A comparison of A₂ adenosine receptor-induced cyclic AMP generation in cerebral cortex and relaxation of pre-contracted aorta

¹Stephen P.H. Alexander, Amanda Losinski, David A. Kendall & Stephen J. Hill

Department of Physiology & Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH

1 A comparative study was carried out between the adenosine receptor mediating a stimulation of cyclic AMP formation in guinea-pig cerebral cortical slices with the adenosine receptor mediating relaxation of phenylephrine precontracted guinea-pig aortic rings.

2 [³H]-cyclic AMP accumulation in [³H]-adenine-prelabelled guinea-pig cerebral cortical slices was stimulated by adenosine and its analogues with the following EC₅₀ values (μM): 5'-N-ethylcarboxamido-adenosine (3.1 ± 0.3) > 2-chloroadenosine (10 ± 2) > adenosine (109 ± 15).

3 2-Chloroadenosine and adenosine elicited maximal responses for [³H]-cyclic AMP accumulation that were 100 ± 7 and 71 ± 6% of the maximal response to 5'-N-ethylcarboxamido-adenosine, respectively. CGS 21680 (100 μM) and DPMA (100 μM) elicited -2 ± 2 and 12 ± 3% of the response to 100 μM 5'-N-ethylcarboxamido-adenosine.

4 Estimation of antagonist potencies at the A₂ adenosine receptor of cerebral cortex showed a rank order of potency (K_i, nM): xanthine amino congener (35 ± 3) > 8-cyclopentyl-1,3-dipropylxanthine (130 ± 22) > PD 115,199 (407 ± 82) > 3,7-dimethyl-1-propargylxanthine (13 ± 2 μM).

5 Adenosine analogues produced long-lasting relaxation of phenylephrine-precontracted aortic rings with the following rank order of potency (EC₅₀ values, μM): 5'-N-ethylcarboxamido-adenosine (0.68 ± 0.06) > 2-chloroadenosine (4.3 ± 0.6) > adenosine (104 ± 13). Maximal relaxations elicited by these agents were 71 ± 3, 98 ± 1, and 100 ± 1%, respectively. CGS 21680 and DPMA at 100 μM elicited smaller relaxations of the precontracted tissues (12 ± 2 and 43 ± 15%, respectively).

6 Antagonism by xanthine derivatives of the 5'-N-ethylcarboxamido-adenosine-induced relaxation of aortic rings showed the following rank order of potency (K_i, nM): xanthine amino congener (17 ± 4) > 8-cyclopentyl-1,3-dipropylxanthine (171 ± 36) > PD 115,199 (341 ± 64) > 3,7-dimethyl-1-propargylxanthine (5520 ± 820).

7 We conclude that the A₂ adenosine receptor mediating relaxation of phenylephrine-contracted aortic rings is an A_{2b} adenosine receptor which exhibits certain minor differences from the A_{2b} receptor which stimulates cyclic AMP accumulation in cerebral cortical slices.

Keywords: Adenosine receptors; xanthines; cyclic AMP; guinea-pig cerebral cortex; guinea-pig aorta

Introduction

Two classes of extracellular adenosine receptor were originally delineated from studies of the effects of adenosine in mouse primary astroglial culture, subserving an inhibition (A₁) and a stimulation (A₂) of adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels (Van Calcar *et al.*, 1979). Radioligand binding to the A₁ receptor has been demonstrated in brain (Schwabe & Trost, 1980; Bruns *et al.*, 1980), adipocyte (Ukena & Schwabe, 1985) and testes (Murphy *et al.*, 1983) to varying extents. A subtype of the A₂ receptor, the high affinity A₂ receptor (Daly *et al.*, 1983) which stimulates adenylyl cyclase activity in membranes from neostriatum, nucleus accumbens and olfactory tubercle (Fredholm, 1977; Wojcik & Neff, 1983) can also be visualised by radioligand binding techniques (Bruns *et al.*, 1986; Jarvis *et al.*, 1989). A second subtype of A₂ receptor, the low affinity A₂ receptor, stimulates cyclic AMP production in most brain regions, but fails to stimulate significantly adenylyl cyclase activity in cell-free preparations (Daly *et al.*, 1983). This subtype has also been recognised in the VA13 human fibroblast cell line, also linked to a stimulation of adenylyl cyclase activity (Bruns *et al.*, 1986). As yet, no selective agonist, antagonist or radioligand binding assay exists for this subtype, making examination of the tissue

distribution of this receptor problematic. These subtypes have been termed A_{2a} and A_{2b} receptors equivalent to the high and low affinity types of the A₂ receptor (Bruns *et al.*, 1986).

Molecular biological approaches to studying adenosine receptors have yielded sequences for A₁ and A_{2a} receptors from dog (Maenhaut *et al.*, 1990; Libert *et al.*, 1991), rat (Mahan *et al.*, 1991; Reppert *et al.*, 1991) and human tissues (Furlong *et al.*, 1992; Libert *et al.*, 1992; Townsend-Nicholson & Shine, 1992). In addition, there are published sequences for an 'A_{2b}' receptor from rat (Stehle *et al.*, 1992) and human tissues (Pierce *et al.*, 1992). Northern blot analysis of the regional distribution of the rat 'A_{2b}' receptor mRNA showed highest density of expression in large intestine, caecum, and urinary bladder, with lower density in the brain and spinal cord (Stehle *et al.*, 1992). Expression of mRNA in the aorta was not examined. Both the rat and human clones, when expressed in COS-6 and Chinese hamster ovary cells, respectively, led to stimulation of cyclic AMP formation upon incubation with the adenosine analogue 5'-N-ethylcarboxamido-adenosine (NECA). However, the former receptor expressed in *Xenopus* oocytes appeared to be coupled to phosphoinositide hydrolysis (Yakel *et al.*, 1993). As the native receptor has only been observed to be coupled to generation of cyclic AMP, there must be some doubt as to the exact nature of this receptor.

¹ Author for correspondence.

A₁ and A₂ classes have been differentiated on the basis of rank order of agonist potency (Hamprecht & van Calcar, 1985; Gurden *et al.*, 1993). Typically N⁶-derivatives of adenosine are more potent than 5'-derivatives at A₁ receptors, and the reverse is true at A₂ receptors. Receptor classification using agonists may, however, be complicated by factors such as receptor reserve or partial activity of agonists, difficulties which the use of selective antagonists would be expected to surmount. The archetypal adenosine receptor antagonists, theophylline and caffeine, show little potential for differentiating between the three receptor types, however, and therefore are not useful as discriminatory tools (Daly *et al.*, 1983). We have used four xanthine-based adenosine antagonists with a range of affinities and reported A₁:A₂ selectivities in radioligand binding assays of rat CNS tissues to characterize the A_{2b} adenosine receptor of guinea-pig cerebral cortical slices linked to cyclic AMP generation. We have compared the pharmacological profile of the A_{2b} receptor with the relaxant A₂ receptor of guinea-pig aorta which has previously also been suggested to be an A_{2b} receptor (Collis *et al.*, 1987; Martin, 1992; Gurden *et al.*, 1993). A preliminary account of some of this work has been presented to the British Pharmacological Society (Losinski *et al.*, 1993).

Methods

Cyclic AMP production in cerebral cortex

Cyclic AMP production was assessed in guinea-pig brain (Hartley strain, 200–600 g, of either sex) as previously described (Alexander *et al.*, 1989). Briefly, cross-chopped slices (350 × 350 µm) of cerebral cortex were prelabelled with [³H]-adenine (74 kBq ml⁻¹) in Krebs Henseleit medium at 37°C, before being washed and pre-incubated in the presence of antagonist in a total volume of 300 µl for 10 min. Except when the effects of adenosine were investigated, the incubation with agonist was carried out in the presence of adenosine deaminase (1 u ml⁻¹) to prevent potential interference from endogenous adenosine. Histamine (0.3 or 1 mM) was also included to amplify responses during the agonist incubation period of 10 min. The incubation was halted by the addition of 200 µl of 1 M HCl containing 25–30 Bq [¹⁴C]-cyclic AMP to act as a recovery marker. [³H]-cyclic AMP was isolated by sequential Dowex 50 – Alumina column chromatography as previously described (Alexander *et al.*, 1989). Analysis of [³H]-cyclic AMP formation was carried out on triplicate samples.

Relaxation of precontracted aortic rings

Guinea-pig aorta was rapidly dissected and freed from connective tissue, cut into 4 mm cylinders and suspended horizontally on thin wires of an isometric apparatus fitted with a Washington Type D transducer (Searle, Kent) under a resting tension of 1 g. Contractile responses were recorded either on a Washington chart recorder (Searle, Kent) or using a Personal Computer equipped with Chart software (CED, Cambs.). The cylinders were allowed to equilibrate for a period of up to 60 min at 37°C in gassed (O₂:CO₂ 95:5) Krebs Henseleit buffer containing indomethacin (10 µM), with occasional changes of medium. Rings were contracted with repeated applications of a submaximally-effective concentration of phenylephrine (4 µM) until a reproducible response was obtained. Cumulative concentration-relaxation curves for the adenosine analogues were constructed in the presence of phenylephrine, discontinuing the addition of agonist when an increase in agonist concentration failed to elicit further relaxation. Agonists were washed out and the tissue allowed to re-equilibrate for a further 60 min before repetition of the contraction-relaxation cycle in the presence of xanthine-based antagonist.

Data analysis and statistics

Data were fitted to a sigmoidal curve using the computer programme GraphPad InPlot (GraphPad, California, U.S.A.). Apparent inhibition constants for the antagonists (*K_i*) from functional assays were calculated as previously described (Alexander *et al.*, 1989) based on the antagonist-induced shift in agonist (unless otherwise indicated, NECA) concentration-response curves. Statistical analyses were carried out using the Student's *t* test.

Chemicals

[¹⁴C]-cyclic AMP (1.6 GBq mmol⁻¹) and [³H]-adenine (888 GBq mmol⁻¹) were obtained from NEN Dupont (Herts.) and Amersham International (Bucks.) respectively. N-[2-(dimethylamino) ethyl]N-methyl-4-(1,3-dipropylxanthine)benzene sulphonamide (PD 115,199) was a gift from Warner-Lambert, U.S.A. Adenosine analogues (including 2-*p*-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamido-adenosine (CGS 21-680) and N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA)) and xanthine derivatives were obtained from Research Biochemicals Incorporated (Semat, Herts.). All other chemicals were from Fisons (Leics.) or Sigma Chemicals (Dorset).

Results

Adenosine receptor-stimulated cyclic AMP production in guinea-pig cerebral cortex

Adenosine analogues were observed to enhance the accumulation of [³H]-cyclic AMP in [³H]-adenine-prelabelled guinea-pig cerebral cortical slice in a concentration-dependent manner. Of the analogues tested, NECA (5'-N-ethylcarboxamido-adenosine) was the most potent, followed by 2CA (2-chloroadenosine) and adenosine (Figure 1, Table 1). The response to adenosine was conducted in the absence of adenosine deaminase (NECA responses in the absence and presence of adenosine deaminase were not significantly different). The response to adenosine showed a reduced maximal effect when compared to NECA and 2CA. In the presence of a maximally-active concentration of adenosine (300 µM), the response to 100 µM NECA was reduced to approximately the same level as the response to adenosine alone (NECA response = 100%; 300 µM adenosine = 82 ± 6%; adenosine + NECA = 81 ± 7%; *n* = 3). The reportedly A₂-selective agents N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA) and 2-*p*-(2-carboxyethyl)phenethylamino-5'-

Figure 1 Stimulation of [³H]-cyclic AMP accumulation in [³H]-adenine-prelabelled guinea-pig cerebral cortical slices by 5'-N-ethylcarboxamido-adenosine (X), 2-chloroadenosine (O) and adenosine (□). Data are means and s.e.mean from single experiments representative of 5–14.

Table 1 Adenosine analogue potency at A₂ adenosine receptors of guinea-pig cerebral cortex and aorta

Agonist	Cerebral cortex			Aorta		
	EC ₅₀ value (μM)	Intrinsic ^a activity (%)	n	EC ₅₀ value (μM)	Intrinsic ^b activity (%)	n
NECA	3.1 ± 0.3	100	14	0.68 ± 0.06	71 ± 3	40
2CA	10 ± 2	97 ± 6	7	4.7 ± 0.6	98 ± 1	5
Adenosine ^c	109 ± 15	71 ± 6	5	104 ± 13	100 ± 1	4
DPMA ^d	—	12 ± 3	3	—	43 ± 15	3
CGS 21680 ^d	—	-2 ± 2	3	—	12 ± 2 ^e	2

^aIntrinsic activity compares the maximal response to each agent (for DPMA and CGS 21680, the response at 100 μM) with that to 100 μM NECA. ^bIntrinsic activity compares the maximal relaxation elicited by each agent as a function of the contractile effect of phenylephrine. ^cThe cyclic AMP response to adenosine was determined in the absence of adenosine deaminase. The response to 100 μM NECA was not significantly altered in the presence of adenosine deaminase. ^dResponses to DPMA and CGS 21680 were examined at a single agonist concentration of 100 μM. Data are means ± s.e.mean (*means ± range) of the indicated number of experiments. For abbreviations, see text.

N-ethylcarboxamidoadenosine (CGS 21680) showed little or no significant stimulatory effects of cyclic AMP formation at concentrations up to 100 μM.

In the presence of fixed concentrations of xanthine-based adenosine receptor antagonists, concentration-response curves to NECA were shifted rightward in an apparently competitive manner (see Alexander *et al.*, 1989), allowing estimation of the apparent inhibition constants for these antagonists (Table 2). A rank order of antagonist potency was observed of xanthine amino congener (XAC) > 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) > N-[2-(dimethylamino)ethyl]-N-methyl-4-(1,3-dipropylxanthine)-benzenesulphonamide (PD 115,199) > 3,7-dimethyl-1-propargylxanthine (DMPX). The concentration-response curve to adenosine was examined in the presence and absence of 40 nM DPCPX (data not shown). This concentration of DPCPX was chosen in order to occupy over 80% of A₁ adenosine receptors in this tissue (based on a K_i for DPCPX of 6 nM; Alexander *et al.*, 1992b) with less than 25% of the A_{2b} receptors occupied (based on a K_i of 130 nM, Table 1). However, this concentration of DPCPX failed to alter significantly either the EC₅₀ value or maximal response of adenosine, indicating little contribution of A₁ receptors to the reduced maximal response of adenosine compared to NECA.

Adenosine receptor-mediated relaxation of guinea-pig aortic rings

We carried out a number of preliminary investigations to determine the optimal conditions for assaying adenosine receptor activity in guinea-pig aortic rings. Analysis of cumulative concentration-response curves for phenylephrine-induced contractions gave an EC₅₀ value of 0.92 ± 0.03 μM with a maximal response of 1.36 ± 0.14 g (*n* = 3). Histamine showed a greater maximal response (2.25 ± 0.18 g; EC₅₀ value 1.21 ± 0.22 μM; *n* = 7); however, the response to phenylephrine was better maintained. In further experiments designed to investigate the relaxant action of adenosine receptor

agonists, 4 μM phenylephrine was used to elicit aortic contractions.

Addition of 3 μM 5'-N-ethylcarboxamidoadenosine (NECA) to precontracted aorta elicited a rapid, maintained relaxation of the aorta (Figure 2). Cumulative concentration-relaxation curves were constructed for the agonists NECA, 2-chloro-adenosine (2CA) and adenosine (Figure 3, Table 1). Interestingly, 2CA and adenosine fully relaxed the tissue, while in

Figure 2 Long-lasting relaxation of a phenylephrine (PE)-contracted guinea-pig aortic cylinder by 300 nM 5'-N-ethylcarboxamidoadenosine (NECA).

Figure 3 Relaxation of phenylephrine-precontracted guinea-pig aortic rings by 5'-N-ethylcarboxamidoadenosine (NECA) (X), 2-chloro-adenosine (2CA, O) and adenosine (□). The relaxant response to 2CA in the presence of a maximally-active concentration of NECA is also shown (●). Data are means and s.e.mean of 4–11 experiments.

Table 2 Antagonist affinities at the A₂ adenosine receptors of cerebral cortex and aorta

Antagonist	Cerebral cortex		Aorta	
	K _i (nM)	n	K _i (nM)	n
XAC	35 ± 3	4	17 ± 4	4
DPCPX	123 ± 21	14	171 ± 36	8
PD 115,199	407 ± 82	5	341 ± 64	6
DMPX	12843 ± 1849	3	5521 ± 824	3

Data are means ± s.e.mean of apparent inhibition constants (calculated as described in Methods) estimated on the indicated number of occasions. For abbreviations, see text.

comparison, the NECA response relaxed the aorta to only 71%. In the presence of a maximally-active concentration of NECA (100 μ M), addition of 2CA elicited a further relaxation of the tissue to completeness (EC_{50} 2CA in the presence of NECA 26 ± 4 μ M, $97 \pm 3\%$ relaxation, $n = 4$). DPMA and CGS 21680 induced smaller responses at 100 μ M (Table 1).

In the presence of fixed concentrations of xanthine-based adenosine receptor antagonists, concentration-response curves to NECA were shifted rightward in an apparently competitive manner (see Alexander *et al.*, 1989), allowing estimation of the apparent inhibition constants for these antagonists (Table 2). Thus, NECA concentration-response curves in the presence of 300 nM XAC, 1 μ M DPCPX, 3 μ M PD 115,199, or 30 μ M DMPX were shifted rightward compared to control NECA responses 22, 14, 11 and 7 fold, respectively. Slopes of the NECA concentration-response curves in the presence of XAC (-1.16 ± 0.08), DPCPX (-1.19 ± 0.15), PD 115,199 (-1.24 ± 0.15) and DMPX (-0.98 ± 0.09) were not significantly different from the control response (-1.06 ± 0.03 , Student's *t* test). Thus, the adenosine receptor of guinea-pig aorta exhibits an antagonist rank order of potency such that XAC > DPCPX > PD 115,199 > DMPX. Preliminary experiments with 2CA as the relaxant agonist in place of NECA produced a similar affinity estimate for the antagonist XAC (100 nM XAC elicited an 11 fold shift in the 2CA concentration-response curve to generate a K_i estimate of 10 nM, $n = 2$, data not shown).

Discussion

Research into the characterisation of adenosine receptors has mainly concentrated on tissues of nervous system origin. In CNS preparations, three subclasses of adenosine receptor are demonstrable, based on changes in levels of the second messenger cyclic AMP (Daly *et al.*, 1983). Thus, A_1 receptors are linked to an inhibition of cyclic AMP formation in both cell-free and slice preparations (Ebersolt *et al.*, 1983; Fredholm *et al.*, 1986). A_2 receptors were subdivided into high and low affinity receptors on the basis of regional localization and demonstration in cell-free or intact slice preparations. These receptors were later renamed A_{2a} and A_{2b} receptors, respectively (Bruns *et al.*, 1986). A_{2a} receptors are almost exclusively located in the neostriatum, nucleus accumbens and olfactory tubercle and stimulate adenylyl cyclase activity in cell-free preparations, while A_{2b} receptors stimulate cyclic AMP formation in intact slice preparations from most brain regions (Daly *et al.*, 1983). Similar adenosine receptor subclasses have been suggested to exist in the periphery (Collis *et al.*, 1987; Martin *et al.*, 1992; Gurden *et al.*, 1993), but a direct comparison of CNS and peripheral adenosine receptors has not been carried out. In the present study, we compare the A_{2b} adenosine receptor of guinea-pig cerebral cortex with the suggested A_{2b} adenosine receptor of guinea-pig aorta. Our data suggest marked similarities in the pharmacological profile of the two receptors, but raise the possibility that the two represent variants of the A_{2b} receptor class.

The A_{2b} receptor of guinea-pig cerebral cortex

Stimulation by adenosine analogues of cyclic AMP formation in guinea-pig cerebral cortex shows a rank order of potency of NECA > 2CA > adenosine >> DPMA $\approx$ CGS 21680. This rank order of NECA, 2CA and adenosine concurs with that observed for stimulation of cyclic AMP generation in rat cerebral cortex (Bazil & Minneman, 1986). In the latter tissue, a reduced maximal response to adenosine was also observed in comparison with NECA and 2CA, raising the possibility that adenosine, the presumed endogenous ligand, is a partial agonist at the A_{2b} receptor. The lack of effect of DPCPX at a concentration chosen to block almost completely A_1 receptors (40 nM) suggests that A_1 receptor-induced inhibition of cyclic AMP generation is not involved in the

reduced maximal response to this agent. The reduced response to 100 μ M NECA observed in the presence of a maximally effective concentration of adenosine adds further evidence for the partial agonist nature of adenosine at the A_{2b} receptor.

DPMA and CGS 21680 have been proposed as A_2 -selective agonists based on radioligand binding assays in rat brain membranes (Bridges *et al.*, 1988; Jarvis *et al.*, 1989); however, it is clear from the present study that they fail to stimulate significantly A_{2b} receptors, and thus may ultimately prove to be A_{2a} -selective.

Recent advances in the synthesis of adenosine receptor antagonists have produced compounds which exhibit high specificity for adenosine receptor subclasses, assessed mainly by use of radioligand binding assays in rat CNS preparations. Thus, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, PD 116,948) was shown to have a 740 fold selectivity for the A_1 against the A_{2a} receptor of rat brain in both radioligand binding and functional adenylate cyclase assays (Lee & Redington, 1986; Bruns *et al.*, 1987a; Lohse *et al.*, 1987). PD 115,199, a benzenesulphonamide derivative of 1,3-dipropylxanthine was shown to have high affinity for rat CNS A_1 and A_{2a} receptors, with, however, little selectivity between the two classes (Bruns *et al.*, 1987b). Xanthine amino congener apparently exhibits high affinity for all three classes of adenosine receptor in the rat, with some A_1 selectivity (van Galen *et al.*, 1992). A further xanthine-based compound, 3,7-dimethyl-1-propargylxanthine has been suggested to be 4 fold A_2 -selective in radioligand binding assays (Ukena *et al.*, 1986; Daly *et al.*, 1986). The rank order of antagonist potency at the A_{2b} adenosine receptor mediating cyclic AMP generation is XAC > DPCPX > PD 115,199 > DMPX. Our recent examination of A_1 and A_{2a} adenosine receptor binding in membranes from guinea-pig brain show antagonist rank order of potencies of DPCPX > XAC > PD 115,199 and PD 115,199 > DPCPX > XAC, respectively (Alexander *et al.*, unpublished observations). The behaviour of these xanthine antagonists at CNS adenosine receptors in the guinea-pig correlates well with data from the rat and may therefore prove to be a useful means of discriminating adenosine receptor subtypes.

A_2 receptor relaxation of guinea-pig aorta

Adenosine and its analogues elicited a long-lasting relaxation of phenylephrine-induced aortic cylinder contraction, with a rank order of potency NECA > 2CA > adenosine > DPMA > CGS 21680. The long-lasting relaxation induced by NECA in the guinea-pig aorta resembles the well-maintained NECA stimulation of cyclic AMP levels in guinea-pig cerebral cortex (Alexander *et al.*, 1992a). Whether the relaxation of guinea-pig aorta induced by NECA is mediated by a stimulation of cyclic AMP levels will be the subject of further investigations. In the aorta, NECA elicited a reduced maximal relaxant response compared to 2CA and adenosine, implying that it may well be a partial agonist in this tissue. In the presence of a maximally-active concentration of NECA, 2CA was able to relax the tissue further; however, the concentration-response curve to 2CA was shifted rightward. This is consistent with both these agents acting through the same receptor.

The xanthine derivatives induced rightward shifts in the NECA concentration-response curve. Calculating apparent inhibition constants from the antagonist-induced shifts indicated a rank order of antagonist potency of XAC > DPCPX > PD 115,199 > DMPX. The similar affinity of XAC for antagonism of NECA- and 2CA-elicited relaxation of phenylephrine-induced contractions is further evidence for an identical site of action of the two agonists.

Molecular biology of A_{2b} receptors

A human ' A_{2b} ' adenosine receptor has been expressed in Chinese hamster ovary cells and exhibited positive coupling

to cyclic AMP generation when stimulated by NECA (EC₅₀ value of 0.9 μ M, c. 10 fold maximal stimulation) and adenosine (1 mM also elicited a c. 10 fold stimulation). CGS 21680, in contrast, failed to elicit a stimulation of cyclic AMP generation in transfected cells. Binding of adenosine receptor radioligands (2-chloro-N⁶-cyclopentyl-[³H]-adenosine, [³H]-CGS 21680 or 5'-N-ethylcarboxamido-[³H]-adenosine) was not supported in cells transfected with this clone. The rat 'A_{2b}' adenosine receptor expressed in COS-6 cells also exhibited a NECA-elicited stimulation of cyclic AMP generation. When this clone was expressed in *Xenopus* oocytes, adenosine evoked an inward current with an EC₅₀ value of ~50 μ M (Yakel *et al.*, 1993). NECA, 10 μ M, elicited a response comparable with that induced by 100 μ M adenosine, while 10 μ M CGS 21680 was ineffective as a stimulus of inward current. The inward current induced by 100 μ M adenosine was reversed by 100 nM (27 $\pm$ 3% inhibition) and 1 μ M DPCPX (95 $\pm$ 4%) and 10 μ M DMPX (75 $\pm$ 5%). The pharmacological profile (albeit very limited) from either of these clones is therefore consistent with the profile of the A_{2b} adenosine receptor from guinea-pig cerebral cortex and the A₂ receptor from the guinea-pig aorta. The potential localization of the

cloned 'A_{2b}' receptors in aortic tissues has unfortunately not been examined. The relative intrinsic activity of NECA and adenosine has also not been examined in these clones.

Concluding remarks

The identical rank order of potency for the selected agonists and antagonists suggests that the A₂ adenosine receptor of guinea-pig aorta is analogous to the A_{2b} adenosine receptor of guinea-pig cerebral cortex. However, adenosine appears to be a partial agonist in the cerebral cortex, whilst behaving as an apparent full agonist in the aorta and the reverse is true for NECA. This is therefore a substantial inconsistency between the tissues. We must conclude that although these receptors exhibit very similar pharmacological profiles, they may well be variants of the A_{2b} adenosine receptor. This question may best be resolved through the application of molecular biological studies.

We thank the Wellcome Trust for financial support and Dr Vince Wilson for useful discussion.

References

- ALEXANDER, S.P.H., CURTIS, A.R., HILL, S.J. & KENDALL, D.A. (1992a). Activation of a metabotropic excitatory amino acid receptor potentiates A_{2b} adenosine receptor-stimulated cyclic AMP accumulation. *Neurosci. Lett.*, **146**, 231–233.
- ALEXANDER, S.P.H., CURTIS, A.R., KENDALL, D.A. & HILL, S.J. (1992b). The affinity of 8-cyclopentyl-1,3-dipropylxanthine at A₁ adenosine receptors of guinea-pig cerebral cortex. *Br. J. Pharmacol.*, **107**, 75P.
- ALEXANDER, S.P.H., KENDALL, D.A. & HILL, S.J. (1989). Differences in the adenosine receptors modulating inositol phosphates and cyclic AMP accumulation in mouse and guinea-pig brain. *Br. J. Pharmacol.*, **98**, 1241–1247.
- BAZIL, C.W. & MINNEMAN, K.P. (1986). An investigation of the low intrinsic activity of adenosine and its analogs at low affinity (A₂) adenosine receptors in rat cerebral cortex. *J. Neurochem.*, **47**, 547–553.
- BRIDGES, A.J., BRUNS, R.F., ORTWINS, D.F., PRIEBE, S.R., SZOTEK, D.L. & TRIVEDI, B.K. (1988). N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine and its uronamide derivatives. Novel adenosine agonists with both high affinity and high selectivity for the adenosine A₂ receptor. *J. Med. Chem.*, **31**, 1282–1285.
- BRUNS, R.F., DALY, J.W. & SNYDER, S.H. (1980). Adenosine receptors in brain membranes: binding of N⁶-cyclohexyl[³H]-adenosine and 1,3-diethyl-8-[³H]phenylxanthine. *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 5547–5551.
- BRUNS, R.F., FERGUS, J.H., BADGER, E.W., BRISTOL, J.A., SANTAY, L.A., HARTMAN, J.D., HAYS, S.J. & HUANG, C.C. (1987a). Binding of the A₁-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat membranes. *Naunyn-Schmied. Arch. Pharmacol.*, **335**, 59–63.
- BRUNS, R.F., FERGUS, J.H., BADGER, E.W., BRISTOL, J.A., SANTAY, L.A. & HAYS, S.J. (1987b). PD 115,199: an antagonist ligand for adenosine A₂ receptors. *Naunyn-Schmied. Arch. Pharmacol.*, **335**, 64–69.
- BRUNS, R.F., LU, G.H. & PUGSLEY, T.A. (1986). Characterization of the A₂ adenosine receptor labeled by [³H]NECA in rat striatal membranes. *Mol. Pharmacol.*, **29**, 331–346.
- COLLIS, M.G., JACOBSON, K.A. & TOMKINS, D.M. (1987). Apparent affinity of some 8-phenyl-substituted xanthines at adenosine receptors in guinea-pig aorta and atria. *Br. J. Pharmacol.*, **92**, 69–75.
- DALY, J.W., BUTTS-LAMB, P. & PADGETT, W. (1983). Subclasses of adenosine receptors in the central nervous system: interaction with caffeine and related methylxanthines. *Cell. Mol. Neurobiol.*, **3**, 69–80.
- DALY, J.W., PADGETT, W.L. & SHAMIM, M.T. (1986). Analogues of caffeine and theophylline: effect of structural alterations on affinity at adenosine receptors. *J. Med. Chem.*, **29**, 1305–1308.
- EBERSOLT, C., PREMONT, J., PROCHIANTZ, A., PEREZ, M. & BOCKAERT, J. (1983). Inhibition of brain adenylate cyclase by A₁ adenosine receptors: pharmacological characteristics and locations. *Brain Res.*, **267**, 123–129.
- FREDHOLM, B.B. (1977). Activation of adenylate cyclase from rat striatum and tuberculum olfactorium by adenosine. *Med. Biol.*, **55**, 262–267.
- FREDHOLM, B.B., JONZON, B. & LINDSTROEM, K. (1986). Effect of adenosine receptor agonists and other compounds on cyclic AMP accumulation in forskolin-treated hippocampal slices. *Naunyn-Schmied. Arch. Pharmacol.*, **332**, 173–178.
- FURLONG, T.J., PIERCE, K.D., SELBIE, L.A. & SHINE, J. (1992). Molecular characterization of a human brain adenosine A₂ receptor. *Mol. Brain Res.*, **15**, 62–66.
- GURDEN, M.F., COATES, J., ELLIS, F., EVANS, B., FOSTER, M., HORNBY, E., KENNEDY, I., MARTIN, D.P., STRONG, P., VARDEY, C.J. & WHEELDON, A. (1993). Functional characterization of three adenosine receptor types. *Br. J. Pharmacol.*, **109**, 693–698.
- HAMPRECHT, B. & VAN CALKER, D. (1985). Nomenclature of adenosine receptors. *Trends Pharmacol. Sci.*, **6**, 153–154.
- JARVIS, M.F., SCHULZ, R., HUTCHISON, A.J., DO, U.H., SILLS, M.A. & WILLIAMS, M. (1989). [³H]CGS 21680, a selective A₂ adenosine receptor agonist directly labels A₂ receptors in rat brain. *J. Pharmacol. Exp. Ther.*, **251**, 888–893.
- LEE, K.S. & REDDINGTON, M. (1986). 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX) inhibition of [³H]N-ethylcarboxamidoadenosine (NECA) binding allows the visualization of putative non-A₁ adenosine receptors. *Brain Res.*, **368**, 394–398.
- LIBERT, F., SCHIFFMANN, S.N., LEFORT, A., PARMENTIER, M., GERARD, C., DUMONT, J.E., VANDERHAEGHEN, J.-J. & VAS-SART, G. (1991). The orphan receptor cDNA RDC7 encodes an A₁ adenosine receptor. *EMBO J.*, **10**, 1677–1682.
- LIBERT, F., VAN SANDE, J., LEFORT, A., CZERNILOFSKY, A., DUMONT, J.E., VASSART, G., ENSINGER, H.A. & MENDLA, K.D. (1992). Cloning and functional characterization of a human A₁ adenosine receptor. *Biochem. Biophys. Res. Commun.*, **187**, 919–926.
- LOHSE, M.J., KLOTZ, K.-N., LINDENBORN-FOTINOS, J., REDDINGTON, M., SCHWABE, U. & OLSSON, R.A. (1987). 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX)- a selective high affinity antagonist radioligand for A₁ adenosine receptors. *Naunyn-Schmied. Arch. Pharmacol.*, **336**, 204–210.
- LOSINSKI, A., HILL, S.J. & ALEXANDER, S.P.H. (1993). A₂ adenosine receptor relaxation of guinea-pig aorta. *Br. J. Pharmacol.*, **108**, 147P.
- MAENHAUT, C., VAN SANDE, J., LIBERT, F., ABRAMOWICZ, M., PARMENTIER, M., VANDERHAEGEN, J.-J., DUMONT, J.E., VASSART, G. & SCHIFFMANN, S. (1990). RDC8 codes for an adenosine A₂ receptor with physiological constitutive activity. *Biochem. Biophys. Res. Commun.*, **173**, 1169–1178.
- MAHAN, L.C., MCVITTIE, L.D., SMYK-RANDALL, E.M., NAKATA, H., MONSMA, F.J., GERFEN, C.R. & SIBLEY, D.R. (1991). Cloning and expression of an A₁ adenosine receptor from rat brain. *Mol. Pharmacol.*, **40**, 1–7.

- MARTIN, P.L. (1992). Relative agonist potencies of C2-substituted analogues of adenosine: evidence for adenosine A_{2b} receptors in the guinea-pig aorta. *Eur. J. Pharmacol.*, **216**, 235–242.
- MURPHY, K.M.M., GOODMAN, R.R. & SNYDER, S.H. (1983). Adenosine receptor localization in rat testes: biochemical and autoradiographic evidence for association with spermatocytes. *Endocrinol.*, **113**, 1299–1305.
- PIERCE, K.D., FURLONG, T.J., SELBIE, L.A. & SHINE, J. (1992). Molecular cloning and expression of an adenosine A_{2b} receptor from human brain. *Biochem. Biophys. Res. Commun.*, **187**, 86–93.
- REPPERT, S.M., WEAVER, D.R., STEHLE, J.H. & RIVKEES, S.A. (1991). Molecular cloning and characterization of a rat A1-adenosine receptor that is widely expressed in brain and spinal cord. *Mol. Endocrinol.*, **5**, 1037–1048.
- SCHWABE, U. & TROST, T. (1980). Characterization of adenosine receptors in rat brain by (–)[³H]N6-phenylisopropyladenosine. *Naunyn-Schmied. Arch. Pharmacol.*, **313**, 179–187.
- STEHLE, J.H., RIVKESS, S.A., LEE, J.J., WEAVER, D.R., DEEDS, J.D. & REPPERT, S.M. (1992). Molecular cloning and expression of the cDNA for a novel A₂-adenosine receptor subtype. *Mol. Endocrinol.*, **6**, 384–393.
- TOWNSEND-NICHOLSON, A. & SHINE, J. (1992). Molecular cloning of a human brain A1 adenosine receptor cDNA. *Mol. Brain Res.*, **16**, 365–370.
- UKENA, D. & SCHWABE, U. (1985). Study of adenosine receptors in intact rat fat cells by radioligand binding. *Pharmacol.*, **30**, 153–159.
- UKENA, D., SHAMIM, M.T., PADGETT, W. & DALY, J.W. (1986). Analogs of caffeine: antagonists with selectivity for A₂ adenosine receptors. *Life Sci.*, **39**, 743–750.
- VAN CALKER, D., MUELLER, M. & HAMPRECHT, B. (1979). Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J. Neurochem.*, **33**, 999–1005.
- VAN GALEN, P.J.M., STILES, G.L., MICHAELS, G. & JACOBSON, K.A. (1992). Adenosine A₁ and A₂ receptors: structure-function relationships. *Med. Res. Rev.*, **12**, 423–471.
- WOJCIK, W.J. & NEFF, N.H. (1983). Differential location of adenosine A₁ and A₂ receptors in striatum. *Neurosci. Lett.*, **41**, 55–60.
- YAKEL, J.L., WARREN, R.A., REPPERT, S.M. & NORTH, R.A. (1993). Functional expression of adenosine A_{2b} receptor in *Xenopus* oocytes. *Mol. Pharmacol.*, **43**, 277–280.

(Received July 28, 1993
 Revised September 24, 1993
 Accepted September 29, 1993)