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Title of dissertation:

PHOSPHODIESTERASE 2 REGULATES EXPRESSION OF THE TYROSINE HYDROXYLASE GENE IN PC12 CELLS

ABSTRACT

Catecholamines (epinephrine, norepinephrine and dopamine) are secreted into the bloodstream mainly and directly from the chromaffin cells of the adrenal medulla and also diffuse from the sites of release from the sympathetic nerve terminals in response to stress agents such as psychological stressors, hypoxia, and hypoglycemia. Catecholamines in the bloodstream act as hormones causing a general mobilization of the body by increase in heart rate, blood pressure and bronchodilation. Excessive and prolonged levels of catecholamines in the bloodstream are harmful to the body, because it can lead to hypertension and heart damage as a result of overloading. Therefore, it is important to understand the mechanisms to prevent excessive synthesis of catecholamines, because it may be useful in design of targeted pharmacotherapies in the future. The first step in the biosynthesis of catecholamines is catalyzed by tyrosine hydroxylase (TH) which is the rate-limiting enzyme in the catecholamine biosynthesis. Changes in the expression of the *TH* gene are one of the major mechanisms determining the long-term synthesis of catecholamines. In stress condition, increased expression of the *TH* gene in chromaffin cells is induced by adenosine and pituitary adenylate cyclase-activating peptide (PACAP). Preliminary results from our Laboratory showed that (rat) atrial natriuretic peptide (rANP) inhibits adenosine-induced expression of the *TH* gene in PC12 cells (*in vitro* model system of chromaffin adrenal cells). Therefore, the main aim of this dissertation was elucidation of the mechanism by which rANP inhibits the signal transduction from adenosine receptor to the expression of the *TH* gene.

The experiments indicated that the rANP inhibits transcription of the *TH* gene induced by both adenosine and PACAP. Next, it was observed that rANP-induced accumulation of cGMP leads to reduction of the adenosine- or PACAP-induced accumulation of cAMP. These results suggested that rANP inhibits the response to adenosine and PACAP *via* the same mechanism. Experiments with the use of phosphodiesterase inhibitors indicated that a phosphodiesterase 2 (PDE2, cGMP-stimulated phosphodiesterase hydrolyzing cAMP) is involved in cross-talk between signaling pathways of rANP and adenosine or PACAP at the level of cyclic nucleotides. Moreover, further studies showed that enhancement of PDE2 hydrolytic activity in PC12 cells stimulated with rANP (acting *via* cGMP) or synthetic activator of PDE2 (5,6-DM-cBIMP) leads to inhibition of the adenosine-induced transcription of the *TH* gene in PC12 cells.

Focusing on the adenosine-induced expression of the *TH* gene, the PDE2-inhibited signaling pathway has been characterized. Results of experiments indicated that PDE2, hydrolysing cAMP, inhibits the activation of protein kinase A (PKA) and transcription factor CREB (a direct target for PKA phosphorylation) in response to adenosine. It was found that the impairment of CREB DNA binding activity leads to much weaker adenosine-induced activation of the *TH* gene promoter. These observations in combination with previous results indicated that PDE2 inhibits PKA- and CREB-dependent expression of the *TH* gene.

Taken together, results of experiments indicate that PDE2 negatively regulates adenosine- or PACAP-induced expression of the *TH* gene in PC12 cells. It is suggested that enhancement of PDE2 hydrolytic activity leads to reduction of the intracellular cAMP level, resulting in a weaker activation of PKA and a lower level of CREB- dependent activation of the *TH* gene in adenosine stimulated PC12 cells.