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A role for mast cells in adenosine A_3 receptor-mediated hypotension in the rat

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1 The adenosine A_3 receptor agonist, N^6-2-(4-aminophenyl)ethyladenosine (APNEA) induces hypotension in the anaesthetized rat. The present experiments were carried out to explore the role of mast cells in the response.

2 Intravenous injection of APNEA (1–30 $\mu g\ kg^{-1}$ to rats in which the A_3 receptor-mediated response had been isolated by pretreatment with 8-(*p*-sulphophenyl) theophylline (8-SPT)), induced dose-related falls in blood pressure accompanied at higher doses by small falls in heart rate. Responses to the mast cell degranulating agent, compound 48/80 (10–300 $\mu g\ kg^{-1}$, i.v.) were qualitatively similar to those to APNEA.

3 Pretreatment with sodium cromoglycate (0.25–20 $mg\ kg^{-1}$, i.v.) induced dose-related, although incomplete, blockade of the hypotensive responses to APNEA. At 20 $mg\ kg^{-1}$, sodium cromoglycate also inhibited the cardiovascular response to compound 48/80 but had no effects on those to the selective A_1 receptor agonist, N^6-cyclopentyladenosine (CPA) or the selective A_{2A} receptor agonist, 2-[*p*-(2-carboxyethyl)phenylamino]-5′-N-ethylcarboxamidoadenosine (CGS 21680). Lodoxamide (0.01–20 mg kg^{-1}) also blocked selectively but incompletely the response to APNEA.

4 The cardiovascular responses to compound 48/80 (10–300 $\mu g\ kg^{-1}$, i.v.) were markedly suppressed in animals which had received repeated doses of the compound by the intraperitoneal route. Similarly APNEA was essentially devoid of cardiovascular activity in such preparations. In contrast, responses to CPA were similar in animals treated repeatedly with compound 48/80 to those obtained in control animals.

5 Plasma and serum histamine concentrations were markedly increased associated with the pronounced hypotensive responses induced by intravenous injections of APNEA (30 or 100 $\mu g\ kg^{-1}$) in the presence of 8-SPT, or compound 48/80 (300 $\mu g\ kg^{-1}$).

6 Taken together the data implicate the mast cell in a key role in adenosine A_3 receptor-mediated hypotension in the anaesthetized rat.

Keywords: Adenosine A_3 receptor; APNEA; compound 48/80; mast cell degranulation; hypotension; histamine

Introduction

The adenosine A_3 receptor agonist, N^6-2-(4-aminophenyl)-ethyladenosine (APNEA) induces dose-related hypotensive responses in both anaesthetized rats (Carruthers & Fozard, 1993a; Fozard & Hannon, 1994) and in pithed preparations with blood pressure raised to normal with a variety of different pressor agents (Fozard & Carruthers, 1993a; Carruthers & Fozard, 1993b). Significantly, the response shows the major features of the recently cloned rat A_3 receptor (Zhou *et al.*, 1992) both with respect to agonist and antagonist pharmacology (Carruthers & Fozard, 1993b; Fozard & Carruthers, 1993a,b; Fozard & Hannon, 1994) and in being suppressed in animals pretreated with pertussis toxin (Carruthers & Fozard, 1993a).

Although both a fall in systemic vascular resistance and a decrease in cardiac output contribute to the hypotension induced in the rat by activation of adenosine A_3 receptors (Salzmann & Fozard, 1994), the actual target cell(s) involved have not been determined. Indeed, it is unknown whether APNEA acts directly on elements of the cardiovascular system or indirectly by inducing transmitter or mediator release. With respect to the latter, a plausible candidate for an intermediary role is the mast cell, which has been shown to express the A_3 receptor (Ali *et al.*, 1990; 1991; Ramkumar *et al.*, 1993) and to be activated by ligands with A_3 receptor agonist properties (Ramkumar *et al.*, 1993). Significantly, both the biochemical events and facilitation of mediator release induced by A_3 receptor-mediated activation of mast cells are pertussis toxin-sensitive (Ali *et al.*, 1990; Qian & McCloskey, 1993). The present experiments were designed to explore the role of mast cells in the hypotensive responses induced by A_3 receptor activation in the rat.

A preliminary account of part of this work has been presented to the British Pharmacological Society (Hannon & Fozard, 1994).

Methods

Animals

Male Sprague-Dawley rats weighing 210–510 g were used throughout. They were supplied by Biological Research Laboratories (Füllinsdorf, Switzerland) and kept under normal laboratory conditions.

Cardiovascular studies

Animals were anaesthetized with pentobarbitone sodium, 60 $mg\ kg^{-1}$, i.p. and set up for recording blood pressure and heart rate and for intrajugular venous administration of drugs as previously described (Carruthers & Fozard, 1993a). After a stabilization period of approximately 15 min, dose-response curves to APNEA, the adenosine A_1 receptor selective agonist, N^6-cyclopentyladenosine (CPA), the adenosine A_{2A} receptor selective agonist, 2-[*p*-(2-carboxyethyl) phenylamino]-5′-N-ethylcarboxamidoadenosine (CGS 21680) or the mast cell degranulating agent, compound 48/80, were established by cumulative bolus injection; the intervals between doses were sufficient to allow a plateau response to develop. In the case of

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APNEA, the A_1/A_2 receptor antagonist, 8-(*p*-sulphophenyl)theophylline (8-SPT) was injected intravenously at a dose of 40 mg kg^{-1} 5 min prior to establishing the agonist dose-response curve in order to 'isolate' the A_3 receptor-mediated component of the response to APNEA (see Carruthers & Fozard, 1993a; Fozard & Carruthers, 1993a; Fozard & Hannon, 1994). Only one agonist dose-response curve was generated per animal.

Sodium cromoglycate or lodoxamide were injected intravenously 3 min before establishing the dose-response curves to the adenosine agonists or compound 48/80. Animals were 'desensitized' to the effects of compound 48/80 by injecting 1 mg kg^{-1}, i.p., four times at 3 h intervals on the day before the experiment and 3 mg kg^{-1}, i.p., 2–3 h prior to anaesthesia on the experimental day. In selected cases, the peritoneal cavities were lavaged with 10 ml of normal saline and microscopical analysis of the numbers of intact granulated mast cells carried out following differential staining with Diff-Quick (Baxter Dade, Switzerland).

Mean values ($\pm$ s.e.mean) from *n* individual experiments are presented.

Measurement of histamine concentrations in serum and plasma

Rats were anaesthetized and prepared for intrajugular venous administration of drugs as described under *Cardiovascular studies* above. A cannula was placed in one common carotid artery for collection of blood. After a stabilization period of approximately 15 min, 19 animals were allocated randomly into 5 groups: Group 1 received 8-SPT (40 mg kg^{-1}, i.v.) 5 min prior to a single i.v. injection of APNEA (30 μg kg^{-1}), group 2 received 8-SPT (40 mg kg^{-1}, i.v.) 5 min prior to an i.v. injection of APNEA, (100 μg kg^{-1}); group 3 received the vehicle for 8-SPT i.v. 5 min prior to an i.v. injection of APNEA (100 μg kg^{-1}); group 4 received 8-SPT 5 min prior to i.v. injection of the vehicle for APNEA; group 5 received vehicle for 8-SPT i.v. 5 min prior to an i.v. injection of vehicle for APNEA. In a second experiment, 8 animals were allocated randomly into two groups: Group 1 received compound 48/80 (300 μg kg^{-1}, i.v.); group 2 received an equivalent volume of vehicle. Two min after drug or vehicle administration, blood was collected for a period of approximately 10 min by allowing it to drip into potassium EDTA-coated or Eppendorff tubes for the preparation of plasma of serum samples, respectively. Samples were stored at $-20°C$ until required for assay.

For assay of histamine, samples were thawed and diluted with Krebs solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2 $NaHCO_3$ 25, dextrose 10; pH 7.4) supplemented with 10 mM HEPES. Histamine concentrations were measured in duplicate for each sample with a commercially available ELISA test system based on avidine-biotin interactions (IBL, Hamburg, Germany). The limit of detection was 0.25 ng ml^{-1} and cross-reactivity to 5-hydroxytryptamine was less than 0.005%.

Measurement of lung function

The method is similar to that described in detail for the guinea-pig by Chapman *et al.* (1992). In brief, rats were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and given gallamine triethiodide (10 mg kg^{-1}, i.m.) to suppress spontaneous respiration. Preparations were maintained at 37°C with a heated pad controlled by a rectal thermistor, and ventilated (8 ml kg^{-1}, 1 Hz) via a tracheal cannula with a mixture of air and oxygen (50:50, v/v). Ventilation was monitored at the trachea by a pneumotachograph (Fleisch 0000, Zabona, Switzerland) connected to a differential pressure transducer (MP 4514871, Validyne, U.S.A.). Coincident pressure changes within the thorax were measured via an intrathoracic cannula, using a differential pressure transducer (MP 4524, Validyne, U.S.A.); blood pressure and heart rate were recorded from the carotid artery with a pressure transducer (P23Dd, Gould, U.S.A.). From measurements of air-flow and transpulmonary pressure, airway resistance (R_L, $cmH_2O\ l^{-1}\ s^{-1}$) was calculated after each respiratory cycle by use of a digital electronic pulmonary monitoring system (PMS, Mumed, London, U.K.). Agonists were injected i.v. as described above.

Compounds used

Pentobarbitone sodium was obtained from Sanofi Santé Animale (Libourne, France). Compound 48/80 (condensation product of N-methyl-*p*-methoxyphenylethylamine with formaldehyde), histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate, gallamine triethiodide, and sodium cromoglycate were obtained from Sigma, Buchs, Switzerland. Lodoxamide (tromethamine) was a gift from the Upjohn Company, Kalamazoo, Mich. U.S.A. N^6-2-(4-aminophenyl) ethyladenosine (APNEA); N^6-cyclopentyladenosine (CPA), 2-[*p*-(2-carboxyethyl)phenylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680) and 8-(*p*-sulphophenyl)theophylline dihydrate (8-SPT) were synthetized at Sandoz, Basel, Switzerland by Dr Fulvio Gadient. The adenosine agonists were dissolved in 50% dimethylsulphoxide in distilled water and diluted immediately before use in 0.9% w/v NaCl. 8-SPT (40 mg) was dissolved in 0.2 ml 0.4 N NaOH and diluted with distilled water to 20 mg ml^{-1}. All other compounds were made up in 0.9% w/v NaCl.

Results

The cardiovascular responses to CPA, CGS 21680, APNEA and compound 48/80: effects of sodium cromoglycate and lodoxamide

The prototype adenosine A_1 receptor agonist, CPA (0.3–10 μg kg^{-1}), induced potent and pronounced hypotensive and bradycardic effects when injected intravenously into anaesthetized rats (Figure 1a). The selective A_{2A} receptor agonist, CGS 21680 (0.3–30 μg kg^{-1}, i.v.), also induced dose-related hypotensive responses which were not accompanied by significant changes in heart rate (Figure 1b). Intravenous injection of APNEA (1–30 μg kg^{-1}) to rats in which the A_3 receptor-mediated response had been 'isolated' by pretreatment with 8-SPT (40 mg kg^{-1}) induced dose-related falls in blood pressure accompanied at higher doses by small falls in heart rate (Figure 1c). Responses to the mast cell degranulating agent, compound 48/80 (10–300 μg kg^{-1}, i.v.), were qualitatively similar to those of APNEA (Figures 1d, 4 and 5).

Pretreatment with sodium cromoglycate, 0.25–20 mg kg^{-1}, i.v. induced dose-related, although incomplete, blockade of the hypotensive responses to APNEA (Figure 1c). The doses of APNEA which reduced blood pressure by 30 mmHg (ED_{30}) were 7.1 ± 0.8 ($n=4$), 9.0 ± 0.5 ($n=4$), 17.2 ± 0.4 ($n=3$), 27.8 ± 0.2 ($n=3$), 35.0 ± 5.7 ($n=6$) μg kg^{-1} for the animals given vehicle or 0.25, 1.25, 5 and 20 mg kg^{-1} of sodium cromoglycate respectively (Figure 1c); a dose of 60 mg kg^{-1} gave no further blockade (ED_{30} 37.8 ± 6.9 μg kg^{-1}, $n=4$). SCG at 20 mg kg^{-1} had no significant effects on the cardiovascular responses induced by CPA (Figure 1a) or CGS 21680 (Figure 1b) but suppressed significantly the response to compound 48/80 at 100 μg kg^{-1} (Figure 1d).

Lodoxamide, 0.01–20 mg kg^{-1}, i.v. also blocked progressively, but incompletely, the responses to APNEA (Figure 2). The doses of APNEA which reduced blood pressure by 30 mmHg (ED_{30}) were 7.1 ± 0.8 ($n=4$), 12.9 ± 2.3 ($n=5$), 23.9 ± 3.6 ($n=4$), 16.3 ± 2.3 ($n=4$), 23.0 ± 2.2 ($n=4$), 24.8 ± 0.8 ($n=3$), 46.2 ± 3.9 ($n=4$) for the animals given vehicle or 0.01, 0.05, 0.25, 1.25, 5 or 20 mg kg^{-1} of lodoxamide, respectively (Figure 2). Lodoxamide, 20 mg kg^{-1} also inhibited responses to compound 48/80 (Figure 1d) but had no effects on the cardiovascular response to CPA (Figure 1a).

Neither sodium cromoglycate nor lodoxamide induced any changes in blood pressure or heart rate *per se*.

Effects of pretreatment with compound 48/80 on the cardiovascular responses to compound 48/80, APNEA and CPA

Rats were given compound 48/80 i.p. at a dose of 1 mg kg^{-1} four times at 3 h intervals on the day before the experiment and at 3 mg kg^{-1} 2–3 h prior to anaesthesia on the experimental day. Mild scratching was evident following the lower dose of compound 48/80; scratching was more pronounced following the higher dose and the animals appeared sedated. In agreement with the literature (Enerbäck & Svensson, 1980), microscopical analysis of the cells obtained following peritoneal lavage of control animals revealed 4.0±0.3% (n=4) of the total cell population to be intact granulated mast cells. In

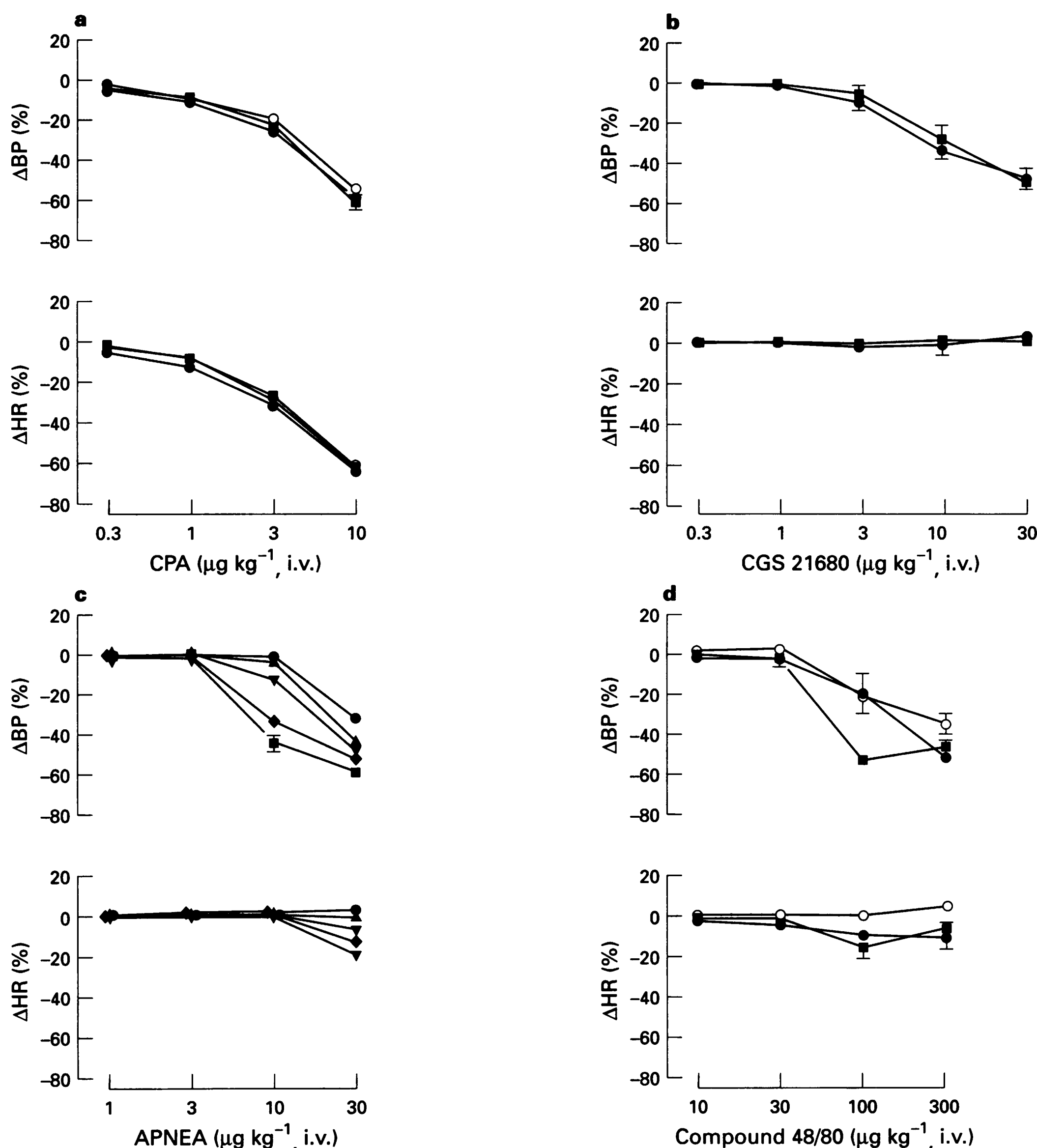

Figure 1 Effects of mast cell stabilizing agents on the cardiovascular responses to N^6-cyclopentyladenosine (CPA) (a), 2-[*p*-(2-carboxyethyl)phenylamino]-5′-N-ethylcarboxamidoadenosine (CGS 21680) (b), N^6-2-(4-aminophenyl)ethyladenosine (APNEA) (c) and compound 48/80 (d) in anaesthetized rats. (■) Vehicle-pretreated controls; (◆), (▼), (▲), (●) animals pretreated with 0.25, 1.25, 5 or 20 mg kg^{-1} sodium cromoglycate (SCG); (○) animals pretreated with 20 mg kg^{-1} lodoxamide (Lod). Points represent mean values (±s.e.mean where these exceed the size of the point) of the number of animals shown in parentheses below. The baseline mean arterial blood pressure (BP) and heart rate (HR) values just prior to starting the agonist injection sequences were:

CPA (vehicle)	134±10 mmHg	446±22 b min^{-1}	(4)
CPA (SCG, 20)	140±4 mmHg	432±7 b min^{-1}	(4)
CPA (Lod, 20)	144±9 mmHg	437±14 b min^{-1}	(4)
CGS 21680 (vehicle)	120±6 mmHg	429±12 b min^{-1}	(6)
CGS 21680 (SCG, 20)	124±6 mmHg	421±27 b min^{-1}	(3)
APNEA (vehicle)	133±5 mmHg	417±11 b min^{-1}	(4)
APNEA (SCG, 0.25)	146±10 mmHg	447±9 b min^{-1}	(4)
APNEA (SCG, 1.25)	152±4 mmHg	430±24 b min^{-1}	(3)
APNEA (SCG, 5)	137±7 mmHg	423±27 b min^{-1}	(3)
APNEA (SCG, 20)	133±3 mmHg	398±22 b min^{-1}	(6)
Co. 48/80 (vehicle)	134±7 mmHg	422±22 b min^{-1}	(4)
Co. 48/80 (SCG, 20)	135±6 mmHg	412±16 b min^{-1}	(4)
Co. 48/80 (Lod, 20)	129±7 mmHg	410±18 b min^{-1}	(5)

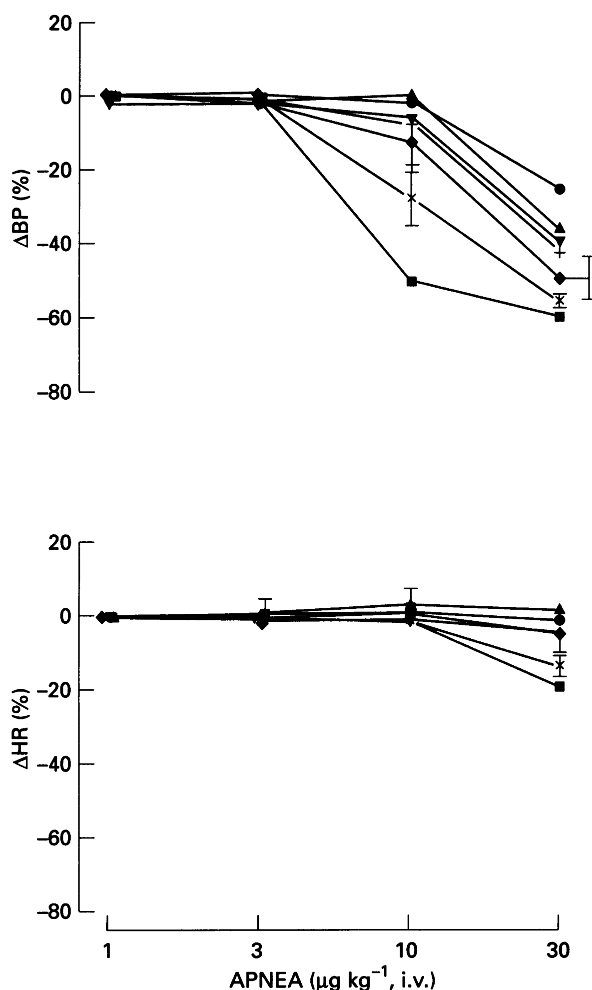

Figure 2 Effects of lodoxamide on the cardiovascular responses to N^6-2-(4-aminophenyl)ethyladenosine (APNEA) in anaesthetized rats. (■) Vehicle-pretreated controls; (X), (▼), (◆), (+), (▲) and (●); animals pretreated with 0.01, 0.05, 0.25, 1.25, 5 or 20 mg kg^{-1} lodoxamide (Lod). Points represent mean values (±s.e.mean where these exceed the size of the point) of the number of animals shown in parentheses below. The baseline mean arterial blood pressure (BP) and heart rate (HR) values just prior to starting the agonist injection sequences were:

APNEA (vehicle)	133±5 mmHg	417±11 b min^{-1}	(4)
APNEA (Lod, 0.01)	148±3 mmHg	444±15 b min^{-1}	(5)
APNEA (Lod, 0.05)	145±9 mmHg	429±21 b min^{-1}	(4)
APNEA (Lod, 0.25)	134±27 mmHg	408±10 b min^{-1}	(4)
APNEA (Lod, 1.25)	133±7 mmHg	381±21 b min^{-1}	(4)
APNEA (Lod, 5)	137±4 mmHg	413±4 b min^{-1}	(3)
APNEA (Lod, 20)	139±2 mmHg	425±22 b min^{-1}	(4)

contrast, no intact granulated mast cells were observed in peritoneal fluid samples from animals treated with compound 48/80. The cardiovascular response to *intravenously* injected compound 48/80 was markedly suppressed in animals which had received repeated doses of the compound by the *intraperitoneal* route (Figure 3a). Similarly, APNEA was essentially devoid of cardiovascular activity in such preparations (Figure 3b). In contrast, the response to CPA did not differ significantly from that obtained in control animals (Figure 3c).

Plasma and serum histamine concentrations following APNEA and compound 48/80

The doses of APNEA (30 and 100 μg kg^{-1}, i.v.) and compound 48/80 (300 μg kg^{-1}, i.v.) chosen for these experiments were expected to give long-lasting (>30 min) falls in blood pressure (see Figures 1, 4 and 5), and this was confirmed in studies in two separate animals with each agent at the doses indicated (data not illustrated). The data are presented in Table 1. The concentrations of histamine measured in plasma and serum in vehicle-treated animals were close to the standard values reported earlier for these tissues by several authors (see review by Tasaka, 1991). The plasma and serum histamine concentrations were not altered following 8-SPT, 40 mg kg^{-1}, i.v. They were, however, in each case, markedly and dose-dependently increased following both APNEA, 30 μg kg^{-1} (75 and 13 fold of the control values, respectively) and APNEA, 100 μg kg^{-1} (158 and 28 fold of the control values, respectively) in the presence of 8-SPT. In animals not treated with 8-SPT, the increase in plasma and serum histamine concentrations induced by APNEA, 100 μg kg^{-1} were not significantly different from those seen in the 8-SPT-treated animals. In a second experiment, compound 48/80, 300 μg kg^{-1} induced a marked and significant ($P<0.01$) increase in the plasma and serum histamine concentrations (2013±116 and 2342±507 ng ml^{-1}, respectively, $n=4$) relative to vehicle-treated control animals (14.5±2.1 and 42.9±1.6 ng ml^{-1}, respectively, $n=4$).

Effects of APNEA and compound 48/80 on lung function

The cardiovascular effects of APNEA, 1–30 μg kg^{-1}, i.v., (injected after 8-SPT, 40 mg kg^{-1}, i.v.), and compound 48/80, 0.1–1 mg kg^{-1}, i.v., were similar in animals instrumented for recording lung function to those seen in experiments where only the cardiovascular effects were monitored (compare Figures 4 and 5 with Figures 1c and 1d). The prominent hypotensive responses to APNEA were not accompanied by changes in airway resistance and this was true irrespective of whether the animals had received prior injections of 5-hydroxytryptamine (5-HT). In contrast, bolus injections of 5-HT induced dose-related increases in airway resistance associated with short-lived hypotensive responses (Figure 4). Compound 48/80 routinely at lower doses (<0.1 mg kg^{-1}, i.v.) induced falls in blood pressure with no accompanying change in airway resistance; at higher doses (>0.3 mg kg^{-1}, i.v.) compound 48/80 induced increases in airway resistance which, however, were not associated with more pronounced falls in blood pressure (Figure 5). Intravenous bolus injections of histamine, 10–100 μg kg^{-1}, induced short-lived falls in blood pressure but had no effects on airway resistance (Figure 5).

Discussion

In the present study, APNEA was used to induce A_3 receptor-mediated hypotensive responses in the anaesthetized rat. Since APNEA is relatively non-selective (Fozard & Carruthers, 1993a), the A_3 receptor component of the cardiovascular response was effectively 'isolated' by pretreatment of animals with the broad spectrum adenosine receptor antagonist, 8-SPT, at a dose well in excess of those required to antagonize A_1, A_{2A} and A_{2B} receptors in this preparation (Fozard & Carruthers, 1993a,b; see also Fozard & Hannon, 1994). The data generated provide strong evidence that the hypotensive response to adenosine A_3 receptor activation in the rat is largely, if not exclusively, a consequence of mediator release from mast cells.

Blockade of the response to APNEA by sodium cromoglycate and lodoxamide

Sodium cromoglycate and lodoxamide are chemically quite distinct yet have in common the property of inhibiting the release of mediators from mast cells in response to antigen challenge or compound 48/80; lodoxamide is significantly more potent (⩽100 fold) than sodium cromoglycate in this respect (Brogden *et al.*, 1974; Johnson *et al.*, 1978). Dose-dependent blockade of the response to APNEA by both com-

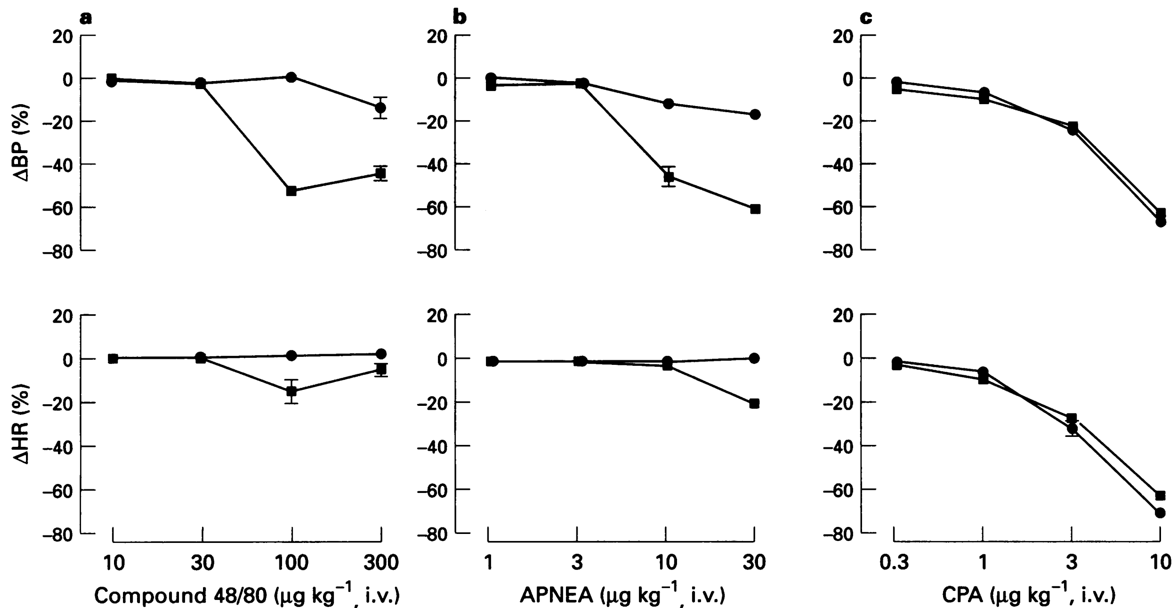

Figure 3 The effects of repeated pretreatment with compound 48/80 on the cardiovascular responses to compound 48/80 (a), N^6-2-(4-aminophenyl)ethyl adenosine (APNEA) (b) and N^6-cyclopentyladenosine (CPA) (c). (■) Controls; (●), animals pretreated with compound 48/80, $1\,mg\,kg^{-1}$, i.p. four times at 3 h intervals on the day before the experiment and $3\,mg\,kg^{-1}$, i.p. 2–3 h prior to anaesthesia on the experimental day. Points represent mean values (±s.e.mean where these exceed the size of the point) of the number of animals shown in parentheses below. The baseline mean arterial blood pressure (BP) and heart rate (HR) values just prior to starting the injection sequences were:

48/80 (control)	134±7 mmHg	422±22 b min^{-1}	(4)
48/80 (48/80 - pretreated)	122±8 mmHg	468±4 b min^{-1}	(5)
APNEA (control)	133±5 mmHg	417±11 b min^{-1}	(4)
APNEA (48/80 - pretreated)	128±4 mmHg	444±9 b min^{-1}	(4)
CPA (control)	134±10 mmHg	446±22 b min^{-1}	(4)
CPA (48/80 - pretreated)	136±8 mmHg	445±20 b min^{-1}	(4)

Table 1 Rat plasma and serum histamine concentrations following 8-SPT and/or APNEA

	Vehicle I[a] + *vehicle II*[b]	*8-SPT 40*[c] + *vehicle II*[b]	*8-SPT 40*[c] + *APNEA 30*[d]	*8-SPT 40*[c] + *APNEA 100*[d]	*Vehicle I*[a] + *APNEA 100*[d]
Plasma (ng ml^{-1})	19.7±2.1 (3)	14.3±2.5 (4)	1073±131* (4)	2253±311* (4)	1803±301* (4)
Serum (ng mg^{-1})	59.5±11.6 (3)	67.9±16.7 (4)	910±168* (4)	1870±249* (4)	1754±54* (4)

Values are means (±s.e.mean) with number of experiments indicated in parentheses.
[a]Vehicle for 8-SPT; [b]vehicle for APNEA; [c]8-SPT 40 mg kg^{-1} given i.v. 5 min prior to APNEA or vehicle for APNEA; [d]APNEA 30 or 100 μg kg^{-1} given 5 min after 8-SPT or vehicle for 8-SPT.
Plasma was prepared from blood collected for approximately 10 min starting 2 min following vehicle II or APNEA administration.
*Significantly different from respective control value, $P<0.05$ (Bonferroni-Holm multiple comparison test).
For abbreviations, see text.

pounds with lodoxamide being clearly the more potent would thus be consistent with a role for mast cells in the response to APNEA. The interpretation would be supported by the fact that sodium cromoglycate and lodoxamide also block the hypotensive response to compound 48/80, a recognised activator of mediator release from mast cells (Paton, 1951; Moran *et al.*, 1962). Of the other possible explanations, non-specific cardiovascular suppression can be ruled out since responses to the selective A_1 receptor agonist, CPA, and the selective A_{2A} receptor agonist, CGS 21680, were unaffected following an optimal dose of sodium cromoglycate. Sodium cromoglycate has been reported to interfere with adenosine binding to rat serosal mast cell membranes (Marquardt & Wasserman, 1985) raising the possibility of a direct interaction with the A_3 receptor. This seems unlikely, however, since blockade by sodium cromoglycate is incomplete and less than that which can be achieved with the surmountable A_3 receptor antagonist, BW-A522 (Fozard & Hannon, 1994). Moreover, when tested in a radioligand binding assay for the cloned rat A_3 receptor, neither sodium cromoglycate nor lodoxamide induced significant displacement at 0.1 mM (K. Jacobson, personal communication; for methodology, see Gallo-Rodriguez *et al.*, 1994). The incomplete blockade of APNEA by sodium cromoglycate and lodoxamide remains to be explained. It is, however, entirely consistent with *in vitro* findings where mediator release inhibition is often incomplete and may show a bell-shaped dose-response relationship (Johnson *et al.*, 1978; Tanizaki *et al.*, 1992).

Blockade of the response to APNEA in animals treated repeatedly with compound 48/80

Repeated treatment with compound 48/80 to achieve depletion of mast cell-derived mediators has been a successful strategy in

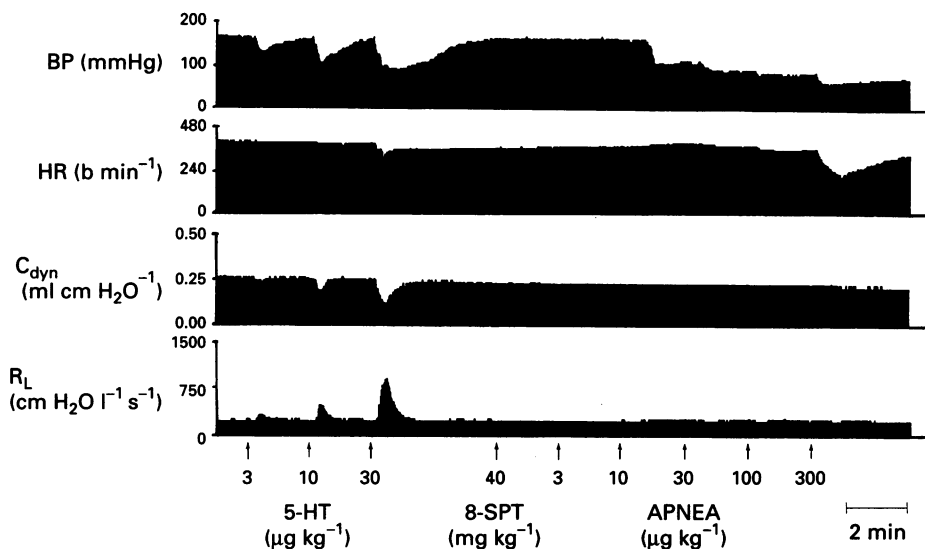

Figure 4 Effects of 5-hydroxytryptamine (5-HT) and N^6-(2-(4-aminophenyl)ethyl adenosine (APNEA) administered after 8-(*p*-sulphophenyl)theophylline (8-SPT) on mean arterial blood pressure (BP), heart rate (HR) and airways compliance (C_{dyn}) and resistance (R_L) in an anaesthetized rat. The record is representative of 4 similar experiments.

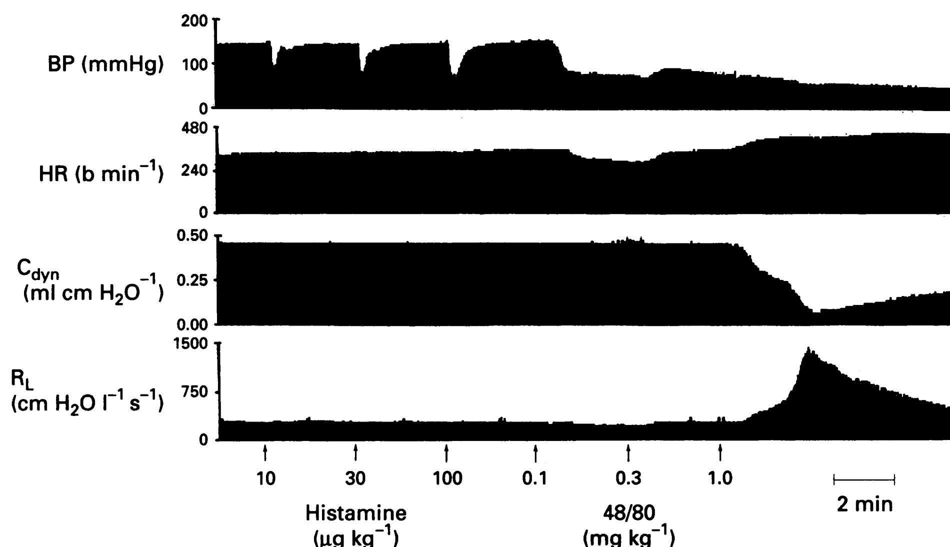

Figure 5 Effects of histamine and compound 40/80 (48/80) on mean arterial blood pressure (BP), heart rate (HR) and airways compliance (C_{dyn}) and resistance (R_L) in an anaesthetized rat. The record is representative of 4 similar experiments.

defining the role of the mast cell in a variety of experimental circumstances (see, for example, Di Rosa *et al.*, 1971; Banks *et al.*, 1990). The pretreatment paradigm used in the present experiments (1 mg kg^{-1}, i.p. four times at 3 h intervals on the day before the experiment and 3 mg kg^{-1}, i.p. 2–3 h prior to anaesthesia on the experimental day) was based on these earlier studies and was clearly effective. Thus, evidence of mast cell degranulation was seen *during* treatment in the form of scratching and elimination of the response to intravenously administered compound 48/80 *after* treatment indicates an eventual extensive depletion of mast cell-derived mediators. Moreover, microscopical analysis of peritoneal lavage fluid revealed a complete absence of intact granulated mast cells following the desensitization protocol with compound 48/80. It bears emphasis that baseline blood pressures and heart rate were within the normal range in the compound 48/80 pretreated animals and the sensitivity to CPA in these animals was unchanged. Hence, suppression of the effect of compound 48/80 in the pretreated animals cannot be attributed to non-specific depression of cardiovascular sensitivity. The fact that under these circumstances, responses to APNEA were suppressed to a similar extent to those to compound 48/80 provides strong evidence of a key role for mast cells in A_3 receptor-mediated hypotension in rats.

Direct evidence for the involvement of mast cells in the cardiovascular responses to APNEA and compound 48/80 in rats

If, in fact, mast cells do play a key role in the cardiovascular responses to APNEA and compound 48/80, then it should be possible to detect evidence of mediator release following administration of these agents. In practice, substantial and dose-related increases in the concentration of the mast cell mediator, histamine, were observed in plasma and serum associated with the sustained hypotensive responses to single doses of 30 and 100 $\mu g\ kg^{-1}$ APNEA; at the highest dose the release of histamine following APNEA was similar to that seen following

compound 48/80, 300 $\mu g\ kg^{-1}$. Since, 8-SPT, used to 'isolate' the A_3 receptor-mediated component of the response to APNEA, neither increased histamine levels *per se* nor modified the response to APNEA, mediator release can be unequivocally attributed to the effects of APNEA.

It bears emphasis that the implication from these *in vivo* studies is that A_3 receptor activation is *per se* sufficient to activate powerfully mast cell mediator release whereas from *in vitro* studies the predominant effect is to augment mediator release induced by stimuli such as allergen and compound 48/80 (Marquardt *et al.*, 1978; Ali *et al.*, 1990; Ramkumar *et al.*, 1993). The reason for this difference remains to be clarified. Nevertheless, there are several examples of mast cell-dependent functional responses to adenosine receptor ligands apparently acting independently of a second activating stimulus, including bronchoconstriction in rats (Pauwels & Van Der Straeten, 1987) and contraction of human bronchi (Björk *et al.*, 1992) and constriction of arterioles from the hamster cheek pouch (Doyle *et al.*, 1994) *in vitro*.

General similarity between responses to APNEA and compound 48/80

The overall similarity in the cardiovascular and histamine releasing profiles of APNEA and compound 48/80 is striking and clearly consistent with a common underlying mechanism of action. To extend the comparison of the properties of APNEA and compound 48/80, we investigated if changes in lung function accompanied the cardiovascular changes. Both similarities and differences between the two compounds were evident. Thus, both APNEA and compound 48/80 produced maximal hypotensive effects at doses which did not alter airways resistance or compliance. However, higher doses of compound 48/80 induced marked bronchoconstriction whereas no effects on pulmonary function were seen with APNEA even at doses 30 times those producing a prominent fall in blood pressure. In the context of an intermediary role for the mast cell it would appear that the mediator(s) released show selectivity for the cardiovascular system over the airways. This may reflect a primary contribution of histamine which in the same model induces prominent hypotensive effects without causing bronchoconstriction. The fact that APNEA has a far more prolonged effect on blood pressure than does histamine alone may simply reflect the quantity and/or time course of histamine released by APNEA. Alternatively, the finding may reflect the concomitant release of other mediators such as the leukotrienes. Studies are currently in progress to define the relative contribution of the different candidate mediators to the cardiovascular response to A_3 receptor activation in the rat. The reason for the difference between APNEA and compound 48/80 in that the latter induces bronchoconstriction at higher doses remains to be determined. The finding does not, of course, preclude a common mechanism (or site) of action at the lower doses and may simply reflect differences in the overall efficacy of the stimulus to mast cell mediator release.

Conclusion

The findings presented in this paper shed new light on the mechanisms underlying adenosine A_3 receptor-mediated hypotensive responses in the anaesthetized rat. Such responses are mimicked closely by the mast cell mediator releasing agent, compound 48/80, and suppressed selectively by sodium cromoglycate and lodoxamide which have mast cell stabilizing properties. Moreover, in rats depleted of mast cell mediators by repeated exposure to compound 48/80 responses to APNEA are selectively suppressed. Finally, hypotensive responses to both APNEA and compound 48/80 are associated with substantial increases in plasma histamine. Taken together, the data implicate the mast cell in a key role in A_3 receptor-mediated hypotension and are entirely consistent with the fact that rat mast cells express the A_3 receptor and are activated by ligands with A_3 receptor agonist properties *in vitro* (Ali *et al.*, 1990; 1991; Ramkumar *et al.*, 1993).

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