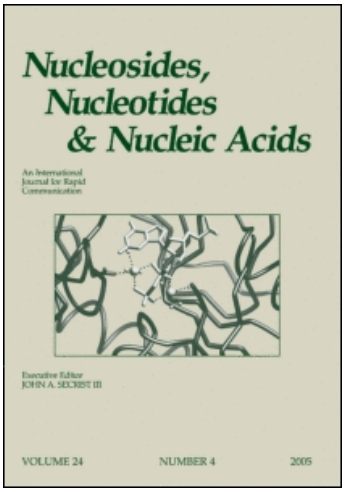

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Synthesis and Cardiac Pharmacology of 2-(AR)Alkoxyadenosines

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SYNTHESIS AND CARDIAC PHARMACOLOGY OF 2-(AR)ALKOXYADENOSINES

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Abstract: Certain relatively large 2-(ar)alkoxy substituents selectively raise the agonist potency of adenosine at the A_2 receptor of coronary artery while lowering activity at the A_1 receptor of AV node.

Certain exocyclic substituents can substantially increase the agonist potency of adenosine at A_1 and A_2 receptors (A_1AR, A_2AR), suggesting that the substituents interact specifically with regions of complementary structure and chemical attributes within the receptors. Although there is a lack of definitive information about the structures of the adenosine receptors, such as that provided by X-ray crystallography, the structure-activity relationships (SAR) of adenosine analogues have generated useful models of the ligand-binding regions. The SAR of the N^6-substituted adenosines have yielded fairly detailed models of the N^6 regions of the A_1AR[1,2] and of the A_2AR[3-5]. Although a large number of C-2 substituted adenosines are known[6-11], the tendency in these reports is to survey a wide assortment of substituents rather than to develop congeneric series. Unfortunately, this information is of limited value in receptor modelling. Here we describe provisional models of the C-2 regions of the A_1AR in the atrioventricular (AV) node and the A_2AR in the coronary arteries of the guinea pig that are based on the SAR of congeneric series of 2-(ar)alkoxyadenosines.

The synthesis of the 2-(ar)alkoxyadenosines consists of the reaction of an alkoxide with either the ethoxymethylidine or the isopropylidine ketal of 2-chloroadenosine[9].

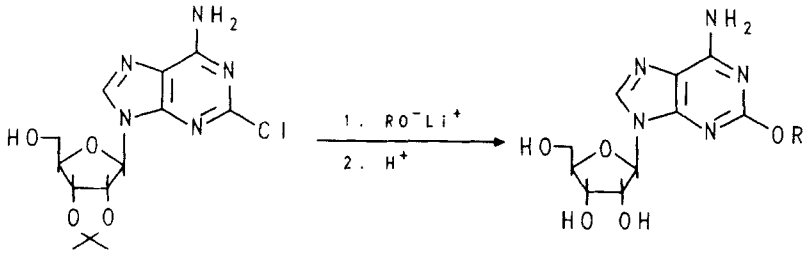


Figure 1. Synthesis of 2-alkoxyadenosines.

After the hydrolysis of the blocking group and purification by reverse phase chromatography, the yield of chromatographically pure product is typically 30-40%. The bioassays of A_1AR and A_2AR agonist potency employ a guinea pig heart perfused by the Langendorff technique and paced at 260 beats/min. Spectrophotometrically standardized solutions of nucleoside are infused directly into the aortic perfusion cannula. The quotient of nucleoside infusion rate (mol/min) divided by coronary flow (L/min) is the agonist concentration in the perfusate. Prolongation of the stimulus-QRS interval measures potency at the A_1AR of the AV node and vasodilation measures potency at the A_2AR in the coronary artery. The index of the A_1AR agonist assay is the EC_{50} of prolongation of the stimulus-QRS interval, defined as one-half the concentration of analogue that causes 2° heart block[12]. Similarly, the EC_{50} of coronary vasodilation is the concentration producing a half-maximum increase of coronary flow[13]. The A_1/A_2 selectivity ratio is the EC_{50} of stimulus-QRS prolongation divided by the EC_{50} of coronary vasodilation.

At the A_1AR, 2-alkoxy and 2-aralkoxy substituents consistently reduce the potency of adenosine, in some instances by as much as 50-fold (Figure 2). Analogues with *sec*-alkyl substituents are even less potent than *n*-alkyl analogues having substituents of the same chain length, suggesting that the receptor contains an alkyl subregion of very limited bulk tolerance immediately adjacent to the subregion that accommodates O-2. The potency of the 2-*iso*alkoxyadenosines and the 2-cyclohexylalkoxyadenosines increases progressively as a function of the number of methylene residues in the alkyl

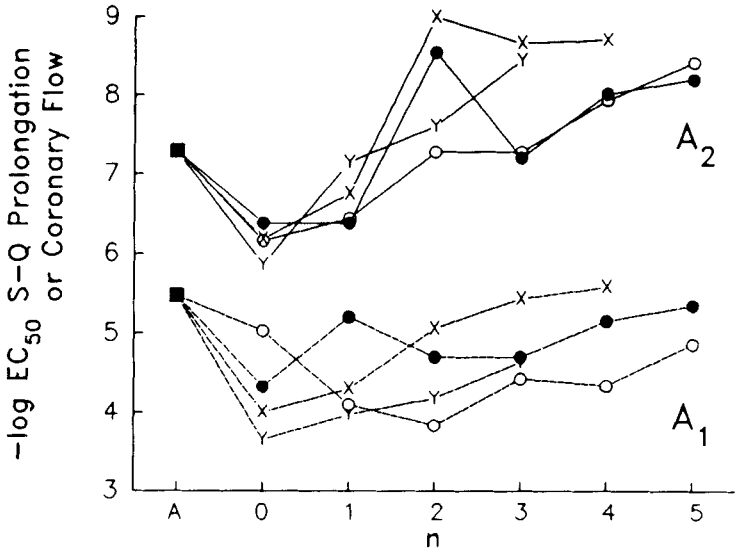
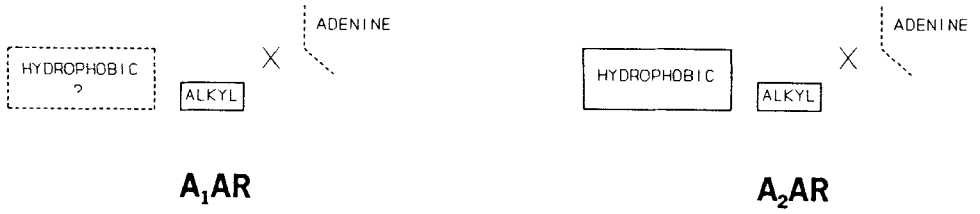


Figure 2. Agonist activity of adenosines having C-2 substituents of the general formula $R(CH_2)_nO$-, where ■ represents adenosine (A) and R is either $-CH_3$ (O), $-CH(CH_3)_2$ (Y), cyclohexyl (X) or phenyl (●). The abscissa represents the number of methylene groups, *n*, interposed between R- and -O-. The ordinate is $-\log EC_{50}$ of either prolongation of the stimulus-QRS interval (lower four curves) or of coronary vasodilation (upper four curves).

chain, but even the most potent of these, 2-(4-cyclohexylbutoxy)adenosine, is no more potent than adenosine itself. The activity of both the 2-*n*-alkoxyadenosines and the 2-phenylalkoxyadenosines seems independent of the size of the C-2 substituent.

At the A_2AR, a phenoxy and also small alkoxy C-2 substituents reduce the potency of adenosine. However, whether the alkyl chain terminates in a $-CH_3$, $-CH(CH_3)_2$, $-C_6H_{11}$, or a $-C_6H_5$ group, lengthening the alkyl chain greatly increases potency as a function of the number of methylene residues in the chain (Figure 2). The most potent analogue in this series is 2-(2-cyclohexylethoxy)adenosine; its EC_{50} is 1 *nM*, a potency 50-fold higher than that of adenosine. The low potencies of adenosines having 2-phenoxy or 2-*sec*-alkoxy substituents suggests that the A_2AR contains an alkyl region of limited bulk tolerance similar to that of the A_1AR. The pronounced augmentation of activity associated with enlarging the 2-substituent, which also



A_1AR A_2AR

Figure 3. Hypothetical models of the C-2 regions of the AV node A_1AR and the coronary artery A_2AR of the guinea pig heart. Features common to both receptors are (*a*) X subregions that accommodates groups such as -NH-, -O- or -S- that link substituents to purine C-2 and (*b*) an alkyl region that is large enough to accommodate a $-CH_2CH_2-$ residue. Whereas studies of single atom/small group C-2 substituents show that occupancy of the X subregion has little direct effect on activity, interaction with the alkyl subregion can significantly lower potency. The A_2AR contains, in addition, a hydrophobic subregion that is large enough to accommodate a naphthyl residue. Occupancy of this region promotes agonist activity in proportion to the size of the substituent. By contrast, the A_1AR either lacks a hydrophobic subregion or, alternatively, the binding of a substituent to it does not promote agonist activity.

increases its hydrophobicity, suggests that the A_2AR contains a hydrophobic subregion adjacent to the subregion of limited bulk tolerance. The fact that 2-(2-cyclohexylethoxy)adenosine is more potent than the phenethyl congener is evidence that this subregion is not flat; indeed, bicycloalkyl substituents that are more rigid and therefore more angular than a cyclohexyl group, for example, a norbornanyl group, are quite active.

RESULTS AND DISCUSSION

Figure 3 depicts models of the C-2 regions of the A_1AR and A_2AR developed from the SAR of 2-(ar)alkoxyadenosines. Both receptors contain an X subregion that accommodates the atom linking the 2-substituent to purine C-2, in this instance an oxygen, and also an alkyl region able to tolerate an ethyl moiety but not alkyl groups of greater lateral bulk. The presence of a hydrophobic region distinguishes the A_2AR from the A_1AR. The lack of a correlation between the activity of the 2-*n*-alkoxy- and 2-phenylalkoxyadenosines and the size of the alkyl chain argues against the existence of a hydrophobic region in the A_1AR.

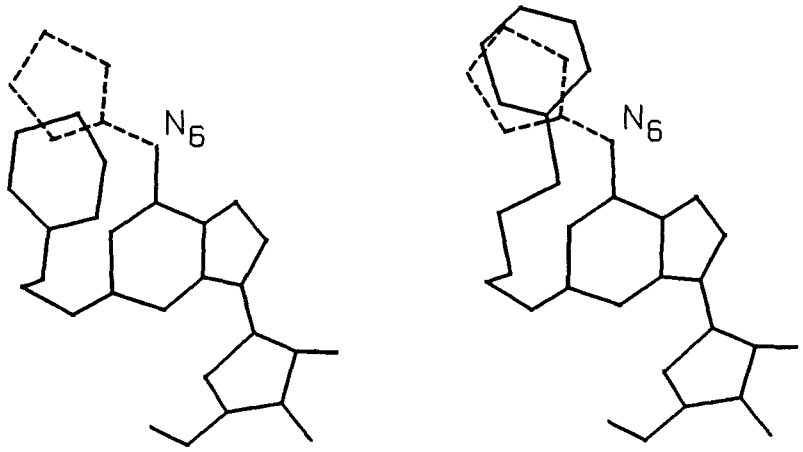


Figure 4. A possible explanation of the parallel relationship between the A_1AR agonist activity of adenosines having branched chain alkoxy C-2 substituents and the sizes of the substituents. The molecules at left and right, respectively, are 2-cyclohexylethoxy- and 2-cyclohexylbutoxyadenosine. The dashed lines represent the cyclopentyl group of N^6-cyclopentyladenosine, which derives its potency as an A_1AR agonist from the interaction of the cycloalkyl group with the N^6 region of the receptor. Note that the flexibility and length of the 2-cyclohexylbutoxy substituent enables it to interact with the N^6 region to a greater extent than the 2-cyclohexylethoxy substituent.

Even though the A_1AR activities of the 2-*iso*alkoxy- and 2-cyclohexylalkoxyadenosines vary directly with the length of the alkyl chain, this is not necessarily evidence of an interaction of those substituents with a hydrophobic subregion. Figure 4 describes an alternative explanation. Studies of molecular models show that chain lengthening enables the longer alkyl groups to reach into the receptor region usually occupied by the *sec*-alkyl and cycloalkyl substituents of N^6-substituted adenosines that are potent A_1AR agonists. This explanation also accounts for the low A_1AR activity of the 2-phenylalkoxyadenosines; inserting a phenyl group into the N^6-alkyl group lowers the potency of an N^6-alkyladenosine at the A_1AR[1]. Presumably, a C-2 substituent containing a phenyl group would interact less well with the N^6 region of the A_1AR than an alkyl substituent.

Investigations of the effect of substitutions in the aryl moiety of the 2-aralkoxyadenosines are not yet complete, but several interesting points emerge. The

high potency of 2-(2-naphthylethoxy)adenosine at the A_2AR (EC_{50} < 1 n*M*) raises the estimated size of the hydrophobic region to at least that of naphthalene. Heteroaryl groups, for example, thiophene, have nearly the same affinity for the hydrophobic region as a phenyl group. Certain phenyl ring substitutions greatly enhance A_2AR potency; for example, the EC_{50} of the coronary vasoactivity of 2-(4-methylphenethoxy)adenosine is <200 p*M*. Owing to the relatively constant affinity of the 2-aralkoxyadenosines for the A_1AR, selectivity varies directly with A_2AR potency. The A_1/A_2 activity ratio of 2-(4-methylphenethoxy)adenosine is > 35,000.

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