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EXPRESSION OF cAMP AND CREB IN THE HUMAN PENIS

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ABSTRACT

The aim of this study is to investigate the expression of adenosine 3',5'-cyclic monophosphate (cAMP) and cAMP-response element-binding protein (CREB) in the human penis as it is known that luteinizing hormone (LH) regulates cellular function mostly through the cAMP signaling pathway and LH receptors are expressed by the penile endothelium. Penile tissue was obtained from three patients during partial or total penectomy due to a rectal cancer with secondary penile metastasis or squamous cell carcinoma of the penis. Immunohistochemistry was used for the detection of cAMP and CREB. Positive immunoreaction for cAMP was present in most cells of superficial, intermedial, and basal layer of urethral epithelium and in fibroblast-like cells (FLC) of interstitial tissue and endothelial cells (EC) of cavernous spaces in corpus spongiosum penis. Positive staining for cAMP was also visible in EC of cavernous spaces and in FLC of interstitial tissue in corpus cavernosum penis. Positive immunoreaction for CREB was present in the superficial and intermedial layer of urethral epithelium, and some positive immunoreaction was also noticed in EC of cavernous spaces and in FLC of interstitial tissue in corpus spongiosum penis. Positive staining was also visible in the EC of cavernous spaces and in fibroplast-like cells of the interstitial tissue in the corpus cavernosum penis. Our results show the presence of cAMP and CREB in the human penis. While LH exerts

its actions through cAMP signaling system and our previous studies have shown the expression of luteinizing hormone/choriogonadotropin (LHCG) receptor in the mouse and human penis, this finding may support the hypothesis that LH could affect the spongious and cavernous tissue of the human penis and thereby influence the development of erectile dysfunction among aging men.

Key Words: *cAMP; corpus cavernosum penis; corpus spongiosum penis; CREB; erectile disturbances; luteinizing hormone*

INTRODUCTION

Human luteinizing hormone (hLH) and human chorionic gonadotropin (hCG) are heterodimeric glycoproteins, consisting of a common α-subunit noncovalently associated with a hormone-specific β-subunit. Both hormones act via human luteinizing hormone receptor (hLHR).[1]

Extracellular messengers, which cannot pass the cell membrane, translate the signals from the plasma membrane to intracellular second messengers via ligand-binding-induded conformation changes in the intracellular parts of the cell surface receptors. The cyclic adenosinemonophosphate (cAMP) is the most important cyclic phosphate related to signal transduction. Synthesized from the adenosine triphosphate by the adenylate cyclase, cAMP mostly mediates its effects by cAMP-dependent protein kinase A (PKA).[2]

By binding to its receptor, LH leads to an increase in the intracellular cAMP level.[3] On Leydig cells, LH binds to its G-protein-coupled receptor and activates adenylate cyclase, which triggers an increase in cAMP levels from the intracellular adenosine triphosphate reserve and expression of the steroidogenic acute regulatory (STAR) protein, which is important for the initiation of steroidogenesis. Steroidogenesis decreases passively by the degradation of cAMP into AMP by phosphodiesterase 4, 8A, and 8B.[4]

In the ovary, both follicle stimulating hormone (FSH) and LH stimulate adenylate cyclase activity, cAMP production, and thereby activation of PKA. Apart from its cytoplasmic effects, PKA translocates to the nucleus and regulates gene expression by phosphorylating transcription factors, especially cAMP-response element-binding protein (CREB). CREB phosphorylation by PKA leads to its interaction with other nuclear regulatory proteins to initiate transcription of cAMP-responsive genes.[5]

CREB is known as a transcription factor that participates in growth factor-dependent cell survival, glucose homeostasis, proliferation, and memory. CREB belongs to the CREB/ATF-1 (activating transcription factor 1)/CREM (CRE modulator) transcription factor family, eliciting responses to a variety of signals, including growth factor and stress.[6]

It has been previously shown that luteinizing hormone/choriogonadotropin receptor (LHCGR) is present in the mouse and human penis.[7,8] It is not known what functions LHCGR may have in the penis, but it is possible that age-associated increased LH levels[9] may directly affect penile tissue and thereby play a crucial role in the development of erectile disorders. While LH exerts its actions through cAMP signaling system, our study shows that cAMP and CREB are present in the human penis tissue.

MATERIALS AND METHODS

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

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

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.

Immunohistochemistry

Samples from corpus cavernosum and corpus spongiosum of penis were embedded in paraffin after fixation in formalin. The 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 nonspecific binding. After blocking, the sections were incubated with the mouse monoclonal antibody to cAMP (ab24851, Abcam) or rabbit monoclonal antibody to CREB (ab32096, Abcam) 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 cAMP was present in most cells of superficial (ESL), intermedial (EIL), and basal layer (EBL) of urethral epithelium and in fibroblast-like cells of interstitial tissue (FLC) and endothelial cells of cavernous spaces (EC) in corpus spongiosum penis (Figure 1a).

Positive staining for cAMP was also visible in EC of cavernous spaces and in FLC of interstitial tissue in corpus cavernosum penis (Figure 1b).

Positive immunoreaction for CREB was present in superficial (ESL) and intermedial layer (EIL) of urethral epithelium, and some positive staining was also noticed in EC of cavernous spaces and in FLC of interstitial tissue in corpus spongiosum penis (Figure 2a). Positive staining was also visible in EC of cavernous spaces and in FLC of interstitial tissue in corpus cavernosum penis (Figure 2b).

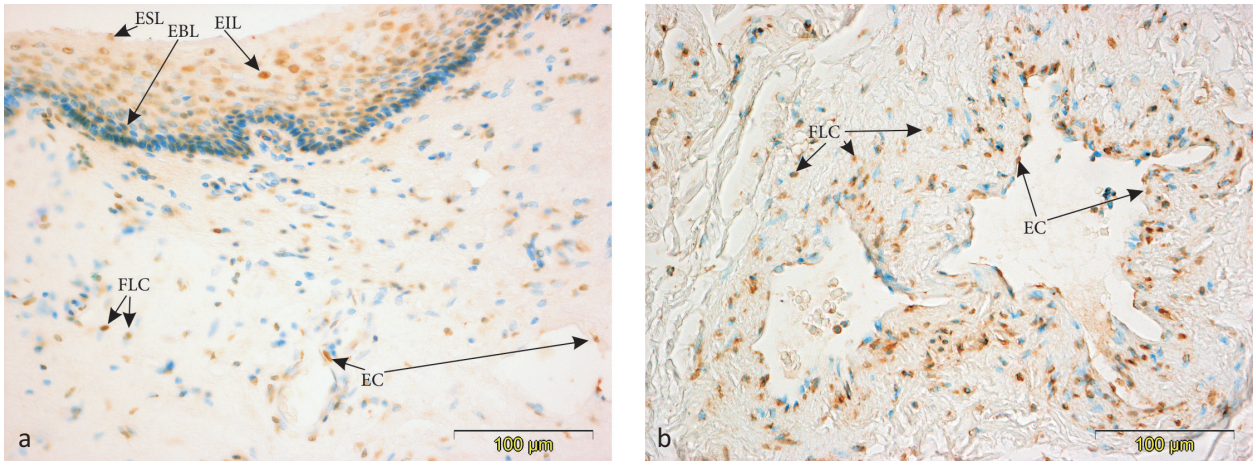

FIG. 1 Expression of cAMP in the human penis. Positive cells are pointed by arrows. Positive immunoreaction is present in most cells of superficial (ESL), intermedial (EIL), and basal layer (EBL) of urethral epithelium, in fibroblast-like cells of interstitial tissue (FLC) and endothelial cells of cavernous spaces (EC) in corpus spongiosum penis (a). Note positive staining for cAMP also in EC of cavernous spaces and in FLC of interstitial tissue in corpus cavernosum penis (b).

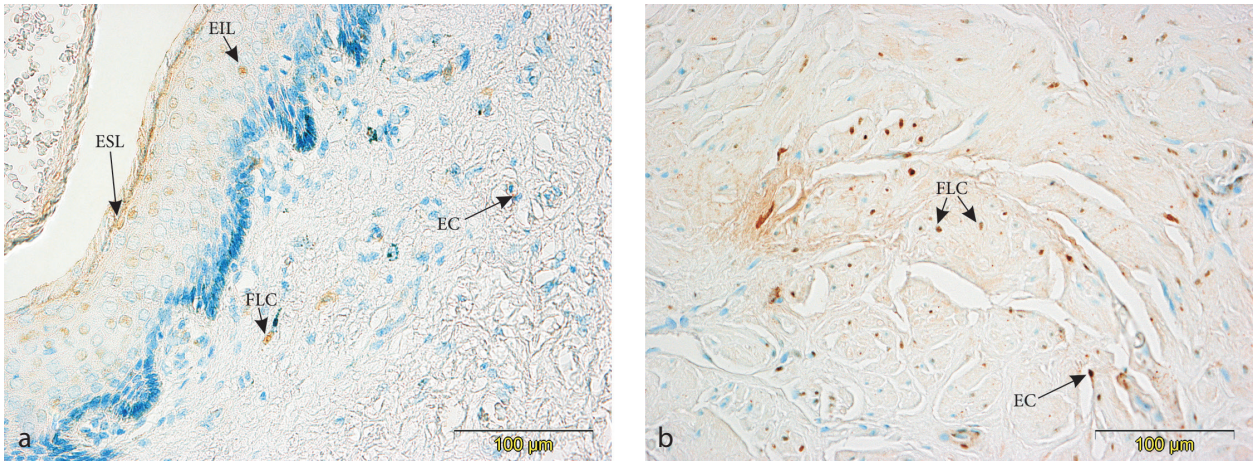

FIG. 2 Expression of CREB in the human penis. Note positive immunoreaction for CREB in superficial (ESL) and intermedial layer (EIL) of urethral epithelium. Some positive staining is visible in endothelial cells of cavernous spaces (EC) and in fibroblast-like cells of interstitial tissue (FLC) in corpus spongiosum penis (a). Positive immunoreaction is also present in EC of cavernous spaces and in FLC of interstitial tissue in corpus cavernosum penis (b).

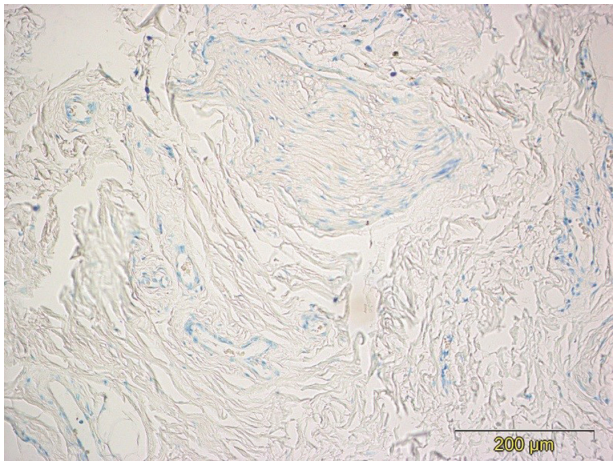

FIG. 3 Negative control. No positive cells were present in negative controls.

No positive cells were visible in negative controls (Figure 3).

DISCUSSION

The present study shows first time positive immunoreaction for cAMP and CREB in human penis tissue. This knowledge, together with our earlier finding on LH expression in the mouse[7] and human[8] penile endothelium, would indicate that LH has yet an unknown role in the human penis. It is possible that the elevated LH levels could regulate the penile tissue of the aging men so that the erectile mechanisms do not function properly. The potential actions of LH in the human penis are supported by the present observations that two components of the adenylate cyclase signal transduction pathway used by the LH receptor, cAMP and CREB, are present in the human penile endothelium.

As the expression of PKA in the human penile arteries has been demonstrated before and on the basis of the figures presented by Waldkirch et al.,[10] and the EC—when compared with the controls given—would seem to express PKA, the present results on the presence of cAMP and CREB in the penile tissue, especially the EC, significantly add to the understanding of the role of the signal transduction from the LH receptor to the nucleus in the penile endothelial cell function. However, as in the rat model erectile function and expression levels of cAMP were previously shown to be significantly lower in the aged than in the younger,[11] the present results would further support the role of

cAMP–CREB pathway in regulation of the erectile function, but by a new mechanism. If the age itself decreases cAMP levels in the penile tissue,[11] the increased LH serum concentrations of the aging men may act as a compensatory mechanism to again increase the levels of cAMP in the penile EC and possibly further to improve the erections.

It is well known that erectile dysfunction is related with many comorbidities and lifestyle factors and that it is a frequent problem among the aging men.[12] According to a prognosis made in 1999, by 2025 roughly 322 million men would be affected by erectile dysfunction worldwide.[13] Many reasons are involved, but the present results suggest that any drug or chemical decreasing the penile endothelial cAMP levels or lowering the serum LH concentrations could possibly cause potency disturbances.

If the decrease in cAMP levels in the penile tissue reported by Cui et al.[11] is due to various genetically regulated aging processes in the penile cells or due to environmental factors and if the age-associated increased LH levels function as a compensatory mechanism to maintain the erectile capacity, then possibly treatment of the impotent men with hCG instead of testosterone should be considered. However, further studies are needed to evaluate this possibility.

CONFLICT OF INTEREST

The authors report no potential conflicts of interest.

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