ELSEVIER

European Journal of Pharmacology 301 (1996) 107–113

ejp

In vivo characterisation of ZM 241385, a selective adenosine A_{2A} receptor antagonist

John R. Keddie, Simon M. Poucher *, Graham R. Shaw, Robert Brooks, Michael G. Collis [1]

Cardiovascular and Metabolism Department, Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, UK

Received 13 November 1995; revised 27 December 1995; accepted 3 January 1996

Abstract

The in vivo characterisation of ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol), a novel, non-xanthine, selective adenosine A_{2A} antagonist is described. In anaesthetised dogs ZM 241385 (i.v.) was 140-fold more potent in attenuating vasodilator responses to exogenous adenosine in the constant flow perfused hind limb than the bradycardic effects. In pithed rats in which blood pressure was supported by angiotensin II infusion, ZM 241385 (10 mg kg^{-1}, i.v.) did not inhibit the hypotensive or bradycardic effects of the A_3/A_1 receptor agonist N^6-2-(4-amino-3-iodophenyl)ethyladenosine (APNEA). In conscious spontaneously hypertensive rats, ZM 241385 (3–10 mg kg^{-1}, p.o.) selectively attenuated the mean arterial blood pressure response produced by exogenous adenosine. No inhibition of the bradycardic effects of adenosine was observed following these doses of ZM 241385. The results indicate that ZM 241385 can be used to evaluate the role of adenosine A_{2A} receptors in the action of adenosine in vivo.

Keywords: Adenosine; Adenosine A_{2A} receptor; ZM 241385; Pharmacology, in vivo

1. Introduction

Adenosine receptors are widely distributed across organs and all cell types are able to produce the nucleoside under conditions of stress. Two functional adenosine receptor subtypes (A_1 and A_2) were first proposed by Londos and Wolff in 1977 (Londos and Wolff, 1977) based upon the ability of adenosine to either inhibit or activate adenylate cyclase and confirmed by subsequent studies (Van Calker et al., 1979; Bruns et al., 1986). The A_2 receptor has been further sub-divided into two classes (Daly et al., 1983) based upon the observations that some A_2 receptors have EC_{50} values for adenosine in the high nanomolar range (A_{2A} subtype) and some in the micromolar range (A_{2B} subtype). The agonist 2-[*p*-(2-carboxyethyl)-phenethylamino]-5′-*N*-ethyl-carboxamidoadenosine) CGS21680 displays higher affinity for the A_{2A} receptor than the A_{2B} (Jarvis et al., 1989) and has been used widely to investigate and characterise the presence of the A_{2A} adenosine receptor subtype both in vitro (Martin, 1992b; Poucher et al., 1995) and in vivo (Norton et al., 1992). More recently, an additional adenosine receptor subtype (A_3) has also been characterised (Zhou et al., 1992).

The particular adenosine receptor subtype responsible for the action of adenosine across a range of tissues and organs has largely been determined by the use of selective agonists. N^6-(*R*-Phenylisopropyl)-adenosine, N^6-cyclohexyl adenosine and N^6-cyclopentyl adenosine have all been used as A_1-selective ligands (Collis and Hourani, 1994), CGS21680 used as an A_{2A}-selective ligand (Jarvis et al., 1989) and more recently aminobenzyl-5′-*N*-methylcarboxamido adenosine (AB-MECA), iodobenzyl-5′-*N*-methylcarboxamido adenosine (IB-MECA) or N^6-2-(4-amino-3-iodophenyl) ethyladenosine (APNEA) as A_3-selective ligands (Olah et al., 1994; Von Lubitz et al., 1995; Fozard and Carruthers, 1993). Adenosine receptor agonist potency ratio, including non-selective agents, has been an effective means of determining the predominant adenosine receptor subtype in a tissue (Collis and Hourani, 1994). However, studies using exogenous agonists do not provide any information regarding the adenosine receptor subtype responsible for the tissues' response to endogenously produced adenosine. This can only be achieved by the use of selective adenosine receptor antagonists. The

* Corresponding author. Tel.: (01625) 512162; fax: (01625) 586707.
[1] Current address: Pfizer Central Research, Sandwich, Kent, UK.

PII S0014-2999(96)00020-9

compound most selective for the A_{2A} adenosine receptor subtype is 4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM 241385). It has high affinity for the A_{2A} adenosine receptor in guinea-pig cardiac vasculature (pA_2 of 9.02) and has 1000-fold $A_{2A}:A_1$ selectivity, 91-fold $A_{2A}:A_{2B}$ selectivity and 500 000-fold $A_{2A}:A_3$ selectivity (Poucher et al., 1995). The present paper describes the in vivo pharmacology of ZM 241385 in rat and dog at cardiovascular adenosine receptors.

2. Materials and methods

2.1. Anaesthetised dog

Female beagles (12–18 kg) were anaesthetised with sodium pentobarbitone (Sagatal, Rhône Merieux, Harlow, UK, 45–50 mg kg^{-1} followed by 6.2–9.4 mg kg^{-1} h^{-1} i.v.). The trachea was intubated and the dogs artificially ventilated (24 cycles min^{-1}, tidal volume 13–15 ml kg^{-1}) with room air. Heart rate was calculated from the Lead II ECG signal. Oesophageal temperature was monitored and maintained at 37.5 ± 0.5°C by a heating blanket. Arterial blood gases and pH were measured (Corning 288 Blood Gas System, Medfield, MA, USA) and maintained within normal ranges by adjustment of tidal volume or by intravenous administration of 8.4% (w/v) sodium hydrogencarbonate as required.

2.2. Effect of ZM 241385 on vascular and cardiac responses to adenosine in the dog

A double lumen catheter was inserted into the left ventricle, via the left common carotid artery, to record left ventricular pressure and for administration of adenosine. Both vagi and the right femoral and sciatic nerves were exposed, ligated and severed. After administration of heparin (1250 U plus 62–94U kg^{-1} h^{-1}, i.v.), the isolated right hind limb was perfused by a pump (Watson-Marlow 502S, Falmouth, UK) at constant flow with blood from the right iliac artery at a mean perfusion pressure of approximately 100 mm Hg. Xamoterol (a β_1-adrenoceptor partial agonist, 1 mg kg^{-1}) and nitrobenzylthioinosine (a purine transport inhibitor, 0.5 mg kg^{-1}) were given (both i.v.) to stabilise heart rate at a high level and to inhibit adenosine uptake, respectively. Adenosine dose-response curves (DRC's; 0.001–0.3 mg kg^{-1}) were performed before and 15 min after dosing ZM 241385 intravenously at either 0.03, 0.1, 0.3, 1.0 or 3.0 mg kg^{-1}.

Adenosine produced dose-dependent reductions in heart rate and hind limb perfusion pressure. Antagonist potency was assessed as the dose required to cause a two-fold rightward shift of the adenosine DRC (DR_2) against heart rate and hind limb perfusion pressure.

2.3. Effect of ZM 241385 on left ventricular inotropic state

Measurement of ventricular contractility was undertaken in a separate group of dogs. Left ventricular (LV) pressure was measured using a Millar mikro tip pressure transducer (model MPC-500, Houston, TX, USA) inserted into the left ventricle via the left carotid artery. The mikro-tip transducer was calibrated in situ and the first differential of the left ventricular pressure wave (LV dP/dt_{max} was derived electronically). Theophylline (3–20 mg kg^{-1} i.v.), ZM 241385 (0.01–10 mg kg^{-1} i.v.) or vehicle (PEG 400/0.1 M NaOH) was administered cumulatively every 10 min and the inotropic actions measured. Antagonist potency was assessed by the degree of blockade of the diastolic blood pressure response to adenosine. A bolus, intravenous dose of adenosine was given to produce a reduction of diastolic blood pressure before antagonist of approximately 60 mm Hg. This bolus administration was repeated 5 min after each dose of vehicle or compound.

2.4. Effect of ZM 241385 on apnea-induced responses in the pithed rat

The in vivo potency of ZM 241385 at A_3 receptors was assessed using the APNEA-mediated hypotension in pithed rats described by Fozard and Carruthers (1993). Male Alderley Park Wistar rats (280–390 g) were anaesthetised with fluothane (4%) and following pithing the blood pressure was maintained by infusion of angiotensin II (0.81 ± 0.06 μg kg^{-1} min^{-1}, i.v., $n = 27$). Drug or vehicle (PEG 400/0.1 M NaOH, 1 ml kg^{-1}, i.v.) was given 10 min after the start of the angiotensin II infusion. Following a further 10 min APNEA (30 mg kg^{-1} i.v., in 1% dimethylsulphoxide (DMSO)-99% saline, 0.5 ml kg^{-1}) was given to each animal (i.e., one single dose per animal).

2.5. Effect of ZM 241385 upon adenosine-mediated bradycardia and tachycardia in conscious spontaneously hypertensive rats

Female Alderley Park spontaneously hypertensive rats (240–260 g) were anaesthetised using a mixture of alphaxolone 9 mg ml^{-1} and alphadolone acetate 3 mg ml^{-1} (0.8–1.0 ml kg^{-1}, Saffan, Pittman-Moore, Uxbridge, UK) injected via the tail vein. The left jugular vein and left common carotid artery were cannulated and the cannulae exteriorised on the dorsal surface of the rat, behind the shoulder. 24 h following surgery each rat was placed in a perspex restraining tube for the assessment of responses to adenosine. The carotid artery cannula was connected to a pressure transducer (Bell and Howell L221, Basingstoke, UK) for measurement of blood pressure and pulse rate (recorded on a Lectromed MT2, St Peter, Jersey). Adenosine infusion (1 mg kg^{-1} min^{-1}, i.v.) was maintained until

stable bradycardic and blood pressure responses were achieved (usually 1–2 min). Rats with a resting diastolic blood pressure of ≤ 120 mm Hg or a diastolic blood pressure reduction to adenosine of ≤ 40 mm Hg were eliminated from the experiment.

Adenosine antagonists were dosed orally in polyethylene glycol 400 (PEG 400, 2 ml kg^{-1}) and the adenosine infusion was repeated 1 h following administration. Effects on the adenosine blood pressure/bradycardic responses were then calculated as percentage inhibition of the adenosine response prior to antagonist.

2.6. Statistical analysis

All values are quoted as the means $\pm$ S.E. Statistical analysis was undertaken using the Student's *t*-test for paired or unpaired data.

2.7. Drugs and compounds used

ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol) was synthesised by Dr Geraint Jones and Mr Peter Caulkett and N^6-cyclohexyl-1,3-dipropylxanthine and 1,3-dipropyl-8-*N*-[2-dimethylamino)ethyl]-*N*-methyl-4-2,3,6,7-tetrahydro-2,6-dioxo)-benzenesulphonamidexanthine (PD115199) were synthesised by Mr Roger James all of Chemistry Department, Zeneca Pharmaceuticals. Adenosine, theophylline, 8-phenyltheophylline (8-PT), 8-(*p*-sulphophenyl)theophylline (8-PST) and nitrobenzythioinosine were obtained from Sigma Chemicals, Poole, UK. N^6-2-(4-Amino-3-iodophenyl)ethyladenosine (APNEA) was obtained from Research Biochemicals Incorporated, St Albans, UK. Xamoterol, was obtained from Zeneca Pharmaceuticals.

ZM241385 was administered intravenously to anaesthetised rats and dogs as a solution in 50% PEG 400/50% 0.1 M NaOH (15 mg ml^{-1} with sonication). Standard antagonists theophylline, 8-PT, N^6-cyclohexyl-1,3-dipropylxanthine and PD115199 were dissolved in a solution of 50% PEG 400-50% 0.1 M NaOH while 8-PST was dissolved in physiological saline.

3. Results

3.1. Effect of ZM 241385 on vascular and cardiac responses to adenosine in the dog

Both bradycardic and hind limb vasodilatation responses to administration of adenosine in the left ventricle were reproducible for at least 2 h after administration of drug vehicle. The bradycardic responses to adenosine (0.03 mg kg^{-1}) before and 2 h after antagonist vehicle were 27 ± 4 and 23 ± 6 beats min^{-1}, respectively (n.s., $n = 3$). Vasodilatation responses of the hind limb were also not

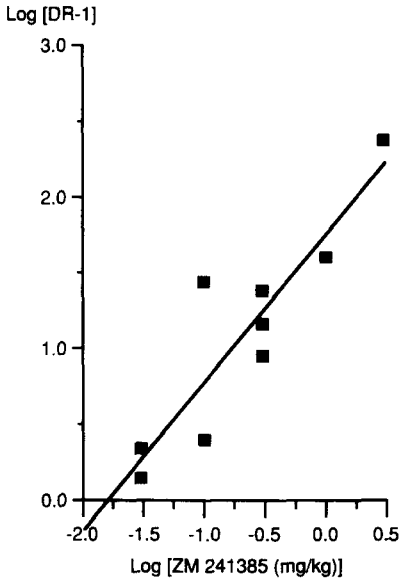

Fig. 1. Each point is derived from the dose ratio value for antagonism of adenosine-mediated reductions in hind limb perfusion pressure obtained 15 min following administration of ZM 241385 (i.v.) to individual dogs. The slope of the regression is 0.975 and the correlation coefficient, r^2 is 0.81288. Constraining the slope to unity gives a DR_2 value of 0.02 ± 0.004 mg kg^{-1}.

altered (42 ± 7 and 37 ± 5 mm Hg, n.s., $n = 3$) following the same dose of adenosine. Administration of ZM 241385 (0.03–3 mg kg^{-1}) had no effect on the perfusion pressure in the hind limb (109 ± 4 and 117 ± 5 mm Hg before and after, respectively, $n = 9$). Drug vehicle had no effect on the responses to adenosine in either the hind limb or the heart. ZM 241385 (0.03–3 mg kg^{-1}) was a potent antagonist producing rightward shifts of the DRC's on hind limb perfusion pressure with minimal antagonism of the bradycardic effects. The log (dose ratio − 1) 15 min following ZM 241385 at 0.03, 0.1, 0.3, 1.0 and 3.0 mg kg^{-1} to individual dogs were 0.24 ($n = 2$), 0.92 ($n = 2$), 1.16 ($n = 3$), 1.6 ($n = 1$) and 2.38 ($n = 1$), respectively. The resultant mean DR_2 against adenosine-mediated hind limb perfusion pressure responses was 0.02 ± 0.004 mg kg^{-1} ($n = 9$ dogs, Fig. 1). Similar calculations for antagonism of the bradycardia, limited to only those dogs which demonstrated dose ratios above unity, resulted in a K_d of 2.8 ± 1.7 mg kg^{-1} ($n = 6$ dogs). Antagonist potencies opposite the vascular and cardiac effects of adenosine show the compound to be 140-fold more potent at inhibiting the vascular effects of adenosine than the bradycardia in dogs. Cumulative addition of ZM 241385 did not, however, produce the expected progressive rightward shifts in adenosine DRCs (Fig. 2). Following a single dose of ZM 241385 the degree of rightward shift of the adenosine DRC in the hind limb rapidly diminished over the 2 h. In one experiment, fifteen min after ZM 241385 at 1 mg kg^{-1} the dose ratio on the hind limb was 42.5 but which declined to a ratio of 3.1 within 2 h of dosing.

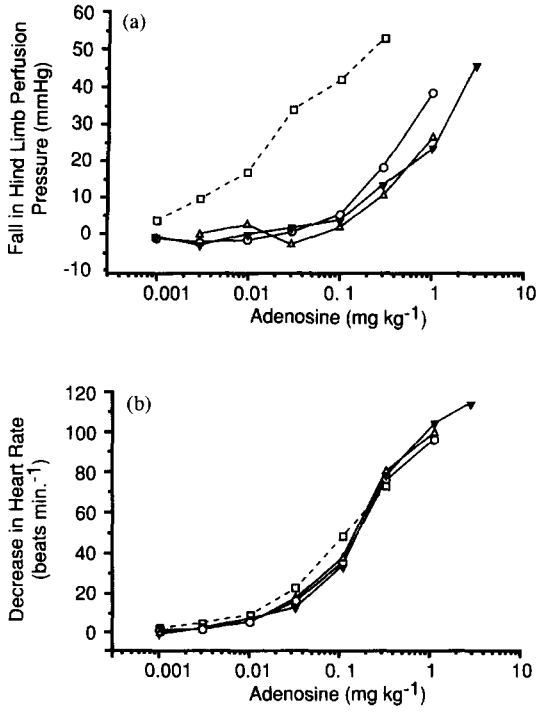

Fig. 2. Representative response to cumulative addition of ZM 241385 in an individual dog. Each curve, showing a reduction in hind limb perfusion pressure (a) and decrease in heart rate (b) was produced following administration of adenosine into the left ventricle. Hind limb perfusion pressure and heart rate responses to adenosine were measured in the presence of the purine transport inhibitor NBTI (0.5 mg kg^{-1}, i.v.) before (□) and 15 min after the cumulative addition of ZM 241385 (0.3, ○; 1.0, △ and 3.0, ▼; mg kg^{-1}, i.v.).

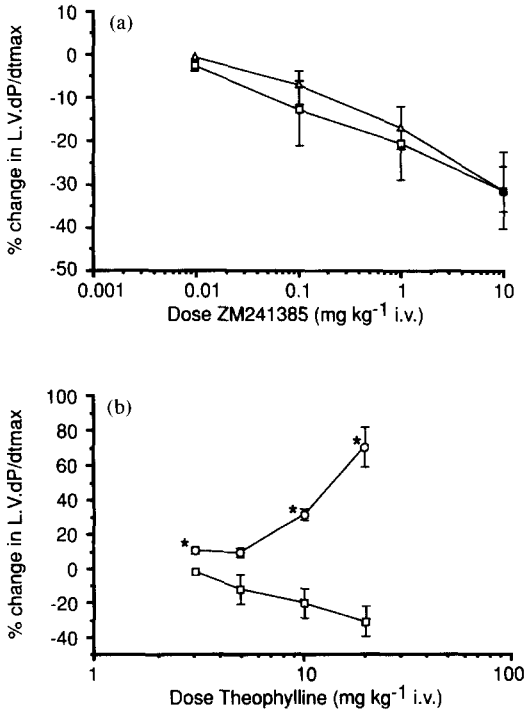

Fig. 3. Each point is the mean ± S.E. of the changes in inotropic state of the left ventricle as assessed by LV dP/dt_{max} following cumulative addition of either vehicle (PEG 400/0.1 M NaOH, $n = 4$; □), ZM 241385 (a; $n = 3$, △) or theophylline (b, $n = 4$, ○). Baseline values of LV dP/dt_{max} (mm Hg s^{-1}) were; PEG 400/NaOH, 3354 ± 402; ZM 241385, 3522 ± 650 and theophylline, 4363 ± 259. These were not significantly different from each other. * $P < 0.002$ vs. vehicle.

3.2. Effect of ZM 241385 on left ventricular inotropic state

ZM241385 demonstrated no positive inotropic action upon the left ventricle at the highest dose studied (10 mg kg^{-1} i.v.) in the anaesthetised dog (Fig. 3a, $n = 3$). In contrast, theophylline (10 and 20 mg kg^{-1}, $n = 4$) demonstrated positive inotropic activity (Fig. 3b). Both ZM 241385 (1 mg kg^{-1}) and theophylline (20 mg kg^{-1}) antagonised the adenosine depressor response in these animals (62 ± 2 mm Hg before either antagonist, $n = 7$) by 88 ± 2% ($n = 3$) and 80 ± 4% ($n = 4$), respectively. ZM 241385 was therefore at least 20-fold more potent than theophylline at antagonising adenosine-mediated responses in this model.

3.3. Effect of ZM 241385 on A_3 receptor-mediated responses in the pithed rat

In angiotensin II-supported pithed rats, APNEA (30 μg kg^{-1}, i.v.) induced a decrease in mean arterial blood pressure of 55 ± 6 mm Hg from a pre drug value of 109 ± 5 mm Hg, and bradycardia of 37 ± 10 beats min^{-1} from 333 ± 16 beats min^{-1} in the vehicle-treated group ($n = 13$). The hypotensive action of APNEA was not inhibited by 8-PST (20 mg kg^{-1}, i.v.), whilst the bradycardic response was reduced. In contrast, ZM 241385 (10 mg kg^{-1}, i.v.) was without effect on either parameter (Table 1).

3.4. Effect of ZM 241385 upon adenosine-mediated bradycardia and tachycardia in conscious spontaneously hypertensive rats

Initial mean arterial pressure for control spontaneously hypertensive rats used in this study was 170 ± 1 mm Hg

Table 1
Effect of ZM 241385 upon APNEA-mediated responses in the angiotensin II-supported pithed rat

Treatment group	Response to APNEA	
	MABP (mm Hg)	Bradycardia (beats min^{-1})
Vehicle ($n = 13$)	55 ± 6	37 ± 10
ZM241385 ($n = 7$)	65 ± 7	28 ± 9
8-PST ($n = 7$)	47 ± 10	4 ± 8 [a]

Mean arterial blood pressure (MABP) and bradycardic responses to APNEA were measured in pithed rats in which the blood pressure was maintained at 117 ± 3 mm Hg by infusion of angiotensin II (0.81 ± 0.06 μg kg^{-1}, i.v., $n = 27$). Responses to APNEA (30 μg kg^{-1}, i.v.) were investigated in the presence of either drug vehicle (PEG 400/0.1 M NaOH), ZM 241385 (10 mg kg^{-1}) or 8-PST (20 mg kg^{-1}). Each value is quoted as the mean ± S.E. [a] $P < 0.05$ versus vehicle using Student's t-test for unpaired data.

Table 2
Effect of adenosine antagonists upon adenosine-mediated responses in the conscious spontaneously hypertensive rat

Compound	Dose (mg kg^{-1}, p.o.)	Inhibition of depressor response (%)	Inhibition of bradycardic response (%)
8-PT	10	44 ± 5 [a]	95 ± 12 [a]
PD115199	10	37 ± 15 [a]	−14 ± 18
8-Cyclohexyl-1,3-dipropylxanthine	0.3	19 ± 9	61 ± 7 [a]
ZM 241385	3	32 ± 8 [a]	−56 ± 51
ZM 241385	10	43 ± 9 [a]	−99 ± 30
Vehicle (PEG 400)	–	4 ± 9	−23 ± 26

Each value is quoted as the mean (± S.E., $n = 8$–27) percentage inhibition of mean arterial blood pressure depression and bradycardia induced by infusion of adenosine (1 mg kg^{-1} min^{-1}, i.v.) to conscious spontaneously hypertensive rats. The responses were measured before and 1 h following oral administration of 8-PT, PD115199, 8-cyclohexyl-1,3-dipropylxanthine, ZM 241385 or vehicle. Initial mean arterial pressure was 170 ± 1 mm Hg ($n = 8$) and 175 ± 3 mm Hg ($n = 27$) for control and ZM 241385 treatment groups, respectively. Mean depressor responses (fall in mean arterial pressure) to adenosine infusion prior to vehicle or ZM 241385 were 65 ± 2 mm Hg ($n = 8$) and 69 ± 3 mm Hg ($n = 27$), respectively. Mean control heart rate was 468 ± 4 beats min^{-1}, with a mean bradycardic response to adenosine of 89 ± 5 beats min^{-1} and 84 ± 8 beats min^{-1} for vehicle and ZM 241385 groups. Statistical analysis undertaken using a Student's t-test (1-tailed), [a] $P < 0.05$ vs. vehicle.

($n = 8$) and 175 ± 3 mm Hg for ZM 241385 treatment groups, $n = 27$). Mean depressor responses (fall in mean arterial pressure) to adenosine infusion prior to oral administration of vehicle or ZM 241385 were 65 ± 2 mm Hg ($n = 8$) and 69 ± 3 mm Hg ($n = 27$), respectively. Similarly, mean control heart rate was 468 ± 4 beats min^{-1}, with a mean bradycardic response to adenosine of 89 ± 5 beats min^{-1} and 84 ± 8 beats min^{-1} for vehicle and ZM 241385 groups. 8-PT (10 mg kg^{-1}, $n = 23$) attenuated both the adenosine-mediated depressor and bradycardic responses by 44 ± 5% and 95 ± 12%, respectively (Table 2). The A_2-selective antagonist PD115199 (10 mg kg^{-1}, $n = 10$) inhibited adenosine-mediated depressor responses but not its bradycardic responses (Table 2). The A_1-selective antagonist 8-cyclohexyl-1,3-dipropylxanthine (0.3 mg kg^{-1}, $n = 6$) resulted in inhibition of the cardiac response only. ZM 241385 (3 and 10 mg kg^{-1}, p.o., $n = 13$ and 14, respectively) inhibited adenosine's blood pressure lowering effect in the absence of an effect upon the bradycardia at 3 mg kg^{-1} but increased the bradycardia to adenosine following 10 mg kg^{-1} (Table 2).

4. Discussion

ZM 241385 administered intravenously to anaesthetised dogs demonstrated potent antagonism to adenosine's vasodilatation effect within the perfused hind limb ($DR_2 = 0.02$ mg kg^{-1}) whereas its antagonism to adenosine's bradycardic action was substantially less ($DR_2 = 2.8$ mg kg^{-1}). Agonist and antagonist potency ratio studies of the dog hind limb model used in this study suggest the adenosine receptor subtype responsible for the vasodilatation is the A_{2A} subtype (Nally et al., 1991). The role of the A_{2A} receptor subtype in responses to adenosine is supported by the finding that CGS21680 produces vasodilatation of the dog hind limb preparation (unpublished observation). The 140-fold selectivity of ZM 241385 for the vascular over cardiac action of adenosine, is less than the 1000-fold $A_{2A}:A_1$ selectivity reported using in vitro preparations (Poucher et al., 1995). However, the specific adenosine receptor subtype found in the sino-atrial node of canine cardiac tissue has yet to be defined completely. Belloni et al. (1989) first reported that the adenosine receptor on the sino-atrial node of canine tissue was probably different from the A_1 cardiac receptor found in the sino-atrial node of guinea pig (Collis, 1983) or rat (Ueeda et al., 1991) in its responses and relative potency to N^6-(*R*-phenylisopropyl)-adenosine (R-PIA) or N^6-cyclopentyladenosine. Martin (1992a) has also reported that the adenosine analogue potency ratios for the dog left atrium are more closely related to those found using the guinea pig aorta than guinea pig atria. This is consistent with the A_{2B} receptor subtype, a finding supported by the studies of Nally et al. (1991) for agonist and antagonist potencies in anaesthetised dogs. Although the adenosine receptor subtype present on canine sino-atrial tissue has not been characterised definitively, the presence of A_{2B} receptors on canine sino-atrial node responsible for the bradycardic effects of adenosine would be consistent with the 140-fold selectivity seen with ZM 241385 since ZM241385 is approximately 100-fold $A_{2A}:A_{2B}$-selective in in vitro preparations (Poucher et al., 1995).

The $A_{2A}:A_1$ selectivity in vivo is supported by the results from the conscious rats. This preparation is capable of detecting both A_{2A}- and A_1-selective antagonist activity of PD115199 and 8-cyclohexyl-1,3-dipropylxanthine, respectively, in our hands. ZM 241385 demonstrated vascular selectivity at inhibiting adenosine actions in spontaneously hypertensive rats at 3 and 10 mg kg^{-1} (p.o.) with no antagonism of adenosine's bradycardic action. Whilst the bradycardic responses in the rat were variable, due to the paradigm using conscious, fully reflexic animals, an inhibition of the bradycardic effect of adenosine was observed following both 8-PT and 8-cyclohexyl-1,3-dipropylxanthine. In fact, in the conscious rat there was a trend for the bradycardic effect of adenosine to be enhanced following ZM 241385. Since the conscious rats had intact cardiovascular reflexes, in the presence of ZM 241385 the enhanced bradycardic effects of adenosine may be accounted for by the reduction or loss of the vagal withdrawal and an increased sympathetic efferent nerve response to the heart elicited by administration of adenosine. Therefore, in the presence of a vascular selective adenosine receptor antagonist, the direct inhibitory action of

adenosine on the sino-atrial node is not opposed by the normal reflex tachycardic response to hypotension. The apparently enhanced variability of the bradycardic response could be attributed to a combination of different sensitivity of the reflex tachycardia, potency of adenosine at the sino-atrial node and differing inter animal potency of ZM 241385 at inhibiting the action of adenosine at vascular adenosine receptors. The weak affinity of ZM241385 for A_1 adenosine receptors in vivo is, however, further supported by the pithed rat studies in which ZM 241385 did not block the bradycardic effect of APNEA (known to be mediated through an A_1 receptor effect of APNEA (Fozard and Carruthers, 1993; Patel et al., 1994) in an areflexic rat following 10 mg kg^{-1} (i.v.). ZM 241385 also did not inhibit the hypotension induced by APNEA, demonstrating a lack of affinity at this dose for the A_3 receptor in the rat (Fozard and Carruthers, 1993) and confirming the in vitro data at this receptor (Poucher et al., 1995). The rat A_3 receptor may be different from the human or sheep A_3 receptor. There is low sequence homology between rat A_3 receptors and that of sheep and human receptor, however (Linden, 1994). Therefore it still remains to be determined whether ZM 241385 has low affinity for the A_3 adenosine receptor across all species.

ZM 241385 has no positive inotropic action in the canine heart at doses up to 10 mg kg^{-1}. Theophylline at 5 mg kg^{-1} and above demonstrated positive inotropic action. ZM 241385 would appear to be an adenosine antagonist which is devoid of positive inotropic action in a similar way to the alkylxanthine, 8-phenyltheophylline (Collis et al., 1984). This lack of positive inotropic action probably relates to the lack of phosphodiesterase inhibitory action previously described for the compound (Poucher et al., 1995).

Fig. 2a demonstrates that a 10-fold increase in the dose of ZM 241385 failed to produce a 10-fold further rightward shift. This was unexpected, but could be explained by a number of reasons, (i) an adenosine receptor subtype, in addition to the A_{2A}, is responsible for the hind limb vasodilatation after antagonist; (ii) the compound being rapidly cleared following intravenous administration; (iii) a combination of both. However, it is known that a rightward shift of over 100-fold can be obtained with a single intravenous dose of 3 mg kg^{-1}, and that a linear 'Schild plot' is obtained when using the antagonism produced after the first dose of ZM 241385 (Fig. 1). Therefore, this result suggests, although not proving conclusively however, that it is unlikely that receptors in addition to the A_{2A} receptor subtype are responsible for the vasodilatation of the dog hind limb produced by adenosine.

Other compounds have been claimed to be selective A_{2A} adenosine receptor antagonists in vivo. 8-(3-Chlorostyryl)caffeine (CSC) is reported to be 22-fold $A_{2A}:A_1$-selective in functional assays in vitro and to have activity in vivo in paradigms believed to reflect A_{2A} activity (locomotor activity in mice, Jacobson et al., 1993). However, as a xanthine, it would be expected that this compound would also have significant phosphodiesterase inhibition activity which would limit its use and no evidence has been reported in favour of in vivo selectivity. More importantly, like several of the adenosine receptor antagonists, the lack of solubility in suitable vehicles (only 1 mg ml^{-1} in 5% DMSO) would be expected to limit the use of CSC. In contrast ZM 241385 can be dissolved at 15 mg ml^{-1} in a vehicle acceptable for in vivo studies. The triazoloquinazoline CGS15943 has also been claimed to be a compound with high affinity and selectivity for the A_2 adenosine receptor and to possess activity in vivo (Ghai et al., 1987). However, the high affinity and selectivity in vitro or the in vivo selectivity claimed by Ghai et al. (1987) have not been confirmed by other groups (Rollins et al., 1994; Patel et al., 1994; Poucher et al., 1995). The potential utility of CGS15943 in the elucidation of the role of endogenous adenosine must therefore be limited.

In conclusion, the results of this study indicate that the A_{2A} adenosine receptor-selective antagonist ZM 241385 is an adenosine receptor antagonist in vivo when given intravenously or orally. ZM 241385 also had little or no activity against responses mediated by either A_1 or A_3 adenosine receptors in the rat and had low potency against bradycardic response to adenosine in the dog. Additional actions commonly seen with the xanthine adenosine antagonist theophylline, such as positive inotropic activity are not observed. However, due to its apparent loss of activity over 2 h following intravenous administration observed in the anaesthetised dog, care must be taken to ensure that sufficient blockade of adenosine receptors is confirmed following single administration, or that dosing regimes are developed to maintain the degree of adenosine receptor antagonism at the required level.

Acknowledgements

We thank Helen Musgrove and Susan Mellor for their technical assistance in undertaking the conscious and anaesthetised rat studies.

References

Belloni, F.L., L. Belardinelli, C. Halperin and T.H. Hintze,1989, An unusual receptor mediates adenosine-induced SA nodal bradycardia in dogs, Am. J. Physiol. 256, H1553.

Bruns, R.F., G.H. Lu and T.A. Pugsley, 1986, Characterisation of the A_2 adenosine receptor labelled by [^{3}H] NECA in rat striatal membranes, Mol. Pharmacol. 29, 331.

Collis, M.G., 1983, Evidence for a A_1 adenosine receptor in the guinea pig atrium, Br. J. Pharmacol. 78, 207.

Collis, M.G. and S.M.O. Hourani, 1994, Adenosine receptor subtypes, Trends Pharmacol. Sci. 14, 360.

Collis, M.G., J.R. Keddie and S.R. Torr, 1984, Evidence that the positive inotropic effects of the alkylxanthines are not due to adenosine receptor blockade, Br. J. Pharmacol. 81, 401.

Daly, J.W., P. Butts-Lamb and W. Padgett, 1983, Subclasses of adenosine receptors in the central nervous system. Interaction with caffeine and related methylxanthines, Cell. Mol. Neurobiol. 1, 69.

Fozard, J.R. and A.M. Carruthers, 1993, Adenosine A_3 receptors mediate hypotension in the angiotensin II-supported circulation of the pithed rat, Br. J. Pharmacol. 109, 3.

Ghai, G., J.E. Francis, M. Williams, R.A. Dotson, M.F. Hopkins, D.T. Cote., F.R. Goodman and M.B. Zimmerman, 1987, Pharmacological characterisation of CGS15943A: a novel nonxanthine adenosine antagonist, J. Pharmacol. Exp. Ther. 242, 784.

Jacobson, K.A., O. Nikodijevic, W.L. Padgett, C. Gallo-Rodriguez, M. Maillard and J.W. Daly, 1993, 8-(3-Chlorostyryl)caffeine (CSC) is a selective A_2-adenosine receptor antagonist in vitro and in vivo, FEBS Lett. 323, 141.

Jarvis, M.F., R. Schulz, A.J. Hutchinson, U.H. Do, M.A. Sills and S.M. Williams, 1989, [^{3}H]CGS21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptor in the brain, J. Pharmacol. Exp. Ther. 251, 888.

Linden, J., 1994, Cloned adenosine A_3 receptors: pharmacological properties, species differences and receptor functions, Trends Pharmacol. Sci. 15, 298.

Londos, C. and J. Wolff, 1977, Two distinct adenosine-sensitive sites on adenylate cyclase, Proc. Natl. Acad. Sci. USA 74, 5482.

Martin, P.L., 1992a, Evidence that adenosine receptors in the dog left atrium are not of the typical A_1 or A_2 adenosine receptor subtype, Eur. J Pharmacol. 214, 199.

Martin, P.L., 1992b, Relative agonist potencies of C^2 substituted analogues of adenosine: evidence for adenosine A_{2B} receptors in the guinea pig aorta, Eur. J. Pharmacol. 216, 235.

Nally, J.E., J.R. Keddie, G.R. Shaw and M.G. Collis, 1991, Investigation of the adenosine receptor responsible for bradycardia and vasodilatation in the dog, Br. J. Pharmacol. 102, 340P.

Norton, E.D., E.K. Jackson, M.B. Turner, R. Virmani and M.B. Forman, 1992, The effects of intravenous infusion of selective adenosine A_1- and A_2-receptor agonists on myocardial reperfusion injury, Am. Heart J. 123, 332.

Olah, M.E., C. Gallo-Rodriguez, K.A. Jacobson and G.L. Stiles, 1994, ^{125}I-4-aminbenzyl-5′-*N*-methylcarboxamidoadenosine, a high affinity radioligand for the rat A_3 adenosine receptor, Mol. Pharmacol. 45, 978.

Patel, M., M.J. Sheehan and P. Strong, 1994, Failure of CGS15943 to block the hypotensive action of agonists acting at the adenosine A_3 receptor, Br. J. Pharmacol. 113, 741.

Poucher, S.M., J.R. Keddie, P. Singh, S.M. Stoggall, P.W.R. Caulkett, G. Jones and M.G. Collis, 1995, The in vitro pharmacology of ZM 241385, a potent, non-xanthine, A_{2A} selective adenosine antagonist, Br. J. Pharmacol. 115, 1096.

Rollins, P.J., S.J. Turner, C.I.O. Akpoguma and K.P. Ray, 1994, Characterisation of human adenosine type 1, 2a and 2b receptors expressed in CHO cells, Biochem. Soc. Trans. 22, 195S.

Ueeda, M., R.D. Thompson, W.L. Padgett, S. Secunda, J.W. Daly and R.A. Olsson, 1991, Cardiovascular actions of adenosines, but not adenosine receptors, differ in rat and guinea pig, Life Sci. 49, 1351.

Van Calker, D., M. Muller and B. Hamprecht, 1979, Adenosine regulates via two different types of receptors, the accumulation of cyclic cAMP in cultured brain cells, J. Neurochem. 33, 995.

Von Lubitz, D.K.J.E., M.F. Carter, S.I. Deutsch, R.C.-S. Lin, J. Mastropaolo, Y. Meshulem and K.A. Jacobson, 1995, The effects of adenosine A_3 receptor stimulation on seizures in mice, Eur. J Pharmacol. 275, 23.

Zhou, Q.-Y., C. Li, M.E. Olah, R.A. Johnson, G.L. Stiles and O. Civelli, 1992, Molecular cloning and characterisation of an adenosine receptor; the A_3 adenosine receptor, Proc. Natl. Acad. Sci. USA 89, 7432.