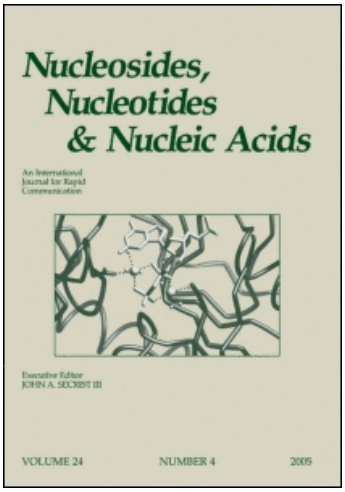

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Ligand Binding to A_1 Adenosine Receptors is Influenced by Protonation

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To cite this Article Hoppe, Edmund and Reddington, Martin(1991) 'Ligand Binding to A_1 Adenosine Receptors is Influenced by Protonation', Nucleosides, Nucleotides and Nucleic Acids, 10: 5, 1139 — 1140
To link to this Article: DOI: 10.1080/07328319108047255
URL: http://dx.doi.org/10.1080/07328319108047255

NUCLEOSIDES & NUCLEOTIDES, 10(5), 1139-1140 (1991)

LIGAND BINDING TO A_1 ADENOSINE RECEPTORS IS INFLUENCED BY PROTONATION

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Abstract: Studies on the pH dependence of ligand binding to A_1 adenosine receptors revealed that protonation of a histidine residue in the binding pocket is accompanied by high affinity agonist binding.

In the last few years it has become clear that binding of hormones to their receptors can be described by the interaction of the ligands with specific amino acid residues in the receptor molecule[1]. It has been shown that many hormones which are positively charged can bind to the receptor by salt-bridging to a negatively charged aspartate residue in the third transmembrane helix. The importance of this salt-bridge for ligand binding can be demonstrated by the fact that neutralisation of this aspartate residue at low pH confers an impairment of hormone binding to its receptor[2].

The mechanism of ligand binding to the A_1 adenosine receptor is thus of interest since adenosine does not possess such a positive charge. Another mechanism apart from salt-bridging must therefore be assumed.

In order to detect amino acid residues on the A_1 adenosine receptor involved in hormone recognition, the pH dependence of ligand binding was examined. A stimulation of binding at low pH was seen in the absence of Mg^{2+} which was most pronounced with the agonists N^6-cyclohexyladenosine (CHA) and R-N^6-phenylisopropyladenosine (R-PIA). This stimulation of [^{3}H]CHA binding was maximal at pH 5, half-

maximal enhancement being seen at pH 6.3. Further investigation with saturation and competition experiments revealed that this effect was due to a shift of receptors with low affinity for agonists to a high affinity state. Binding of the antagonist 8-cyclopentyl-1,3-[^{3}H]dipropylxanthine ([^{3}H]DPCPX) was also increased by decreasing its Kd at low pH.

The pKa of 6.3 in the stimulation of [^{3}H]CHA binding suggests an involvement of a histidine- or, alternatively, an aspartate/glutamate residue in a hydrophobic environment. Chemical modification of carboxyl residues failed to alter the stimulation of [^{3}H]CHA binding by low pH. On the other hand, chemical modification of histidine residues has been shown to abolish ligand binding to A_1 adenosine receptors due to the presence of a histidine residue in the binding pocket[3]. Therefore, the pKa of this histidine residue in the binding pocket of the A_1 adenosine receptor was determined by pH-dependent photooxidation in the presence of methylene blue. Inactivation of [^{3}H]CHA binding sites by photooxidation could be prevented by preincubation of membranes with theophylline or R-PIA or by an increase in H^+-concentration. The pH-dependence revealed a pKa of 6.3 for this histidine residue which was not significantly different from the pKa obtained in the stimulation of [^{3}H]CHA binding. From these data it can be concluded that protonation of a histidine residue in the binding pocket of the A_1 adenosine receptor is accompanied by an induction of high affinity agonist binding. It is tempting to speculate that this effect is due to a hydrogen bond from the protonated histidine residue in the binding pocket to the adenosinergic ligand.

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