A Steric and Electrostatic Comparison of Three Models for the Agonist/Antagonist Binding Site on the Adenosine A_1 Receptor

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Several models have been described in the literature to explain the similarity of interaction of adenosine receptor agonists and antagonists with the binding site of the receptor. Besides the superposition of the nitrogen atoms of adenosine and xanthine (the "standard" model), two other models have been described: one in which xanthine is rotated around its "horizontal" axis before superposition ("flipped") and one in which the adenosine N^6-region and the xanthine C8-region are superimposed ("N^6-C8"). In this study we compared the steric and electrostatic properties of these models. The flipped model tends to show higher percentages of overlap for the positive electrostatic potentials and the N^6–C8 model yielded predominantly a slightly higher overlap for the negative electrostatic potentials, although these differences were rather small. Since the N^6-region in adenosine and the C8-region in xanthine are coinciding in this model, the N^6–C8 model yielded a much larger overlap in van der Waals volume than the other two models. The N^6–C8 model seems therefore to be the more probable model, also because the interactive groups point in the same direction for both adenosine and xanthine analogues. We determined the geometries of both the adenosine N^6-substituents and the xanthine 8-substituents in earlier studies. The N^6–C8 model causes a coincidence of these separately determined conformations.

Introduction

Ligands for the two subtypes of adenosine receptors (A_1 and A_2) are able to induce a wide range of physiological effects. Adenosine agonists may be therapeutically applicable as hypotensives, antiarrhythmics, platelet aggregation inhibitors, anticonvulsants, and hypnotics.[1-5] All adenosine agonists designed up to now are structurally very similar to adenosine itself. Substitution at the N^6-position of adenosine (Figure 1a) enhances its affinity for the adenosine A_1 receptor and thus the A_1 selectivity of the ligand.[6] This has led to potent adenosine A_1 agonists, such as N^6-cyclopentyladenosine (CPA, Figure 1b). The enhancement of A_1 affinity by N^6-substitution is also dependent on the configuration of chiral substituents. *R*-PIA (N^6-1-phenyl-2-propyladenosine), for example, is 45-fold more potent than its stereoisomer *S*-PIA.[6]

The adenosine antagonists cause facilitation of the atrioventricular conduction, renal vasodilatation, stimulation of the central nervous system, and bronchodilation.[7] Adenosine receptor antagonists include a wide variety of chemical classes. The xanthines, of which theophylline (1,3-dimethylxanthine, Figure 1c) and caffeine (1,3,7-trimethylxanthine) are the prototypes, have been extensively modified to obtain compounds with high affinity. Affinity enhancement of xanthines can be obtained by alkyl substituents at the positions 1 and 3, and by substitution at the 8-position.[8] Substitution at the 8-position of xanthines enhances not only their affinity but also their A_1 selectivity.[9] An example of a xanthine antagonist with a high affinity for the A_1 receptor is 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, Figure 1d). Some other classes of adenosine antagonists are 9-methyladenines, [1,2,4]triazolo[1,5-*c*]quinazolines, 7*H*-pyrazolo[4,3-*d*]pyrimidin-7-ones, and 1*H*-imidazo[4,5-*c*]quinolin-4-amines.[10-13] Adenosine agonists and antagonists bind to the same receptor binding site.[14] Therefore a certain superposition of adenosine agonist and antagonist structures is conceivable and may represent the way the ligands bind to the receptor binding site with respect to each other. At first sight a superposition of the four nitrogen atoms of both ring systems seems most obvious (Figure 2a). We will refer to this superposition as the "standard" model. Recently two other models have been postulated. Van Galen et al. have designed a model, which we will call the "flipped" model.[15] The positions of atoms N1, N3, N7, and N9 of the xanthine ring coincide in this model with

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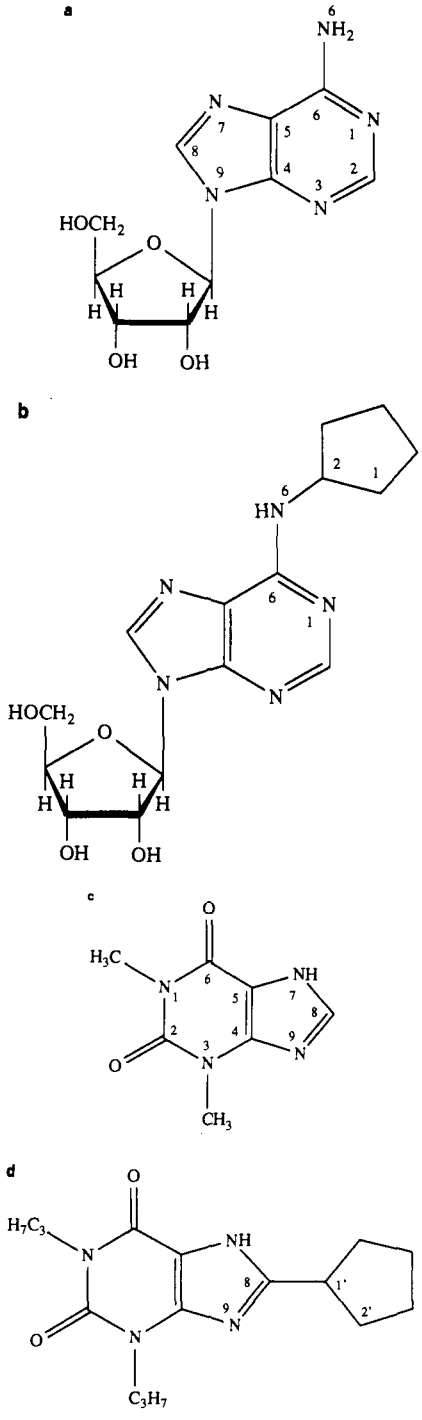

Figure 1. Four adenosine receptor ligands: (a) adenosine, (b) N^6-cyclopentyladenosine (CPA), (c) theophylline, and (d) 1,3-dipropyl-8-cyclopentylxanthine (DPCPX).

C2, C6, N9, and N7, respectively, in adenosine (Figure 2b). This model is based on 2-dimensional electrostatic, steric, and lipophilic similarities of these classes of ligands. Peet et al. have postulated a superposition in which the C8-substituents of xanthine analogues overlap with the N^6-substituents of adenosine.[16] In this model N1, N3, and

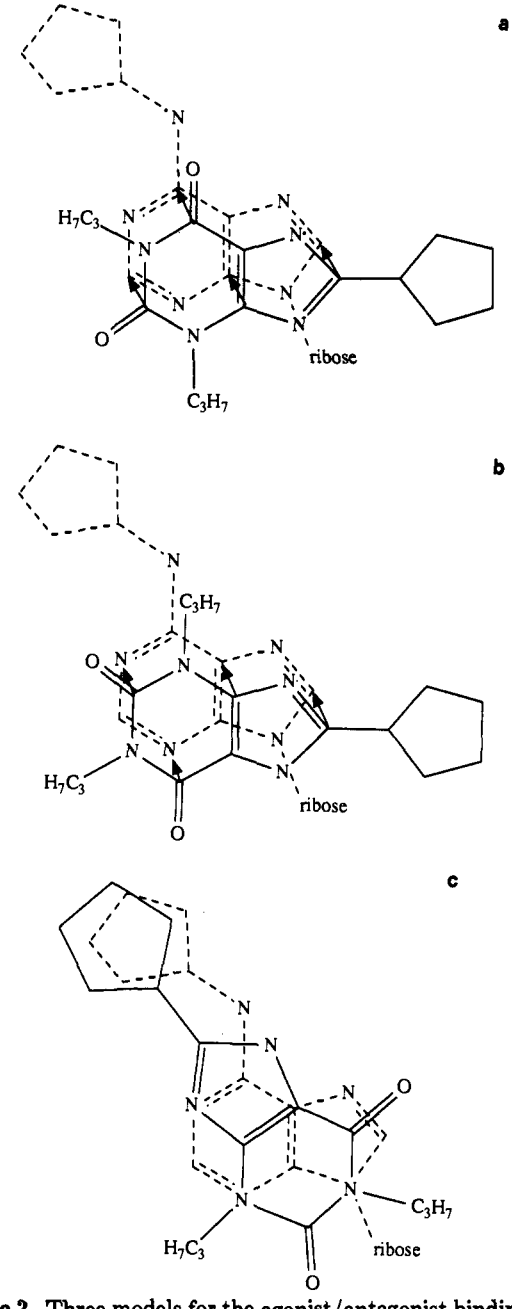

Figure 2. Three models for the agonist/antagonist binding site on the adenosine A_1 receptor: (a) standard, (b) flipped, (c) N^6–C8.

N9 of the xanthine ring coincide with N9, N3, and N1, respectively, of adenosine (Figure 2c). This model, which we will refer to as the "N^6–C8" model, is based on analogous (stereo)chemical requirements of groups at the adenosine N^6-position and xanthine C8-position and on the energy contour surfaces for a water probe.

The purpose of this study is to compare these three models with equal molecular modeling methods and to determine the (relative) likelihood of these three models.

Modeling

The three models mentioned in the introduction, i.e. standard, flipped, and N^6–C8, were compared in two respects: the similarity in van der Waals volume of the

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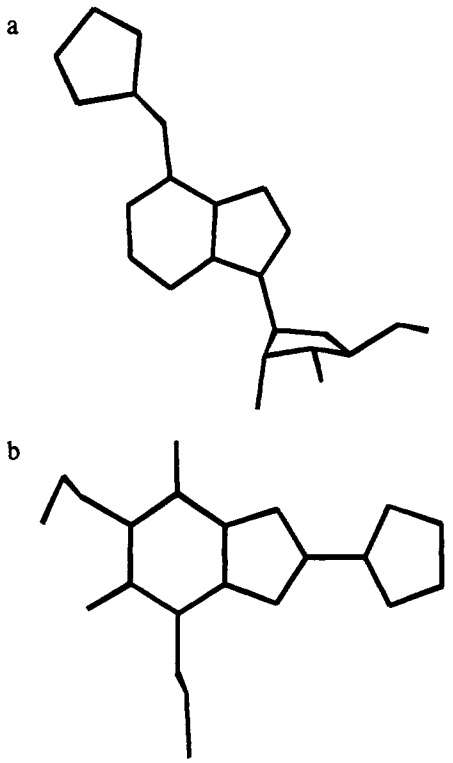

Figure 3. The ligand conformations used in this study (hydrogen atoms not displayed): (a) CPA in the −75° conformation at the N^6-position, determined by van Galen et al.,[17] (b) DPCPX in the C8-conformation of 330°, determined by van der Wenden et al.[18]

agonists and antagonists and their similarity in electrostatic potentials. The direction of interactive groups, such as groups that can give rise to formation of a hydrogen bond or to lipophilic interactions, was also considered.

The comparison of the three models by superposition of agonists and antagonist has been carried out for four pairs of ligands: adenosine/theophylline, N^6-cyclopentyladenosine (CPA)/1,3-dipropyl-8-cyclopentylxanthine (DPCPX), N^6-phenyladenosine/1,3-dipropyl-8-phenylxanthine, and N^6-benzyladenosine/1,3-dipropyl-8-benzylxanthine. The latter pairs, substituted at the N^6- or C8-position, were chosen, because the conformations of these substituents were available from computer graphic studies. Van Galen et al. determined the conformation of the adenosine N^6-substituents.[17] They ascertained the conformation of the N1–C6–N^6–C^2 dihedral angle to be + or −75 ± 10°, and the C6–N^6–C^2–C^1 dihedral angle 60 ± 5° (Figure 3a). We investigated the conformation of C8-substituents of xanthine analogues.[18] Thus, the torsion angle N9–C8–C1′–C2′ was determined to be approximately 220° for phenyl substituents and 330° for cycloalkyl substituents (Figure 3b).

Methods

The affinities of the N^6-substituted adenosine derivatives were determined by Daly et al.[6] with [^{3}H]CHA (N^6-cyclohexyladenosine) on rat cerebral cortical membranes. The mean K_i values[18] of the 8-substituted xanthine analogues were determined in membrane preparations of rat cerebral cortex or in whole rat brain with [^{3}H]*R*-PIA or [^{3}H]CHA as radioligands, as well as those determined in rat fat cells with [^{3}H]*R*-PIA as radioligand, were used. The affinities determined by those assays are similar.[19,20]

The programs used were run on a Vax 11/785 computer and a Convex C-120 minisupercomputer. A Pericom MX 7200 color display was used for visualization. The hardware and software used were made available by the CAOS/CAMM Center in Nijmegen, the Netherlands.

The atomic charges were calculated with the semiempirical molecular orbital program MOPAC,[21,22] version 4.10, based on AMPAC 1.0 and MOPAC 4.0. In MOPAC, the AM1 (Austin Method 1) Hamiltonian was used. MOPAC had also been used in the determination of the conformation of the N^6-region of adenosine[17] and the xanthine C8-region.[18] The structures were manipulated using the modeling package Chem-X (Chemical Design Ltd, Oxford, England) updated to the April 1990 version. Chem-X was also used in the calculation of van der Waals volume and electrostatic potential maps.

The method used to calculate the maps is described extensively in the Chem-X reference manual.[23] A short description of this method is outlined below. First, the maps are calculated for the separate superimposed structures. This is done by the construction of an imaginary grid (25 × 25) around the molecule. Initially, each grid point is assigned a value of zero. Subsequently, the value of the property due to the specified atoms is calculated at each point on the lattice and added to that point. The contours are calculated by interpolating between the points in the (display) lattice. These so-called simple maps are combined by logical (i.e. nonarithmetic) operations into complex maps, to compare the structures. A significance value is required for this operation to determine which values are "true" and which are "false". Thus the regions of similarity are calculated by a logical "and" operation on the simple maps, and the regions of dissimilarity are calculated by a logical "exclusive or" operation.

The corresponding regions of two molecules with an electrostatic potential (EP) of + and −5 kcal/mol will be calculated below as an example. The significance value should be 5 (kcal/mol) for this operation, because this is the value of interest. First, the EPs of both molecules are calculated separately. Subsequently, these values are compared for each grid point. This comparison is done by logically "anding" the EPs at the grid points, because we want to determine the regions of similarity. If both EPs at a grid point are larger than +5 or both smaller than −5 kcal/mol (the significance value), both values are "true"; thus the most extreme value is assigned to this grid point. Otherwise, i.e. if (at least) one molecule has an EP between −5 and +5 kcal/mol, or if the EPs have opposite signs, the value zero will be assigned to the grid point. The regions for which the EPs of both molecules meet the significance value will be shown if the EPs of + and −5 kcal/mol are drawn.

In this way, contours of spatial properties, like van der Waals volume (in Å^3) or EP energy (in kcal/mol) of the investigated structures are calculated and displayed. The maps are constructed 3-dimensionally around the molecules. The contours appear as cages on such a 3-dimensional map and the volumes of these cages are calculated.

We compared the electrostatic overlap of the models for six EPs, viz. 20, 10, 5, −5, −10, and −20 kcal/mol. For the comparison of the similar and dissimilar areas of volume and EP, we compared

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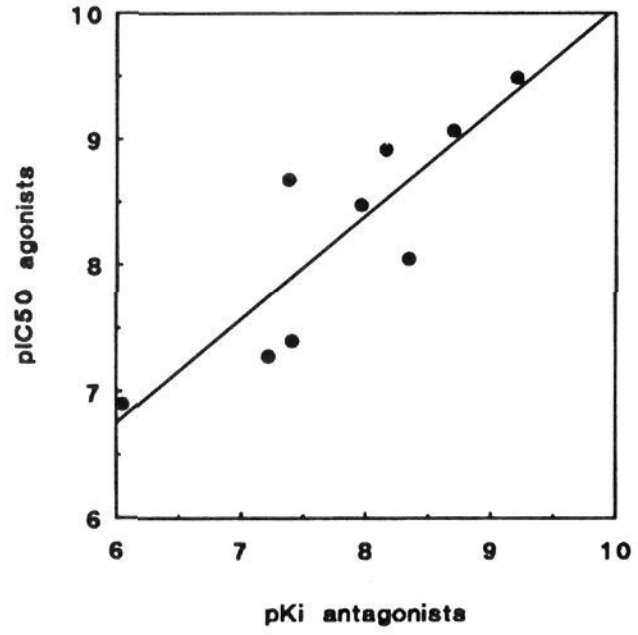

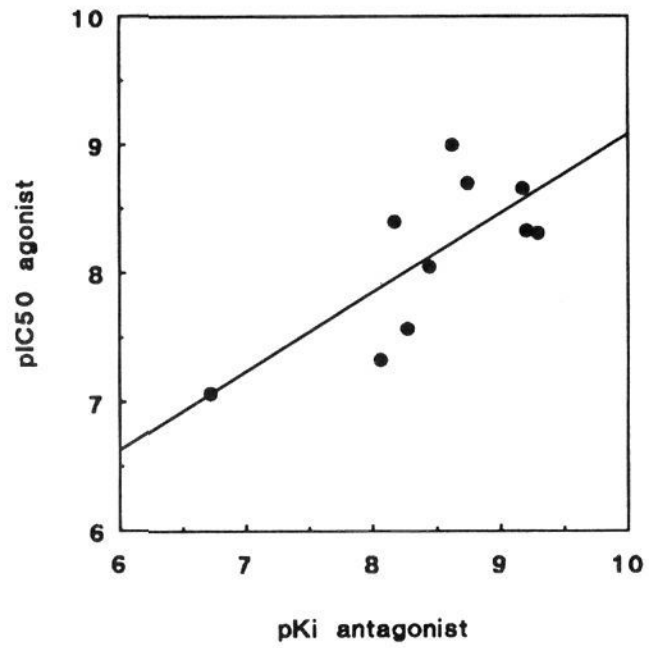

Figure 4. Linear regressions on agonist and antagonist affinities: (a) N^6-substituted adenosine and 8-substituted 1,3-dipropylxanthines (Table I); correlation coefficient = 0.86; slope = 0.82; intercept = 1.81. (b) N^6-Phenyl substituted adenosine and 8-phenyl substituted 1,3-dimethylxanthines (Table II); correlation coefficient = 0.74; slope = 0.61; intercept = 2.94.

the size of the volume of these areas. The absolute amount of overlap between two ligands was calculated by

$$\text{total overlap} = \text{common volume ligand}_1 + \text{ligand}_2 \text{ (in Å}^3\text{)}$$

The relative amount of overlap between two ligands was calculated by the following equation:

$$\%\ \text{overlap} = \frac{2 \times (\text{common volume ligand}_1 + \text{ligand}_2)}{(\text{volume ligand}_1 + \text{volume ligand}_2)} \times 100\%$$

Results

A high correlation beween the effects of adenosine N^6-substituents and xanthine C8-substituents on the affinity should be found if they bind the same receptor subregion. Therefore linear regression was carried out on the negative logarithms of the affinities of adenosines, substituted at the N^6-position,[6] and 1,3-dipropylxanthines, substituted at the 8-position,[16,18] with equal substituents (Table I). The variance of this linear regression, r^2, is 0.74 (Figure 4a); this means that the affinities show a tendency in which substituents have an equal influence on the affinity of adenosine agonists and xanthine antagonists. The logarithms of the affinities of N^6-phenyladenosines[6] and 1,3-dimethyl-8-phenylxanthines,[9] both substituted at the phenyl ring (Table II), were also examined by linear regression. This led to a variance of 0.55 (Figure 4b). The 1,3-dimethyl-8-phenylxanthines were used in the regression instead of the 1,3-dipropylxanthines, because sufficient

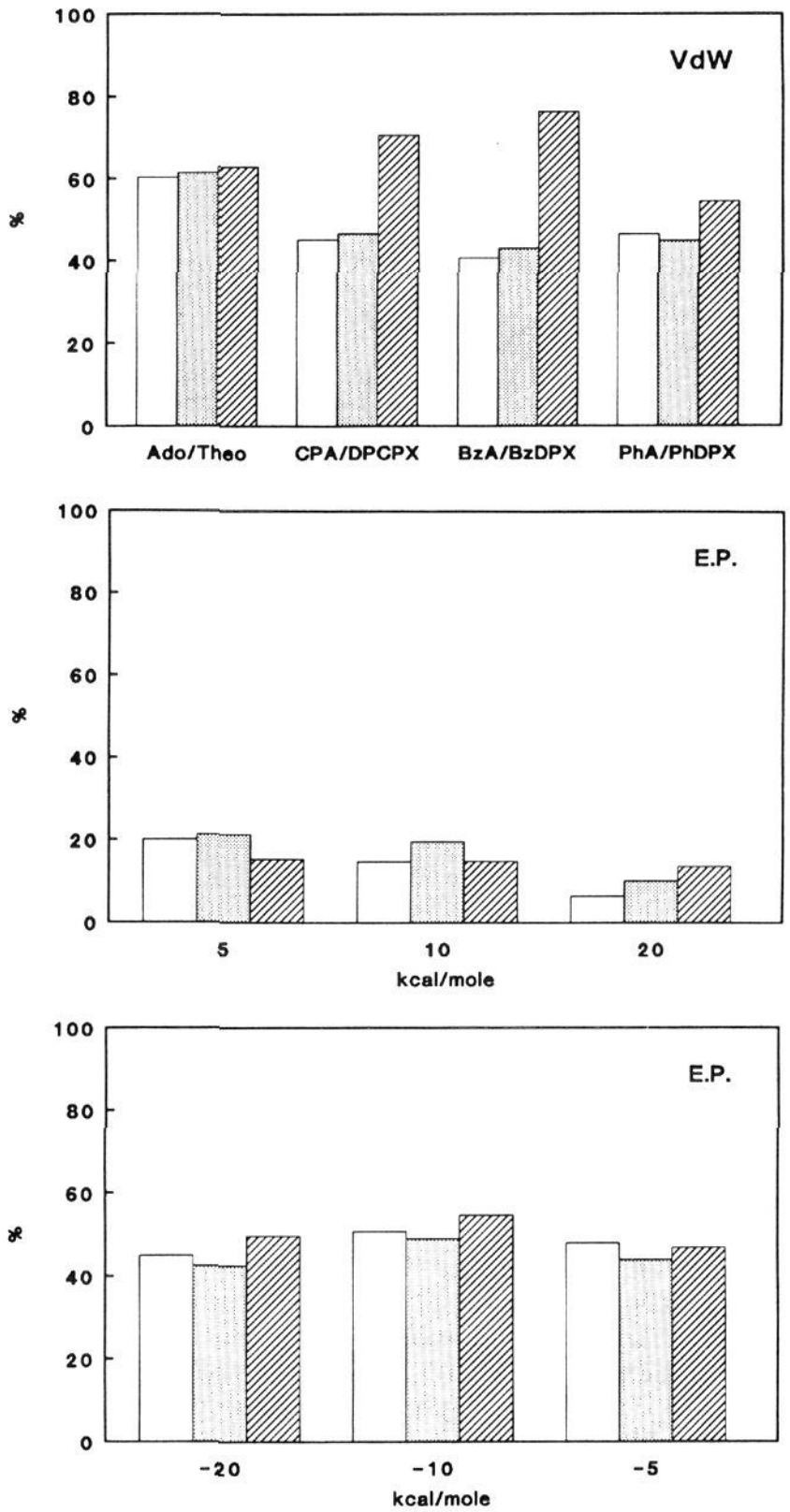

Figure 5. A comparison of the steric and electronic properties of the three agonist/antagonist binding site models (standard, white; flipped, dotted; N^6–C8, shaded): (a) Percentage overlap between the van der Waals volumes of four pairs of substituted adenosines and 1,3-dipropylxanthines (Ado = adenosine, Theo = theophylline, CPA = N^6-cyclopentyladenosine, DPCPX = 1,3-dipropyl-8-cyclopentylxanthine, BzA = N^6-benzyladenosine, BzDPX = 1,3-dipropyl-8-benzylxanthine, PhA = N^6-phenyladenosine, PhDPX = 1,3-dipropyl-8-phenylxanthine), (b) mean percentage overlap for the calculated positive electrostatic potentials (EP) (+5, +10, and +20 kcal/mol) of the four pairs of ligands, (c) mean percentage overlap for the calculated negative electrostatic potentials (EP) (−5, −10, and −20 kcal/mol) of the four pairs of ligands.

data on 1,3-dipropyl-8-phenylxanthine substituents are lacking. The rather low value for the variance indicates a differing influence of the phenyl substituents on the affinity of the adenosines and xanthines.

Van Galen et al. determined two possible conformations for the N^6-substituents.[17] The N1–C6–N^6–C^2 dihedral angle can have a value of + or −75 ± 10° according to their calculations. The N^6-substituted adenosines were fitted on the C8-substituted xanthines with the N1–C6–N^6–C^2 dihedral angle close to the −75° conformation, because this torsion angle coincides with the determined conformations of the 8-substituted xanthines in the N^6–C8 model. The

Table I. The Adenosine A_1 Receptor Affinities of N^6-Substituted Adenosines and 8-Substituted 1,3-Dipropylxanthines, Substituted with Equal Groups

substituent	pK_i of 8-substituted 1,3-dipropylxanthine	pIC_{50} of N^6-substituted adenosine
cyclopentyl	9.21[a]	9.49[c]
cyclohexyl	8.70[a]	9.07[c]
p-methoxyphenyl	8.34[a]	8.05[c]
(*R*)-1-phenyl-2-propyl	8.16[b]	8.92[c]
phenyl	7.96[a]	8.48[c]
methylcyclohexyl	7.41[a]	7.40[c]
cyclopropyl	7.38[a]	8.68[c]
(*S*)-1-phenyl-2-propyl	7.22[b]	7.28[c]
benzyl	6.05[a]	6.90[c]

[a] pK_i calculated from the mean K_i from several authors, taken from van der Wenden et al.[18] [b] pK_i calculated from the K_i, determined by Peet et al.[16] [c] pIC_{50}, determined by Daly et al.[6]

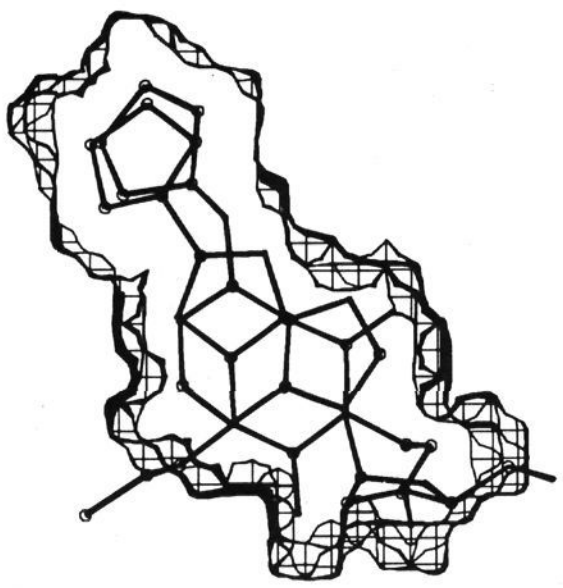

Figure 6. Opened lattice of the regions of similarity of the van der Waals volumes of CPA and DPCPX in the N^6–C8 superposition (hydrogen atoms not pictured).

comparison of the van der Waals volumes of the four pairs of ligands has led to the relative amounts of overlap, depicted in Figure 5a. The N^6–C8 model led to larger regions of coincidence than the other models. The three models show small differences in percentages overlap for the EPs. Which model shows the best overlap varies with the chosen EP. The mean percentages of overlap as calculated for the positive EPs for the four models are pictured in Figure 5b. It can be seen that the flipped model tends to show somewhat higher percentages of overlap for the positive EPs, except for the 20 kcal/mol EP, and the standard model tends to show percentages of overlap that are slightly lower. Figure 5c contains the mean percentages of overlap as calculated for the negative EPs. Here, the N^6–C8 model shows slightly higher percentages of overlap than the other two models, while those of the flipped model tend to be lower.

The N^6–C8 model has a larger overlap of van der Waals volumes of the ligands (Figures 5a and 6). This volume overlap was examined for all three models in further detail with respect to the groups overlapping, because the coinciding volume of the adenosine and xanthine analogues seems to be the major difference between the models. This analysis has been carried out for CPA and DPCPX. For all three models the main contribution to the overlap is the (partial) coincidence of the adenine and xanthine ring system. The heterocyclic moieties are corresponding for 89.8 Å^3 (67.1%), for the standard model, with a total overlap of 133.7 Å^3 (Figure 7a). The overlap between the nucleobase fragment and the xanthine core structure is 82.0 Å^3, 58.1% of the total overlap of 141.2 Å^3, for the flipped model (Figure 7b). The N^6–C8 model shows a

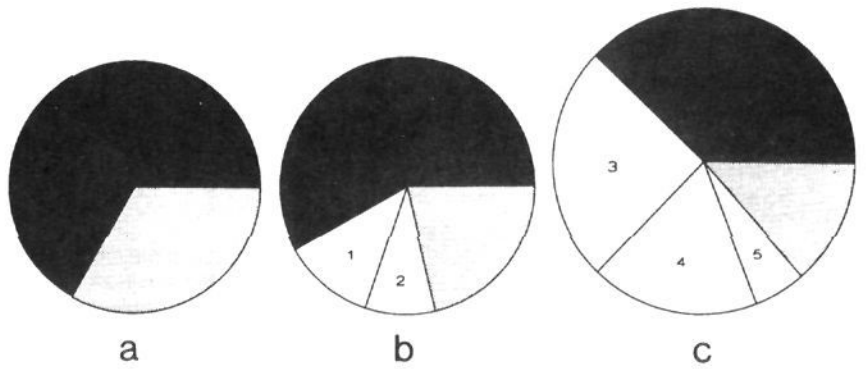

Figure 7. Distribution of the van der Waals overlap for the agonist/antagonist binding site models. The black regions represent the contribution of the adenine and xanthine ring systems, the dotted regions the unspecified smaller regions of overlap: (a) standard model, (b) flipped model, (c) N^6–C8 model for (1) 1-propyl of DPCPX/adenine ring system of CPA, (2) cyclopentyl ring of DPCPX/ribose moiety of CPA, (3) cyclopentyl rings of DPCPX and CPA, (4) 1-propyl of DPCPX/ribose moiety of CPA, (5) xanthine ring system of DPCPX/ribose moiety of CPA. The relative size of the circles is in proportion to the absolute amount of overlap for the models.

somewhat smaller coinciding of the ring systems: 76.5 Å^3, only 38.3% of the total overlap of 199.9 Å^3 (Figure 7c). Besides the overlap in heterocyclic systems, the standard model has only small contributions in overlap of other groups. The flipped model has two other considerable contributions to the overlap: the DPCPX 1-propyl group, corresponding with the adenine skeleton, and the overlap between the DPCPX cyclopentyl ring and the ribose ring of CPA. These regions are 16.5 Å^3 (11.7%) and 12.8 Å^3 (9.0%), respectively. The low percentage of coincidence for the ring systems for the N^6–C8 model indicates that other groups are very important in the overlap of the van der Waals volumes. The coinciding cyclopentyl moieties of CPA and DPCPX (an overlap of 49.9 Å^3, 24.9%) and the 1-propyl group of DPCPX overlapping the ribose ring of CPA (35.8 Å^3, 17.9%) contribute to the corresponding volumes. A somewhat smaller contribution is made by the overlap between the CPA ribose ring and the xanthine core structure of DPCPX, which accounts for 11.1 Å^3 (5.5%).

Discussion and Conclusions

The choice between the three models is not obvious per se, because it is not evident from both steric and electrostatic properties which model should be preferred. Therefore, it is a matter of debate as to which model is considered more "true". The most important factors that govern the binding of a ligand to a receptor are the sterical fit to the binding site of the receptor, the electrostatic complementarity of the ligands, i.e. the opposite EP of the receptor and ligands, the proximity of lipophilic regions, and the optimal interaction geometry for interactive groups. These factors will be considered consecutively below for the three models.

Clear differences between the three models can be seen in the overlap of the van der Waals volumes of the adenosine agonists and xanthine antagonists, at least when an adenosine N^6- and a xanthine C8-substituent are present. The standard model and the flipped model both produce considerably less overlap than the N^6–C8 model. Of course it has to be kept in mind that, by using similar substituents in this investigation, this result can be somewhat biased unintentionally. Adenosine and theophylline themselves hardly show any difference in overlap for the three models. The addition of the N^6-group to adenosine and the 1-, 3-, and 8-groups to xanthine provide the additional overlap of the N^6–C8 model. On the other hand, the conformations of the adenosine N^6-substituents and the 1,3-dipropylxanthine C8-substituents coincide so remarkably well that

Table II. Adenosine A_1 Receptor Affinities of Substituted N^6-Phenyladenosines and Substituted 1,3-Dimethyl-8-phenylxanthines, Substituted with Equal Groups

phenyl substituent	pK_i of 1,3-dimethyl-8-phenylxanthine[a]	pIC_{50} of N^6-phenyladenosine[b]
p-methyl	9.29	8.31
p-methoxy	9.20	8.34
p-chloro	9.19	8.66
p-fluoro	8.74	8.70
m-fluoro	8.62	9.00
o-methyl	8.44	8.05
m-methyl	8.27	7.57
o-fluoro	8.17	8.40
m-methoxy	8.06	7.33
o-methoxy	6.72	7.07

[a] pK_i calculated from the K_i, determined by Bruns et al.[9] [b] pIC_{50}, determined by Daly et al.[6]

this corresponding geometry causes the real contribution to the overlap. It should be borne in mind that the conformations of the adenosine N^6-substituents[17] and the xanthine 8-substituents[18] were determined separately, independent of each other.

The three models differ little in their electrostatic potential overlap. The standard and the N^6–C8 model seem to be slightly better than the flipped model for the negative EPs. Varying aspects can be seen for the positive EPs. The overlap is somewhat higher for the flipped model than for the other models, except for the N^6-benzyladenosine/8-benzyl-1,3-dipropylxanthine overlap; the N^6–C8 model is slightly preferred for those ligands. Thus, no choice can be made, based on the EP only.

The third aspect, the proximity of lipophilic regions, is better fulfilled in the N^6–C8 model. The lipophilic regions of the ligands correspond better in this model than in the other models, again by the overlap between the adenosine N^6-substituents and the xanthine C8-substituents. The lipophilic 1-propyl group, on the other hand, corresponds with the hydrophilic ribose moiety of adenosine and should be able to bind the same receptor subregion (see Figure 6). The influence of phenyl substituents should be equal for N^6-phenyladenosine and 1,3-dipropyl-8-phenylxanthine, if those phenyl rings coincide in the receptor binding site. However, the affinities of adenosine and xanthine analogues show little correlation (Table II), probably because those rings do not coincide completely but are somewhat shifted and turned with respect to each other. As a consequence of this turn, the direction in which the phenyl substituents point toward the receptor is somewhat different. Considering these aspects, the N^6–C8 model can also be preferred for the proximity of lipophilic regions.

The interactions between ligands and the adenosine A_1 receptor are probably governed by hydrogen bonds. Both the adenosine and the xanthine ring system have groups that can form hydrogen bonds, by donation or acceptance. Two groups have investigated the adenosine A_1 receptor by chemical modifications of amino acids and ligand protection experiments. Klotz et al. investigated the ligand binding site of adenosine A_1 receptors in rat brains.[24] Garritsen et al. explored those in calf brains.[25] Modification of histidyl residues with diethyl pyrocarbonate (DEP) induced a decrease of ligand binding. Klotz et al. found that the decrease of agonist binding could be prevented in part by the presence of an agonist, but not by an antagonist and vice versa. From these results it was concluded that the adenosine A_1 binding site should contain at least two histidine moieties in the ligand binding site, one involved in agonist binding and one involved in antagonist binding. With two different histidyl residues involved in binding the interacting, hydrogen-bond-forming, groups of agonists and antagonists do not have to point in the same direction. Both the standard and the flipped model direct the sites, which we determined to be the most probable sites for hydrogen-bonding interaction,[26] in different directions. Only in the N^6–C8 model both regions of the agonists and antagonists are positioned in the same direction. In this model the adenosine N^6-hydrogen and the xanthine N7-hydrogen, both possible hydrogen-bond donors, point in the same direction. In addition two possible hydrogen-bond acceptors, i.e. adenosine N7 and xanthine O6, are near each other. The results of the investigation of Klotz et al. and our results seem to be in contradiction with each other, but do not necessarily have to be so. The protection found by Klotz et al. was not absolute. The presence of an agonist during DEP treatment could only preserve 60% of the agonist binding, 20% more binding than without an agonist present. However the presence of an antagonist maintained 45–50% of the agonist binding. Similar results were found for the antagonist binding. Therefore it is possible that agonists and antagonists bind the same histidyl residue in the receptor binding site. In addition, it is conceivable that other, distinct histidyl residues play a role as well in the binding of agonists and antagonists.

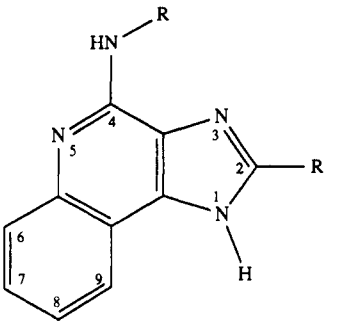

Figure 8. General formula of the 1*H*-imidazo[4,5-c]quinolin-4-amines.

Van Galen et al. determined two possible conformations for the N1–C6–N^6–C^2 dihedral angle: + or −75 ± 10°.[17] A rational choice between those two conformations is now possible, because the conformation of −75° corresponds with the 330° dihedral angle of 8-alkyl-substituted xanthines in the N^6–C8 model.

Some problems still warrant further investigation. Both authors of the described models have synthesized compounds that fit in a special way in these models.

Van Galen et al. have synthesized the 1H–imidazo[4,5-c]quinolin-4-amines, which they substituted at the 2- and 4-positions (Figure 8).[13] Hydrophobic substitution led to an increase of the affinity, with phenyl at the 2-position and cyclopentyl at the 4-position as optimal. According

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to their model, these substituents would coincide with the adenosine N^6-position and the xanthine C8-position. The 2-phenyl ring would overlap with the adenosine C8-region in the N^6–C8 model.

Peet et al. substituted the 8-position of 1,3-dipropylxanthine with a 1-phenyl-2-propyl group, analogous to *R*- and *S*-PIA.[16] This substitution led to a nanomolar affinity, the *R* enantiomer being significantly more potent. This stereochemical similarity of the adenosine N^6-position and the xanthine 8-position indicates a binding to the same receptor region or to two almost identical subregions of the receptor.

Two attempts to add a ribose moiety to the xanthine ring, either to convert the xanthines to agonists or to improve their affinity, have been described in the literature. Clanachan has tested theophylline 9–riboside as an adenosine receptor ligand.[27] He found that theophylline 9-riboside has no affinity for the adenosine receptor. Therefore, it is not possible for the 9-ribose ring to accommodate the binding site to which the adenosine ribose moiety binds. Van Galen et al. synthesized some xanthine 7-ribosides to test their flipped model.[28] The xanthine 7-ribosides appeared to be adenosine A_1 receptor antagonists, but somewhat less potent (3–10 times) than the corresponding xanthines. The effects of substituents at the 1- and 3-positions of the xanthine 7-ribosides agree with known structure–affinity relationships for xanthines. Obviously, the xanthine N7-region can accommodate a relatively large ribose moiety with a limited loss of affinity. This conclusion seems to be in favor of their flipped model.

From the computational investigations described in this paper it can be concluded that the N^6–C8 model seems to be the more probable model, because of the good sterical and conformational fit. An extension of this model, however, will be necessary to fit all (classes of) antagonists in this model. In this respect it should be noted that the schematic map of van Galen et al.,[15] indicating the steric, electrostatic, and hydrophobic requirements for antagonist binding, was derived independent of the assumed relative orientations of adenosine versus the xanthines. Therefore, this map may still be an accurate representation of the antagonist A_1 binding site, notwithstanding our conclusion that the N^6–C8 model is preferred over the flipped model. Further investigations and biochemical data will be necessary to solve these issues completely. The recent elucidation of the amino acid sequence of the adenosine receptor[29,30] will provide more insight and knowledge of the "real" interactions of ligands with the receptor.

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Registry No. N^6-Cyclopentyladenosine, 41552-82-3; N^6-cyclohexyladenosine, 36396-99-3; N^6-(*p*-methoxyphenyl)adenosine, 29204-77-1; N^6-[(*R*)-1-phenyl-2-propyl]adenosine, 38594-96-6; N^6-phenyladenosine, 23589-16-4; N^6-(methylcyclohexyl)adenosine, 97826-39-6; N^6-cyclopropyladenosine, 97374-48-6; N^6-[(*S*)-1-phenyl-2-propyl]adenosine, 38594-97-7; N^6-benzyladenosine, 4294-16-0; 8-cyclopentyl-1,3-dipropylxanthine, 102146-07-6; 8-cyclohexyl-1,3-dipropylxanthine, 106686-66-2; 8-(*p*-methoxyphenyl)-1,3-dipropylxanthine, 101126-73-2; 8-[(*R*)-1-phenyl-2-propyl]-1,3-dipropylxanthine, 130324-52-6; 8-phenyl-1,3-dipropylxanthine, 85872-53-3; 8-(methylcyclohexyl)-1,3-dipropylxanthine, 138314-04-2; 8-cyclopropyl-1,3-dipropylxanthine, 108653-60-7; 8-[(*S*)-1-phenyl-2-propyl]-1,3-dipropylxanthine, 130324-53-7; 8-benzyl-1,3-dipropylxanthine, 108670-88-8; N^6-(*p*-methylphenyl)adenosine, 29204-54-4; N^6-(*p*-chlorophenyl)-adenosine, 29204-62-4; N^6-(*p*-fluorophenyl)adenosine, 29305-74-6; N^6-(*m*-fluorophenyl)adenosine, 29204-64-6; N^6-(*o*-methylphenyl)adenosine, 29204-55-5; N^6-(*m*-methylphenyl)adenosine, 29204-56-6; N^6-(*o*-fluorophenyl)adenosine, 29204-63-5; N^6-(*m*-methoxyphenyl)adenosine, 29204-59-9; N^6-(*o*-methoxyphenyl)-adenosine, 29204-58-8; 1,3-dimethyl-8-(*p*-methylphenyl)xanthine, 57196-70-0; 1,3-dimethyl-8-(*p*-methoxyphenyl)xanthine, 967-42-0; 1,3-dimethyl-8-(*p*-chlorophenyl)xanthine, 29064-02-6; 1,3-dimethyl-8-(*p*-fluorophenyl)xanthine, 57281-09-1; 1,3-dimethyl-8-(*m*-fluorophenyl)xanthine, 85872-67-9; 1,3-dimethyl-8-(*o*-methylphenyl)xanthine, 85884-03-3; 1,3-dimethyl-8-(*m*-methylphenyl)xanthine, 85872-61-3; 1,3-dimethyl-8-(*o*-fluorophenyl)-xanthine, 85872-56-6; 1,3-dimethyl-8-(*m*-methoxyphenyl)xanthine, 85872-64-6; 1,3-dimethyl-8-(*o*-methoxyphenyl)xanthine, 85872-55-5.

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