EVALUATION OF SIGNALING PATHWAY OF A2A ADENOSINE RECEPTOR IN MIN 6 PANCREATIC B CELLLINE

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Abstract -

Objective

Chronic exposure of pancreatic β-cells to high glucose leads to the decrease of regeneration and consequently impaired insulin secretion. So, finding new agents or pathways can be helpful to prevent β-cells failure. Recent studiesrevealed some aspects of extracellular signaling of AdenosineP1 receptors (A1, A2a, A2b and A3)in many physiopathological conditions of β-cells.Investigations on A2a receptor showed promising results. However intracellular signaling of A2a receptor in β-cells isn't clear. Here, we evaluated the effects of A2a receptor activation in viability and insolinotrophic features in a mouse pancreatic β-cell line, MIN 6. Many critical components of intracellular signaling pathway of this receptoralso evaluated.

Methods

At first we evaluated A2a expression in MIN 6 cells by qRT-PCR. Then, we analyzed the effect of pharmacological A2a receptor agonist, CGS 21680 (0.01- 100 μM), on *in vitro*MIN 6 proliferation by Brdu assay. Accumulation of cAMP was evaluated by Sandwitch-based ELISA. Calcium fluctuations probed by Fura-2 am. Insulin secretion was assayed by specific mouse-insulin ELISA at (0.01-10 μM). Inhibitor-1 gene expression levels, as a key controller of protein phosphatase 1 (PP-1) in A2a signaling pathway, measured by qRT-PCR.

Results

A2a expression in MIN 6 cells confirmed. According to the Brdu assay results, pharmacological activation of A2a receptor can increase MIN 6 proliferation significantly.As expected, activation of this receptor can increase intracellular levels of cAMP. CGS 21680 can also change intracellular levels of Calcium. However, higher doses of CGS 21680 needed to increase insulin secretion from MIN 6 cells.According to our results, 10 μM of this agonist needed to increase insulin secretion. Inhibitor-1 gene expression levels are decreased significantly by CGS 21680.

Conclusions

We showed that A2a receptor signaling has an important role in β-cell proliferation and functionality.Activation of this receptor by CGS 21680 agonist can increase MIN 6 β-cell expansion and insulin secretion. Activation of this receptor has a potent effect on Inhibitor-1 gene expression, which indicate delicate control of PP-1 role in β-cell proliferation and functionality. Further evaluation of intracellular signaling pathways of this receptor needed.

Keywords - β-cell, Adenosine, A2a receptor, diabetes.