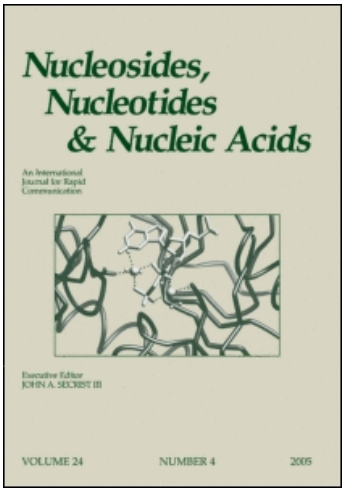

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P_2-Purinoceptor Mediated Inhibition of Adenylate Cyclase Activity

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P_2-PURINOCEPTOR MEDIATED INHIBITION OF ADENYLATE CYCLASE ACTIVITY

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In 1972 Burnstock[1] proposed that adenosine 5'-triphosphate (ATP) is the principal neurotransmitter in non-adrenergic, non-cholinergic nerves through an interaction with P_2-purinergic receptors. In 1985 Burnstock and Kennedy[2] suggested a subdivision of this class of receptors into a P_{2x}- and a P_{2y}-subclass on the basis of rank order of potency of various ATP-analogues in pharmacological studies. The potency order on the P_{2x}-purinoceptor is α,β-methyleneATP (AMP.CPP) = β,γ-methyleneATP (AMP.PCP) > ATP = 2-methylthioATP (2MeSATP), whereas the potency order on the P_{2y}-purinoceptor is 2MeSATP >> ATP > AMP.CPP = AMP.PCP. Derivatization of ATP at various positions resulted in a more pronounced definition of the structure-activity requirements for both subtypes of the purinoceptor. The P_{2x}-purinoceptor is rather tolerant to modifications at the ribose moiety and the phosphate chain and the activity of adenine modified analogues is not affected. On the P_{2y}-purinoceptor the activity almost disappears when ribose-modified analogues are applied, but modification of the adenine ring results in enhancement, and modification of the phosphate chain results in a small decrease in activity.

Since most of the data is based on functional pharmacology, very little is known about signal transduction mechanisms and biochemical effects related to stimulation of these receptors. We investigated the possibility that one of the receptor subtypes is coupled to the second messenger system adenylate cyclase[3]. We found that various adenine nucleotides, either modified at the adenine ring, the

ribose moiety or the phosphate chain, inhibited forskolin-stimulated adenylate cyclase activity in membranes of rat hepatocytes, in a dose-dependent manner.

Since we used membranes, where the internal and external conditions are the same, and since ATP acts both as a neurotransmitter for the P_2-purinoceptor and as a substrate for adenylate cyclase, we investigated the liability of these analogues to cyclization by adenylate cyclase. No such influence could be demonstrated except for two adenine nucleotides that demonstrated no inhibitory activity. Of the eight compounds used adenine-9→1'-arabinofuranoside 5'-triphosphate (arabinoseATP) was the most active, with an IC_{50}-value of 26 ± 3 μM and an availability as substrate relative to ATP of 0.2 ± 0.2 %, whereas adenosine-5'-(2-O-thiodiphosphate) (ADPβS) stimulated the adenylate cyclase and had an availability as substrate of 32.1 ± 3.7 %.

To exclude interference with adenylate cyclase we performed studies on intact cells as well. It was shown that in intact cells (viability ≥ 85%) adenylate cyclase could also be inhibited by adenine nucleotides in a dose-dependent manner. The most potent adenine nucleotides were ATP and ADP with an IC_{25}, i.e. the ligand concentration that inhibits maximal cAMP production by 25%, of 21 ± 5 μM resp. 15.8 μM. Generally, the potency of the nucleotides tested on whole cells appeared to be decreased by a factor 3 to 4 relative to the potency on membranes. Of the analogues tested the most potent was 3'-deoxyATP with an IC_{25} of 156 ± 19 μM.

From these data we conclude that the rank order of potency for purinoceptor mediated inhibition of adenylate cyclase does not fully match any of the functional pharmacological subclasses. Furthermore, considering the tolerance of the receptor to modifications at the ribose moiety we suggest that the P_2-purinoceptor coupled to adenylate cyclase in the rat hepatocyte resembles the P_{2x}-subtype.

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