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Antagonist pharmacology of adenosine A_{2B} receptors from rat, guinea pig and dog

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Abstract

We have sought evidence for species differences between adenosine A_{2B} receptors by comparing the potencies of eight adenosine receptor antagonists, representing four different chemical classes, at the native adenosine A_{2B} receptors which mediate relaxation of smooth muscle from rat colon, guinea pig aorta and dog saphenous vein. In all three assays, the antagonists caused parallel rightward shifts in the concentration–response curves to NECA and there was no depression of the maximum responses. There were highly significant correlations between the pK_B values on each of the three receptors. However, the pK_B values of 8-SPT (8-*p*-(sulphophenyl)theophylline), XAC (8-[-[[[[(2-aminoethyl)amino]-carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine), CGS 15943 (9-chloro-2,2-(furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine) and CGH 2473 *N*-[4-(3,4-dichloro-phenyl)-5-pyridin-4-yl-thiazol-2-yl]-acetamide) for the dog receptor exceeded by at least 0.5 log units the pK_B values at the rat and guinea pig sites. Our data indicate species differences between the rat and guinea pig adenosine A_{2B} receptors on the one hand and the dog adenosine A_{2B} receptor on the other with respect to antagonist pharmacology.
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Keywords: Adenosine A_{2B} receptor; Species difference; Adenosine receptor antagonist; Colon, rat; Aorta, guinea pig; Saphenous vein, dog

1. Introduction

Adenosine exerts its manifold biological effects by interacting with four G-protein coupled receptors designated A_1, A_{2A}, A_{2B} and A_3 (Ralevic and Burnstock, 1998; Fredholm et al., 2001). These receptors have been cloned from a number of species and their structures determined (Fredholm et al., 2000; Alexander et al., 2001). In the case of the adenosine A_1 receptor, and particularly the adenosine A_3 receptors, structural differences between species manifest as major differences in agonist and antagonist pharmacology (Klotz et al., 1991; Tucker and Linden, 1993; Ji et al., 1994; Klotz, 2000; Fredholm et al., 2001).

The A_{2B} receptor has been cloned from human (Pierce et al., 1992), rat (Rivkees and Reppert, 1992) and mouse (Marquardt et al., 1994). The overall structural homology between the human and rat or mouse adenosine A_{2B} receptors is 86% to 87% whereas the rat and mouse share 96% sequence homology. There have been few studies which have compared systematically the pharmacology of adenosine A_{2B} receptors from different species. However, similarities in pharmacological profiles have been reported between the A_{2B} receptors in fibroblast cell lines of human and murine origin (Brackett and Daly, 1994) and between the cloned human receptor expressed in Chinese hamster ovary (CHO) cells and the native A_{2B} receptor from guinea pig brain (Alexander et al., 1996).

The purpose of the present studies was to seek further evidence for pharmacological similarities or differences between A_{2B} receptors of different species. To this end, we have compared the affinities of eight adenosine receptor antagonists from four distinct chemical classes at the native A_{2B} receptors which mediate relaxation of longitudinal muscle from rat colon (Peachey et al., 1999), relaxation of the smooth muscle from the guinea pig aorta (Martin, 1992) and dog saphenous vein (Hargreaves et al., 1991).

2. Methods

2.1. Animals

Male Brown Norway rats weighing 200–250 g and male Dunkin–Hartley guinea pigs weighing 300–500 g were

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supplied by Charles River, L'Arbresle, France. Saphenous veins were obtained from male and female beagle dogs being euthanised as control animals in toxicology studies. All experiments with animal tissues were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinaeramt, Basel-Stadt).

2.2. Rat A_{2B} receptor assay. Longitudinal muscle from colon

The distal colon was rapidly removed from rats killed by exposure to carbon dioxide and placed in Tyrode solution of the following composition (mM): NaCl, 136.9; KCl, 2.8; $MgSO_4$, 2.1; $CaCl_2$, 1.8; NaH_2PO_4, 0.3; $NaHCO_3$, 11.9; glucose, 5.6. The longitudinal muscle and the muscularis mucosa were separated as described by Bailey and Hourani (1992). Segments of longitudinal muscle (10 mm long, 3 mm wide) were set up for recording isotonic tension in 10-ml organ baths containing Tyrode solution at 37 °C bubbled with 95% O_2/5% CO_2. Resting tension was maintained at 0.5 g. After a stabilisation period of 1 h, during which time the tissues were repeatedly washed, a supramaximal concentration of bethanechol (0.1 mM) was added to the bath. After repeated washing during 1–1.5 h, bethanechol (0.1 mM) was added to the bath followed again by washout. The tissue was then exposed a third time to bethanechol (0.1 mM) followed 20 min later by establishment of a relaxant concentration–response curve to NECA (10 nM–0.1 mM) in the presence of the bethanechol. After repeated washing, a second concentration–response curve to NECA in the presence of bethanechol was established. Antagonists or vehicle (dimethylsulphoxide [DMSO], final bath concentration 0.2% which had no effect on the responsiveness of the tissues) were included in the Tyrode solution 30 min prior to establishment of the second concentration–response to NECA. Relaxant effects were expressed relative to the maximal response to bethanechol. The dissociation constant of the antagonist–receptor complex, K_B, was calculated from the equation: $([A^*]/[A]) - 1 = ([B]/K_B)$; where $[A^*]/[A]$ is the ratio of concentrations of agonist giving an equal response in the presence and in the absence of a given concentration of antagonist, B (Furchgott, 1972).

2.3. Guinea pig A_{2B} receptor assay. Aorta

The method was that described by Martin (1992) with minor modifications. Animals were killed by a blow to the head. The thoracic aorta was removed, cleared of fat and connective tissue and cut into ring segments approximately 4 mm long. Rings were mounted in 10-ml organ baths in modified Krebs' solution of the following composition (mM): NaCl, 118; KCl, 4.8; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11, gassed with 95%O_2/5% CO_2 at 37 °C and under a resting tension of 2 g. After a stabilisation period of 1 h, during which time the tissues were repeatedly washed, the tissue was contracted with phenylephrine (3 μM). After repeated washing for approximately 1 h, phenylephrine (3 μM) was added to the bath followed again by washout. The sequence was repeated a third time. The tissue was then exposed a fourth time to phenylephrine (3 μM) followed 20 min later by establishment of a relaxant concentration–response curve to NECA (10 nM–0.1 mM) in the presence of the phenylephrine. After repeated washing, a second concentration–response curve to NECA in the presence of phenylephrine was established. Antagonists or vehicle (DMSO, final bath concentration 0.2% which had no effect on the responsiveness of the tissues) were included in the Krebs' solution 30 min prior to establishment of

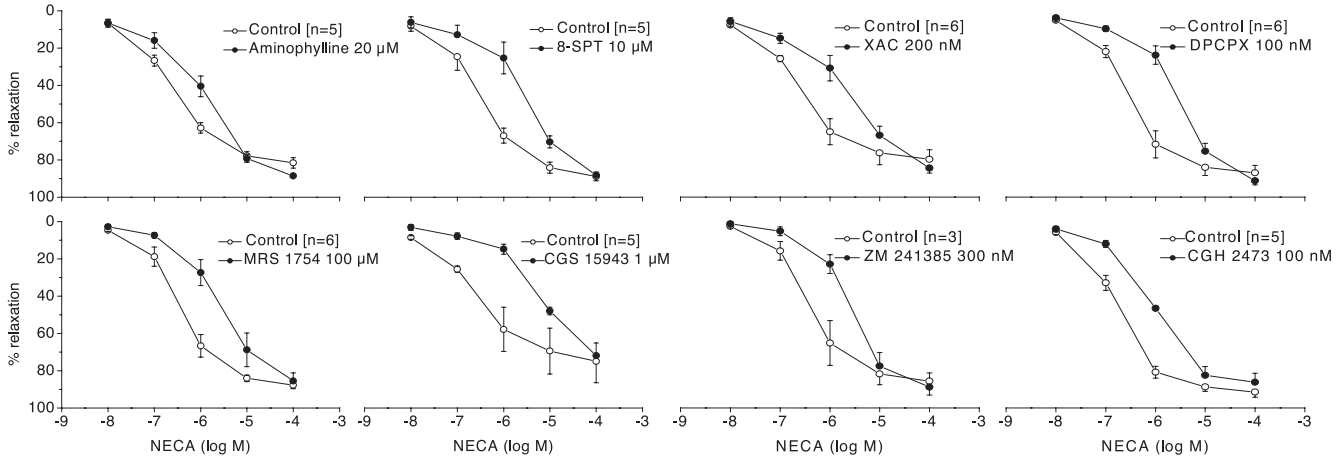

Fig. 1. The effects of adenosine receptor antagonists on relaxant responses to NECA induced in longitudinal muscle strips from rat colon contracted with bethanechol (0.1 mM). Two concentration–response curves to NECA were established. The antagonists were applied at the concentrations indicated 30 min before establishing the second concentration–response curve to NECA. Administration of vehicle had no effect on the sensitivity to NECA (data not illustrated). Mean values ± S.E.M. of n experiments are presented.

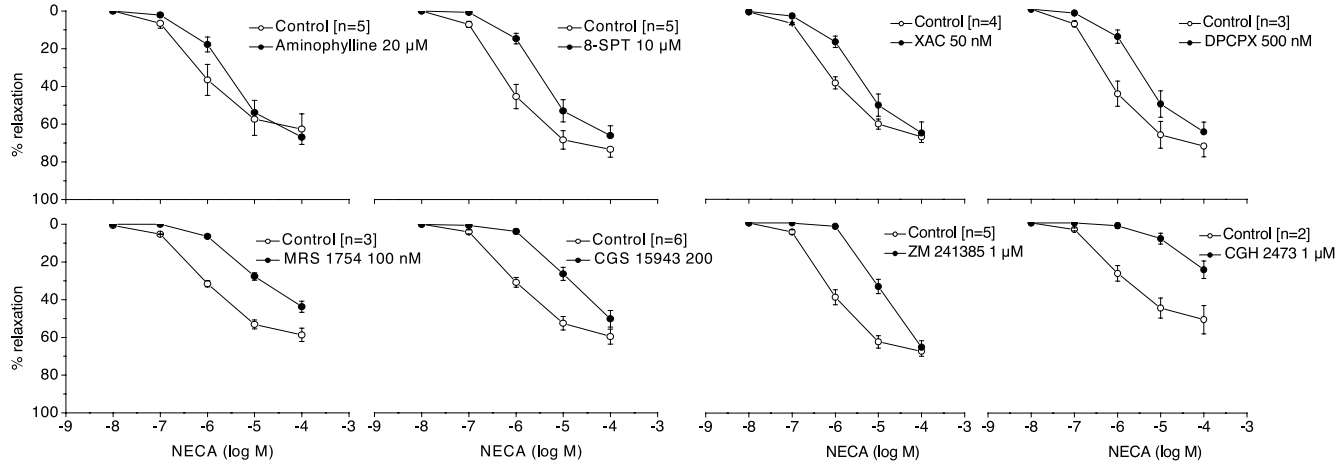

Fig. 2. The effects of adenosine receptor antagonists on relaxant responses to NECA induced in rings of guinea pig aorta contracted with phenylephrine (3 μM). Two concentration–response curves to NECA were established. The antagonists were applied at the concentrations indicated 30 min before establishing the second concentration–response curve to NECA. Administration of vehicle had no effect on the sensitivity to NECA (data not illustrated). Mean values ± S.E.M. of *n* experiments are presented.

the second concentration–response to NECA. Relaxant effects were expressed relative to the maximal response to phenylephrine.

2.4. Dog A_{2B} receptor assay. Saphenous vein

A section of saphenous vein was removed from beagle dogs killed with an overdose of pentothal sodium and placed in modified Krebs' solution of the composition described above. Strips approximately 5 mm long were prepared and mounted in 10-ml organ baths in modified Krebs' solution (for details, see above), gassed with 95% O_2/5% CO_2 at 37 °C and under a resting tension of 2 g. The experimental procedure was identical to that used for the guinea pig aorta (see above).

2.5. Materials

Aminophylline, bethanechol (carbamy-β-methyl choline chloride), phenylephrine hydrochloride, 8-[-[[[[(2-aminoethyl)amino]-carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine (XAC), 9-chloro-2,2-(furanyl)[1,2,4]triazolo [1,5-c]quinazolin-5-amine (CGS 15943), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 8-*p*-(sulphophenyl)theophylline (8-SPT) and 5′-*N*-ethyl-carboxamidoadenosine (NECA), were obtained from Sigma/RBI, Switzerland. 4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385) was from Tocris, UK. *N*-(4-Cyano-phenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-2,3,4,5,6,7-hexahydro-1*H*-purin-8-yl)-phenoxy]-acetamide (MRS 1754) and *N*-[4-(3,4-dichloro-phenyl)-5-pyridin-4-yl-

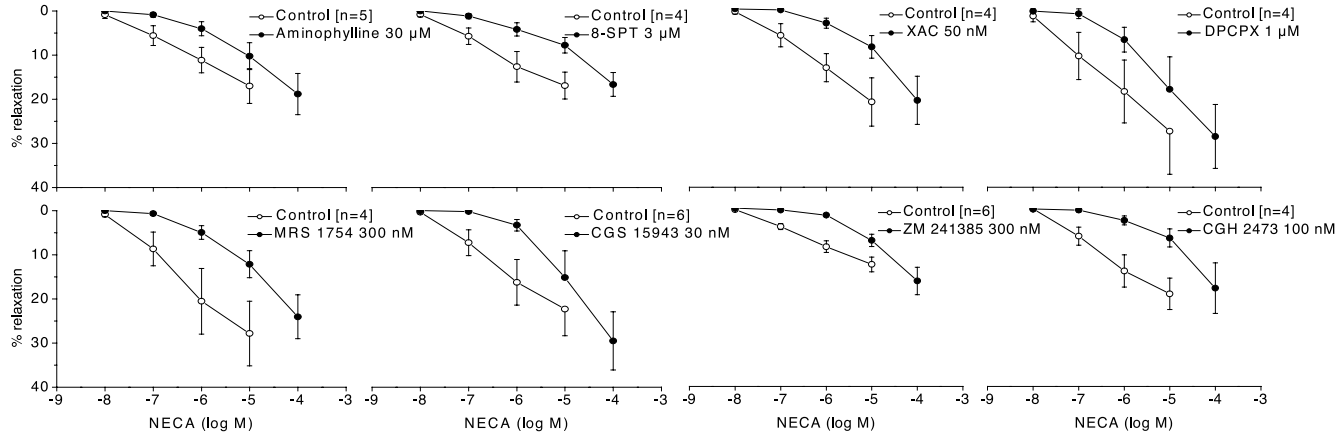

Fig. 3. The effects of adenosine receptor antagonists on relaxant responses to NECA induced in strips of dog saphenous vein contracted with phenylephrine (3 μM). Two concentration–response curves to NECA were established. The antagonists were applied at the concentrations indicated 30 min before establishing the second concentration–response curve to NECA. Administration of vehicle had no effect on the sensitivity to NECA (data not illustrated). Mean values ± S.E.M. of *n* experiments are presented.

thiazol-2-yl]-acetamide (CGH 2473) were synthesised at Novartis Horsham Research Centre, UK. The adenosine receptor antagonists were dissolved in DMSO.

2.6. Data analysis

All data are presented as means ± S.E.M. The negative logarithms of the K_B values (pK_B) are presented. Student's *t*-test with Bonferroni correction was used to compare pK_B values from the different tissues. A P value <0.05 was considered significant.

3. Results

CGH 2473 is a novel broad spectrum adenosine receptor antagonist (K_i values at the human A_1, A_{2A}, A_{2B} and A_3 receptors are 2.5, 585, 5 and 0.1 nM, respectively (Press et al., 2002). The concentrations of antagonists used were chosen to induce a minimum shift of threefold in the concentration–response curves to NECA. In the rat and guinea pig adenosine A_{2B} receptor assays, the inhibitory concentration–response curves to NECA were obtained over the range 100 nM–100 μM and were classically sigmoid attaining a maximum inhibition of the induced tone of between 80–90% and 50–60%, respectively. All the antagonists caused parallel rightward shifts in the concentration–response curves to NECA and there was no evidence for depression of the maximum response (Figs. 1 and 2). On dog saphenous vein, NECA also induced relaxation from 100 nM. However, the concentration–response curves were shallow and the maximum relaxation obtained at 10 μM varied between 10% and 28% (Fig. 3). Nevertheless, all the antagonists were able to shift the concentration–response curves to NECA to the right and, allowing for the variability in some of the groups, broadly in parallel.

There was generally good agreement between the tissues with respect to the antagonist pK_B values (Table 1) and regression analysis revealed highly significant correlations between each of the three receptors (rat/guinea pig, $r=0.988$, $P<0.0001$; rat/dog, $r=0.911$, $P=0.002$; guinea pig/dog, $r=0.940$ $P=0.0005$). However, the correlation between the rat and guinea pig receptors was clearly stronger than the correlation between the dog receptor and either the rat or guinea pig receptors. Indeed, there were no significant differences between the pK_B values for any of the antagonists at the rat or guinea pig receptors. In contrast, the pK_B values of 8-SPT (8-*p*-(sulphophenyl)theophylline), XAC (8-[-[[[[(2-aminoethyl)amino]-carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine), CGS 15943 (9-chloro-2,2-(furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine) and CGH 2473 (*N*-[4-(3,4-dichloro-phenyl)-5-pyridin-4-yl-thiazol-2-yl]-acetamide) for the dog receptor exceeded by at least 0.5 log units—and, with one exception, significantly—the pK_B values at the rat and guinea pig receptors (Table 1).

4. Discussion

We present here the pK_B values for eight nonselective adenosine receptor antagonists from four different chemical classes generated to explore the differences or similarities between the adenosine A_{2B} receptors of rat, guinea pig and dog. We have used the longitudinal muscle strip of rat colon, the guinea pig aorta and the dog saphenous vein. The relaxant responses to NECA of these three tissues have been shown to be mediated through the adenosine A_{2B} receptor (Hargreaves et al., 1991; Martin, 1992; Peachey et al., 1999). In our own preliminary experiments, we showed that the prototype A_{2A} receptor agonist, CGS21680 (2-*p*-(2-Carboxyethyl)-phenethylamino-5′-*N*-ethylcarboxyamidoadenosine), had no relaxant effects on the tissues at concentrations up to 1 μM (data not illustrated); hence, a relaxant effect of NECA via adenosine A_{2A} receptors can be ruled out.

All the compounds behaved as surmountable antagonists on the three tissues and the range of affinities spanned three log units. These conditions are appropriate to allow the pharmacological definition of a receptor site and differences between receptors, if they exist, should be detected (Furchgott, 1972; Fredholm et al., 2001). In practice, the antagonist pK_B values were broadly similar between the different assays and highly significant coefficients of correlation were obtained. On the face of it, therefore, these data indicate that

Table 1
pK_B values for blockade of adenosine A_{2B} receptor from different species

	Rat	Guinea pig	Dog
Aminophylline	5.19 ± 0.16 (4) [5]	4.96 ± 0.11 (3)	5.51 ± 0.34 (5)
8-SPT	5.80 ± 0.06 (3) [5][a]	5.66 ± 0.13 (5)[a]	6.65 ± 0.18 (4)
XAC	7.37 ± 0.15 (4)[a]	7.75 ± 0.24 (4)	8.45 ± 0.33 (4)
DPCPX	6.70 ± 0.14 (5)	6.84 ± 0.07 (4)	6.88 ± 0.26 (4)
MRS 1754	7.78 ± 0.14 (3) [5]	8.04 ± 0.14 (3)	7.91 ± 0.20 (4)
CGS 15943	7.18 ± 0.08 (5)[a]	7.63 ± 0.13 (6)[a]	8.44 ± 0.10 (6)
ZM 241385	7.19 ± 0.11 (3)	7.06 ± 0.04 (5)	7.54 ± 0.18 (6)
CGH 2473	7.51 ± 0.10 (5)[a]	7.83 ± 0.08 (3) [4][a]	8.51 ± 0.24 (4)

Values are means (± S.E.M.) of data from the number of animals shown in parentheses. Numbers in square brackets indicate the total number of tissues used. Chemical names of the compounds are given in Methods.

[a] $P<0.05$ that the value differs significantly from the dog value.

the antagonist pharmacology of the adenosine A_{2B} receptors is similar between the three species.

Closer inspection of the data, however, reveals that the correlation between the affinities for the rat and guinea pig A_{2B} receptor was clearly stronger than the correlation between the dog and either the rat or guinea pig receptors. Indeed, there were no significant differences between the pK_B values for any of the antagonists at the A_{2B} receptors of the rat and guinea pig indicating that the receptors are very closely similar, if not identical with respect to antagonist pharmacology. In contrast, the pK_B values for 8-SPT, XAC, CGS 15943 and CGH 2473 for the dog receptor exceeded, by at least 0.5 log units—and, with one exception, significantly—the pK_B values at the other sites. Furchgott (1972) has suggested that a difference of 0.5 log units in the pK_B value obtained with a competitive antagonist in different assays can be taken as preliminary evidence that different receptors/receptor subtypes may mediate the responses. The fact that in our study four compounds representing three different chemical classes yielded pK_B values at the dog adenosine A_{2B} receptor greater than 0.5 log units different from the values on the rat and guinea pig receptors indicates that the adenosine A_{2B} receptor subtype in the dog may be different from that in the rodent species.

It bears emphasis that the effects of NECA on the dog tissue are relatively modest, the error bars are quite large and the concentration–response curves are not entirely sigmoid. However, despite this, the shifts in the curves were broadly parallel and K_B values could be calculated by the method described. Importantly, the significant differences between the dog K_B values and the values obtained in the rat and guinea pig tissues were obtained with XAC, 8-SPT and CGH 2473 where the data were least variable and the shifts were closest to parallel. Thus, it seems unlikely that experimental difficulties can account for the differences between the pK_B values of dog and rodent.

There have been few systematic comparisons of adenosine A_{2B} receptor pharmacology across species (Feoktistov and Biaggioni, 1997). Similar antagonist pharmacology using functional readouts has, however, been claimed between human and mouse (Brackett and Daly, 1994) and between human and guinea pig (Alexander et al., 1996) A_{2B} receptors. It was of interest, therefore, to compare the pA_2 or pK_B values at the human A_{2B} receptors published by these authors and from the literature for the antagonists we have used (see Table 2). Data for six of the antagonists are available. The coefficients of correlation between these values and the rat, guinea pig and dog data in Table 1 were: rat/human, $r=0.988$, $P<0.0001$; guinea pig/human, $r=0.984$, $P=0.0001$; dog/human, $r=0.941$ $P=0.007$ which is consistent with their broad similarity. It bears emphasis, however, that the correlation of the human with the rat and guinea pig sites was stronger than that with the dog. There was close similarity between the pK_B values for the six compounds on the human, rat and guinea pig sites. Interestingly, however, as was the case for the rat and guinea pig receptors, the pK_B values for 8-SPT, XAC and CGS 15943 for the human receptor were lower by at least 0.5 log units than the equivalent values from the dog. Thus, in support of the earlier findings (Brackett and Daly, 1994; Alexander et al., 1996), the human adenosine A_{2B} receptor shows antagonist pharmacology similar to that of the rat and guinea pig receptors. Our data suggest the dog receptor is different from the human receptor in this respect.

Table 2
Antagonist pharmacology of human adenosine A_{2B} receptors (literature values)

	pA_2/pK_B	n
Theophylline	5.32 ± 0.07[a]	4
8-SPT	5.92[a]	1
XAC	7.89 ± 0.02[b]	3–5
DPCPX	7.16 ± 0.12[b]	
CGS 15943	7.75 ± 0.09[b]	
ZM 241385	7.88 ± 0.05[c]	3

[a] Brackett and Daly (1994).
[b] Alexander et al. (1996).
[c] De Zwaart et al. (1999).

Finally, the majority of the antagonists we have investigated are used as tools to define the involvement of adenosine receptor subtypes in biological responses (Ralevic and Burnstock, 1998; Alexander et al., 2001). Our data provide reference values for these agents for A_{2B} receptor affinities of rat, guinea pig and dog, species widely used in pharmacological investigations.

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