Abstract

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Title of diploma thesis: Studium of gene regulation of nucleoside transporters in BeWo cell line

Nucleoside transporters (NTs) localized in syncytiotrophoblast control placental uptake of nucleosides. Dysregulation of NTs can disrupt nucleoside homeostasis with a negative consequences on placental and fetal development and can lead to a change in placental pharmacokinetics of nucleoside-derived drugs. Therefore, understanding the expression and function of NTs is necessary for effective and safe pharmacotherapy during pregnancy. The aim of this diploma thesis was to study the adenylate cyclase (AC) activated regulatory pathways of gene expression of concentrative nukleoside transporter 2 (CNT2). For this purpose, qRT-PCR and *in vitro* accumulation assays using the model substrate [^{3}H]-adenosine were employed. The human placental choriocarcinoma-derived BeWo cell line has been exposed to an AC activator, forskolin (50 μM), and/or inhibitors of AC/cAMP/PKA, AC/cAMP/MAPK (MEK1/2, p38 MAPK) signaling pathways, PKA inhibitor, KT 5720 (5 μM), an inhibitor of MEK1/2, U0126 (10 μM) and an inhibitor of p38 MAPK, SB202190 (10 μM). The application of inhibitors *blocked* the increase in expression of CNT2 gene caused by forskolin, but revealed no effect on CNT2 mRNA levels in FSK-nontreated cells. All tested inhibitors caused decrease in [^{3}H]-adenosine uptake (1 min incubation), but in longer 15 min incubation this effect was observed only for KT 5720. In conlusion, inhibition of cAMP/PKA, cAMP/MEK1/2, cAMP/p38 MAPK pathways seems to block the effect of forskolin on CNT2, and these inhibitors likely affect CNT2 distribution into the cytoplasmic membrane. We hypothesize that the decreased levels of CNT2 in the membrane affects the rate of adenosine uptake, but not its final intracellular concentration over longer time periods. In addition to these findings we have also reported that the concentration of glucose or fetal serum in the culture medium *doesn't* affect CNT2 gene expression. This work contributed to the unterstanding of the molecular mechanism of CNT2 regulation in the BeWo cell line.