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Analysis of adenylate cyclase subtypes which couple to D1 dopamine receptor in SK-N-MC human neuroblastoma cells.

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Dopamine is one of the neurotransmitters of the central nervous system. The dopaminergic system is known to be involved in the regulation of motor and motivated behaviors, such as those based on reward. Degeneration of the dopaminergic system is proposed to be implicated in schizophrenia, depression, and Parkinson's disease.

The dopamine receptors, which transduce dopamine signals into cells, are seven - transmembrane proteins that bind to GTP binding proteins, leading to regulation of adenylate cyclases (ACs). Among the reported five dopamine receptors, D1 dopamine receptor stimulates ACs through binding to the stimulatory GTP-binding protein (Gs), resulting in increase in intracellular cyclic AMP. The dopaminergic system, like other receptor - mediated signal transduction systems, is known to be regulated at the receptor level and the Gs level, but the regulation at the AC level is not yet defined.

ACs are twelve - transmembrane enzymes which are stimulated by Gs to convert ATP to cyclic AMP (cAMP). Recently, cDNA sequences of ten subtypes of AC have been reported, which have 50-90% cDNA sequence homology between each other. All of the ten subtypes are activated by Gs and forskolin, but the regulation by other cytosolic or membrane - bound factors is subfamily specific.

To find out the role of subtype-specific properties of AC in the regulation of receptor-mediated signal transduction and specific coupling of AC to the receptors, SK-N-MC human neuro-

blastoma cell lines which express D1 dopamine receptor and  $\beta$  adrenergic receptor endogenously were used. Reverse transcription (RT) -PCR analysis of RNA extracted from the cells and cDNA sequencing of RT-PCR products revealed that this cell expressed mRNA of three distinct ACs, two of which had a 95.7% and 86.5% homology to rat AC type III and VI respectively, and one was identified as human AC type VII.

AC type III in the membrane fraction of olfactory tubercle was activated in a calcium-(38.1  $\mu$ M)-calmodulin-dependent manner, whereas AC in the membrane fraction of SK-N-MC cells was not. These results suggest that there is no expression of AC type III as a functional protein in these cells. Stimulation of AC activity by protein kinase C, which is a characteristic of AC type VII, was detected when either D1 dopamine receptor or  $\beta$  adrenergic receptor was stimulated, indicating the coupling of AC type VII with both of the receptors. Inhibition of the activity by high calcium concentration and protein kinase A is the characteristic of AC type VI. In SK-N-MC cells, calcium (2 mM) inhibited both D1 dopamine receptor and  $\beta$  adrenergic receptor-stimulated AC activity, showing a possible coupling of AC type VI to both receptors. The involvement of protein kinase A on AC regulation was determined by examining homologous and heterologous desensitization in the presence and absence of a protein kinase A inhibitor. Apparent heterologous desensitization of the D1 dopamine receptor system was observed by pretreatment of a  $\beta$  adrenergic receptor agonist, which

was reversed by inhibition of protein kinase A activity, while homologous desensitization was not affected by the protein kinase A inhibitor. On the other hand,  $\beta$  adrenergic receptor system underwent only homologous desensitization, which also was not affected by the protein kinase A inhibitor.

Conclusively, SK-N-MC cells express AC type VI and VII, which couple to both D1 dopamine receptor and  $\beta$  adrenergic receptor, but D1 dopamine receptor rather than  $\beta$  adrenergic receptor seems to couple dominantly to AC type VI.

### The effect of palytoxin in cultured porcine adrenal medullary cells

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(Summary of Graduation thesis written under direction of Dr. Y. Nakazato)

1. The effect of palytoxin (PTX) was studied in cultured porcine adrenal medullary cells with whole-cell voltage clamp techniques and measurement of intracellular  $\text{Na}^+$  concentration ( $[\text{Na}^+]_i$ ).

2. At a holding potential of  $-70$  mV, PTX caused dose-dependent increases (0.1–100 nM) in inward currents and  $[\text{Na}^+]_i$ .

3. PTX-induced inward current was inhibited by the replacement of extracellular  $\text{Na}^+$  with  $\text{Cs}^+$ ,  $\text{Li}^+$  or  $\text{Tris}^+$ . The order of inhibitory potency was  $\text{Tris}^+ \gg \text{Li}^+ > \text{Cs}^+$ . In  $\text{Na}^+$ ,  $\text{Cs}^+$  or  $\text{Li}^+$  solution, the voltage-current relationship was linear and the reversal potential was about 0 mV. On the other hand, in  $\text{Tris}^+$  solution, the voltage-current relationship showed the outward rectification and the reversal potential was about  $-30$  mV.

4. In the absence of extracellular  $\text{Ca}^{2+}$  or in the presence of  $\text{Ca}^{2+}$  together with  $\text{Mn}^{2+}$  (2.5 mM), PTX (10 nM) failed to evoke an inward

current.  $\text{Mn}^{2+}$  slightly inhibited inward currents which were induced by PTX.

5. In the pretreatment with ouabain (0.1 mM), an inhibitor of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, PTX did not induce any inward currents. Ouabain had no effect on inward currents which PTX had induced. Amiloride, an inhibitor of  $\text{Na}^+/\text{H}^+$  exchanger, inhibited PTX-induced inward current in a dose-dependent manner (0.01–1 mM).

6. The amplitude of PTX-induced inward current was increased by intracellular adenine nucleotide. The order of potency was  $\text{ATP} \cong \text{ADP} > \text{no adenine nucleotide} \cong \beta, \gamma$ -methyleneadenosine-5'-triphosphate.

7. The developing rate of PTX-induced inward current was increased by the flash photolysis of caged-ATP.

8. These results suggest that PTX induces non-selective monovalent cation channels by causing conformational changes in  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase at some states which depend on intracellular ATP or ADP in cultured porcine adrenal medullary cells.