Summary

Cu-GnRH complex preserves amino acid sequence of native GnRH but contains a Cu^{2+} ion stably bounded to the nitrogen atoms including that of the imidazole ring of His^2 and amide of $pGlu^1$ and His^2. Previous research focused on various aspects of Cu-GnRH activity, indicated that coordination of the Cu^{2+} ion resulted in changes of GnRH molecule conformation and in consequence generated a gonadoliberin analog with a specific, different that non-complexed molecule, biological activity. So far it has been established that Cu-GnRH complex binds with an enhaced affinity to GnRH receptor, is more potent than native GnRH stimulator of LH and FSH release *in vivo* and *in vitro* and exhibites less susceptibility for degradation by hypothalamic and pituitary proteolytic enzymes. Of special interest, however, is that Cu-GnRH is able to activate cAMP/PKA signaling in anterior pituitary cells *via* intracellular calcium mechanism. It is now well recognized that activation of intracellular signalling pathways leads to specific changes exerted at the transcription level. So far, these aspect of Cu-GnRH activity remained unknown.

The aim of this study was to (i) evaluate protein kinase A (PKA) activity in the pituitary gland after the stimulation by Cu-GnRH and PACAP $_{1-38}$ *ex vivo*; (ii) determine mRNA expression of *Egr1*, *Nr5a1*, *Ctnnb1*, *Lhb*, *Fshb*, *Gnrhr*, *Adcyap1r1*, *Prkaca*, *Prkg1*, *Nos1*, *Fst* i *Nr4a1* genes, after intraventricular infusions of Cu-GnRH and PACAP $_{1-38,}$ (iii) examine whether pattern of pituitary stimulation affects selected genes mRNA expression after (iv) define whther pharmacological changes in GnRHR and PAC1 receptors activity modulate Cu-GnRH effects exerted at the transcriptional as well as hormone release levels.

Cu-GnRH and PACAP $_{1-38}$ – stimulated PKA activity in isolated anterior pituitary gland was determined using ELISA method. Real-timePCR method was applied to evaluate specific genes mRNA expression in anterior pituitary of ovariectomized and i.c.v micronjected with Cu-GnRH or PACAP $_{1-38}$ female rats. Serum LH and FSH concentration was determined by specific RIA method.

Obtained data indicate that both Cu-GnRH and PACAP $_{1-38}$ similarly increased PKA activity within respective, 1 h long, isolated anterior pituitary stimulation. Cu-GnRH effects exerted on gonadotrope axis transcriptional acivity were dependent on exogenous

pulse - frequency pattern of pituitary stimulation. Cu-GnRH applied at frequency 2 pulses/hour/5 h significantly enhanced *Egr1*, *Nr5a1 and Nos1*, *Prkaca* mRNA expression whereas complex given as 1 pulse/hour/5 h was effective only for stmulation *Egr1* transcription. Cu-GnRH effects were also dependent on GnRHR as well as PAC1 receptors activity. In the presence of GnRH antagonist (antide), Cu-GnRH-induced *Egr1*, *Prkaca* and *Nr4a1* mRNA expression was significantly diminished whereas in the presence of PAC1 receptor antagonist (PACAP $_{6-38}$) a decrease of complex-stimulated *Egr1*, *Nr5a1*, *Nos1*, *Prkaca* and *Nr4a1* transcriptional activity were observed. Both antide and PACAP $_{6-38}$ also down-regulated a stimulatory effect of Cu-GnRH exerted on gonadotropin release *in vivo*. An enhanced *Egr1* and *Nr5a1* mRNA expression were found after exogenous PACAP$_{1-38}$ microinjections and these effects were not dependent on pattern of pituitary stimulation. In contrast, PACAP $_{1-38}$ was able to induce *Nos1* mRNA expression only when administered in a pulsatile pattern whereas its single bolus was effective in activation of *Prkaca* and *Nr4a1* trancription as well as both gonadotropin release.

Obtained results revealed Cu-GnRH ability to affect selected gonadotrope network genes trancriptional activity. Cu-GnRH intracellular efficiency required GnRHR and PAC1 receptors activation and adequate frequency-dependent pulsatile pituitary stimulation pattern. The same pattern of genes activated by Cu-GnRH as well as PACAP$_{1-38}$ in the anterior pituitary glad of female rats *in vivo* indicates cAMP/PKA pathway involvement in mediating both peptides effects.

Keywords: Cu-GnRH, PACAP $_{1-38}$, cAMP, PKA, anterior pituitary gland, GnRHR, PAC1, gonadotropin axis genes.