Role of mineralocorticoid receptor in regulation of activity of pituitary corticotropic cell and its implication in treatment of Cushing's disease.

Glucocorticoids are steroid hormones secreted by adrenal gland that play a vital role in the regulation of various physiological processes. They act through binding two intracellular proteins - glucocorticoid (GR) and mineralocorticoid (MR) receptors. These receptors are present in various body tissues and exert tissue-specific effects of glucocorticoids. Both receptors act as transcription factors - proteins that regulate the expression of genes. Upon activation they form two-protein complexes (dimers) composed either of two molecules of GR or MR (homodimers) or heterodimers composed of GR and MR. The composition of the dimer acting as functional transcription factor influences which genes are regulated and affects the response of the cell.

Secretion of glucocorticoid (cortisol) by adrenal gland is tightly regulated and forced by ACTH produced by pituitary. Prolonged high secretion of cortisol cause various clinical signs and symptoms. This hypercortisolemia is commonly caused by neuroendocrine pituitary corticotroph tumor and in this case is diagnosed as Cushing's disease (CD). Negative feedback via hypothalamic–pituitary–adrenal axis is responsible for negative regulation of glucocorticoid secretion. Glucocorticoids act on hypothalamus and directly on pituitary cells (corticotrophs) to reduce the secretion of ACTH. The mechanism of action of glucocorticoids on pituitary cells is relatively poorly understood and the influence of MR in the functioning of GR in corticotroph cells is unknown. Importantly, targeting GR is a therapeutical option in treatment of CD. However, direct action of the drug on the receptors in pituitary corticotrophs may play unfavorable role in the response. Our previous results on the expression of GR and MR in corticotroph pituitary tumors indicate that both receptors are involved in regulation of ACTH secretion. The hypothesis is that in pituitary corticotroph cells MR modifies the action of GR and influences the direct adrenal - pituitary negative feedback.

The study is aimed to determine the response of pituitary corticotroph cells to glucocorticoids depending on whether they act only on GR or on both cooperating receptors(GR and MR). The role of GR in the presence of MR or GR alone in the regulation of the expression of the genes regulated by glucocorticoids is of special interest.

Murine AtT-20/D16v-F2 cells – the only available cell line model of pituitary corticotrophs will be used for most of the experiments. These cell express GR but they lack MR protein. The variant of these cells with MR expression will be generated at the first step of the project and then original cells (with GR only) and the cells with both receptors will be subjected to experiments to compare their response to glucocorticoids. The pattern of GR-binding sites in genomic DNA and expression of genes depending on the expression of GR alone or the co-expression of GR and MR will be determined.

AtT-20/D16v-F2 cells are mouse cells, which are very useful for in vitro studies for many reasons. Due to some interspecies differences between mouse and human physiology using murine model cause some limitations of our study. In order to get the results conclusive for human cells, apart from using AtT-20/D16v-F2 cells, some of the experiments will be also performed on primary cultures of human corticotrophs obtained from pituitary tumors causing CD. These experiments will include evaluation of the effect of synthetic modulator of MR (selective MR antagonist, esaxerenone) on human corticotroph cells.

We expect that the results of the project will allow to describe the influence of glucocorticoids on negative regulation of pituitary corticotroph cells secretory activity and will show the difference in the role of GR and MR. Since glucocorticoids play a specific role in regulation of pituitary corticotrophs as part of negative feedback, this knowledge is important for better understanding the mechanism of treatment of CD patients with GR antagonists.