SL102 Modulation of Calcium Signaling and Neuronal Gene Expression by Adenosine Receptors
Adensoine 수용체에 의한 칼슘 신호와 유전자 발현의 조절

김경태
포항공과대학교 생명과학과

PC12 cells are neurosecretory cells which release catecholamines upon external stimulations. Both P2 purinoceptors and voltage-sensitive calcium channels (VSCCs) play important roles in catecholamine secretion by increasing $[Ca^{2+}]_i$ in the cells. In addition, the presence of typical A_{2A} adensoine receptors and their activation of adenylyl cyclase are well demonstrated in PC12 cells. Since adenosine receptors are known to modulate a variety of physiological functions, it is likely that the activity of P2 purinoceptors and VSCCs are regulated by the A_{2A} receptors.

At first we investigated the regulatory role of A_{2A} receptors in the P2 purinoceptor-mediated calcium signaling. When PC12 cells were treated with CGS21680, a specific agonist of A_{2A} receptors, extracellular ATP-induced $[Ca^{2+}]_i$ rise was inhibited by ~20%. Since ATP can induce $[Ca^{2+}]_i$ increase via several pathways, it was determined which pathway is sensitive to CGS21680. CGS21680 inhibited the portion of Ca^{2+} influx induced by ATP. However, CGS21680 did not affect thapsigargine-induced $[Ca^{2+}]_i$ rise. In addition, the CGS21680-induced inhibition was completely blocked by reactive blue-2, suggesting that nonselective cation channels (P2X) are inhibited by the A_{2A} receptors. The CGS21680 effect was mimicked by forskolin and dibutyryl-cAMP and blocked by staurosporine and R_p-cAMPS. The data suggest that activation of A_{2A} receptors inhibits ATP-induced Ca^{2+} influx through nonselective cation channels via protein kinase A (PKA) in PC12 cells.

The role of the A_{2A} receptor in regulating VSCCs was also investigated. Both L- and N-type VSCCs were inhibited by CGS21680 treatment. Cholera toxin (CTX) treatment for 24 hours completely eliminated the CGS21680 potency. Similar inhibitory effects on VSCCs were obtained by membrane-permeable activators of PKA. These effects were blocked by R_P-cAMPS, a PKA inhibitor. The data suggest that activation of the A_{2A} receptor leads to inhibition of VSCCs via a CTX-sensitive G protein and PKA. Although A_{2A} receptors induce inhibition of VSCCs as well as ATP-induced Ca^{2+} influx, the two inhibitory effects are clearly distinct from each other.

Finally we investigated the role played by A_{2A} receptors in the gene expression of rat tyrosine hydroxylase (TH), a key enzyme in the biosynthetic pathway of catecholamines. CGS21680 caused TH mRNA levels to increase to more than twice the level of the untreated control. Transient transfection analysis demonstrated that the transcription of the TH gene was markedly enhanced upon treatment with CGS21680. Mutational analysis of the 5' upstream region of the TH gene revealed that the cAMP response element (CRE) at -45 to -38 bp was responsible for the CGS21680-induced effect. Gel mobility shift assays revealed that six CRE-specific DNA-protein complexes were formed, and the amounts of three of them were significantly increased by treatment with CGS21680. Co-transfection with an expression vector containing PKA inhibitor markedly decreased the CGS21680 effect. The results suggest that stimulation of the A_{2A} receptor leads to an elevated expression of the TH gene by changing the binding pattern of DNA binding proteins that interact with CRE through activation of PKA.

These results suggest that A_{2A} adenosine receptors play a critical role in the regulation of $[Ca^{2+}]_i$ homeostasis and biosynthesis of neurotransmitters.