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Mast cell degranulation following adenosine A_3 receptor activation in rats

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Abstract

The present studies were carried out to provide further evidence for the hypothesis that the hypotensive response to adenosine A_3 receptor activation in the anaesthetized rat involves mediator release from mast cells. Male Sprague-Dawley rats were anaesthetized and given just supramaximal hypotensive doses of either the non-selective A_3 receptor agonist, N^6-2-(4-aminophenyl)ethyladenosine (APNEA: 100 μg/kg, preceded by the A_1/A_2 receptor antagonist, 8-*p*-(sulphophenyl)theophylline, to 'isolate' the A_3 receptor-mediated component of the response), the mast cell degranulating agent, compound 48/80 (300 μg/kg) or the vehicle for APNEA, intravenously. Blood was withdrawn from a carotid artery between 2 and 10 min after the injection and plasma and serum histamine concentrations measured. Samples of connective tissue (surrounding the abdominal musculature), thymus, mesenteric lymph node, kidney, skin and diaphragm were removed for histological analysis. The plasma and serum histamine concentrations were markedly and significantly higher in the APNEA- or compound 48/80-treated animals compared to vehicle-treated controls. Consistent with this, a substantially greater proportion of mast cells was seen to be undergoing degranulation in all tissues removed from animals treated with APNEA or compound 48/80 compared to those from rats treated with vehicle. Thus, adenosine A_3 receptor activation results in rapid mast cell degranulation in the anaesthetized rat. The data provide further evidence of a key role for the mast cell in adenosine A_3 receptor-mediated hypotension in this species.

Keywords: Adenosine A_3 receptor; Hypotension; Mast cell; Histamine; APNEA (N^6-2-(4-aminophenyl)ethyladenosine); Compound 48/80

1. Introduction

Low doses of N^6-2-(4-aminophenyl)ethyladenosine (APNEA) induce hypotension in the rat which is resistant to blockade by selective antagonists for adenosine A_1, A_{2A} and A_{2B} receptors (Fozard and Carruthers, 1993a; Carruthers and Fozard, 1993a). Significantly, the response shows similar pharmacology to the cloned adenosine A_3 receptor (Zhou et al., 1992; Linden, 1994) with respect to agonist relative potencies (Fozard and Carruthers, 1993a) and in being susceptible to blockade by 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)-1-propylxanthine (BW-A522; Fozard and Hannon, 1994).

Recently, we presented evidence implicating the mast cell in the hypotensive response to A_3 receptor activation in the rat (Hannon et al., 1995): responses to APNEA were mimicked by the mast cell degranulating agent, compound 48/80, absent in animals desensitized by repeated injections of compound 48/80, suppressed by the selective inhibitors of mast cell degranulation, cromoglycate and lodoxamide, and associated with a marked and dose-dependent rise in the plasma histamine concentrations.

We now provide histological evidence that mast cells in a number of tissues are indeed degranulated following administration of APNEA to rats. The effects of APNEA in the tissues studied resemble qualitatively those of compound 48/80 although there are quantitative differences between the two agents with respect to the extent of degranulation induced in individual tissues.

A preliminary account of these experiments has been given to the EPHAR Meeting, Milan, June 1995 (Fozard et al., 1995).

2. Materials and methods

Male Sprague-Dawley rats weighing 421–543 g and supplied by Biological Research Laboratories (Füllinsdorf, Switzerland) were anaesthetized with pentobarbitone

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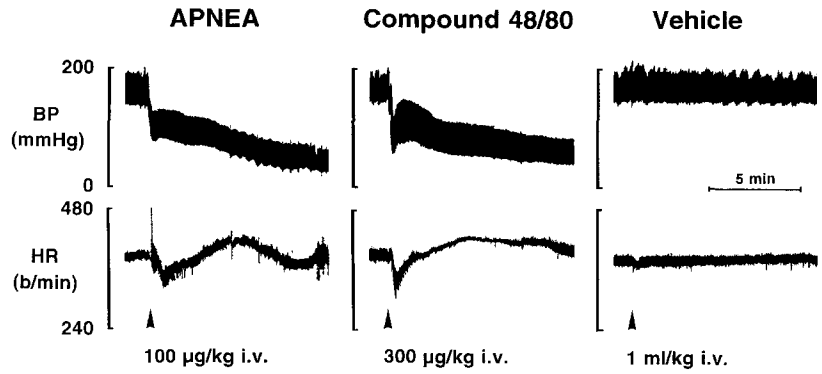

Fig. 1. Experimental records illustrating the effects of APNEA, compound 48/80 and the vehicle for APNEA given intravenously (i.v.) on blood pressure (BP) and heart rate (HR) in anaesthetised rats. The effects are representative of four, four and three similar experiments for APNEA, compound 48/80 and vehicle, respectively.

sodium, 60 mg/kg and prepared for intrajugular venous administration of drugs and collection of blood from one cannulated common carotid artery as previously described (Hannon et al., 1995). After a stabilisation period of approximately 15 min, 12 animals were allocated randomly to three groups: group 1 received 8-*p*-(sulphophenyl)theophylline (8-SPT), 40 mg/kg, i.v. 5 min prior to a single i.v. injection of APNEA, 100 μg/kg; group 2 received compound 48/80, 300 μg/kg; group 3 received the vehicle for APNEA. Two minutes 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 or serum samples, respectively. Samples were stored at −20°C until assayed for histamine by means of a commercially available ELISA test system (IBL, Hamburg, Germany).

Samples of connective tissue (surrounding the abdominal musculature), mesenteric lymph node, skin, thymus, diaphragm and kidney were removed for histological analysis at the end of the blood sampling period and fixed in phosphate-buffered formalin. Paraffin sections were prepared and stained with toluidine blue. The degree of mast cell degranulation was scored 'blind' as follows: 0 = essentially intact mast cells with no, or only marginal, signs of degranulation; 1 = mast cell showing unequivocal signs of degranulation; 2 = degranulated mast cell with no cell body visible. Mast cells illustrating this scoring system can be seen in Fig. 2. Four sections were scored in total for each tissue from the four rats. The aim was to score a minimum of 100 mast cells per section, i.e. a total of 400 cells in the four sections. However, because in the kidney the number of mast cells is low, four sections was not sufficient to reach 400 cells; in contrast, because there were many mast cells in the skin up to 500–800 cells were scored. The absolute numbers of cells scored in the analysis are given in the legend to Fig. 3.

APNEA and 8-SPT were synthesized by Dr. Fulvio Gadient at Sandoz, Basel, Switzerland. Compound 48/80 was obtained from Sigma, Buchs, Switzerland. APNEA

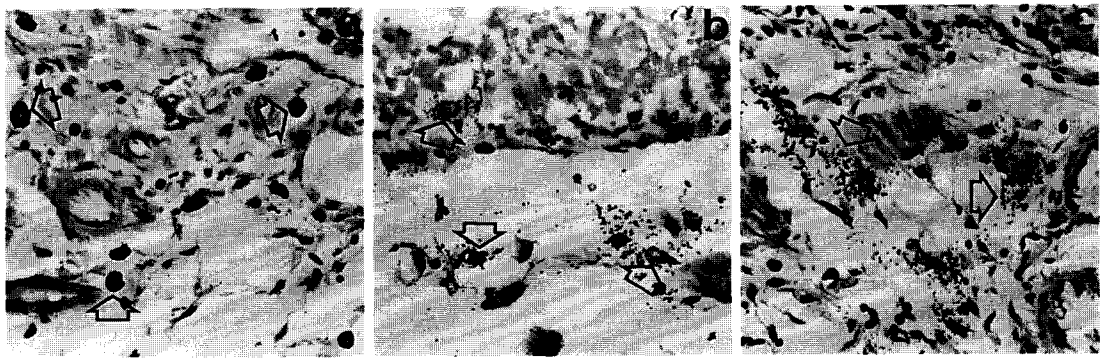

Fig. 2. Presence of mast cells in the connective tissue surrounding the mesenteric lymph node and the adjacent medulla area of the lymph node. (a) Control lymph node from vehicle-treated animal showing intact mast cells (score 0, three cells indicated by the arrows); (b) lymph node from APNEA-treated animal showing an intact mast cell and some partially degranulated mast cells (score 1, three cells indicated by the arrows); (c) lymph node from compound 48/80-treated animal showing almost completely degranulated mast cells with loss of cell morphology (score 2, two examples indicated by the arrows). Metachromatic staining (toluidine blue) of a paraffin section of formalin-fixed material, ×250.

was 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. Compound 48/80 was made up in 0.9% w/v NaCl.

Mean values (±S.E.M.) of $n = 4$ individual animals

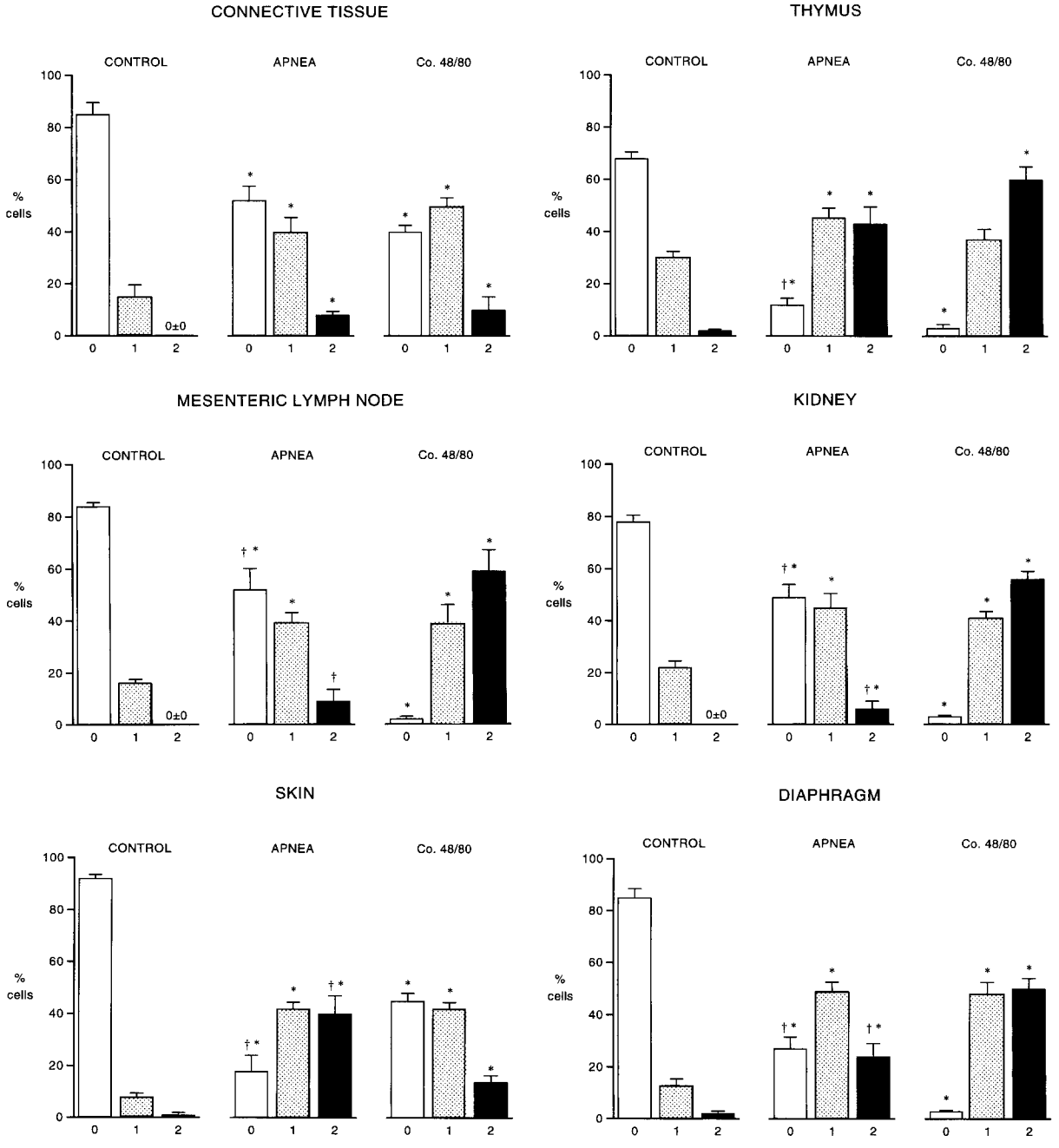

Fig. 3. The degranulation status of mast cells in different tissues following administration of APNEA, compound 48/80 or the vehicle for APNEA to anaesthetized rats. % cells = percentage of mast cells per tissue (mean ± S.E.M. of four sections from four rats) with a status defined according to the following scale: 0 = essentially intact mast cells with no, or only marginal, degranulation; 1 = mast cell showing unequivocal signs of degranulation; 2 = degranulated mast cell with no cell body visible. The absolute numbers of cells included in the analysis were:

	Connective tissue	Thymus	Mesenteric lymph node	Kidney	Skin	Diaphragm
APNEA	372	399	400	221	801	340
Compound 48/80	387	400	400	289	773	411
Vehicle	315	345	374	165	620	231

* $P < 0.05$ that the value differs from that of vehicle-treated animals; † $P < 0.05$ that the value differs from that of compound 48/80-treated animals (Student's unpaired t-test).

are presented. Details of the statistical analyses are given in the text (histamine levels) or legend to Fig. 2 (histology). A P value < 0.05 was considered significant.

3. Results

The doses of APNEA (100 μg/kg) and compound 48/80 (300 μg/kg) chosen for these studies give just supramaximal falls in blood pressure when administered cumulatively to anaesthetized rats (Hannon et al., 1995). Confirmation that such doses give substantial, sustained and similar hypotensive responses under the present experimental conditions is presented in Fig. 1.

The mean plasma histamine concentrations were 14.5 ± 2.1 ng/ml ($n = 4$) in the vehicle-treated animals and markedly and significantly ($P < 0.001$; Bonferroni-Holm Multiple Comparison Test) higher following APNEA (1369 ± 247 ng/ml, $n = 4$) or compound 48/80 (2013 ± 116 ng/ml, $n = 4$). The mean serum histamine levels changed similarly being 42.9 ± 3.1 ng/ml in the vehicle-treated group and 1429 ± 392 and 2342 ± 878 ng/ml in the APNEA- and compound 48/80-treated groups, respectively.

Within the total population of mast cells in tissue sections, the proportion of intact or marginally degranulated mast cells in animals treated with vehicle ranged from 68 ± 3% in thymus to 92 ± 2% in skin ($n = 4$). In contrast, a substantial proportion of mast cells showed degranulation in tissue sections from animals treated with APNEA or compound 48/80 (Figs. 2 and 3). The effects of the two drugs were quantitatively similar in connective tissue and thymus although the extent of degranulation was markedly greater in the latter. However, the effects of compound 48/80 were significantly more pronounced than those of APNEA in mesenteric lymph node, kidney and diaphragm; in contrast, APNEA was clearly the more potent agent in inducing degranulation in the skin (Fig. 3).

4. 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 and 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 and Carruthers, 1993a, b). Our earlier data (Hannon et al., 1995) showed that 8-SPT neither increased histamine levels per se nor modified the response to APNEA; thus, the increase in mast cell mediator release and, by implication, the mast cell degranulating effects can be unequivocally attributed to APNEA.

The present histological findings provide the first direct evidence that activation of A_3 receptors results in mast cell degranulation in vivo. They extend the studies of Doyle et al. (1994) who provided similar direct evidence that mast cell degranulation can be triggered by A_3 receptor activation in perfused cheek pouch arteries from the hamster in vitro. The heterogeneity of the degranulation response to APNEA and compound 48/80 is perhaps to be expected in view of the many observations that mast cells from different species and even diverse tissues within a given animal are functionally heterogeneous (Pearce, 1986; Church et al., 1994). In this context, to define the heterogeneity of the response of human mast cells to selective A_3 receptor stimulation would be of considerable interest.

Our data also suggest that the blood pressure fall in response to A_3 receptor stimulation in the rat reflects mainly, if not exclusively, the release of mast cell mediators. Thus, plasma histamine levels, a marker of mast cell mediator release, are elevated at doses similar to those which induce the cardiovascular changes (Hannon et al., 1995; Fig. 1). Second, responses to A_3 receptor activation are abolished selectively in animals depleted of their mast cell mediators by repeated dosing with compound 48/80 (Hannon et al., 1995). Experiments are currently in progress using selective antagonists of known mast cell mediators to provide further support for this conclusion.

Finally, our data have significance for the interpretation of results obtained with certain adenosine receptor agonists in in vivo studies in rats and possibly other species. Thus, if xanthine-resistant hypotension is used as an indicator of A_3 receptor activation in vivo then the selectivity of a number of nominally selective A_1 receptor agonists is substantially less with respect to the A_3 receptor than either the A_{2A} or the A_{2B} receptor subtypes (Fozard and Carruthers, 1993a; Carruthers and Fozard, 1993b). It is certainly possible that mast cell mediators would contribute at higher doses to the cardiovascular effects of such compounds which are difficult to reconcile with a high selectivity for A_1 over A_2 receptors (Trivedi et al., 1991). Recent in vivo studies with the selective A_3 receptor agonist, N^6-(3-iodobenzyl)adenosine-5′-*N*-methylcarboxamide (IB-MECA), implicate the A_3 receptor in behavioral depression in mice (Jacobson et al., 1993) and post-ischaemic brain damage (Von Lubitz et al., 1994) and seizure susceptibility (Von Lubitz et al., 1995) in gerbils. However, scratching in mice (blocked by the 5-hydroxytryptamine/histamine receptor antagonist, cyproheptadine) and long-lasting hypotension in gerbils suggests that extensive mast cell degranulation is occurring under the conditions of these experiments. It follows that the effects observed are likely to reflect the polypharmacology of A_3 receptor activation plus the effects of the mediators released from mast cells.

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