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Molecular modeling of adenosine receptors. The ligand binding site on the rat adenosine A_{2A} receptor

Ad P. IJzerman^{a,*}, Eleonora M. van der Wenden^a, Philip J.M. van Galen^b, Kenneth A. Jacobson^b

^aLeiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, PO Box 9502, 2300 RA Leiden, The Netherlands ^bNIH/NIDDK, Laboratory of Bioorganic Chemistry, Bldg 8, Rm B1A-17, Bethesda, MD 20892, USA

Abstract

The amino acid sequence of the rat adenosine A_{2A} receptor and the atomic coordinates of bacteriorhodopsin were combined to generate a three-dimensional model for the adenosine A_{2A} receptor. This model consists of seven amphipathic α -helices, forming a pore that is rather hydrophilic compared to the hydrophobic outside of the protein. Subsequently, a highly potent and selective ligand for this receptor, 2-(cyclohexylmethylidenehydrazino)adenosine (SHA 174), was docked into this cavity. A binding site is proposed that takes into account the conformational characteristics of the ligand. Moreover, it involves two histidine residues that were shown to be important for ligand coordination from chemical modification studies. Subsequently, the deduced binding site was used to model other potent ligands, including 8-(3-chlorostyryl)caffeine, a new A₂-selective antagonist, that could all be accommodated consistent with earlier biochemical and pharmacological findings. Finally, some thoughts on how adenosine receptor activation might proceed are put forward, based on structural analogies with the enzyme family of serine proteases.

Keywords

Molecular modeling; Adenosine receptor; Receptor binding and activation; Bacteriorhodopsin; Histidine residue

1. Introduction

The superfamily of G protein-coupled receptors encompasses over 300 cloned and sequenced members, and this number is still rapidly growing. Among them are the adenosine receptor subtypes – A₁, A_{2A}, A_{2B} and A₃ – some of them originating from various species (see also Fig. 1). Also adenosine receptors show the characteristic secondary structure for all G protein-coupled receptors, i.e., seven predominantly hydrophobic

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*Correspondence to: A.P. IJzerman, LACDR - medicinal chemistry PO Box 9502 2300RA Leiden, The Netherlands tel: (31)-71-274651, fax: (31)-71-274277.

stretches of 20–25 amino acids that could span the cell membrane as α -helices (see also Fig. 2) (Libert et al., 1989; Chern et al., 1992; Fink et al., 1992).

Recently, we have reported on the molecular modeling of the canine adenosine A₁ receptor, which was based on the atomic coordinates of bacteriorhodopsin (IJzerman et al., 1992). Hibert and co-workers (Hibert et al., 1991; Trumpp-Kallmeyer et al., 1992) were the first to analyze in depth the opportunities and pitfalls associated with this approach. Although met with scepticism, the models developed appear to be useful in understanding the putative molecular basis of receptor-ligand recognition, in particular when combined with and adjusted to available pharmacological and structure-activity relationship (SAR) data.

Selective ligands to characterize the adenosine A_{2A} receptor have been scarce, unlike A₁-selective ligands. Only quite recently truly A₂ selective agonists have become available (Francis et al., 1991; Ueeda et al., 1991a, 1991b; Niiya et al., 1992a, 1992b), whereas A₂ selective antagonists were still lacking. With the disclosure of 8-styrylxanthines as A₂-selective antagonists, however, this gap has been largely filled now (Shimada et al., 1992; Jacobson et al., 1993; for some examples of structures see also Fig. 3). The receptor itself has been subjected to chemical modification from which two histidine residues (indeed present in the transmembrane domains, viz. helices VI and VII) appeared important for ligand binding (Jacobson et al., 1992). This recent, novel information together with our previous experience with modeling the adenosine A₁ receptor, convinced us such a receptor modeling approach was timely for the adenosine A_{2A} receptor. The model, as developed in this report, is not meant to represent the definitive structural characterization of the receptor. Hopefully, however, it will generate tangible concepts dealing with ligand binding and receptor activation, to be validated by further experimentation.

2. Computational methods

All model building, docking, energy minimizations and molecular dynamics calculations were carried out using the software package BIOGRAF, versions 2.2 and 3.1 (Molecular Simulations, Waltham, MA, USA). All manipulations were performed on a Silicon Graphics 4D/25GT workstation.

2.1. Homology model building

The nucleotide sequences for the adenosine receptors, other G protein-coupled receptors, and bacteriorhodopsin were accessed from the GenBank data base. The implied amino acid sequences were generated and further sequence analysis was performed with the aid of the Sequence Analysis Software Package (Devereux et al., 1984) running on a Convex C-240 computer. The prediction of the transmembrane sections was based on the Kyte-Doolittle method (1982) with a 9-residue window. Multiple sequence alignment was performed with the pile-up program in the Sequence Analysis Software Package, with no gaps within the transmembrane domains allowed. The rationale behind this limitation is that these residues which are conserved throughout a family, and certainly between closely related receptors like the adenosine receptor subtypes, are likely to serve similar functions. The introduction of only a one-residue gap would already cause a major spatial reorientation (a 100° shift) of the residues to follow.

A three-dimensional model structure of the seven transmembrane domains of the rat adenosine A_{2A} receptor was constructed by using the atomic coordinates of the bacterial protein bacteriorhodopsin (Brookhaven Protein Data Bank, code 1BRD; Henderson et al., 1990). The amino acid residues of the seven transmembrane α -helices were 'mutated' on the computer screen to provide an initial structure of the adenosine A_{2A} receptor. It should be mentioned here that there is virtually no amino acid homology between bacteriorhodopsin and G protein-coupled receptors. However, all helices in both proteins are of similar size, and we used this feature for the alignment of helical residues in the adenosine A_{2A} receptor (Fink et al., 1992) with those present in 1BRD as follows (bacteriorhodopsin/adenosine A_{2A} receptor): helix I: 10–32/5–27; helix II: 39–61/41–63; helix III: 79–100/74–95; helix IV: 107–127/118–138; helix V: 137–157/171–191; helix VI: 168–191/230–253; helix VII: 202–225/262–285. Although other, slightly different alignments are conceivable, the further manipulations with the α -helices of the adenosine A_{2A} receptor (rotations, energy minimizations and molecular dynamics) allow the amino acid side chains to 'scan' ample space in the receptor architecture. Hence, the initial, rather arbitrary, alignment appeared to be of little importance for the eventual model.

Subsequently, the helices of the adenosine A_{2A} receptor were rotated in such a way that most conserved, most hydrophilic (in particular the histidine residues in helices VI and VII), and all charged residues were located at the 'inside' of the receptor. Rotations were constrained to spin about the helical axes as defined by the 1BRD structure. Translational motions were not allowed. The orientation of the helices relative to each other remained virtually unchanged during this process. The rationale here was that hydrophobic residues, now mainly located at the outside of the receptor, will interact with the lipid environment.

2.2. Energy minimizations and docking of ligands

Throughout all the calculations default values for the various parameters in the BIOGRAF molecular mechanics Dreiding force field (Mayo et al., 1990) were used. The Dreiding force field does not employ a distance-dependent dielectric constant (ϵ). The default value for ϵ was 1.0, unless indicated otherwise. Hydrogen atoms bound to non-chiral carbon atoms were not represented explicitly, in order to save computer time. Hydrogen atoms bound to chiral carbon atoms (i.e., C α -atoms, and those on threonine and isoleucine residues), however, had to be included. Otherwise, correct stereochemistry could not always be preserved during minimization and dynamics procedures. Conjugate gradient energy minimizations were continued until the rms energy gradient was less than 0.1 kcal/mol.Å. First, the initial adenosine A_{2A} receptor structure as described above was energy minimized with all peptide backbone atoms kept fixed. In this way unfavorable nonbonded contacts (cut off distance: 9Å) were eliminated from the side chains of the amino acid residues, and the adenosine A_{2A} receptor proline residues were allowed to adopt a configuration as appropriate as possible. Second, the freely movable atoms were relaxed by a 25 ps (adiabatic) molecular dynamics run. A constant temperature of 300K was maintained during the runs by velocity scaling. A time step of 0.001 ps was used, with updates of the nonbonded pair list after every 50 time steps. Eventually, another energy minimization was applied, resulting in an optimized receptor model with the overall appearance of bacteriorhodopsin. A similar strategy was used in the presence of ligands.

SHA 174 (2-(cyclohexylmethylidenehydrazino)adenosine; Fig. 3) was used as ligand of choice for docking purposes, since it combines high potency with a rather rigid side chain compared to other known A₂ selective agonists (Niiya et al, 1992a). Its structure was built and minimized, starting from the crystal structure of adenosine, as retrieved from the Cambridge Crystallographic Database (Allen et al., 1979). The other compounds used in the present study were also built and energy minimized in BIOGRAF.

The energy values were calculated as follows: $E_{\text{interaction}} = E_{\text{complex}} - (E_{\text{receptor}} + E_{\text{ligand}})$. As an example, data for the preferred complex of the receptor and SHA 174 were: $-71 \text{ kcal/mol} = 269 \text{ kcal/mol} - (255 + 85) \text{ kcal/mol}$. In this calculation, the receptor stands for the amino acids that have (some of) their atoms within 4 Å from the ligand. The energy values do not correspond to the 'real' energetic values in an absolute way. They can only be compared to each other in terms of 'more' or 'less' favorable states. The energy values cannot be used to calculate exact values of affinities between molecules out of interaction energies, since changes in entropy and solvation effects are not taken into account.

3. Results

3.1. Adenosine A_{2A} receptor topology

The alignment of the rat adenosine A_{2A} receptor with other rat adenosine receptor subtypes as well as some other G protein-coupled macromolecules (the rat m3 muscarinic acetylcholine receptor and rhodopsin) is shown in Fig. 1. Only the seven helical domains are given (H I–H VII). Several residues are identical within the adenosine receptor subclass only, such as a glutamic acid residue (E) in helix I, and a histidine (H) in helix VII. Other residues are present in virtually all G protein-coupled receptors, e.g., an aspartic acid (D) in helix II.

A two-dimensional representation of the adenosine A_{2A} receptor is given in Fig. 2. Three extracellular and three cytoplasmic loops connect the transmembrane domains. The N-terminus is located on the extracellular side, whereas the C-terminus is in the intracellular compartment. A more detailed analysis of structure-function relationships for the adenosine receptors, e.g., sites for glycosylation, phosphorylation and G protein interaction, has been described elsewhere (Van Galen et al., 1992).

For the construction of a three-dimensional model of the adenosine A_{2A} receptor we used the atomic coordinates of bacteriorhodopsin (see Methods). Since no data for the non-membrane parts of this protein (i.e., loops and tails) are available, we refrained from modeling equivalent parts of the adenosine A_{2A} receptor. There are as many prolines in the adenosine A_{2A} receptor transmembrane domains as in the corresponding regions in bacteriorhodopsin (5 in both proteins), but some on different positions, which renders the topology of the A_{2A} receptor slightly different from bacteriorhodopsin, since prolines may act as 'helix benders'. Due to the procedure followed ('mutation' with subsequent minimization), however, the adenosine A_{2A} receptor retains the overall appearance of bacteriorhodopsin, with only slight changes in helical architecture.

3.2. Docking of A₂ selective agonists

The A₂ selective compound SHA 174 (Fig. 3) was used as a starting ligand for docking purposes. It was preferred over the reference A₂ selective agonist CGS 21680 (Fig. 3), since the latter is conformationally far less constrained in the C2-side chain. From energy minimizations with molecular mechanics on SHA 174 only, two main conformations emerged, one with the C2-side chain oriented 'upwards', i.e., in the direction of the exocyclic amino function (N⁶) on the purine ring ($E = 75$ kcal/mol), the other with the C2-side chain pointing 'downwards' ($E = 71$ kcal/mol). Although the receptor bound conformation might differ from these two, they were used as starting conformations. Since the two histidine residues in helices VI and VII are important for ligand binding in both the adenosine A₁ and the adenosine A_{2A} receptor, it was reasoned that the model derived for the adenosine A₁ receptor-ligand interaction could be used as a starting point as well. Thus, SHA 174 was docked in the adenosine A_{2A} receptor topology similar to the docking of N⁶-cyclopentyladenosine in the adenosine A₁ receptor architecture (see also Computational methods). It readily turned out that SHA 174 with its C2-side chain pointing downwards (i.e., to the intracellular side of the protein) could not be accommodated in that case by the receptor. The cavity within the receptor has funnel-like characteristics, just as in bacteriorhodopsin, the diameter of the pore at the extracellular side being larger than at the intracellular side. An energetically favorable orientation could be obtained with the C2-side chain pointing to the extracellular side of the protein, close to helices III, IV and V. Subsequent molecular dynamics calculations allowed a further 'fine-tuning', on which all figures and examples of calculations are based. Fig. 4 is a representation of SHA 174 surrounded by the seven α -helices (backbones shown only), as seen from the extracellular side. Fig. 5 shows the details of the interaction between SHA 174 and the two histidine residues in helices VI and VII, respectively. The non-protonated nitrogen atom of His²⁴⁵ forms a hydrogen bond with the exocyclic N⁶-H in SHA 174. His²⁷³ may form a double hydrogen bond, namely with the 2'- and 3'-OH groups in ribose as proton donors. The ligand binding site on the adenosine A_{2A} receptor (arbitrarily defined as the amino acid residues with atoms within 4 Å from SHA 174) is represented as a stereo drawing in Fig. 6. Apart from the histidines there are other hydrophilic residues in close proximity to the hydrophilic purine and ribose moieties of SHA 174. The amino group of the carboxamide function in Asn²⁴⁸ interacts with N1 of the purine ring system. The non-protonated nitrogen atom in the (hydrophilic) hydrazino part of the C2-side chain of SHA 174 interacts with the OH group in Ser¹²⁹. Finally, Ser²⁷⁶ and the 5'-OH group of the ribose moiety appear to interact, again via hydrogen bonding. In contrast, the hydrophobic cyclohexyl group of SHA 174 is indeed in a lipophilic environment. Ile¹³², Phe¹⁷⁷ and Cys²⁴⁹ surround the cyclohexyl group within a distance of 4 Å. Upon binding, both the receptor and SHA 174 change their conformation in order to maximize the interaction strength. Since we started the docking procedure with the receptor and the ligand in their energetically optimized conformations, these changes led to increases in intramolecular energy content, namely 25 kcal/mol for the receptor and 10 kcal/mol for SHA 174. The interaction energy of the complex is -71 kcal/mol, resulting in a decrease in total van der Waals repulsion energy of 36 kcal/mol.

Similarly, A₂ selective agonists with longer C2-substituents were docked into the receptor. Fig. 7 shows how PAPA-APEC (see Fig. 3), a functionalized congener of CGS 21680, might

interact with the adenosine A_{2A} receptor model. Its extended substituent easily ‘climbs up’ the cavity to reach the extracellular space with little or no steric hindrance. Also CGS 21680 itself can be accommodated by the receptor. Its carboxylic terminus might have some favorable interaction with Cys²⁴⁹. A structurally less related A₂ selective agonist, N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl] adenosine (DPMA, see Fig. 3), interacts favorably as well (Bridges et al., 1988). Both *R*- and *S*-isomers, which differ only slightly in their A₂ receptor affinity, are readily tolerated by the adenosine A_{2A} receptor model. In this case the two phenyl substituents on the asymmetric carbon atom in the N⁶-substituent can be interchanged to yield the two stereoisomers.

3.3. Docking of A₂ selective antagonists

Very recently, a new series of A₂ receptor-selective antagonists has been described (Shimada et al., 1992; Jacobson et al., 1993). The most selective member of this class, all 8-styrylxanthines, is 8-(3-chlorostyryl)caffeine (CSC, see Fig. 3). The conformational characteristics of this compound allow ample interactions within the agonist receptor binding domain. The rather hydrophilic xanthine moiety is surrounded by largely hydrophilic residues. Trp²⁴¹, by virtue of its indole N-H fragment, may favorably interact through hydrogen bonding to the carboxy function (C6 = O) in the caffeine analog. Also, His²⁴⁵, Asn²⁴⁸ and His²⁷³ are in close proximity. The hydrophobic 3-chlorostyryl substituent is buried in a hydrophobic domain, consisting of Ile¹³², Tyr¹⁷⁴, Phe¹⁷⁷, Cys²⁴⁹, and Phe²⁵². This substituent has considerable overlap with the N⁶-substituent in the A₂ selective agonist DPMA (see above).

3.4. Receptor activation vs. receptor binding

With the docking of both agonists and antagonists to receptors, the question arises of how activation of the receptors by agonists could proceed. Since the basic difference between adenosine receptor agonists and antagonists resides in the presence of a ribose moiety, it is conceivable that the histidine–ribose interaction as described above could play a role in adenosine receptor activation. Merely as a speculation, the possibilities of proton transfer from the ribose group to the histidine residue in helix VII (His²⁷³) were mimicked by analyzing the methanol-4-methylimidazole interaction as a model. In Table 1 energy data according to the DREIDING II force field are gathered that describe two extremes, viz. the uncharged methanol-4-methylimidazole complex versus the charged (but neutral) methanolate-4-methylimidazolium complex. As an example (dielectric constant $\epsilon = 5$, a value generally considered relevant to a protein environment), the gain in interaction energy for the uncharged complex is 6.4 kcal/mol (1.9 + 14.8 – 10.3), and 10.7 kcal/mol for the charged one. Moreover, the charged complex appears to be more stable (8.3 kcal/mol) than the uncharged one (10.3 kcal/mol). Apparently, the transfer of a proton in this typical interaction is possible, thereby drastically changing the electrostatic characteristics of the groups involved. Calculations at a higher level of sophistication (semi-empirical or ab initio approach) proved unsatisfactory. The interaction between the two charged species (methanolate and 4-methylimidazolium) is extremely improbable, due to the implicit *in vacuo* conditions that apply to these calculations. By the inclusion of explicit water molecules, shielding the two opposite charges, such calculations might be done, but these were considered to be beyond the scope of this study.

4. Discussion

4.1. Adenosine A_{2A} receptor topology

The sequence alignment of G protein-coupled receptors is a matter of some debate. The pivotal suggestion that this important receptor family bears structural homology to bacteriorhodopsin, a proton pump present in the cell wall of *Halobacterium halobium*, has been an impetus to our current understanding of receptor structure. On the other hand, the same suggestion has caused considerable confusion since there is virtually no sequence (i.e., amino acid) homology between the two types of proteins.

Nowadays, with over 300 members of G protein-coupled receptors cloned and sequenced, there is little need to rely on the amino acid sequence of bacteriorhodopsin. The class in itself includes ample information on, e.g., conserved residues within subclasses, likely boundaries of transmembrane domains etc. As an example, the occurrence of an aspartic acid residue halfway the third α -helix is typical for cationic neurotransmitter receptors. It will take some time, however, to process all intrinsic, 'hidden' information from these primary sequences in a statistical way in order to derive more definitive clues concerning receptor structure and function.

From the selection of sequences in Fig. 1, it appears that adenosine receptor subtypes share residues that are conserved in all G protein-coupled receptors as well as amino acids that are typical for this particular receptor class. A prominent example of a residue unique to all adenosine receptors is a histidine residue located midway on helix VII. In helix VI an additional histidine residue occurs that is fairly specific for adenosine receptors as well. It is also present in some peptide receptors (e.g., receptors for tachykinins, bombesin and endothelin), and appears to be missing (i.e., substituted for a serine) in the A₃ receptor subtype (Zhou et al., 1992). These authors found that prototypic adenosine receptor agonists still bind to this receptor, whereas antagonists – mainly xanthines – do not, a further indication that the two histidines are involved in ligand binding.

The atomic coordinates (3D structure) of bacteriorhodopsin have been determined experimentally (Henderson et al., 1990). However, its structural map has a resolution of 3.5 Å at the best. Also, the Kyte-Doolittle algorithm used to determine the outer boundaries of the helices in the adenosine A_{2A} receptor (and, hence, their size) is rather crude, which may warrant adjustments in the future (Fasman and Gilbert, 1990). Nevertheless, the helices in bacteriorhodopsin as derived from the 3D structure are similar (not identical) in size to the ones in the adenosine receptors according to the Kyte-Doolittle method. In the bacteriorhodopsin structural data file neither intra- nor extracellular domains are present, although they make up a large part of the protein molecule. Obviously, the nature and size of tails and loops will influence overall receptor architecture, and, as a consequence, the ligand binding site, to an extent that is unknown. The packing of the helices (both in bacteriorhodopsin and in the receptor model), however, is very tight, enabling even the smallest loops to span the distance between two helices.

Despite these uncertainties it is our firm belief, also for reasons discussed in greater detail earlier (IJzerman et al., 1992) that the structure of bacteriorhodopsin could serve as a

template to construct 3D models of G protein-coupled receptors. Here, we should like to repeat that one should be cautious in doing so, although some quite stimulating results have been reported so far (Lewell, 1992; Nordvall and Hacksell, 1993). In the present study on the adenosine A_{2A} receptor, it appeared that, as for the adenosine A₁ receptor, a bacteriorhodopsin-like structure is very well compatible with all the different amino acids that are present in the adenosine A_{2A} receptor. Such a model also allows the visualization of a putative ligand binding site and suggests some possibilities for activation of the receptor, which will be discussed below. Very recently, Henderson and co-workers reported on the 3D structure of mammalian rhodopsin, which shares greater sequence homology with (other) G protein-coupled receptors. Its overall appearance is that of bacteriorhodopsin, although the helices might be more tilted relative to each other. The spatial characteristics of the retinal binding domain, being more or less in the middle of the pore, are not very different between rhodopsin and bacteriorhodopsin. Unfortunately, no atomic coordinates are yet available, due to the low resolution of the rhodopsin structure (Baldwin, 1993; Schertler et al., 1993).

4.2. The ligand binding site

Both agonists and antagonists that are A₂ selective have been docked into our adenosine A_{2A} receptor model. Although the pore formed by the seven helices is rather large, the combined structural information of the various ligand classes, left only limited ambiguity with respect to possible binding modes. Furthermore, the biochemical evidence, that also on the adenosine A_{2A} receptor, the two histidine residues mentioned above are critical for ligand interaction, led to a model that is largely compatible to the one derived for the adenosine A₁ receptor. The discovery that SHA 174, a conformationally relatively restricted compound, is a potent and A₂ selective agonist, proved to be of great value here, since its C2 side chain could only be directed into a hydrophobic pocket of similar (but not identical) nature as had been found for the so-called N⁶ region in the adenosine A₁ receptor. This finding could also explain why ligands in which both N⁶ and C2 substituents have been introduced, do not have higher affinities than the monosubstituted analogs (Thompson et al., 1991).

Some N⁶-substituted agonists, such as DPMA, are A₂ selective too. The bulky N⁶ substituent of this compound may very well overlap with the styryl moieties in C8-substituted xanthines that are also A₂ selective. This provides evidence for the general validity of the so-called N⁶/C8 model (Van der Wenden et al., 1992) to explain the competitive interaction between agonists and antagonists on both adenosine A₁ and A_{2A} receptors.

The two histidine side chains, together with some other hydrophilic residues, are close to the purine-ribose moiety as present in all agonists. Analogous to our adenosine A₁ receptor model, His²⁷³ (helix VII) interacts with the two hydroxyl groups in the ribose via hydrogen bonding, whereas His²⁴⁵ (helix VI) is near the exocyclic amino group (N⁶) in the purine base, as is Asn²⁴⁸. From the N⁶/C8 model it follows that His²⁴⁵ is also important for antagonist binding, whereas His²⁷³ is not. This is in good agreement with the finding that the absence of a histidine in helix VI, as in mutated adenosine A₁ receptors (Olah et al., 1992) or in the wild-type A₃ receptor (Zhou et al., 1992), precludes antagonist binding. The proposed interactions with the histidine residues are only feasible with the unprotonated

form of these amino acids. In a very recent report addressing this issue, Allende et al. (1993) concluded that the pK_a values of the histidines involved could be between 6.0 and 7.4. This suggests that at physiologic pH the majority of the histidine residues would, indeed, be unprotonated upon binding.

4.3. Selectivity with respect to other adenosine receptor subtypes

Interestingly, the hydrophobic cyclohexyl group in SHA 174 interacts with Ile^{132} , just like the cyclopentyl group in CPA (N^6 -cyclopentyladenosine) interacts with the corresponding Val^{138} in the adenosine A_1 receptor. This apparently small and minor difference might induce A_1/A_2 receptor selectivity in compounds. A similar 'switch', through point mutation studies, from Leu to Val in cholecystokinin receptors from different species was proven entirely responsible for ligand selectivity (Beinborn et al., 1993). On the other hand, subtle differences in overall architecture (e.g., a different 'tuning' of the seven helices) could also account for ligand/receptor selectivity. In this respect, as has been pointed out by Jacobson et al. (1993), the adenosine A_{2A} receptor might have as many as four disulfide bridges between and within the extracellular loops, thereby limiting the conformational freedom of the connected transmembrane domains in the adenosine A_{2A} receptor compared to the adenosine A_1 receptor.

Although less extensively studied, the differences in ligands' affinities for adenosine A_{2A} and A_{2B} receptors are equally remarkable. In particular, adenosine analogs with large substituents in the 2-position were highly selective agonists for adenosine A_{2A} receptors, as was the antagonist 8-(3-chlorostyryl)caffeine (Brackett and Daly, 1994). In the ligand binding site, as defined in Fig. 6, there are two differences between adenosine A_{2A} and A_{2B} receptors. Ser^{129} is substituted for an alanine residue in the adenosine A_{2B} receptor, as is Leu^{244} for a valine. Since Ser^{129} is close to the C2-side chain of the agonists, this residue might indeed be pivotal for A_{2A}/A_{2B} selectivity.

4.4. Receptor activation

As has been pointed out in Results, His^{278} coordinates the ribose group present in adenosine receptor agonists. When both ribose-hydroxyl groups are removed, as in 2',3'-dideoxy- N^6 -cyclohexyladenosine (Lohse et al., 1988), receptor activation is no longer observed, the compound effectively being an antagonist with modest affinity. In this particular case, the double hydrogen bond between the ribose and the histidine is completely lost, suggesting that adenosine receptor agonism has at least part of its molecular origin here. Are there additional lines of evidence? In both bacteriorhodopsin and mammalian rhodopsin a lysine residue is at the corresponding position on helix VII. This lysine residue can form a protonated Schiff's base linkage with the retinal chromophore. Subsequently, proton transfer may take place, as the subsequent trigger of the opsins' activation process (Khorana, 1988; Khorana, 1992). Additional histidine residues appear to be involved too, which are thought to be effective upon their protonation (Weitz and Nathans, 1992). Hibert and co-workers (Hibert et al. 1991; Trumpp-Kallmeyer et al., 1992), in a thorough analysis of their receptor modeling efforts, noticed that in cationic neurotransmitter receptors a tyrosine residue, again in the same position in helix VII, could be involved in the activation and signal transduction

of these G protein-coupled receptors. In conclusion, there is convergent evidence for a role of His²⁷⁸ in adenosine receptor activation.

Could proton transfer play a role as a trigger to this process on the adenosine receptor, as it does in the opsins? The energy data in Table 1 show that a proton transfer from ribose to histidine is feasible. It should be stressed here that the force field employed can only yield a rough approximation of the two extremes in this gradual process of proton translocation. More important, however, is the question whether such processes do occur in 'biochemical reality'. An intriguing parallel is seen with the functioning of the catalytic triad in serine proteases, a large and diverse enzyme family. The triad usually consists of the residues Asp-His-Ser, catalyzing the hydrolysis of peptide bonds. The serine's hydroxy group is always within hydrogen-bonding distance of the (nonprotonated) nitrogen of the imidazole ring system of the histidine residue. The other (protonated) ring nitrogen atom is hydrogen-bonded to the carboxylate moiety of the aspartic acid residue (Branden and Tooze, 1991). Speculatively, the ribose group could serve a similar purpose as the serine does, also since 2'- or 3'-deoxyribose (even more resembling a serine) replacing an intact ribose in adenosine derivatives, leads to receptor activation. As an example, 3'-deoxy-R-PIA is a fairly potent adenosine receptor agonist (Taylor et al., 1986).

Which carboxylate group in the receptor may be available for proton transfer? There are only two carboxylic acid groups in the transmembrane regions of adenosine receptors. One is an aspartate in helix II that is present in (virtually) all G protein-coupled receptors and indeed implicated in regulatory aspects of receptor activation, e.g., modulation by Na⁺ ions. However, due to its presence in all receptors it is improbable that this residue is directly linked to the histidine that occurs in adenosine receptors only. The other acidic residue is a glutamate in helix I (Glu¹⁰ in the adenosine A_{2A} receptor), that is entirely specific for the adenosine receptors. This glutamate residue is in the transsectional plane of the ribose-OH groups and His²⁷³, and can be brought in the vicinity (within hydrogen-bonding distance) of the histidine residue. A putative Asp/Glu-His hydrogen bond would resemble such bonds observed in the crystal structures of many proteins (Christianson and Alexander, 1990). Finally, energy calculations on the catalytic triad, more elaborate and sophisticated than the one described in the Results section, also strongly supported a (single) proton transfer mechanism with concomitant electrostatic stabilization through the aspartic acid residue (Warshel et al., 1989).

5. Conclusion

In this report we described a model for the ligand binding site on the adenosine A_{2A} receptor. Two histidine and two serine residues, all hydrophilic residues, coordinate the hydrophilic purine riboside. The C2 substituents are generally accommodated in a hydrophobic region that is resembling the so-called N⁶ region found on the adenosine A₁ receptor. Particular, although speculative, attention was paid to the possible mechanism of receptor activation. Some analogies with the catalytic triad in serine proteases were drawn, with suggestions for further biochemical (e.g., point mutation) experiments. Hopefully, combined efforts in the apparently rather distinct disciplines of computational chemistry and

molecular biology, will lead to validation and optimization of the proposed model, with subsequent consequences for the more rational design of new chemical entities.

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H I		H II	
A1	AYIGIEVLIALVSVPGNVLVIWA	FIVSLAVADVAVGALVIPLAILI	
A2a	VYITVELAIAVLAILGNVLVCWA	FVVSLLAAADIAVGVLAIPIFAITI	
A2b	LYVALELVIAALAVAGNVLVCAA	FLVSLATADVAVGLFAIPFAITI	
A3	TYVTMEAAIGLCAVVGNNMLVIWV	FIVSLALADIAVGVLVIPLAIAV	
M3	FIAFLTGFALVTIIGNILVIVA	FLLSLACADLIIGVISMNLFETTY	
Rh	MLAAYMFLIMLGFPINFLTLYV	ILLNLAVADLFMVFGGFTTTLYT	
H III		H IV	
A1	CLMVACPVLIILTQSSILALLAIA	RAAVAIAGCWILSLVVGLTPMFG	
A2a	CLFFACFVLVLTQSSIFSLAIA	RAKGIIAICWVLSFAIGLTPMLG	
A2b	CLFLACFVLVLTQSSIFSLAVA	RARGIIAVLWVLAFGIGLTPFLG	
A3	CLFMSCVLLVFTHASIMSLAIA	RIWLFLGLCWLVSFVLVGLTPMFG	
M3	CDLWLSIDYVASNASVMNLLVIS	RRGVMIGLAWVISFVLWAPAILF	
Rh	CNLEGFFATLGGEIALWSLVVLA	HAIMGVAFTWVMALACAAPPLVG	
H V		H VI	
A1	MEYMVYFNFFVWVLPPLLLMVLIIYL	LALILFLFALSWLPLHIINCITLF	
A2a	MNYMVYFNFFAFVLLPPLLLMLAIYL	LAIIVGLFALCWLPPLHIINCFTFF	
A2b	MSYMVYFNFFGCVLPPLLIMMVIYI	LAMIVGIFALCWLPVHAINCITLF	
A3	LDYMVFFSFITWILIPLVVMCIYYL	LFLVLFLFALCWLPPLSIINFVSFY	
M3	EPTITFGTAIAAFYMPVTIMTILYW	LSAILLAFIITWTPYNIMVLVNTF	
Rh	NESFVIYMFVVHFIIPLIVIFFCYG	VIIMVIAFLICWLPYAGVAFYIFT	
H VII			
A1	SILIIYIAIFLTHGNSAMNPVYAFRI		
A2a	PWLMYLAIILSHSNSVVPFIYAYRI		
A2b	KWVMNVAILLSHANSVVPFIYAYRN		
A3	EIAMCLGILLSHANSMMNPVYACKI		
M3	KTYWNLGYWLCYINSTVNPVCYALCN		
Rh	PIFMTIPAFFAKTSAVYNPVIYIMMN		

Fig. 1.

A comparison of the transmembrane amino acid sequences of rat adenosine receptors (A₁, A_{2A}, A_{2B} and A₃) with the similar sequences of the rat m₃ muscarinic acetylcholine receptor and rhodopsin. No gaps were allowed in these segments. Nucleotide sequences were retrieved from the GenBank data base.

Fig. 2.

Two-dimensional representation of the rat adenosine A_{2A} receptor. The N-terminus is located on the extracellular side, and the C-terminus on the cytosolic side. Putative glycosylation sites are in the second extracellular loop.

Fig. 3.
Structures of various adenosine receptor ligands having high affinity at adenosine A_{2A} receptors.

Fig. 4.
The binding of SHA 174 to the rat adenosine A_{2A} receptor (direction of view from outside to inside the cell). The receptor backbone only is shown, without side chains.

Fig. 5.
The interaction of SHA 174 with the two histidine residues on helices VI and VII, respectively. Possible hydrogen bonds are indicated by dashed lines.

Fig. 6.
Stereo representation of the proposed SHA 174 binding site on the rat adenosine A_{2A} receptor. The amino acids, of which the side chains are shown only, have been numbered consecutively. For reasons of clarity the two histidines (see figure 5) have been omitted. The amino acids are: Ile¹³² (1); Cys²⁴⁹ (2); Asn²⁴⁸ (3); Phe¹⁷⁷ (4); Leu²⁴⁴ (5); Ser¹²⁹ (6); Trp²⁴¹ (7); Ser²⁷⁶ (8); Val⁸¹ (9); Leu⁸⁴ (10); Val²⁷⁷ (11). All amino acids are within 4 Å from SHA 174.

Fig. 7.
The binding of PAPA-APEC to the rat adenosine A_{2A} receptor (overview). Only the helical backbones are shown. Top of figure represents the extracellular side.

Table 1

Energy values (in kcal/mol, at different dielectric constants) for (i) methanol and 4-methylimidazole, and (ii) methanolate and 4-methylimidazolium, and their respective complexes

	$\epsilon = 1$	$\epsilon = 5$	$\epsilon = 10$
Methanol	8.3	1.9	1.1
4-Methylimidazol	9.2	14.8	15.5
Complex	9.2	10.3	10.4
Methanolate	0.0	0.0	0.0
4-Methylimidazolium	24.8	19.0	18.3
Complex	-8.8	8.3	10.4