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SPECIAL REPORT

Adenosine A_3 receptors[1] mediate hypotension in the angiotensin II-supported circulation of the pithed rat

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The cardiovascular effects of N^6-2-(4-aminophenyl)ethyladenosine (APNEA), which when radiolabelled with ^{125}I shows high affinity for the newly described adenosine A_3 receptor, have been investigated in the angiotension II-supported circulation of the pithed rat. APNEA induces hypotensive responses which are unaffected by high doses (20–40 mg kg^{-1}) of the broad spectrum, adenosine receptor antagonist, 8-(*p*-sulphophenyl)theophylline (8-SPT). 8-SPT-resistant falls in blood pressure are also seen, in the absence of bradycardia, with 5'-N-ethylcarboxamidoadenosine (NECA) and the **R**- and **S**-enantiomers of N^6-phenylisopropyladenosine (PIA). Xanthine insensitivity, high potencies of APNEA, NECA and **R**-PIA, and an enantiomeric selectivity favouring **R**- over **S**-PIA are distinguishing features of the adenosine A_3 receptor. We suggest that hypotension in the pithed rat may be a functional correlate of this site.

Keywords: 5'-N-ethylcarboxamidoadenosine (NECA); **R**-N^6-phenylisopropyladenosine (**R**-PIA); **S**-N^6-phenylisopropyladenosine (**S**-PIA); N^6-2-(4-aminophenyl)ethyl adenosine (APNEA); hypotension; A_3 adenosine receptors

Introduction Recently, Zhou *et al.* (1992) described the cloning, expression and functional characterization of a novel adenosine receptor which they designated A_3. The A_3 receptor can be labelled with high affinity by the A_1 receptor agonist radioligand, [^{125}I]-APNEA (N^6-2-(4-amino-3-iodophenyl)ethyladenosine. We here report that low doses of APNEA induce hypotension in pithed rats with their blood pressures raised to normal by angiotension II. Significantly, the response shows the major features of the A_3 receptor in being resistant to blockade by 8-(*p*-sulphophenyl)theophylline (8-SPT), a xanthine-based, broad spectrum, adenosine receptor antagonist, mimicked by low doses of 5'-N-ethylcarboxamidoadenosine (NECA) and **R**-N^6-phenylisopropyladenosine (**R**-PIA) and manifesting enantiomeric selectivity favouring **R**-PIA over **S**-PIA.

Methods Male Sprague-Dawley rats weighing 284–389 g, supplied by Biological Research Laboratories (Füllinsdorf, Switzerland) were anaesthetized with sodium pentobarbitone, 60 mg kg^{-1}, i.p., and pithed as described previously (Mir *et al.*, 1989). Rats were artificially respired with pure oxygen (Palmer Pump, 1 ml 100 g^{-1}; 60 strokes min^{-1}) and catheters inserted into the right carotid artery for recording blood pressure and heart rate and into the jugular veins for drug administration. Body temperature was maintained at 37°C by means of a heated table and lamp. All parameters were displayed on a Beckman R-SIIA polygraph.

Following pithing, blood pressure was routinely raised to 100–120 mmHg by infusion of angiotensin II, 0.2–0.5 μg kg^{-1} min^{-1} and a 15 min stabilization period was allowed before any further intervention. Dose-response curves to the adenosine receptor agonists were established by cumulative bolus injection, the intervals between doses being sufficient to allow a plateau response to develop. In the experiments with the non-selective A_1/A_2 receptor antagonist, 8-SPT, either 8-SPT, 20 or 40 mg kg^{-1} was injected intravenously 5 min before establishing the dose-response curves to the agonists. Only one agonist dose-response curve was generated per animal.

The following compounds were used: N^6-2-(4-aminophenyl)ethyladenosine (APNEA), gift of Professor R. Olsson, University of South Florida, Tampa, Fla, U.S.A.; 5'-N-ethylcarboxamidoadenosine (NECA), **R**- and **S**-N^6-phenylisopropyladenosine (**R**- and **S**-PIA), Sigma, Buchs, Switzerland; angiotensin II in the form of the amide analogue, Ciba-Geigy, Basel, Switzerland; 8-(*p*-sulphophenyl)theophylline (8-SPT), synthesized by Dr F. Gadient at Sandoz, Basel, Switzerland; sodium pentobarbitone (Sagatal), May and Baker Ltd., Dagenham, U.K. Final dilutions of all drugs were prepared in 0.9% NaCl.

Results Cumulative intravenous administration of APNEA, 1–30 μg kg^{-1} induced dose-related falls in blood pressure in the angiotensin II supported circulation of the pithed rat accompanied at the higher doses by short-lived (<1 min) falls in heart rate (Figure 1a). NECA, 0.3–10 μg kg^{-1}, **R**-PIA, 1–30 μg kg^{-1} and **S**-PIA, 10–300 μg kg^{-1} also induced dose-related falls in blood pressure (Figure 1b–d); in each case these were accompanied by bradycardia (Figures 1b–d) which was sustained relative to that seen with APNEA.

8-SPT, injected intravenously at a dose of 20 mg kg^{-1}, abolished the fall in heart rate induced by APNEA but did not influence the fall in blood pressure; a similar result was obtained following 40 mg kg^{-1} (Figure 1a). Following 40 mg kg^{-1} 8-SPT, the bradycardia following NECA, **R**-PIA and **S**-PIA was essentially abolished. In contrast, falls in blood pressure persisted in the presence of 8-SPT although the dose-response curves were displaced to the right at the ED_{50} level by 4.9, 2.7 and 1.5 fold for NECA, **R**-PIA and **S**-PIA, respectively (Figure 1b–d, Table 1).

Discussion The major finding of the present study is that APNEA, which when radiolabelled with ^{125}I shows high affinity for the newly described A_3 adenosine receptor (Zhou *et al.*, 1992), induced hypotensive responses in the pithed rat which are resistant to blockade by the broad spectrum adenosine receptor antagonist, 8-SPT. The response is not dependent on the use of angiotensin II to raise blood pressure since APNEA manifests similar hypotension in pithed rats infused

[1] Footnote: There is confusion in the literature with respect to the term 'adenosine A_3 receptor'. In this paper 'adenosine A_3 receptor' refers to the novel, structurally defined, adenosine receptor cloned from rat brain by Xhou *et al.* (1992) and not to the quite different 'A_3 receptor' suggested on pharmacological grounds to be present in certain peripheral neurones (Ribeiro & Sebastião, 1986).

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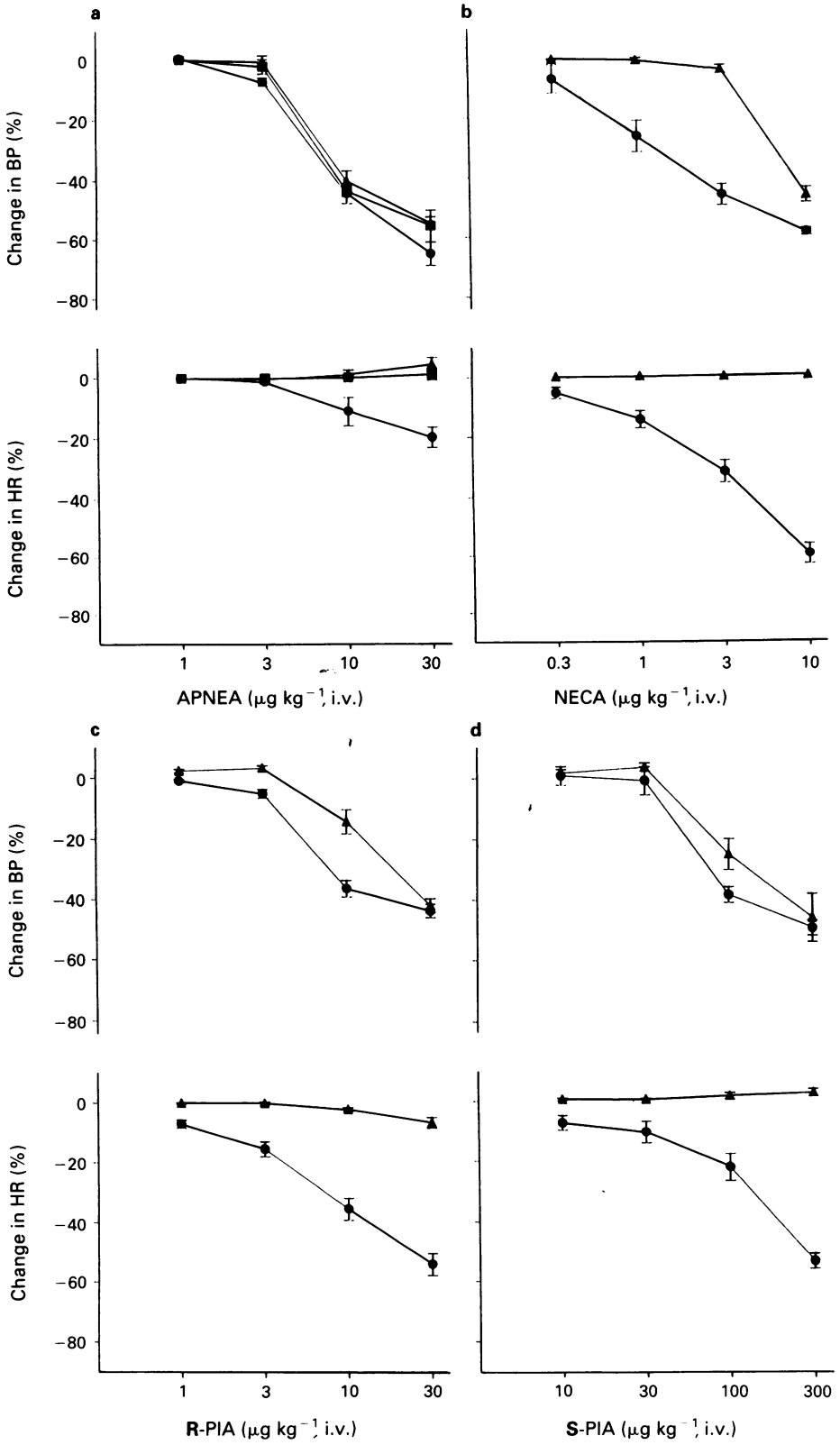

Figure 1 Effects of 8-(*p*-sulphophenyl)theophyline (8-SPT) on the cardiovascular responses to N^6-2-(4-aminophenyl)ethyl adenosine (APNEA) (a), 5'-N-ethylcarboxamidoadenosine (NECA) (b), **R**-N^6-phenylisopropyladenosine (**R**-PIA) (c) and S-PIA (d) in pithed rats with blood pressure supported by angiotensin II: (●) vehicle-treated controls; (■) animals treated with 20 mg kg^{-1} 8-SPT; (▲) animals treated with 40 mg kg^{-1} 8-SPT. The mean baseline blood pressure (BP) and heart rate (HR) values just prior to starting the injection sequences were:

APNEA	(vehicle)	110 ± 4	280 ± 6	(4)
APNEA	(8-SPT; 20)	110 ± 3	293 ± 7	(4)
APNEA	(8-SPT; 40)	108 ± 2	285 ± 7	(4)
NECA	(vehicle)	102 ± 3	299 ± 10	(4)
NECA	(8-SPT; 40)	102 ± 1	293 ± 9	(4)
R-PIA	(vehicle)	103 ± 5	267 ± 10	(4)
R-PIA	(8-SPT; 40)	113 ± 3	288 ± 6	(9)
S-PIA	(vehicle)	106 ± 4	283 ± 11	(4)
S-PIA	(8-SPT; 40)	108 ± 3	287 ± 12	(4)

Table 1 Cardiovascular effects of adenosine receptor agonists and their blockade by 8-(*p*-sulphophenyl)theophyline) (8-SPT)

	Control			*8-SPT-treated*[b]			
	BP, ED_{30}[a] ($nmol\ kg^{-1}$)	*HR, ED_{30}*[a] ($nmol\ kg^{-1}$)	n	*BP, ED_{30}*[a] ($nmol\ kg^{-1}$)	*HR, ED_{30}*[a] ($nmol\ kg^{-1}$)	*BP* Shift[c]	n
APNEA	16 ± 1	>78	4	20 ± 2	>259	1.2	4
NECA	3.7 ± 0.9	7.8 ± 1.5	4	18.2 ± 0.7	>88	4.9	4
R-PIA	21 ± 2	20 ± 3	4	56 ± 6	>259	2.7	9
S-PIA	198 ± 19	337 ± 58	4	292 ± 35	>778	1.5	4

[a]Dose to lower BP and HR by 30%; [b]40 mg kg^{-1} given i.v. 5 min prior to first dose of agonist; [c]Ratio: BP, ED_{30} (control)/BP, ED_{30} (8-SPT); For abbreviations, see text.

with phenylephrine (unpublished data). The high doses of 8-SPT used are sufficient to block the bradycardia induced by NECA and **R**-PIA which is known to result from activation of the A_1 receptor of the sino-atrial node (Linden, 1991). Moreover, a dose of 40 mg kg^{-1} 8-SPT blocked hypotension in the pithed rat induced by 2-[*p*-(2-carboxyethyl)phenylethylamino]-5'N-ethylcarboxamidoadenosine (CGS 21680), a selective A_{2A} receptor agonist (Hutchinson *et al.*, 1989), by 19 fold (Fozard & Carruthers, 1993). Thus, the fall in blood pressure induced by APNEA is not a consequence of activation of either A_1 or A_{2A} receptors. Further, an effect on A_{2B} receptors can be excluded since 8-SPT is at least as active at this particular subtype as at either the A_1 or the A_2 receptors (Bruns *et al.*, 1986; Hargreaves *et al.*, 1990).

Apart from a high affinity for APNEA, the principal pharmacological characteristics of the A_3 receptor are xanthine insensitivity, high affinities of the A_1/A_2 receptor agonist, NECA, and the nominally selective A_1 receptor agonist, **R**-PIA and an enantiomeric selectivity favouring **R**-PIA over **S**-PIA (Zhou *et al.*, 1992). It bears emphasis that low doses of NECA and **R**-PIA induce dose-related falls in blood pressure in the presence of a dose of 8-SPT adequate to block A_1, A_{2A} and A_{2B} receptors and that **S**-PIA is some 6 fold less active than **R**-PIA in this respect.

In conclusion, our data indicate that hypotension in the angiotensin II-supported circulation of the pithed rat is a functional correlate of the A_3 adenosine receptor and confirm the activity of the classical adenosine receptor agonists, NECA and **R**-PIA, at this site. It is significant that the selectivity of the nominally A_1 receptor selective agonist, **R**-PIA, is substantially less when comparisons are made with the A_3 receptor rather than either of the A_2 receptor subtypes. Moreover, since a number of claimed A_1 selective agonists induce 8-SPT-resistant hypotension in our model (Carruthers *et al.*, 1992), significant activity at this site may be a widespread property among adenosine receptor agonist ligands traditionally used to discriminate between adenosine A_1 and A_2 receptor subtypes. Clearly, a redefinition of the value of such tools and a reappraisal of conclusions made on the basis of their use would seem appropriate.

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