–490.
28. Edwards BJ, Li J: Endocrinology of menopause. Periodontol 2000 2013;61(1): 177–194.
29. Elben A, Bao S, Lei ZM, Nakajima ST, Rao CV: The presence of functional luteinizing hormone/chorionic gonadotropin receptors in human sperm. J Clin Endocrinol Metab 2001;86:2643–2648.
30. El-Sakka AI, Lue TF: Physiology of penile erection. Scientific World Journal 2004;4 Suppl 1:128–134.
31. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB: Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol 1994;151(1):54–61.
32. Foresta C, Calogero AE, Lombardo F, Lenzi A, Ferlin A: Late-onset hypogonadism: beyond testosterone. Asian J Androl 2015;17:236–238.
33. Gallego MJ, Porayette P, Kaltcheva MM, Bowen RL, Vadakkadath Meethal S, Atwood CS: The pregnancy hormones human chorionic gonadotropin and progesterone induce human embryonic stem cell proliferation and differentiation into neuroectodermal rosettes. Stem Cell Res Ther 2010;1(4):28.
34. Gartner LP: Textbook of Histology. Elsevier 2016.

35. Ghinea N, Milgrom E: A new function for the LH/CG receptor: transcytosis of hormone across the endothelial barrier in target organs. Semin Reprod Med 2001; 19(1):97–101.
36. Ghinea N, Mai TV, Groyer-Picard MT, Milgrom E: How protein hormones reach their target cells. Receptor-mediated transcytosis of hCG through endothelial cells. J Cell Biol 1994;125(1):87–97.
37. Grzesik P, Teichmann A, Furkert J, Rutz C, Wiesner B, Kleinau G, Schülein R, Gromoll J & Krause G. Differences between lutropin-mediated and choriogonadotropin-mediated receptor activation. FEBS J 2014;281:1479–1492.
38. Gurung P, Jialal I: Physiology, Male Reproductive System. SourceStatPearls [Internet]. Treasure Island (FL): StatPearls Publishing 2019.
39. Hattori MA, Nishida N, Takesue K, Kato Y, Fujihara N: FSH suppression of nitric oxide synthesis in porcine oocytes. J Mol Endocrinol 2000;24(1):65–73.
40. Hearn MT, Gomme PT: Molecular architecture and biorecognition processes of the cystine knot protein superfamily: part I. The glycoprotein hormones. J Mol Recognit 2000;13(5):223–78.
41. Herr F, Baal N, Reisinger K, Lorenz A, McKinnon T, Preissner KT *et al*.; HCG in the regulation of placental angiogenesis. Results of an in vitro study. Placenta 2007;28:5–93.
42. Hotaling JM, Waggott DR, Goldberg J, Jarvik G, Paterson AD, Cleary PA et al.: Pilot genome-wide association search identifies potential loci for risk of erectile dysfunction in type 1 diabetes using the DCCT/EDIC study cohort. J Urol 2012; 188:514–520.
43. Hsieh CH, Liu SP, Hsu GL, Chen HS, Molodysky E, Chen YH, Yu HJ: Advances in understanding of mammalian penile evolution, human penile anatomy and human erection physiology: clinical implications for physicians and surgeons. Med Sci Monit 2012;18(7):RA118–125.
44. Hsu GL, Brock G, Martinez-Pineiro L, Nunes L, von Heyden B, Lue TF: The three-dimensional structure of the human tunica albuginea: anatomical and ultrastructural levels. Int J Impotence Res 1992;4:117–129.
45. Huhtaniemi, I: Late-onset hypogonadism: current concepts and controversies of pathogenesis, diagnosis and treatment. Asian J Androl 2014;16:192–202.
46. Huhtaniemi I, Alevizaki M: Gonadotrophin resistance. Best Pract Res Clin Endocrinol Metab, 2006;20(4):561–76.
47. Härkönen K, Huhtaniemi I, Mäkinen J, Hübler D, Irjala K, Koskenvuo M, Oettel M, Raitakari O, Saad F, Pöllänen P: The polymorphic androgen receptor gene CAG repeat, pituitary-testicular function and andropausal symptoms in ageing men. Int J Androl 2003;26:187–194.
48. Kahle W, Leonhardt H, Platzer W: Colour Atlas and Textbook of Human Anatomy 4th Revised edition. Thieme Publishing Group 1993.
49. Kerns SL, Ostrer H, Stock R, Li W, Moore J, Pearlman A, et al.: Genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of erectile dysfunction in African-American men after radiotherapy for prostate cancer. Int J Radiat Oncol Biol Phys 2010;78:1292–1300.
50. Kerns SL, Stock R, Stone N, Buckstein M, Shao Y, Campbell C et al.: A 2-stage genome-wide association study to identify single nucleotide polymorphisms associated with development of erectile dysfunction following radiation therapy for prostate cancer. Int J Radiat Oncol Biol Phys 2013;85:21–28.

51. Kinjo K, Sandoval S, Sakamoto KM, Shankar DB: The role of CREB as a proto-oncogene in hematopoiesis. Cell Cycle 2005;4(9):1134–1135.
52. Kirby RS: Impotence: diagnosis and management of male erectile dysfunction. BMJ 1994;308(6934):957–961.
53. Kossack N, Simoni M, Richter-Unruh A, Themmen AP, Gromoll J: Mutations in a novel, cryptic exon of the luteinizing hormone/chorionic gonadotropin receptor gene cause male pseudohermaphroditism. PLoS Med 2008;5:88.
54. Laisk T, Haller-Kikkatalo K, Laanpere M, Jakovlev U, Peters M, Karro H *et al*.: Androgen receptor epigenetic variations influence early follicular phase gonadotropin levels. Acta Obstet Gynecol Scand 2010;89(12):1557–63.
55. Latronico AC, Arnhold IJ: Inactivating mutations of the human luteinizing hormone receptor in both sexes. Semin Reprod Med 2012; 30:382–386.
56. Lee DM, Ulubaev A, Tajar A, Pye SR, Pendleton N, Purandare N *et al*.: Endogenous hormones, androgen receptor CAG repeat length and fluid cognition in middle-aged and older men: results from the European Male Ageing Study. Eur J Endocrinol 2010;162(6):1155–1164.
57. Lei ZM, Reshef E, Rao CV: The expression of human chorionic gonadotropin/luteinizing hormone receptors in human endometrial and myometrial blood vessels. J Clin Endocrinol Metab 1992;75:651–659.
58. Li CH, Pedersen KO: Physicochemical characterization of pituitary follicle-stimulating hormone. J Gen Physiol 1952;35(4):629–637.
59. Licht P, von Wolff M, Berkholz A, Wild L: Evidence for cycle-dependent expression of full-length human chorionic gonadotropin/luteinizing hormone receptor mRNA in human endometrium and decidua. Fertil Steril 2003;79 (Suppl 1):718–723.
60. Ludwig W, Phillips M: Organic causes of erectile dysfunction in men under 40. Urol Int 2014;92(1):1–6.
61. Manna PR, Dyson MT, Eubank DW, Clark BJ, Lalli E, Sassone-Corsi P et al.: Regulation of steroidogenesis and the steroidogenic acute regulatory protein by a member of the cAMP response-element binding protein family. Mol Endocrinol 2002;16(1):184–99.
62. Mazina O, Allikalt A, Tapanainen JS, Salumets A, Rinken A: Determination of biological activity of gonadotropins hCG and FSH by Förster resonance energy transfer based biosensors. Sci Rep 2017;7:42219.
63. Mayr B, Montminy M: Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat Rev Mol Cell Biol 2001;2(8):599–609.
64. McFarland KC, Sprengel R, Phillips HS, Köhler M, Rosemblit N, Nikolics K, Segaloff DL, Seeburg PH. Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. Science 1989;245(4917):494–499.
65. Morgentaler A: Male impotence. Lancet 1999;354(9191):1713–1718.
66. Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C: Prevalence of hypogonadism in males aged at least 45 years: the HIM study. Int J Clin Pract 2006;60(7):762–9.
67. Nishida N, Hattori M-A, Takesue K, Kato Y, Fujihara N: Critical role of nitric oxide in expression of porcine LH receptor at transcription and post-transcription levels. Exp Clin Endocrinol Diabetes 2000;108(6):424–9.
68. Phillips RJ, Tyson-Capper Née Pollard AJ, Bailey J, Robson SC, Europe-Finner GN: Regulation of expression of the chorionic gonadotropin/luteinizing hormone

receptor gene in the human myometrium: involvement of specificity protein-1 (Sp1), Sp3, Sp4, Sp-like proteins, and histone deacetylases. J Clin Endocrinol Metab 2005;90:3479–3490.
69. Pierce JG, Parsons TF: Glycoprotein hormones: structure and function. Annu Rev Biochem 1981;50:465–495.
70. Prast J, Saleh L, Husslein H, Sonderegger S, Helmer H, Knöfler M: Human chorionic gonadotropin stimulates trophoblast invasion through extracellularly regulated kinase and AKT signaling. Endocrinology 2008;149:979–987.
71. Prins J, Blanker MH, Bohnen AM, Thomas S, Bosch JL: Prevalence of erectile dysfunction: a systematic review of population-based studies. Int J Impot Res 2002;14(6):422–432.
72. Priyanka S, Medhamurthy R: Characterization of cAMP/PKA/CREB signaling cascade in the bonnet monkey corpus luteum: expressions of inhibin-alpha and StAR during different functional status. Mol Hum Reprod 2007;13(6):381–390.
73. Puett D, Angelova K, da Costa MR, Warrenfeltz SW, Fanell F: The luteinizing hormone receptor: insights into structure-function relationships and hormone-receptor-mediated changes in gene expression in ovarian cancer cells. Mol Cell Endocrinol 2010;329:47–55.
74. Puett D, Li Y, DeMars G, Angelova K, Fanelli F: A functional transmembrane complex: the luteinizing hormone receptor with bound ligand and G protein. Mol Cell Endocrinol 2007;260–262:126–136.
75. Rahman NA1, Rao CV: Recent progress in luteinizing hormone/human chorionic gonadotrophin hormone research. Mol Hum Reprod 2009;15(11):703–11.
76. Rao CV: Multiple novel roles of luteinizing hormone. Fertil Steril 2001;76(6): 1097–1100.
77. Rao CV, Li X, Toth P, Le ZM, Cook VD: Novel expression of functional human chorionic gonadotropin/luteinizing hormone receptor gene in human umbilical cords. J Clin Endocrinol Metab 1993;77:1706–1714.
78. Rey RA, Grinspon RP, Gottlieb S, Pasqualini T, Knoblovits P, Aszpis S *et al*.: Male hypogonadism: an extended classification based on a developmental, endocrine physiology-based approach. Andrology 2013;1(1):3–16.
79. de Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer et al.: Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature 1998;396(6710):474–477.
80. Ross M H, Pawlina W: Histology: A Text and Atlas: With Correlated Cell and Molecular Biology. Lippincott Williams and Wilkins 2011.
81. Rousseau-Merck MF, Atger M, Loosfel H, Milgrom, E, Berger R: The chromosomal localization of the human follicle-stimulating hormone receptor gene (FSHR) on 2p21-p16 is similar to that of the luteinizing hormone receptor gene. Genomics 1993;15:222–224.
82. Rousseau-Merck MF, Misrahi M, Atger M, Loosfelt H, Milgrom E, Berger R: Localization of the human luteinizing hormone/choriogonadotropin receptor gene (LHCGR) to chromosome 2p21. Cytogenet Cell Genet 1990;54:77–79.
83. Saare M, Belousova A, Punab M, Peters M, Haller K, Ausmees K *et al*.: Androgen receptor gene haplotype is associated with male infertility. Int J Androl 2008; 31(4):395–402.
84. Sasaki Y, Ladner DG, Cole LA: Hyperglycosylated hCG the source of pregnancy failures. Fertil Steril 2008;89:1871–1786.

85. Segaloff DL: Regulatory processes governing the cell surface expression of LH and FSH receptors. Subcell Biochem 2012;63:113–129.
86. Shamloul R, Ghanem H: Erectile dysfunction. Lancet 2013;381(9861):153–65.
87. Solomon H, DeBusk RF, Jackson G: Erectile dysfunction: the need to be evaluated, the right to be treated. Am Heart J 2005;150(4):620–626.
88. Szczerba A, Białas P, Pięta PP, Jankowska A: hCG – related molecules and their measurement.Ginekol Pol 2016;87(1):65–70.
89. Stenman UH, Tiitinen A, Alfthan H, Valmu L: The classification, functions and clinical use of different isoforms of HCG. Hum Reprod Update 2006;12(6):769–84.
90. Stocco DM, Clark BJ: Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 1996;17(3):221–244.
91. Stojkov NJ, Baburski AZ, Bjelic MM, Sokanovic SJ, Mihajlovic AI, Drljaca DM *et al.*: In vivo blockade of α1-adrenergic receptors mitigates stress-disturbed cAMP and cGMP signaling in Leydig cells. Mol Hum Reprod 2014;20:77–88.
92. Zamberlam G, Sahmi F, Price CA: Nitric oxide synthase activity is critical for the preovulatory epidermal growth factor-like cascade induced by luteinizing hormone in bovine granulosa cells. Free Radic Biol Med 2014;74:237–44.
93. Zeleznik AJ, Somers JP: Regulation of the Primate Corpus Luteum: Cellular and Molecular Perspectives. Trends Endocrinol Metab 1999;10:189–193.
94. Tortora G J: Principles of Human Anatomy 7th Revised edition. John Wiley & Sons Inc 1995.
95. Toth P, Li X, Rao CV, Lincoln SR, Sanfilippo JS, Spinnato JA 2nd et al.: Expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human uterine arteries. J Clin Endocrinol Metab 1994;79:307–315.
96. Tourkova IL, Witt MR, Li L, Larrouture Q, Liu L, Luo J et al.: Follicle stimulating hormone receptor in mesenchymal stem cells integrates effects of glycoprotein reproductive hormones. Ann N Y Acad Sci 2015;1335:100–109.
97. Vasan SS.: Synopsis of results from European male ageing study. J Midlife Health 2010;1: 93–94.
98. Veräjänkorva E, Laato M, Pollanen P: Analysis of 508 infertile male patients in south-western Finland in 1998–2000: hormonal status and factors predisposing to immunological infertility. Eur J Obstet Gynecol Reprod Biol 2003;111(2):173–8.
99. Vermeulen A: Andropause. Maturitas 2000;34(1):5–15.
100. Wagner G, Saenz de Tejada I: Update on male erectile dysfunction. BMJ 1998; 316(7132):678–682.
101. Waldkirch ES, Ückert S, Sigl K, Satzger I, Geismar U, Langnäse K et al.: Expression of cyclic AMP-dependent protein kinase isoforms in human cavernous arteries: functional significance and relation to phosphodiesterase type 4. J Sex Med 2010;7:2104–2111.
102. Yan K, Gao LN, Cui YL, Zhang Y, Zhou X. The cyclic AMP signaling pathway: Exploring targets for successful drug discovery (Review). Mol Med Rep 2016; 13:3715–3723.
103. Yu XH, Zhao J, Zhang SC, Li JH, Zheng JB, Li Z et al.: The impact of age, BMI and sex hormone on aging males' symptoms and the international index of erectile function scores. Aging Male 2017;20(4):235–240.

SUMMARY IN ESTONIAN

Luteiniseeriva hormooni (LH) retseptori esinemine peenises ja selle võimalik roll erektsioonihäirete patogeneesis

Sissejuhatus

Seoses elanikkonna vananemisega arenenud riikides on erektsioonihäiretega seotud uuringud kaasaja teaduses aktuaalseks teemaks.

European Male Ageing Study kohaselt esines erektsioonihäireid 30%-l uuritavatest. Esinemissagedus oli kõrgem vanemates vanuserühmades, eriti 70-aastaste ja vanemate meeste seas (64%) (Vasan, 2010). Kuni 1990. aastate alguseni arvati, et erektsioonihäired on seotud loomuliku vananemisprotsessiga, kuid ulatuslikud uuringud selles valdkonnas on näidanud, et see pole seotud ainult vanusega (Solomon jt, 2005).

Märkimisväärselt suurel osal vanemaealistest meestest esineb erinevate suguhormoonide taseme muutusi. Neist levinum on subkliiniline hüpogonadism (seerumi LH > 6.0 U/l, seerumi testosteroon > 9.8 nmol/l), mida esineb 23%-l üle 40-aastastest meestest ning mille esinemissagedus tõuseb proportsionaalselt vanusega (Härkönen jt, 2003; Veräjänkorva jt, 2003). P. Härköneni (2003) uurimisrühm Turku Aging Male keskusest on leidnud statistiliselt olulise positiivse korrelatsiooni LH taseme tõusu ja potentsi languse vahel. Võimalik, et vanemaealiste meeste kõrgenenud LH tase mõjutab peenise kude ning võib seeläbi olla seotud erektsioonihäirete tekkemehhanismidega. Selle eelduseks on LH retseptori esinemine peenises.

Ajuripatsi eessagara gonadotropiin LH on üks tähtsamaid sugunäärmete talitluse reguleerijaid, mis avaldab toimet objektrakkudele nende rakumembraanil olevate retseptorite kaudu. LH retseptorid kuuluvad G-proteiinide kaudu toimivate, seitse korda rakumembraani läbivate retseptorite geeniperekonda (Ascoli jt, 2002). LH retseptor on avastatud mitmetes ekstragonadaalsetes kudedes, kuid selle olemasolu peenises pole varem tõestatud.

Uurimistöö eesmärgid

Uurimistöö põhieesmärgiks on selgitada LH retseptori ja LH signaali ülekandmisega seotud molekulide olemasolu peenises.

Sellest tulenevalt on eesmärgid järgmised:

1. Määrata LH retseptori esinemine hiire peenise koes.
2. Selgitada, kas LH retseptor on olemas inimese peenise koes.
3. Määrata kahe LH signaali ülekandmisega seotud molekuli, cAMP-i ja CREB-i, olemasolu inimese peenise koes.
4. Analüüsida saadud tulemuste põhjal, kas vanemaealiste meeste kõrgenenud seerumi LH tase võib mõjutada peenise spongioos- ja kavernooskeha funktsiooni ja olla seotud erektsioonihäirete patogeneesiga.

Materjal ja meetodid

LH retseptori määramiseks hiire peenises kasutati järgnevaid uurimismeetodeid: immunohistokeemia, Western blotting ja kvantitatiivne RT-PCR. Peenise ja testise koe doonoritena kasutati Balb/c hiiri. Immunohistokeemias kasutati 14 hiire kude (6–7 nädala vanused), Western blottingus 20 hiire kude (10–12 nädala vanused) ning kvantitatiivses RT-PCR-s 8 hiire kude (15 nädala vanused).

LH retseptori määramiseks inimese peenises kasutati kolme Tampere Ülikooli Haigla patsiendi peenise kude. Kahel patsiendil (66- ja 64-aastane) oli diagnoositud pärasoole vähk sekundaarsete peenise metastaasidega ning neile teostati täielik penektoomia. 83-aastasel patsiendil oli diagnoositud peenise skvamoosrakuline kartsinoom ning teostati osaline penektoomia. Uurimismeetodina kasutati immunohistokeemiat.

LH retseptori ja LH signaali ülekandjate (cAMP ja CREB) määramiseks peenise spongioos- ja kavernooskoes kasutati samade patsientide peenise kude, kellel sai teostatud LH retseptori uuring. Uurimismeetodina kasutati immunohistokeemiat.

Uurimuse tulemused

Positiivne immunoreaktsioon LH retseptorile esineb hiire peenise ureetra epiteelis ning peenise spongioos- ja kavernooskeha kavernoossete tühimike endoteelirakkudes. Positiivsed tulemused leiti kõikides uuritud peenistes. Negatiivsetes kontrollides positiivset immunoreaktsiooni ei esine. Testise kude kasutati positiivseks kontrolliks ning saadi positiivne immunoreaktsioon LH retseptorile Leydigi rakkudes ja seemnetorukeste kesksetes osades valendiku lähedal.

Positiivsed tulemused saadi ka Western blottingu ning kvantitatiivse RT-PCR-i uuringutega.

Inimese peenise koes saadi positiivne immunoreaktsioon kõikides uuritud peenistes. Positiivne immunoreaktsioon esineb peenise spongioos- ja kavernooskeha kavernoossete tühimike endoteelirakkudes ja spongiooskeha interstitsiaalse koe fibroblastoidsetes rakkudes. Positiivne immunoreaktsioon on nähtav ka *glans penis*'es ureetra epiteeli pindmise, vahepealse ja basaalse kihi rakkudes, kavernoossete tühimike endoteelirakkudes ning interstitsiaalse koe fibroblastoidsetes rakkudes. Positiivne immunoreaktsioon esineb ka kapillaariseinte endoteelirakkudes. Negatiivsetes kontrollides positiivset immunoreaktsiooni ei leidu.

Positiivne immunoreaktsioon cAMP-ile esineb enamikes ureetra epiteeli pindmise, vahepealse ja basaalse kihi rakkudes ning interstitsiaalse koe fibroblastoidsetes rakkudes. Positiivne immunoreaktsioon on nähtav ka peenise spongioos- ja kavernooskeha kavernoossete tühimike endoteelirakkudes ja kavernooskeha interstitsiaalse koe fibroblastoidsetes rakkudes.

Positiivne immunoreaktsioon CREB-ile esineb ureetra epiteeli pindmises ja vahepealses kihis ning mõningal määral peenise spongiooskeha kavernoossete tühimike endoteelirakkudes ja interstitsiaalse koe fibroblastoidsetes rakkudes.

Positiivne reaktsioon on nähtav ka peenise kavernooskeha kavernoossete tühimike endoteelirakkudes ning interstitsiaalse koe fibroblastoidsetes rakkudes.

Negatiivsetes kontrollides positiivset immunoreaktsiooni ei esine.

Järeldused

1. Käesolev uuring näitab selgelt LH retseptori olemasolu hiire peenise koes.
2. LH retseptor esineb inimese peenise koes.
3. Kaks LH signaali ülekandmisega seotud molekuli, cAMP ja CREB, esinevad inimese peenise koes.
4. Lähtudes asjaolust, et peenises on olemas nii LH retseptor kui ka selle signaaliülekande molekulid, on võimalik, et LH reguleerib peenise spongioos- ja kavernooskeha funktsiooni ja võib seeläbi osaleda erektsioonihäirete patogeneesis.

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PUBLICATIONS

CURRICULUM VITAE

Name: Helen Zirnask
Place of Birth: Kiviõli, Estonia
Date of Birth: July 2th, 1981
Citizenship: Estonian
Address: Department of Anatomy, University of Tartu, Ravila 19, 50411 Tartu Estonia
Tel: +372 737 4253
E-mail: helen.zirnask@ut.ee

Education:

2009–	PhD student, University of Tartu, Faculty of Medicine
1999–2008	University of Tartu, Faculty of Medicine
1996–1999	Hugo Treffner Gymnasium

Professional employment:

2008–	University of Tartu, Department of Anatomy, Assistant

Professional organisations:
Anatomical Society of Finland

Scientific work:
5 scientific articles published in international journals; 4 poster presentations at international or local conferences.

Publications:

1. Zirnask, Helen; Pöllanen, Pasi; Suutre, Siim; Kuuslahti, Marianne; Kotsar, Andres; Pakarainen, Tomi; Kokk, Kersti (2019). Expression of LHCG receptors in the human penis. Aging Male 2018;15:1–6.
2. Zirnask, Helen; Pöllanen, Pasi; Suutre, Siim; Kuuslahti, Marianne; Kotsar, Andres; Pakarainen, Tomi; Kokk, Kersti (2019). Expression of cAMP and CREB in the human penis. Journal of Men`s Health 2019;15:e12–e17.
3. Kokk, Kersti; Kuuslahti, Marianne; Keisala, Tiina; Purmonen, Sami; Kaipia, Antti; Tammela, Teuvo; Orro, Helen; Helle Evi, Simovart; Pöllanen, Pasi (2011). Expression of LH Receptors in the Mouse Penis. Journal of Andrology 2011;32:49–54.
4. Orro, Helen; Kokk, Kersti (2012). Erectile dysfunction etiology and hormonal changes. Papers on Anthropology, XXI, 194–200.
5. Kokk, K.; Kuuslahti, M.; Keisala, T.; Purmonen, S.; Kaipia, A.; Tammela, T.; Orro, H.; Simovart, H.E.; Pöllänen, P. (2010). Immunohistochemical detection of the luteinizing hormone receptors in penile tissue. Papers on Anthropology, XIX, 203–210.

ELULOOKIRJELDUS

Nimi: Helen Zirnask
Sünnikoht: Kiviõli, Eesti
Sünniaeg: 02.07.1981 a
Kodakondsus: Eesti
Aadress: anatoomia osakond, Tartu Ülikool, Ravila 19, 50411 Tartu, Eesti
Telefon: +372 737 4253
e-mail: helen.zirnask@ut.ee

Haridus:

2009–	Tartu Ülikool, arstiteaduskond, doktoriõpe
1999–2008	Tartu Ülikool, arstiteaduskond
1996–1999	Hugo Treffneri Gümnaasium

Teenistuskäik:

2008–	Tartu Ülikool, anatoomia instituut, assistent

Osalemine seltsides ja ühendustes:
Soome Anatoomide Selts

Teadustöö:
Ilmunud 5 teadusartiklit rahvusvahelise levikuga, 4 posterettekannet rahvusvahelistel ja kohalikel konverentsidel.

Publikatsioonid:

1. Zirnask, Helen; Pöllanen, Pasi; Suutre, Siim; Kuuslahti, Marianne; Kotsar, Andres; Pakarainen, Tomi; Kokk, Kersti (2019). Expression of LHCG receptors in the human penis. Aging Male 2018;15:1–6.
2. Zirnask, Helen; Pöllanen, Pasi; Suutre, Siim; Kuuslahti, Marianne; Kotsar, Andres; Pakarainen, Tomi; Kokk, Kersti (2019). Expression of cAMP and CREB in the human penis. Journal of Men's Health 2019;15:e12–e17.
3. Kokk, Kersti; Kuuslahti, Marianne; Keisala, Tiina; Purmonen, Sami; Kaipia, Antti; Tammela, Teuvo; Orro, Helen; Helle Evi, Simovart; Pöllanen, Pasi (2011). Expression of LH Receptors in the Mouse Penis. Journal of Andrology 2011;32:49–54.
4. Orro, Helen; Kokk, Kersti (2012). Erectile dysfunction etiology and hormonal changes. Papers on Anthropology, XXI, 194–200.
5. Kokk, K.; Kuuslahti, M.; Keisala, T.; Purmonen, S.; Kaipia, A.; Tammela, T.; Orro, H.; Simovart, H.E.; Pöllänen, P. (2010). Immunohistochemical detection of the luteinizing hormone receptors in penile tissue. Papers on Anthropology, XIX, 203–210.

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