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Failure of CGS15943A to block the hypotensive action of agonists acting at the adenosine A_3 receptor

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1 Adenosine receptor agonists were evaluated for their activity at the putative adenosine A_3 receptor which mediates a 'xanthine-resistant' hypotensive response in the anaesthetized rat. The compounds tested were: the A_1/A_3 receptor agonist, N-[2-(4-aminophenyl)ethyl]adenosine (APNEA), the non-selective adenosine receptor agonist, 5'-N-ethylcarboxamidoadenosine (NECA), the adenosine A_1 receptor-selective agonists, N-[(1S,*trans*)-2-hydroxycyclopentyl]adenosine (GR79236) and N^6-cyclopentyl adenosine (CPA), the A_{2a} receptor-selective agonists, 2-[[2-[4-(2-carboxyethyl) phenyl] ethyl] amino]-N-ethylcarboxamidoadenosine (CGS21680) and 2-phenylaminoadenosine (CV1808), and the moderately A_{2b} selective agonist, N-[(2-methylphenyl)methyl]adenosine (metrifudil).

2 In confirmation of literature findings, APNEA (1–1000 nmol kg^{-1}) induced hypotension and bradycardia; the hypotension was not blocked by pretreatment with the xanthine antagonist, 8-*P*-sulphophenyltheophylline (8-sPT; 40 mg kg^{-1}, i.v.), whereas the bradycardia was attenuated. The non-xanthine antagonist, 9-fluoro-2-(2-furyl)-5,6-dihydro [1,2,4]triazolo{1,5-c}-quinazin-5-imine (CGS15943A; 3 mg kg^{-1} i.v.), also attenuated the bradycardia without affecting the hypotension.

3 The adenosine A_1 receptor-selective agonists, GR79236 and CPA, both produced dose-dependent falls in blood pressure and heart rate which were antagonized by 8-sPT (40 mg kg^{-1}) and CGS15943A (3 mg kg^{-1}).

4 The adenosine A_{2a} receptor-selective agonists, CGS21680 and CV1808, produced only a hypotensive response which was antagonized by 8-sPT (40 mg kg^{-1}) and to a much greater extent by CGS15943A (3 mg kg^{-1}), consistent with the response being mediated solely by A_{2a} receptors.

5 The modestly A_{2b} receptor-selective agonist, metrifudil, produced a dose-dependent fall in blood pressure and at higher doses a fall in heart rate. The hypotension induced by metrifudil was not antagonized by either 8-sPT (40 mg kg^{-1}) or CGS15943A (3 mg kg^{-1}) even though the bradycardia was abolished, suggesting that this agonist activates the putative A_3 receptor.

6 The non-selective adenosine receptor agonist, NECA, produced a hypotension and bradycardia that was attenuated by 8-sPT (40 mg kg^{-1}), confirming previous work. The non-xanthine antagonist, CGS15943A (3 mg kg^{-1}), also attenuated the hypotension and bradycardia. The bradycardia was blocked to a much greater extent, suggesting that NECA may therefore induce hypotension partly by activating the putative A_3 receptor.

7 In conclusion, we have confirmed that the putative A_3 receptor mediating hypotension in the anaesthetized rat is not blocked by 8-sPT, and further shown that it is not blocked by CGS15943A. The A_{2a} agonists CGS21680 and CV1808 showed no discernible activity at the A_3 receptor, whereas APNEA, NECA, CPA and metrifudil appear to activate this receptor. The adenosine A_1 receptor agonist, GR79236, shows considerable selectivity for the A_1 receptor but may activate the A_3 receptor at high doses.

Keywords: Adenosine A_3 receptor, GR79236; CGS21680; CV1808; APNEA; hypotension; anaesthetized rat

Introduction

In addition to the A_1, A_{2a} and A_{2b} sub-types of adenosine receptors, the characteristics of which we have recently evaluated in functional studies (Gurden *et al.*, 1993), adenosine A_3 receptors have recently been cloned from rat (Zhou *et al.*, 1992), sheep (Linden *et al.*, 1993) and human (Salvatore *et al.*, 1993) sources.

There is at present very little work described in the literature that addresses the nature of the physiological processes which adenosine A_3 receptors may control. However, in recent publications it has been suggested that activation of these receptors induces a hypotensive effect in both pithed and anaesthetized rats (Fozard & Carruthers 1993a; Fozard & Hannon, 1994). The features of the response which suggest that it is mediated by A_3 receptors are (i) its occurrence in response to administration of N-[2-(4-aminophenyl)ethyl] adenosine (APNEA; Fozard & Carruthers, 1993a), (ii) its resistance to blockade by 8-(*P*-sulphophenyl]theophylline (8-sPT) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX; Fozard & Carruthers, 1993a; Carruthers & Fozard, 1994), (iii) its inhibition by pertussis toxin (Carruthers & Fozard, 1993) and (iv) its inhibition by (albeit high doses of) the antagonist BW-A522 (3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)-1-propylxanthine; Fozard & Hannon, 1994). An interesting feature of these studies has been the observation that other adenosine derivatives routinely used in the study of adenosine receptors, including 5'-N-ethylcarboxamidoadenosine (NECA) and R-phenylisopropyladenosine (R-PIA), also have affinity for A_3 receptors and induce an 8-sPT-resistant hypotension in rats (Fozard & Carruthers, 1993a).

In our recent work (Gurden *et al.*, 1993) we identified the A_1-receptor selective agonists, GR79236 (N-[(1S,*trans*)-2-hydroxycyclopentyl] adenosine) and CPA (N^6-cyclopentyl adenosine), the modestly A_{2b} receptor-selective agonist, metrifudil (N-[(2-methylphenyl)methyl] adenosine) and the A_{2a}-receptor selective agonists CGS21680 (2-[[2-[4-(2-carboxyethyl) phenyl] ethyl] amino]-N-ethylcarboxamidoadenosine) and CV1808 (2-phenylaminoadenosine) as being particularly useful in the receptor classification of adenosine receptor-mediated responses. Accordingly, the aims of this study were

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to investigate the extent to which GR79236, metrifudil and CV1808 are able to elicit an 8-sPT-resistant hypotensive response in the anaesthetized rat, and to confirm the findings of Fozard & Carruthers (1993b) for CPA and CGS21680. In addition, we have also investigated the effects of pretreatment with CGS15943A (9-fluoro-2-(2-furyl)-5,6-dihydro [1,2,4] triazolo{1,5-c}-quinazin-5-imine), a potent non-xanthine adenosine receptor blocking drug which shows a 6 fold selectivity for A_1 receptors over A_2 receptors (Williams *et al.*, 1987), on cardiovascular responses to APNEA, NECA, CPA, GR79236, metrifudil, CGS21680 and CV1808. A preliminary account of this work has been published in abstract form (Patel *et al.*, 1994).

Methods

Female AH/A Wistar rats weighing 190–250 g were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}), and a cannula inserted via a tracheostomy to facilitate spontaneous respiration. The right external jugular vein was cannulated for the continuous infusion of a maintenance dose of sodium pentobarbitone (100 μg kg^{-1} min^{-1}) and the left external jugular vein was cannulated for the administration of drugs (agonists and antagonists). The right carotid artery was cannulated with a heparinized catheter (50 unit ml^{-1} heparin in 0.9% w/v NaCl solution; i.d. = 0.50 mm) for measurement of blood pressure. The heart rate was derived from the blood pressure record. Body temperature was maintained at 37°C by means of a heated mat and a lamp, and all parameters were displayed on a Devices M19 chart recorder.

After a 10–15 min stabilization period, two priming doses of adenosine (300 nmol kg^{-1}) were administered as bolus doses in 0.1 ml volume with a time interval of 3 min. After a further 10 min stabilization period, dose-response curves to agonists were constructed by cumulative bolus injection, the intervals between doses being sufficient to allow a plateau response to develop. In the experiments with the adenosine receptor antagonists, either 8sPT (40 mg kg^{-1}) or CGS15943A (1–10 mg kg^{-1}), drug or vehicle was injected intravenously 5 min before establishing dose-response curves to the agonists. All drugs were given in a 0.1 ml dose volume. Only one agonist dose-response curve was generated per animal.

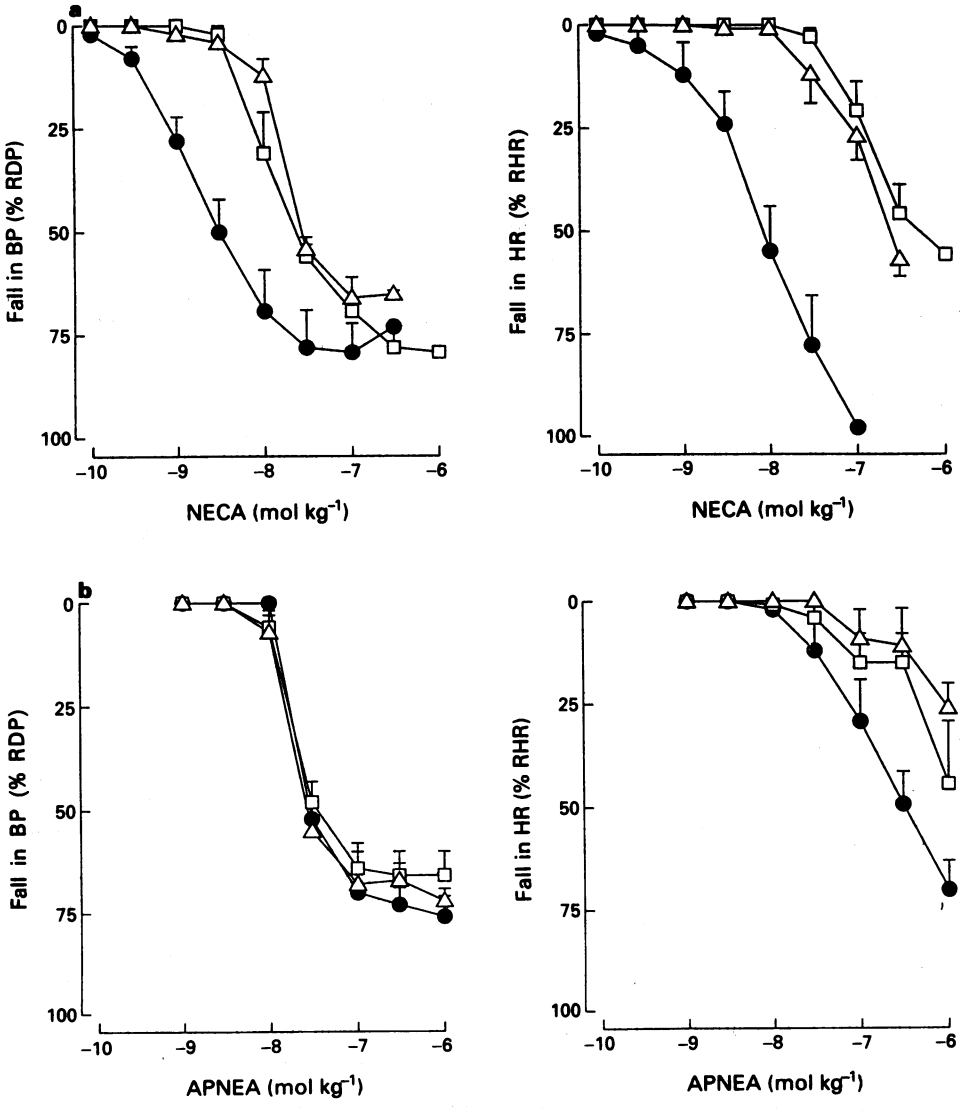

Figure 1 Effects of pretreatment with 8-sPT or CGS15943A on the cardiovascular responses to NECA (a) and APNEA (b) in the anaesthetised rat; (●) untreated controls; (□) animals pretreated with 8-sPT (40 mg kg^{-1}); (△) animals pretreated with CGS15943A (3 mg kg^{-1}) given 5 min prior to the agonist dose-response curve (DRC). The resting diastolic blood pressure (BP) and resting heart rate (HR) values just prior to starting the agonist DRC were as follows. NECA (control); 140 ± 6 mmHg, 473 ± 14 beats min^{-1} (n = 4); NECA (8-sPT): 102 ± 14 mmHg, 452 ± 22 beats min^{-1} (n = 5); NECA (CGS15943A): 119 ± 6 mmHg, 448 ± 13 beats min^{-1} (n = 4); APNEA (control): 109 ± 10 mmHg, 440 ± 34 beats min^{-1} (n = 4); APNEA (8-sPT): 130 ± 12 mmHg, 420 ± 22 beats min^{-1} (n = 5); APNEA (CGS15943A): 131 ± 4 mmHg, 457 ± 18 beats min^{-1} (n = 4). For abbreviations, see text.

Drugs

The following compounds were used: adenosine (Sigma Chemical Co.); CV1808 (2-(phenylamino)adenosine) and CGS21680 (2-[[2-(4-[2-carboxyethyl)phenyl]ethyl] amino] -*N*-ethylcarboxamindoadenosine) and 8-*p*-sulphophenyltheophylline (batches from both Research Biochemicals Inc and Chemistry Division, Glaxo Research and Development); NECA (5'-N-ethylcarboxamidoadenosine), CPA (N^6-cyclopentyladenosine), metrifudil (N-[(2-methylphenyl)methyl] adenosine), CGS15943A (9-fluoro-2-(2-furyl)-5,6-dihydro [1,2,4]triazolo{1,5-c}-quinazin-5-imine), APNEA (N-[2-(4-aminophenyl)ethyl] adenosine) and GR79236 (N-[(1S, *trans*)-2-hydroxycyclopentyl] adenosine) were synthesized in the Chemistry Division (Glaxo Research and Development).

GR79236, CPA, NECA, APNEA, metrifudil and CGS21680 were dissolved in 50 µl 1 M HCl and made up to volume in 0.9% (w/v) NaCl solution (saline). CV1808 was dissolved in 200 µl 1 M HCl + 200 µl 1 M NaOH and made up to volume in saline. A stock concentration of 100 µmol ml^{-1} was used for the agonists. The antagonists, 8sPT and CGS15943A, were prepared in dimethylsulphoxide (DMSO); administration of either 8sPT or CGS15943A did not alter resting blood pressure or heart rate. Administration of 0.1 ml DMSO vehicle produced a transient fall in blood pressure and heart rate which returned to pre-dose levels within 2 min.

Analysis of data

Hypotension was measured as the fall in diastolic pressure in mmHg, and expressed as a percentage of the resting diastolic pressure. Bradycardia was measured as the fall in heart rate in beats min^{-1}, and expressed as a percentage of the resting heart rate. The effective dose required to produce a fall in blood pressure of 40% or a fall in heart rate of 40% (ED_{40} BP and ED_{40} HR respectively) were expressed in nmol kg^{-1} and were determined by graphical interpolation. Dose-ratios (DR) were determined by dividing the ED_{40} value obtained from the antagonist pretreated groups by the ED_{40} value obtained from the control groups. Data are presented as the arithmetic mean ± s.e.mean, or geometric mean with 95% confidence interval as appropriate, from 3 or more animals per dose group. Significant differences between treatment groups in ED_{40} values were determined with Student's *t* test for unpaired data. Resting diastolic blood pressure and heart

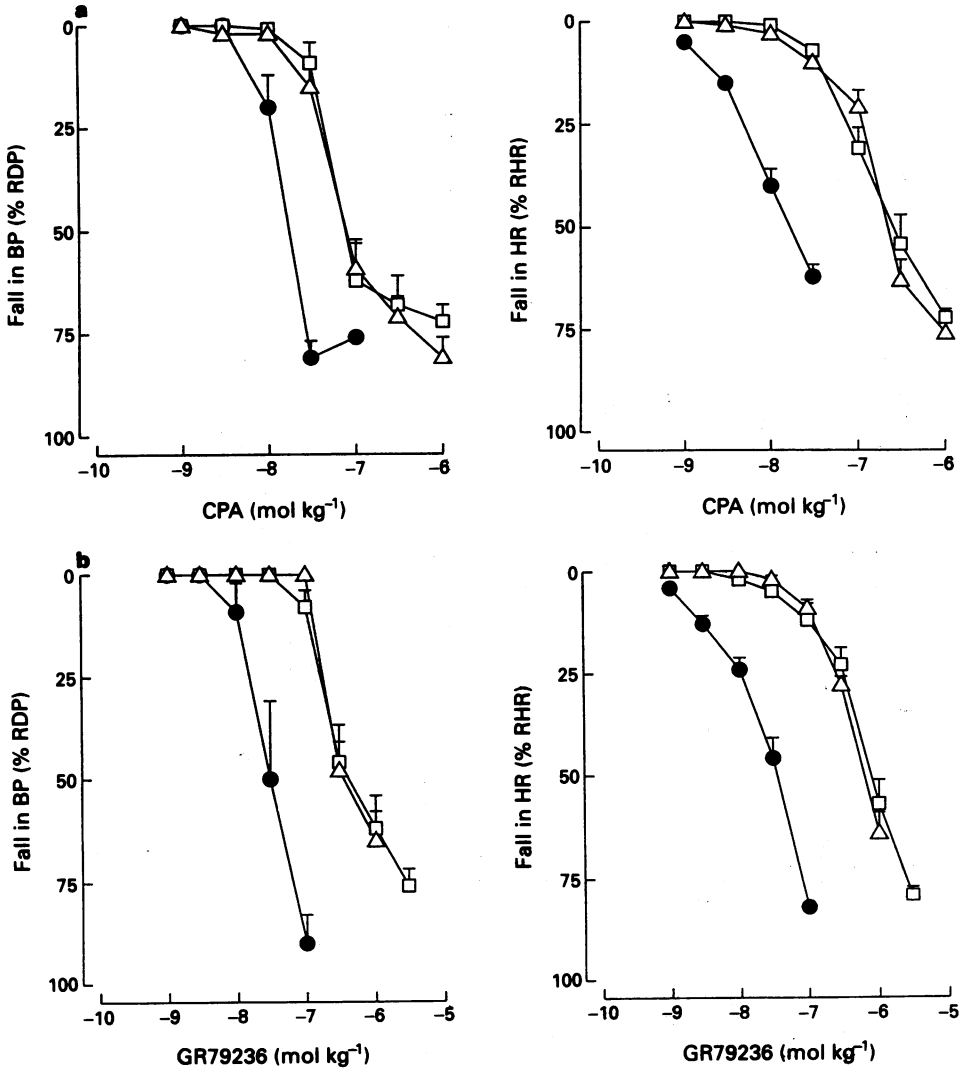

Figure 2 Effects of pretreatment with 8-sPT or CGS15943A on the cardiovascular response to CPA (a) and GR79236 (b) in the anaesthetized rat; (●) untreated controls; (□) animals pretreated with 8-sPT (40 mg kg^{-1}); (Δ) animals pretreated with CGS15943A (3 mg kg^{-1}) given 5 min prior to the agonist dose-response curve (DRC). The resting diastolic blood pressure (BP) and resting heart rate (HR) values just prior to starting the agonist DRC were as follows: CPA (control); 135 ± 10 mmHg, 455 ± 19 beats min^{-1} ($n = 4$); CPA (8-sPT): 136 ± 23 mmHg, 433 ± 25 beats min^{-1} ($n = 4$); CPA (CGS15943A): 119 ± 15 mmHg, 385 ± 24 beats min^{-1} ($n = 4$); GR79236 (control): 114 ± 23 mmHg, 460 ± 27 beats min^{-1} ($n = 4$); GR79236 (8-sPT): 125 ± 4 mmHg, 404 ± 20 beats min^{-1} ($n = 5$); GR79236 (CGS15943A): 123 ± 3 mmHg, 460 ± 9 beats min^{-1} ($n = 4$). For abbreviations, see text.

Table 1 Cardiovascular effects of adenosine agonists and their blockade by 8-sPT and CGS15943A

	Control		*8-sPT-treated*[c]					*CGS15943A-treated*[d]				
	BP ED_{40}[a] (nmol kg^{-1})	*HR* ED_{40}[a]	*BP* ED_{40}[a] (nmol kg^{-1})	*HR* ED_{40}[a]	*Dose-ratio* *BP*[b]	*HR*[b]	n	*BP* ED_{40}[a] (nmol/kg^{-1})	*HR* ED_{40}[a]	*Dose-ratio* *BP*[b]	*HR*[b]	n
NECA	2 (0.5–10)	11 (3–41)	12 (6–23)	323* (82–1272)	6	29	5	21 (16–27)	149* (92–243)	3	14	4
APNEA	25 (14–43)	164 (44–615)	27 (19–38)	774* (497–1205)	1	5	5	27 (6–120)	>880*	1	>7	4
CPA	14 (8–21)	19 (4–100)	72 (28–180)	156* (61–401)	5	8	4	62 (39–98)	178* (118–268)	5	10	4
GR79236	25 (9–72)	23 (14–37)	307* (104–896)	496* (328–750)	12	21	5	302* (131–697)	466* (315–689)	12	20	4
CGS21680	7 (4–12)	>1000	90* (27–298)	>1000	13	–	5	947* (498–1799)	>3000	135	–	4
CV1808	21 (8–51)	>1000	941* (497–1779)	>10000	45	–	5	3009* (1214–7459)	>10000	143	–	5
Metrifudil	18 (16–21)	649 (432–976)	61 (30–128)	10000	4	–	7	37 (22–62)	10000	2	–	5

Values are mean + 95% confidence interval.
[a]dose to lower BP and HR by 40%; [b]BP or HR ED_{40} (control)/BP or HR after antagonist; [c]40 mg kg^{-1} or [d]3 mg kg^{-1} given 5 min prior to agonist DRC
*denotes the means are significantly different from controls, $P<0.05$.

Table 2 Cardiovascular effects of APNEA and GR79236 and their blockade by CGS15943A

	Control		*CGS15943A-treated*[c]					*CGS15943A-treated*[d]				
	BP ED_{40}[a] (nmol kg^{-1})	*HR* ED_{40}[a]	*BP* ED_{40}[a] (nmol kg^{-1})	*HR* ED_{40}[a]	*Dose-ratio* *BP*[b]	*HR*[b]	n	*BP* ED_{40}[a] (nmol/kg^{-1})	*HR* ED_{40}[a]	*Dose-ratio* *BP*[b]	*HR*[b]	n
APNEA	25 (14–43)	164 (44–615)	35 (10–122)	658 (187–2315)	1	4	3	44 (29–68)	5811* (2422–13940)	2	35	4
GR79236	25 (9–72)	23 (14–37)	145* (66–340)	124* (83–184)	6	5	4	544* (226–1310)	1115* (771–1612)	24	48	4

Values are mean + 95% confidence interval.
[a]dose to lower BP and HR by 40%; [b]BP or HR ED_{40} (control)/BP or HR after antagonist; [c]1 mg kg^{-1} or [d]10 mg kg^{-1} given 5 min prior to agonist DRC
*denotes the means are significantly different from controls, $P<0.05$.

rate values for each group of experiments are given in the figure legends.

Results

The resting diastolic blood pressure of a representative group of anaesthetized rats was 126 ± 3 mmHg, with a resting heart rate of 417 ± 14 beats min^{-1} ($n = 15$). Two priming doses of adenosine (300 nmol kg^{-1}) administered as single intravenous bolus doses produced a fall in blood pressure of 43 ± 4 mmHg, associated with a fall in heart rate of 65 ± 5 beats min^{-1}, and 46 ± 4 mmHg, with a fall in heart rate of 71 ± 7 beats min^{-1}, for the first and second priming dose respectively.

NECA and APNEA

The non-selective adenosine receptor agonist NECA (0.1–300 nmol kg^{-1}), and the A_1/A_3 agonist APNEA (1–1000 nmol kg^{-1}), produced dose-dependent falls in blood pressure and heart rate (Figure 1a,b). The antagonist 8-sPT (40 mg kg^{-1}) and CGS15943A (3 mg kg^{-1}) produced a smaller rightward displacement of the dose-response curve (DRC) to the hypotensive effect of NECA than to the bradycardic effect of NECA (Figure 1a; Table 1). Even more notably, the antagonists 8-sPT (40 mg kg^{-1}) and CGS15943A (3 mg kg^{-1}) were without effect on the hypotension observed with APNEA. However, both antagonists were able to produce a rightward displacement of the DRC to the bradycardia obtained with APNEA (Figure 1b; Table 1).

A_1 receptor-selective agonists, CPA and GR79236

The A_1-selective agonists, CPA and GR79236 (0.1–300 nmol kg^{-1}), also produced a dose-dependent fall in blood pressure and heart rate. Both agonists produced hypotension and bradycardia at approximately equieffective doses (Table 1). The antagonists 8-sPT (40 mg kg^{-1}) and CGS15943A (3 mg kg^{-1}) produced slightly smaller rightward displacements of the DRC to the hypotensive effects of GR79236 and CPA than to their bradycardic effects, although this difference was only significant for CPA ($P < 0.05$) (Figure 2a,b, Table 1).

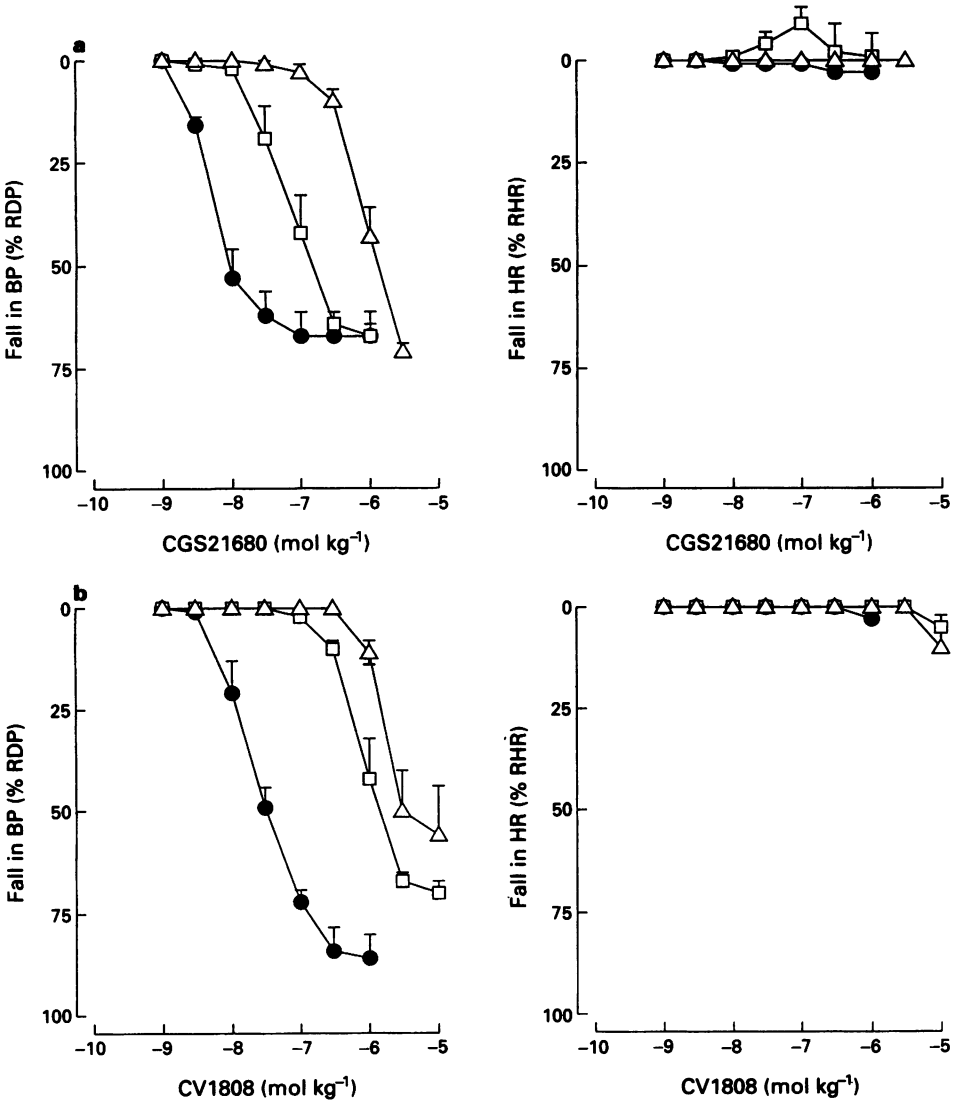

Figure 3 Effects of pretreatment with 8-sPT or CGS15943A on the cardiovascular responses to CGS21680 (a) and CV1808 (b) in the anaesthetized rat; (●) untreated controls; (□) animals pretreated with 8-sPT (40 mg kg^{-1}); (△) animals pretreated with CGS15943A (3 mg kg^{-1}) given 5 min prior to the agonist dose-response curve (DRC). The resting diastolic blood pressure (BP) and resting heart rate (HR) values just prior to starting the agonist DRC were as follows: CGS21680 (control); 112 ± 10 mmHg, 435 ± 20 beats min^{-1} ($n = 4$); CGS21680 (8-sPT): 132 ± 8 mmHg, 382 ± 17 beats min^{-1} ($n = 5$); CGS21680 (CGS15943A): 141 ± 7 mmHg, 427 ± 10 beats min^{-1} ($n = 4$).CV1808 (control): 106 ± 14 mmHg, 380 ± 25 beats min^{-1} ($n = 4$); CV1808 (8-sPT): 143 ± 7 mmHg, 428 ± 19 beats min^{-1} ($n = 5$); CV1808 (CGS15943A): 117 ± 4 mmHg, 372 ± 14 beats min^{-1} ($n = 5$). For abbreviations, see text.

A_{2a} receptor-selective agonists, CGS21680 and CV1808

The adenosine A_{2a} receptor selective agonists, CGS21680 (0.1–100 nmol kg^{-1}) and CV1808 (1–1000 nmol kg^{-1}), both produced a dose-dependent fall in blood pressure, with no significant effect on heart rate. Following administration of either 8-sPT (40 mg kg^{-1}) or CGS15943A (3 mg kg^{-1}), a rightward displacement of the DRC to the hypotensive effect of CGS21680 was observed, with CGS15943A (3 mg kg^{-1}) producing a much greater antagonism of this effect. A modest tachycardia was observed following 8-sPT treatment (Figure 3a; Table 1). Similarly, both 8-sPT (40 mg kg^{-1}) and CGS15943A (3 mg kg^{-1}) produced an antagonism of the hypotensive effect of CV1808, with CGS15943A being the more potent of the two antagonists (Figure 3b, Table 1).

A_{2b} receptor-selective agonist, metrifudil

The adenosine A_{2b} receptor-selective agonist, metrifudil (0.01–10 μmol kg^{-1}), also produced a dose-dependent hypotension and bradycardia. The metrifudil-induced hypotension occurred at lower doses than those causing bradycardia (Table 1). The hypotension induced by metrifudil was not significantly antagonized by either 8-sPT (40 mg kg^{-1}) or CGS15943A (3 mg kg^{-1}) (Figure 4a, Table 1); however, the bradycardia produced by metrifudil was attenuated by both antagonists (Figure 4b; Table 1).

Antagonism of the effects of APNEA and GR79236 by CGS15943A (1–10 mg kg^{-1})

The hypotension induced by APNEA has already been demonstrated to be resistant to blockade by both 8-sPT (40 mg kg^{-1}) and CGS15943A (3 mg kg^{-1}) (Figure 1a). This effect of APNEA was also resistant to blockade by a higher dose of CGS15943A (10 mg kg^{-1}) (Figure 5a; Table 2). However, CGS15943A (1–10 mg kg^{-1}) produced a dose-dependent rightward displacement of the APNEA-induced bradycardia (Figure 5a; Table 2). In contrast, CGS15943A (1–10 mg kg^{-1}) produced dose-dependent rightward displacements of the DRC to both the hypotension and bradycardia induced by GR79236 (Figure 5b; Table 2).

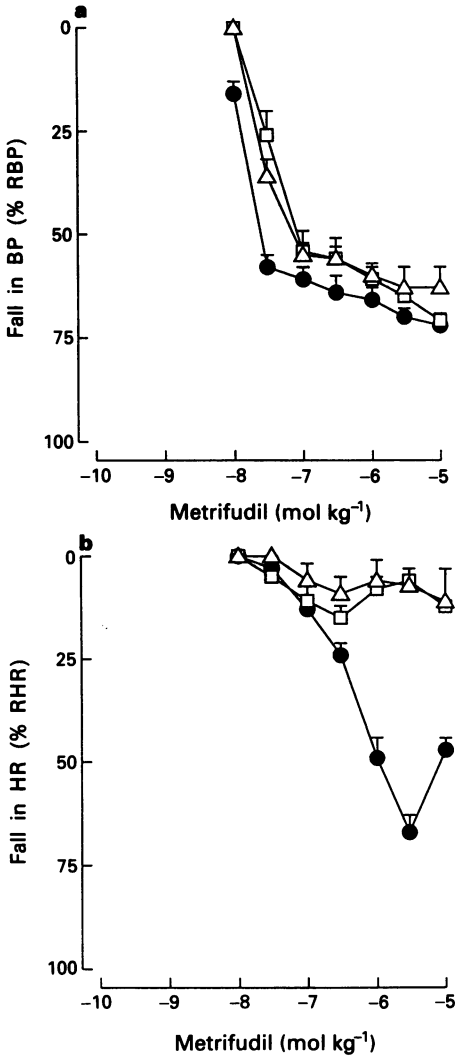

Figure 4 Effects of pretreatment with 8-sPT or CGS15943A on the cardiovascular responses to metrifudil in the anaesthetized rat; (●) untreated controls; (□) animals pretreated with 8-sPT (40 mg kg^{-1}); (△) animals pretreated with CGS15943A (3 mg kg^{-1}) given 5 min prior to the agonist dose-response curve (DRC). The resting diastolic blood pressure (BP) (a) and resting heart rate (HR) (b) values just prior to starting the agonist DRC were as follows. Metrifudil (control); 111 ± 9 mmHg, 370 ± 21 beats min^{-1} ($n = 4$); metrifudil (8-sPT): 147 ± 5 mmHg, 427 ± 12 beats min^{-1} ($n = 7$); metrifudil (CGS15943A) 134 ± 7 mmHg, 406 ± 22 beats min^{-1} ($n = 5$). For abbreviations, see text.

Discussion

The hypotension observed in the presence of blockade of A_1 and A_2 receptors in pithed or anaesthetized rats has been postulated to be an *in vivo* functional correlate for the adenosine A_3 receptor (Fozard & Carruthers, 1993a; Fozard & Hannon, 1994). In the present study we have attempted to characterize further the putative A_3 receptor mediating a hypotensive response in the anaesthetized rat to a range of adenosine agonists in the presence of A_1/A_2 receptor blockade with the xanthine antagonist 8sPT, or the non-xanthine antagonist CGS15943A (Ghai *et al.*, 1987). The initial characterization of CGS15943A suggested that this antagonist had a much greater affinity for adenosine receptors of the A_2 than the A_1 receptor subtype (Ghai *et al.*, 1987). However, recent studies in CHO cells transfected with human A_1, A_{2a} or A_{2b} receptors suggest that its selectivity is modest. For example, A_{2a} to A_1 selectivity may be as little as 5 fold and A_{2a} to A_{2b} selectivity only 20 fold (Rollins *et al.*, 1994) and so it may be more accurate to consider CGS15943A as an adenosine receptor antagonist with limited selectivity. Indeed, CGS15943A has previously been described as a non-selective adenosine receptor antagonist (Merkel *et al.*, 1993).

In the presence of a high dose of 8sPT, the agonists CPA, APNEA and NECA produced a fall in blood pressure at doses lower than those required for an effect on heart rate. In this respect, our results confirm the findings of Fozard and co-workers (1993a,b, 1994) suggesting that these agonists may cause hypotension by activating the A_3 adenosine receptor. Furthermore, the hypotensive response to APNEA remained unaffected after A_2/A_1 receptor blockade by high doses of CGS15943A (3 and 10 mg kg^{-1}) which clearly attenuated the effects of APNEA on heart rate. Thus the A_3 receptor appears to have little affinity for CGS15943A.

In the presence of CGS15943A (3 mg kg^{-1}), the selective A_1 receptor agonist CPA was found to mediate a hypotensive response at doses lower than those required to produce bradycardia. This is similar to the results obtained for CPA in the presence of a high dose of 8-sPT (Figure 2a, Table 1) and confirms the findings of Fozard & Carruthers (1993b). In contrast, under the same conditions GR79236 produced hypotension only at doses that also produced bradycardia. However, in the presence of a higher dose of CGS15943A (10 mg kg^{-1}), GR79236 may produce hypotension in the absence of significant bradycardia, although the dose-ratios for the falls in blood pressure and heart rate were not significantly different. It is unlikely that GR79236 produces a hypotensive response by activation of vascular A_2 receptors.

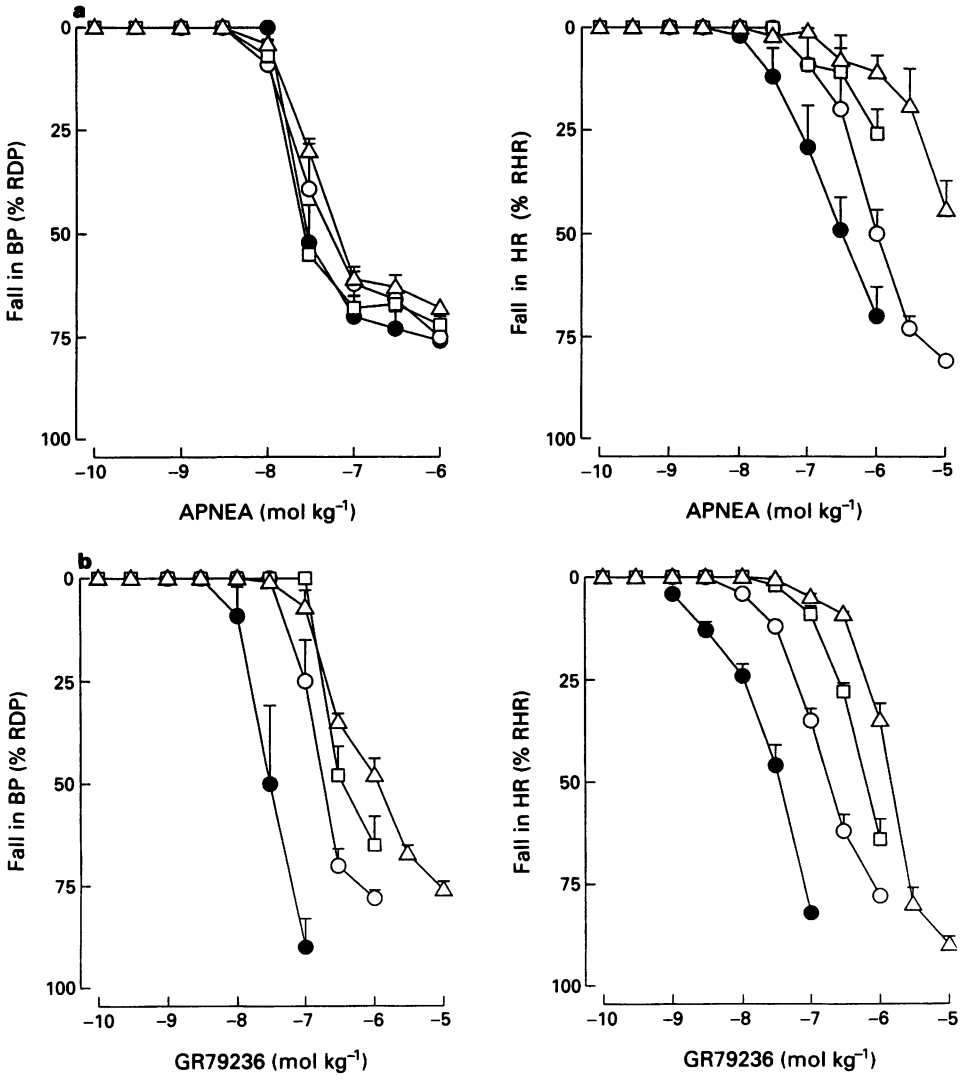

Figure 5 Effects of pretreatment with CGS15943A on the cardiovascular responses to APNEA (a) and GR79236 (b) in the anaesthetized rat; (●) untreated controls; (□) animals pretreated with CGS15943A (1 mg kg^{-1}); (□) CGS15943A (3 mg kg^{-1}),(△) CGS15943A (10 mg kg^{-1}) given 5 min prior to the agonist dose-response curve (DRC). The resting diastolic blood pressure (BP) and resting heart rate (HR) values just prior to starting the agonist DRC were as follows. (a) APNEA (control); 109 ± 10 mmHg, 440 ± 34 beats min^{-1} ($n = 4$); CGS15943A (1 mg kg^{-1}): 145 ± 9 mmHg, 370 ± 15 beats min^{-1} ($n = 3$); CGS15943A (3 mg kg^{-1}): 131 ± 5 mmHg, 457 ± 18 beats min^{-1} ($n = 4$); CGS15943A (10 mg kg^{-1}): 139 ± 19 mmHg, 402 ± 14 beats min^{-1} ($n = 4$). (b) GR79236 (control): 114 ± 22 mmHg, 460 ± 27 beats min^{-1} ($n = 4$); CGS15943A (1 mg kg^{-1}): 140 ± 6 mmHg, 412 ± 5 beats min^{-1} ($n = 4$); CGS15943A (3 mg kg^{-1}): 123 ± 3 mmHg, 460 ± 9 beats min^{-1} ($n = 4$): CGS15943A (10 mg kg^{-1}): 120 ± 10 mmHg, 385 ± 9 beats min^{-1} ($n = 4$).

Recent studies have shown that GR79236 has 300 fold selectivity for A_1 over A_2 receptors (Gurden *et al.*, 1993), and furthermore, both 8-sPT and CGS15943A were effective antagonists of the A_{2a}-mediated hypotensive response to CGS21680 and CV1808. Therefore it is possible that GR79236 may activate the A_3 receptor at high doses.

The modestly selective A_{2b} receptor agonist, metrifudil, also produced a profound hypotension with smaller effects on heart rate. The fall in blood pressure induced by metrifudil was not antagonized by either 8sPT or CGS15943A at doses that blocked A_{2a}-mediated responses, and since CGS15943A has approximately equal affinity for A_{2a} and A_{2b} receptors (Rollins *et al.*, 1994), the hypotensive response cannot be explained by an action at A_{2b} receptors. These data suggest that metrifudil may produce a hypotensive effect by the activation of the putative A_3 receptor and so is probably of limited value in studying A_{2b} receptors *in vivo*. Although the hypotension induced by metrifudil was resistant to blockade by 8sPT and CGS15943A, in a similar manner to that described for APNEA, the response was qualitatively different from that observed with APNEA. The response was slower in onset and had a longer duration to attainment of a maximum response. This qualitative difference between metrifudil and APNEA is not fully understood and merits further investigation.

In conclusion, our results not only support the findings of Fozard and co-workers but extend the characterization of agonists that have efficacy at this putative A_3 receptor to include metrifudil. Since the hypotensive response mediated by the A_3 receptor was also resistant to the non-xanthine antagonist CGS15943A, it is clear that the A_3 receptor can no longer be described as a xanthine-insensitive receptor. Indeed, the xanthine antagonist, BW-A522, has been shown to antagonize the response to APNEA in a surmountable manner, albeit with somewhat lower potency than would be expected from its affinity for the human and sheep A_3 receptor (Fozard & Hannon, 1994). The results also suggest that CGS15943A is a more potent antagonist than 8sPT at blocking both A_2 and A_1 receptors and would therefore be a more appropriate antagonist with which to study A_3-receptor-

mediated events in preparations where mixed populations of adenosine receptors exist. Finally, our study suggests that the selective A_1 receptor agonist, GR79236, and the A_{2a} receptor agonists, CGS21680 and CV1808, are without significant effect at the putative A_3 receptor and would therefore be suitable tools with which to investigate this novel receptor.

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