Cloning, Expression and Pharmacological Characterization of Rabbit Adenosine A₁ and A₃ Receptors

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ABSTRACT

The role of adenosine A₁ and A₂ receptors in mediating cardioprotection has been studied predominantly in rabbits, yet the pharmacological characteristics of rabbit adenosine A₁ and A₂ receptor subtypes are unknown. Thus, the rabbit adenosine A₂ receptor was cloned and expressed, and its pharmacology was compared with that of cloned adenosine A₁ receptors. Stable transfection of rabbit A₁ or A₂ cDNAs in Chinese hamster ovary-K1 cells resulted in high levels of expression of each of the receptors, as demonstrated by high-affinity binding of the A₁/A₂ adenosine receptor agonist N³-(4-aminomethyl)adenosine (125I-ABA). For both receptors, binding of 125I-ABA was inhibited by the GTP analog 5'-GTP (Gpp(NH)p) and forskolin-stimulated cyclic AMP accumulation was inhibited by the adenosine receptor antagonist ABA was inhibited by the GTP analog 5'-GTP (Gpp(NH)p) and forskolin-stimulated cyclic AMP accumulation was inhibited by the adenosine receptor antagonist (R)-phenylisopropyladenosine (PIA). The rank orders of potency of adenosine receptor antagonists for inhibition of 125I-ABA binding were as follows: rabbit A₁, N³-cyclopentyladenosine > (R)-phenylisopropyladenosine > N⁶-ethylcarboxamidoadenosine > I-ABA > N⁶-2-(4-aminophenyl)ethyladenosine > N⁶-(3-iodobenzyl)adenosine-5'-N-methyluronamide > N⁶-(4-aminomethyl)adenosine; rabbit A₂, N³-(4-aminobenzyl)adenosine-5'-N-methyluronamide > I-ABA > N⁶-ethylcarboxamidoadenosine > N⁶-2-(4-aminophenyl)ethyladenosine > N⁶-(3-iodobenzyl)adenosine-5'-N-methyluronamide.

Adenosine receptors have been shown to mediate a wide variety of physiological functions in the cardiovascular and central nervous systems and to modulate allergic and immunological responses (Liu et al., 1994; von Lubitz et al., 1994; Beaven et al., 1994; Jacobson et al., 1995). The G protein-coupled adenosine receptors have been classified into A₁, A₂a, A₂b and A₃ subtypes, based on pharmacological and functional studies of cloned receptors (Olah and Stiles, 1995). In contrast to A₁ and A₂ receptors, the recently cloned A₃ receptors from rats, sheep and humans show much lower homology among species at the amino acid level. In addition, recent pharmacological characterization of cloned A₃ receptors has revealed the existence of remarkable differences in the affinity of adenosine agonists and antagonists for A₃ receptors of different species, apparently reflecting their lower degree of homology (Zhou et al., 1992; Salvatore et al., 1993; Linden, 1994; Ji et al., 1994).

Both adenosine A₁ and A₃ receptors are negatively coupled to adenylate cyclase and have been hypothesized to mediate the cardioprotective effects of adenosine. The A₁/A₃ hypothesis is based primarily on recent studies using rabbit models of cardioprotection (Liu et al., 1991, 1994; Armstrong and Ganote, 1994, 1995; Rice et al., 1996). Because recombinant rabbit A₁ and A₃ receptors had not yet been expressed and pharmacologically characterized, the interpretation of data in those studies was based on the affinity of adenosine agonists and antagonists for cloned rat, sheep and human adenosine A₁ and A₃ receptors. However, given the documented differences in A₃ adenosine receptor pharmacology among cloned rat, sheep and human A₃ receptors, it is possible that a poor correlation also exists between the affinities of aden-
...encestexistbetweenrabbitA1andA3receptorsandA1andA3receptorsinmediatingcardioprotectioninrabbits.Thus,thepharmacologicalcharacterizationofrecombinantrabbitA1andA3adenosine receptorsiscriticalforthedeterminationoftheirrolesinrabbitmodelsofcardioprotection.

Therefore, we describe the cloning and stable expression of the rabbit A3 receptor and the stable expression of the previously cloned rabbit A1 receptor (Bhattacharya et al., 1993). In addition, we provide the first in-depth pharmacological characterization of functional rabbit A1 and A3 receptors. The results of this study illustrate that pharmacological differences exist between rabbit A1 and A3 receptors and A1 and A3 receptors reported for other species. Consequently, these data will be important in the design and interpretation of future studies evaluating the role of A1 and A3 receptors in rabbit models of adenosine-mediated cardioprotection.

**Methods**

**Materials.** Restriction endonucleases and other molecular biological reagents were purchased from New England Biolabs. All molecular biological methods were performed according to kit protocols provided by the manufacturers or according to the protocols of Sambrook et al. (1989). The rabbit A1 adenosine receptor cDNA was a generous gift from Dr. Samita Bhattacharya (University of Virginia) and Dr. William S. Spielman (Michigan State University). All cloned sequences were obtained from GenBank. CHO-K1 cells were obtained from the American Type Culture Collection (Rockville, MD). DMEM/F-12 culture medium and fetal calf serum were obtained from Gibco-BRL (Grand Island, NY). I-ABA and IB-MECA were synthesized at Pfizer Central Research (Groton, CT). 125I-ABA was prepared by New England Nuclear (Boston, MA). BWA1433 [3-dipropyl-8-(4-acrylate)phenylxanthine] was a gift from Glaxo-Wellcome (Research Triangle, NC). ADA was obtained from Boehringer Mannheim (Indianapolis, IN). Reference adenosine compounds and RO-20-1724 [4-[(3-butoxy-4-methoxyphenyl)methyl]-2-imidazolidinone] were obtained from Research Biochemicals International (Natick, MA). Gpp(NH)p was obtained from Sigma Chemical Co. (St. Louis, MO).

**PCR cloning.** One microgram of rabbit lung poly(A)+ RNA (Clontech) was primed with random hexamers and reverse-transcribed according to the GeneAmp RNA PCR core kit protocol (Perkin Elmer). The rabbit A3 receptor was then cloned using a four-step nested PCR approach, as described in figure 1. Degenerate oligonucleotide primers (indicated by a lowercase “d”) were based on an alignment of published human, rat and sheep A3 receptor sequences (Zhou et al., 1992; Salvatore et al., 1993; Linden et al., 1993). The remaining primers were chosen from validated rabbit A3 sequences. The primers were as follows: dRA3-1F, tcatactgtctctggtrgtgc (292–311); dRA3-1R, gcattaagccaatggag (846–825); dRA3-2F, tgtggctgtctggtrgtgc (394–414); dRA3-2R, cactagcggtctgtgcagga (834–814); dRA3-3R, gaccttgaggttgctgaa (695–675); RA3-4R, ctctggaaacagttcgcg (635–616); dRA3-3F, atstgtgkergagatgccg (%87 to %69); RA3-4F, cactatgacgaaacttttg (609–628); RA3-5F, tgtggctgtctggagctg (785–802); dRA3-5R, gsgramtstgaaagttagctag (1005–984); RA3-6F, gctagctcgctgtgcagga (%29 to %11); RA3-6R, gtgggagaccctctgg (983–965), with b = cgt, d = agt, h = act, k = m, n = acg, r = ag, s = cg, v = aeg, w = at and y = ct. Primer positions are indicated in parentheses, with nucleotide 1 corresponding to the first adenine of the initiator methionine (atg), except for primers dRA3-3F and dRA3-5R, whose numbering corresponds to the published human A3 receptor untranslated sequences. The first PCR of each nested set (fig. 1, steps 1–3) was carried out according to the GeneAmp PCR reagent protocol (Perkin Elmer) for 30 cycles of 30 sec at 94°C, 30 sec at 50°C and 45 sec at 72°C, followed by a single cycle of 6 min at 72°C. A second nested PCR was then performed under higher stringency conditions, using 2 μl of a 1:500 dilution of the primary PCR mixture for 30 sec at 94°C, 30 sec at 55°C and 30 sec at 72°C, followed by a single cycle of 6 min at 72°C. PCR products were agarose gel-purified by using a Gene Clean kit (Bio 101) and were subcloned into a pGEM-T vector by using a Promega pGEM-T cloning kit. Plasmid DNA from independent clones was isolated with a Wizard MiniPrep DNA purification system (Promega) and subjected to automated sequencing on an Applied Biosystems 373-A sequencer. For each nested PCR product, three independent clones were sequenced with two to four overlapping sequence passes on both complementary DNA strands.

The full-length rabbit A3 receptor cDNA (fig. 1, step 4) was generated by reverse transcription-PCR as above, using nondegenerate PCR primers RA3-6F and RA3-6R designed from nested PCR product-verified sequences. PCRs were performed for 30 cycles of 45 sec at 94°C, 45 sec at 59°C and 60 sec at 72°C, followed by a single cycle of 6 min at 72°C, and the PCR product was subcloned into pGEM-T. The full-length sequence was verified by three or four overlapping sequence passes on three independent clones.

**Expression studies.** For stable expression studies, the entire coding region of the rabbit A3 receptor cDNA (RabA3) was subcloned as a NotI-EcoRV fragment into the expression vector pcDNA3 (Invitrogen), creating the vector pcDNA3RabA3. The rabbit A1 receptor cDNA (RabA1) was subcloned as a SpH1/blunt-ended with Klenow-EcoRI fragment into pcDNA3, creating pcDNA3RabA1. Both plasmids were confirmed by sequencing and restriction analysis. Adenosine receptor expression plasmids (20 μg) were transfected into CHO-K1 cells grown in DMEM/F-12 medium with 10% fetal calf serum, using a calcium phosphate mammalian cell transfection kit (5 Prime-3 Prime). Stable transfecteds were obtained by selection in 1997

**Rabbit A1 and A3 Receptors**

Fig. 1. PCR strategy for cloning the rabbit A3 receptor. Degenerate (indicated by a lowercase “d”) and sequence-verified primers used in each PCR are shown. Products from first (hatched bar) and second (solid bar) nested PCRs are shown, with untranslated sequences indicated as lines. Solid bars represent nucleotide sequences, which were verified by extensive sequencing.
complete medium containing 500 µg/ml active neomycin (G418), and individual clones were screened for expression by 125I-ABA binding.

**Receptor membrane preparation.** CHO-K1 cells stably transfected with either RabA1 or RabA3 were washed with PBS (Ca2+/Mg2+-free) and then detached with 1.0 mM EDTA/PBS. Cells were collected by centrifugation at 300 × g for 5 min, the supernatant was discarded and the cell pellet was resuspended in incubation buffer consisting of 50 mM Tris, 120 mM NaCl, 10 mM MgCl2, 5 mM KCl, 2 mM CaCl2, 0.1 mM phenylmethylsulfonyl fluoride, 100 µM bacitracin, 10 µg/ml leupeptin, 100 µg/ml DNase I and 2 U/ml ADA, pH 7.4. Crude cell membranes were prepared by repeated aspiration through a 21-gauge needle, collected by centrifugation at 60,000 × g for 10 min and stored in incubation buffer at −80°C.

**125I-ABA binding.** Binding reactions (10–20 µg of membrane protein) were carried out for 1 hr at room temperature in 250 µl of incubation buffer containing 0.01 to 100 nM 125I-ABA (2200 Ci/mmol) and the appropriate concentration of compound. The reaction was stopped by rapid filtration with ice-cold PBS, through glass fiber filters (presoaked in 0.6% polyethyleneimine), using a Tomtec 96-well harvester (Tomtec, Orange, CT). Filters were counted in a Wallac Microbeta liquid scintillation counter (Wallac, Gaithersburg, MD).

**Data analysis.** Equilibrium dissociation constants (Kd) and receptor densities (Bmax) were calculated by fitting specific binding data, by nonlinear least-squares regression analysis, to the following equation: 125I-ABA bound = Bmax × [125I-ABA]/Kd + [125I-ABA]. Inhibitory constants (Ki) were calculated by fitting binding data, by nonlinear least-squares regression analysis, to the following equation: % inhibition = 100 × [1 + ([log(Compound)] / Ki)]. C (IC50) is log(Compound) at 50% inhibition and D is the Hill coefficient. At the 0.1 nM concentration of 125I-ABA used in competition experiments (70-fold less than the 125I-ABA Kd for rabbit A1 receptors and 90-fold less than the 125I-ABA Kd for rabbit A3 receptors), IC50 = Ki (Chang and Prusoff, 1973). Inhibitory constants for rabbit A1 and A3 receptors are reported as K values.

**Results**

**Cloning of the rabbit A3 receptor.** A rabbit A3 receptor cDNA (RabA3) was identified by reverse transcription of rabbit lung mRNA, followed by a nested PCR strategy using degenerate oligonucleotides derived from an alignment of rat, human and sheep A3 sequences. Rabbit A3 mRNA was also identified in rabbit liver, brain and heart mRNA by reverse transcription-PCR (data not shown). RabA3 contained an open reading frame encoding a protein of 320 amino acids. Hydrophobicity analysis of the deduced amino acid sequence showed that the protein contained seven hydrophobic domains (fig. 2) characteristic of G protein-coupled receptors, including the adenosine receptor family. Computer analysis revealed that RabA3 had the highest amino acid identity with human, sheep and rat A3 receptors, i.e., 76%, 75% and 68%, respectively (fig. 3). In contrast, identity between RabA3 and the only other published rabbit adenosine receptor, A1, was 46%.

**Stable expression of rabbit A1 and A3 receptors.** To confirm the identity of the rabbit A1 and A3 receptors, RabA1, and RabA3 were stably transfected into CHO-K1 cells, which do not express endogenous adenosine receptors. Figure 4 illustrates the concentration-dependent binding of 125I-ABA to rabbit A1 and A3 receptors. For both receptors the data were best fit by a model assuming the interaction of 125I-ABA with a single high-affinity state of the receptor. The Kd and Bmax values (mean ± S.E., n = 3) were 2 ± 0.2 nM and 2 ± 0.2 pmol/mg protein, respectively, for the rabbit A1 receptor and 9 ± 3 nM and 10 ± 3 pmol/mg protein, respectively, for the rabbit A3 receptor. Specific binding at the Kd for each receptor represented >90% of the total binding. No specific 125I-ABA binding was observed in nontransfected CHO-K1 cell membranes (data not shown).

**Functional coupling of cloned rabbit receptors.** The high-affinity binding of 125I-ABA to rabbit A1 and A3 recep-
tors suggested that they were functionally coupled to a G protein second messenger system. Figure 5A illustrates the effect of the stable GTP analog Gpp(NH)p on $^{125}$I-ABA binding to rabbit A$_1$ and A$_3$ receptors. Gpp(NH)p reduced $^{125}$I-ABA binding to both receptors by approximately 75%, suggesting that the majority of the expressed receptors are coupled to G proteins. To determine whether the cloned rabbit receptors are functionally coupled to adenylate cyclase, we examined the effect of the A$_1$-selective agonist (+)-PIA on forskolin-stimulated cAMP accumulation in CHO-K1 cells stably transfected with either RabA$_1$ or RabA$_3$. (+)-PIA produced a concentration-dependent inhibition of forskolin-stimulated cAMP accumulation, with $IC_{50}$ values of 0.4 nM and 25 nM for rabbit A$_1$ and A$_3$ receptors, respectively (fig. 5B).

Pharmacological characterization of cloned rabbit receptors. The pharmacology of cloned rabbit A$_1$ and A$_3$ receptors was characterized using reference adenosine agonists and antagonists. Figure 6 illustrates the concentration-dependent inhibition of $^{125}$I-ABA binding to rabbit A$_1$ and A$_3$ receptors by the A$_1$-selective agonist (+)-PIA (Fig. 6A) and the A$_3$-selective agonist IB-MECA (Fig. 6B). (+)-PIA and IB-MECA demonstrated selective inhibition of binding to rabbit A$_1$ and A$_3$ receptors, respectively [(+)-PIA, 50-fold; IB-MECA, 15-fold]. The $K_i$ values of reference adenosine...
agonists and antagonists for inhibition of $^{125}$I-ABA binding to rabbit A1 and A3 receptors. Rabbit A1 and A3 receptors are summarized in Table 1. The agonist rank orders of potency were as follows: rabbit A1, CPA = (R)-PIA > N-ethylcarboxamidoadenosine ≥ I-ABA ≥ N$^\text{6}$-2-(4-aminophenyl)ethyladenosine ≥ IB-MECA ≥ ABA; rabbit A3, IB-MECA ≥ I-ABA ≥ N-ethylcarboxamido-

<table>
<thead>
<tr>
<th>Adenosine agonists</th>
<th>Rabbit A1</th>
<th>Rabbit A3</th>
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<tr>
<td>IB-MECA</td>
<td>30 ± 3</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>I-ABA</td>
<td>4 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>NECA$^b$</td>
<td>2 ± 0.1</td>
<td>27 ± 9</td>
</tr>
<tr>
<td>APNEA</td>
<td>6 ± 1</td>
<td>47 ± 11</td>
</tr>
<tr>
<td>(R)-PIA</td>
<td>1 ± 0.2</td>
<td>49 ± 8</td>
</tr>
<tr>
<td>CPA</td>
<td>40 ± 3</td>
<td>169 ± 23</td>
</tr>
<tr>
<td>ABA</td>
<td>329 ± 80</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>BWA1433</td>
<td>746 ± 105</td>
<td>746 ± 105</td>
</tr>
<tr>
<td>DPCPX</td>
<td>1,120 ± 145</td>
<td>1,120 ± 145</td>
</tr>
<tr>
<td>8-SPT</td>
<td>37,797 ± 4,409</td>
<td>37,797 ± 4,409</td>
</tr>
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$^a$ Kᵢ values for inhibition of $^{125}$I-ABA binding were determined as described in “Methods.” Data are presented as mean ± S.E. of three to six experiments.

$^b$ NECA, N-ethylcarboxamidoadenosine; APNEA, N$^\text{6}$-2-(4-aminophenyl)ethyladenosine; XAC, xanthine amine congener; 8-SPT, 8-(p-sulfophenyl)theophylline.

TABLE 1

Kᵢ values of adenosine agonists and antagonists for rabbit A₁ and A₃ receptors

Discussion

By using a nested PCR approach, we have cloned and expressed the rabbit adenosine A₃ receptor. Several lines of evidence support the conclusion that RabA₁ and RabA₃ encode for the rabbit A₁ and A₃ receptor, respectively. First, we observed pmol/mg levels of high-affinity $^{125}$I-ABA binding to CHO-K1 cells after the stable transfection of the rabbit cDNAs (fig. 4). Second, the stable GTP analog Gpp(NH)p inhibited $^{125}$I-ABA binding by 75% (fig. 5A). Third, forskolin-stimulated cAMP accumulation in cells transfected with RabA₃ was selectively inhibited by the A₃-selective adenosine receptor agonist (R)-PIA (fig. 5B). These observations are characteristic of the expression of A₁ and A₃ receptors that have been reported for other species (Zhou et al., 1992; Salvatore et al., 1993; Linden, 1994), and they indicate that the expressed rabbit receptors are negatively coupled to adenylate cyclase via G proteins.

The identities of the rabbit cDNAs and the expressed proteins are further supported by our pharmacological characterization experiments. Several of the reference compounds used in this study were chosen because of their reported selectivity for A₁ or A₃ receptors in other species. For example, (R)-PIA and CPA are selective A₁ agonists, DPCPX has been widely reported as an A₁-selective antagonist and IB-MECA is an A₃-selective agonist in rats (Collis and Hourani, 1993; Galio-Rodriguez et al., 1994; Olah and Stiles, 1995). In the present study, demonstration of the appropriate selectivity of these compounds for inhibition of $^{125}$I-ABA binding to CHO-K1 cells stably transfected with RabA₁ or RabA₃ (table 1) supports the identity of the RabA₁- and RabA₃-expressed proteins as A₁ and A₃ receptors, respectively.

The rabbit A₃ receptor is structurally similar to other cloned A₃ receptors in terms of its size (320 amino acids) and seven-transmembrane domain topography. It shares 59% overall identity in amino acid sequence with the A₃ receptors of other species (rat, sheep and human) (fig. 3) and contains the highly conserved, potential N-linked glycosylation sites at Asn-4 and Asn-161 (fig. 2) that are found in all A₃ receptors but are absent in A₁, A₂a, and A₂b receptors. RabA₃ also contains a cysteine residue in the carboxyl terminus (Cys-304 in the rabbit sequence) that is found in A₃, A₁ and A₂b receptors of all species but is absent in the A₂a subtype; it represents a potential palmitoylation site involved in the formation of a fourth intracellular loop (Linden, 1994).

The similarity of the rabbit A₃ receptor (59%) to other A₃ receptors is high enough for it to be included in the A₃ family (fig. 3). However, these receptors appear quite diverse, compared with the identity observed among A₁ receptors (74% for chicken, rat, guinea pig, human, bovine and rabbit receptors). A phylogenetic analysis of all A₃ receptors demonstrates that, whereas the rabbit receptor is not highly related...
to either the rat, sheep or human receptor, it is more closely related to the human receptor than to the rat A3 receptor (data not shown). The apparent phylogenetic divergence of A3 receptors is supported by differences in the affinities of certain adenosine analogs for rabbit A3 receptors, compared with other species (see below).

Although the rank orders of potency of adenosine agonists and antagonists for RabA1- and RabA2-transfected cells are mostly consistent with those reported for A1 and A3 receptors in other species (Tucker and Linden, 1993; Linden, 1994; Olah and Stiles, 1995), the absolute affinities of certain compounds for rabbit A1 and A3 receptors, compared with other species, are clearly different. The most striking illustration of the differences in affinities was observed with the xanthine (Sauer et al., 1994), the absolute affinities of certain compounds for rabbit A1 receptors (Ji et al., 1994) or A3 receptors (Linden et al., 1994). Thus, the A1-selective profile of BWA1433 (200 nM) inhibited ischemic preconditioning in the present study. However, in other species, such as rabbits, BWA1433 has recently been reported as a potent antagonist of cloned sheep A3 receptors (Linden et al., 1993) or A1 receptors (Linden et al., 1994; Olah and Stiles, 1995). Thus, with cloned rabbit A3 receptors (K_i = 746 nM), BWA1433 with a greater than sheep or human A3 receptors, respectively. In addition, we observed high-affinity binding of BWA1433 to rabbit A1 receptors (K_i = 3 nM), which contrasts with its K_i value of 122 nM for inhibition of 125I-ABA binding to CHO cells stably expressing cloned sheep A3 receptors (Salvatore et al., 1994), or K_i = 40 nM (R. J. Hill and S. P. Kennedy, unpublished observations). Thus, the A3-selective profile of BWA1433 in rabbits contrasts with its nonselective A1/A3 profile in humans, thus providing additional evidence for species differences in A3 adenosine receptor pharmacology.

The species differences in A3 receptor pharmacology observed in the present study and reported by others (Linden, 1994; Ji et al., 1994) are particularly relevant to recent studies that examined the role of A3 receptors in rabbit models of adenosine-dependent cardioprotection (Armstrong and Ganote, 1994, 1995; Liu et al., 1994). For example, Liu et al. (1994) suggested the involvement of A3 receptors in mediating cardioprotection, based in part on their observation that BWA1433 (200 nM) inhibited ischemic preconditioning in isolated rabbit hearts. The pharmacological data (from other species) available to those investigators suggested that this concentration of BWA1433 should have antagonized both A1 and A3 receptors; thus, this finding supported the involvement of an A3 receptor in mediating preconditioning. However, the present study demonstrates that BWA1433 is in fact highly selective for rabbit A1 receptors (K_i = 3 nM) and is only weakly active at rabbit A3 receptors (K_i = 746 nM). Thus, 200 nM BWA1433 would not be expected to have a significant effect on rabbit A3 receptor activation, while maximally inhibiting rabbit A1 receptor activation. Consequently, the inhibition of preconditioning by 200 nM BWA1433 that was observed by Liu et al. (1994) could be interpreted as supporting the involvement of adenosine A1 receptors, rather than A3 receptors. These observations provide additional support for the use of receptor pharmacological data for the same species in which the role of A3 receptors is examined.

In conclusion, this study provides the first in-depth pharmacological characterization of functional, cloned, rabbit A1 and A3 receptors. The results will facilitate further examination of the roles of A1 and A3 receptors in adenosine-mediated cardioprotection in rabbits, by allowing the selection of appropriate A1- and A3-selective concentrations of adenosine agonists and antagonists. This is particularly important because rabbit models of cardioprotection are used extensively to identify novel A1- and A3-selective compounds that may have therapeutic utility.

Acknowledgments

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References


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### Table 2

**Species** | **K_i at Adenosine Receptors** | **Adenosine A1** | **Adenosine A3** | **A1/A3**
--- | --- | --- | --- | ---
**Rabbit** | 3 | 746 | 40<sup>a</sup> | 2.5<sup>a</sup>
**Human** | 120<sup>a</sup> | 55<sup>a</sup> | 0.004<sup>a</sup> | 0.004<sup>a</sup>
**Sheep** | ND<sup>a</sup> | 21<sup>a</sup> | ND<sup>a</sup> | ND<sup>a</sup>

<sup>a</sup> Inhibition of 0.1 nM 125I-ABA binding to membranes prepared from CHO cells stably expressing cloned human A1 receptors (R. J. Hill and S. P. Kennedy, unpublished observations).

<sup>b</sup> Inhibition of 0.1 nM 125I-ABA binding to membranes prepared from CHO cells stably expressing cloned human A2 receptors (Salvatore et al., 1993).

<sup>c</sup> Inhibition of 0.1 nM 125I-ABA binding to membranes prepared from human embryonic kidney 293s cells stably expressing cloned human A3 receptors (R. J. Hill and S. P. Kennedy, unpublished observations).

<sup>d</sup> ND, not determined.


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