BINDING THERMODYNAMICS AT THE HUMAN A_3 ADENOSINE RECEPTOR

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Thermodynamic parameters of the binding equilibrium of drugs to their receptors have aroused increasing interest in the recent years due to the information they may provide on binding mechanisms. Because of the very low receptor concentration (1-100 fmol/mg of tissue for a typical neurotrasmitter) direct microcalorimetric measurements cannot be performed. The only method praticable for receptor studies is that based on binding constant measurements at different temperatures, followed by van't Hoff analysis. Until now, such studies have been carried out on several receptor systems such as -adrenoceptor[1], opiate[2], benzodiazepine[3], dopamine[4], nicotine[5] and adenosine A_1[6] and A_{2A}[7] receptors.
Adenosine is an endogenous modulator acting as an autacoid via interaction with four types of G protein-coupled receptors: A_1, A_{2A}, A_{2B} and A_3. A_3 receptors are widely distributed in human body suggesting their involvement in regulating many physiological functions. Recently, we have performed a pharmacological and biochemical characterization of [^{3}H]MRE 3008F20 binding to the human A_3 receptors transfected in CHO cells[8]. Furthermore, we have shown that MRE 3008F20 binding to human A_3 receptors is enthalpy- and entropy-driven[8]. A_3 receptors exist in equilibrium between two different conformations, which correspond to a high and a low affinity binding state; antagonists bind equally well to both receptor states whereas agonists discriminate between them.
This study is the first to deal with the binding thermodynamics of adenosine agonists and antagonists to the human A_3 adenosine receptor transfected in CHO cells.
The thermodynamic parameters ÄG°, ÄH° and ÄS° of the binding equilibrium of the six adenosine receptors agonists (IB-MECA, Cl-IB-MECA, AB-MECA, NECA, R-PIA, S-PIA) and five antagonists (MRE 3008F20, MRE 3020F20, MRE 3005F20, CGS 15943 and DPCPX) at adenosine A_3 receptors were determined by means of affinity measurements at six different temperatures (4, 10, 15, 20, 25 and 30°) and van't Hoff analysis. Affinity constants were measured on CHO cells transfected with the human A_3 receptor by inhibition assays of the binding of the selective A_3 antagonist [^{3}H]MRE 3008F20. Van't Hoff plots were linear for agonists and antagonists ; this implies that the $ÄC°_p$ (the standard specific heat difference of the equilibrium) for the binding equilibrium approximates zero in all cases, or, in other words, the ÄH° is not significantly affected by temperature variations in the range investigated (4-30°). This phenomenon seems to indicate that the conformational changes needed to produce the pharmacological effect are relatively small in this class of macromolecules, most probably because larger modificationswould make the association of the receptor with the cell membrane unstable. This point of view is strongly supported by the findings that all membrane receptors so far studied are chacaterized by essentially linear van't Hoff plots with the only known exception of the binding of insulin to its receptor.
It is of interest the extrathermodynamic interdependence of ÄH° and -T ÄS° for the A_3 receptor, clearly apparent from scatter plot of ÄH° *versus* ÄS° where all the experimental

points appear to be arranged along the same diagonal line. This phenomenon is named Enthalpy-Entropy compensation and it seems to be a common feature in all case of drug receptor binding. It can be attribuited to the solvent reorganization that accompanies the receptor binding process in diluted solution. In fact, while ÄG° values are most probably determined by the features of the ligand-receptor binding process, ÄH° and T ÄS° values appear strongly affected by the rearrangements occurring in the solvent.

The thermodynamic parameters fall in the range 21 ≤ ÄH° ≤ 67 kJmol^{-1} and 208 ≤ ÄS° ≤ 410 J(K mol^{-1}) for agonists and -52 ≤ ÄH° ≤ -9 kJmol^{-1} and 16 ≤ ÄS° ≤ 81 J(K mol^{-1}) for antagonists, showing that agonist binding is always totally entropy-driven while antagonists binding is enthalpy- and entropy-driven. Agonists and antagonists are thermodynamically discriminated for all adenosine receptors studied so far, and in this light we suggest that the similarity in thermodynamic parameters probably reflects a common mechanism of ligand-receptor interaction for all adenosine receptor subtypes.

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