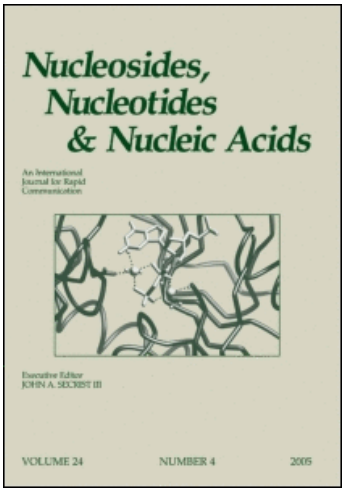

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Gerwins, Pär , Nordstedt, Christer and Fredholm, Bertil B.(1991) 'GTP and IAP Shifts Adenosine A_1-Agonist Binding in Smooth Muscle Membranes to the Low Affinity State, Similar to the one Found in Intact Cells', Nucleosides, Nucleotides and Nucleic Acids, 10: 5, 1173 — 1174
To link to this Article: DOI: 10.1080/07328319108047264
URL: http://dx.doi.org/10.1080/07328319108047264

NUCLEOSIDES & NUCLEOTIDES, 10(5), 1173-1174 (1991)

GTP AND IAP SHIFTS ADENOSINE A_1-AGONIST BINDING IN SMOOTH MUSCLE MEMBRANES TO THE LOW AFFINITY STATE, SIMILAR TO THE ONE FOUND IN INTACT CELLS.

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Abstract:Adenosine A_1 receptors in the smooth muscle cell line DDT_1 MF-2 were characterized by radioligand binding using the antagonist [^{3}H]-8-cyclopentyl-1,3-dipropylxanthine ([^{3}H]DPCPX) as the ligand. Binding properties of adenosine agonists and antagonists to both intact cells and membranes were investigated.

Results

Binding characteristics for [^{3}H]DPCPX were similar in intact DDT_1 MF-2 cells (1) and in membranes prepared from these cells (K_D value of approximately 1 nM). The maximum binding amounted to 183 fmol/10^6 intact cells and 344 fmol/mg of membranes.

To characterize the receptor, competition curves for [^{3}H]DPCPX binding using several adenosine agonists and antagonists were performed. Adenosine receptor antagonists appeared to bind to a single class of binding site both in membranes and intact cells. The order of potency was (membranes, intact cells): 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (0.5, 0.9 nM) = CGS 15943A (1.1, 0.7 nM) > 8-cyclopentyl-1,3-dimethylxantine (8-CPT) (10, 4.9 nM) > 8-(p-sulfophenyl)-theophylline (8-PST) (690, 144 nM) > 3-isobutyl-1-metylxanthine (IBMX) (2.5, 1.4 μM) > theophylline (7.3, 3.7 μM). Competition curves using adenosine agonists in membranes were best described by a two-site rather than a one-site model. In intact cells, only a single site was detected. The equilibrium K_D values for intact cells were similar to the low affinity K_D values in membranes (K_L). The order of potency was (K_L membranes, K_D intact cells): N^6-cyclopentyladenosine (CPA) (41, 58 nM) ≥ (-)-N^6-phenylisopropyladenosine (R-

PIA) (93, 77 nM) ≥ N^6-cyclohexyl adenosine (CHA) (147, 144 nM) > 5'-N-ethylcarboxamido adenosine (NECA) (362, 445 nM) > 2-chloroadenosine (2-CADO) (644, 801 nM) > adenosine (intact cells only) (1.6 μM) > 2-phenylaminoadenosine (CV 1808) (16.2, 11.2 μM).

Treatment of cells with pertussis toxin (PTX) ADP-ribosylated G-proteins and eliminated the high affinity agonist binding in membranes, but did not affect binding to intact cells. The addition of GTP (100 μM) also shifted the competition curves from bi- to monophasic curves in membranes.

Adenosine receptor agonists inhibited the formation of adenosine cyclic monophosphate (cAMP) induced by isoprenaline (IC_{50} for R-PIA approximately 1 nM). This inhibition could be prevented with adenosine receptor antagonists. Pretreatment with PTX also reversed these effects and actually revealed functional A_2 receptors as shown by the formation of cAMP induced by NECA.

Conclusions

The smooth muscle cell line DDT_1 MF-2 from Syrian hamster vas deferens expresses adenosine A_1 receptors. Agonist binding shows that the A_1 receptor is present in two affinity states in membrane preparations whereas in intact cells only the low affinity state is present. This is probably due to the fact that cells have sufficient amount of GTP to rapidly shift agonist binding to the low affinity state. Antagonists bound to a single site both in intact cells and in membranes. Membranes from cells treated with pertussis toxin or addition of GTP to membranes shifts agonist binding to low affinity. The A_1 receptors are negatively coupled to adenylate cyclase via a pertussis sensitive G protein. The A_2 receptors could stimulate the accumulation of cAMP but only after treatment with pertussis toxin. Adenosine is half as potent as 2-Chloroadenosine.

Supported by the Swedish Medical Research Council, Karolinska Institutet, the Swedish Cancer Association and Astra AB.

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