Suppression of Adenosine A₃ Receptor-Mediated Hypotension and Mast Cell Degranulation in the Rat by Dexamethasone

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ABSTRACT

Dexamethasone increases the expression of adenosine A₃ receptors and augments degranulation in response to their activation in the rat basophilic leukemia cell line, RBL-2H3. We have studied the effects of dexamethasone on mast cell activation induced by Aᵢ receptor stimulation in vivo. Administration of the Aᵢ receptor agonist APNEA [N⁶-2-(4-aminophenyl)ethyladenosine; 10–30 µg kg⁻¹, i.v.] to anesthetized Sprague-Dawley rats induced falls in blood pressure. Pretreatment with dexamethasone (1 mg kg⁻¹, i.p., ~24 h) blocked the hypotensive response to APNEA but not those induced by the Aᵢ receptor agonist N⁶-cyclopentyladenosine, the Aᵢₐ receptor agonist 2-[(p-(2-carboxyethyl)phenylamino)-5'-N-ethylcarboxamido]adenosine, or the mast cell degranulating agent compound 48/80 (100–300 µg kg⁻¹, i.v.). APNEA (10 and 30 µg kg⁻¹, i.v.) and compound 48/80 (100 and 300 µg kg⁻¹, i.v.) increased plasma histamine concentrations dose dependently. Pretreatment with dexamethasone significantly inhibited the increases induced by the lower doses of each compound. APNEA induced degranulation of mast cells in thymus but not in skin or skeletal muscle, whereas compound 48/80 induced degranulation in each tissue. Pretreatment with dexamethasone inhibited APNEA-induced degranulation of mast cells in the thymus and slightly, yet significantly, reduced degranulation induced by compound 48/80. Thus, in contrast to the findings in RBL-2H3 cells in vitro, in the whole animal, dexamethasone down-regulates the response of the mast cell to Aᵢ receptor activation. The qualitatively similar effects on compound 48/80 suggest that dexamethasone suppresses mast cell responsiveness by modulating site(s) downstream from the adenosine A₃ receptor, possibly at the level of the Gᵢ family of trimeric GTP-binding proteins.

Several studies have demonstrated an increase in the functional response to activation of adenosine A₃ receptors in rat basophilic leukemia (RBL-2H3) cells following exposure to dexamethasone (Collado-Escobar et al., 1990a,b; Qian and McCloskey, 1993; Ramkumar et al., 1995). An initial analysis of the response led to the suggestion that enhanced responsiveness was due in part to an increased A₃ receptor number (Collado-Escobar et al., 1990b). Direct evidence in support of this was provided by Ramkumar and colleagues (1995), who demonstrated that the augmented response to A₃ receptor activation following dexamethasone was associated with increases in both the level of mRNA and the number of A₃ receptors. Moreover, the levels of the Gᵢ family of GTP-binding proteins were also increased following dexamethasone treatment, raising the possibility that such increases might contribute, at least in part, to the enhanced response to A₃ receptor activation (Ramkumar et al., 1995). To date, up-regulation of A₃ receptor responsiveness by dexamethasone has been described only in studies with the RBL-2H3 mast cell line, and the data obtained may not reflect the regulation of this receptor in the whole animal. It was, therefore, of interest to explore the effects of dexamethasone on the responsiveness of mast cells to Aᵢ receptor activation under more physiological conditions in vivo. The adenosine A₃ receptor agonist N⁶-2-(4-aminophenyl)ethyladenosine (APNEA) induces hypotension in the anesthetized rat (Carruthers and Fozard, 1993a,b; Fozard and Hannon, 1994). The response is associated with a widespread degranulation of tissue mast cells and a substantial increase in plasma histamine (Hannon et al., 1995; Fozard et al., 1996). Pharmacological evidence points to mast cell activation as the primary mechanism contributing to A₃ receptor-mediated hypotension in the rat (Hannon et al., 1995; Van Schaick et al., 1996).

Here, we describe the effects of dexamethasone on the hypotension, histamine release, and degranulation of tissue mast cells induced by APNEA. Comparisons have been made with the effects on compound 48/80, a polycationic mast cell activator that interacts directly with the Gᵢ/G₄ families of trimeric GTP-binding proteins (Mousli et al., 1990; Tomita et al., 1991; Chahdi et al., 2000).

ABBREVIATIONS: APNEA, N⁶-2-(4-aminophenyl)ethyladenosine; 8-SPT, 8-(p-sulfophenyl) theophylline; CPA, N⁶-cyclopentyladenosine; CGS 21680, 2-[(p-(2-carboxyethyl) phenylamino)-5'-N-ethylcarboxamido]adenosine; BP, blood pressure; HR, heart rate.
Experimental Procedures

Animals. Male Sprague-Dawley rats, supplied by Biological Research Laboratories (Füllinsdorf, Switzerland) and weighing 196 to 410 g, were used throughout. Groups of up to five animals were housed in sawdust-lined drawer cages (approximately 560 × 335 × 200-mm) and kept at an ambient temperature of 22 ± 2°C under 12-h normal phase light/dark cycles. All experiments were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinaeramt, Basel-Stadt).

Cardiovascular Studies. Animals were anesthetized with pentobarbitone sodium (60 mg kg\(^{-1}\), i.p.) and set up for recording blood pressure and heart rate and intrajugular venous administration of drugs, as previously described (Hannon et al., 1995). After a stabilization period of approximately 15 min, dose-response curves to APNEA, the selective adenosine A\(_1\) receptor agonist \(N^\circ\)-cyclopentyladenosine (CPA), the selective adenosine A\(_2A\) receptor 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 each dose were sufficient to allow a plateau response to develop. In the case of APNEA, the A\(_1\)/A\(_2A\)/A\(_3\) Receptor antagonist 8-(p-sulfophenyl)theophylline (8-SPT) was injected intravenously at a dose of 40 mg kg\(^{-1}\) 5 min prior to establishing the agonist dose-response curve to “isolate” the A\(_3\) receptor-mediated component of the response to APNEA (see Carruthers and Fozard 1993a; Fozard and Carruthers 1993; Fozard and Hannon, 1994). Only one agonist dose-response curve was generated per animal.

Measurement of Plasma Histamine Concentrations and Histological Analysis. Rats were pretreated with vehicle (saline, 1 ml kg\(^{-1}\), i.p.) or dexamethasone (1 mg kg\(^{-1}\), i.p.) 24 h prior to being anesthetized and prepared for intrajugular venous administration of drugs, as described above. A cannula was placed in the right carotid artery for blood collection. A stabilization period of approximately 15 min was started after completion of all surgery. A total of 29 animals were allocated into 6 groups: group 1 was pretreated with the vehicle for dexamethasone (saline, 1 ml kg\(^{-1}\), i.p.) and 24 h later received APNEA (10 \(\mu\)g kg\(^{-1}\), i.v., followed 5 min later by 20 \(\mu\)g kg\(^{-1}\) i.v.); group 2 was pretreated with dexamethasone (1 mg kg\(^{-1}\), i.p.) and 24 h later received APNEA (10 \(\mu\)g kg\(^{-1}\), i.v., followed 5 min later by 20 \(\mu\)g kg\(^{-1}\) i.v.); group 3 was pretreated with vehicle for dexamethasone (saline, 1 ml kg\(^{-1}\), i.p.) and 24 h later received compound 48/80 (100 \(\mu\)g kg\(^{-1}\), i.v., followed 5 min later by 200 \(\mu\)g kg\(^{-1}\), i.v.); group 4 was pretreated with dexamethasone (1 mg kg\(^{-1}\), i.p.) and 24 h later received compound 48/80 (100 \(\mu\)g kg\(^{-1}\), i.v., followed 5 min later by 200 \(\mu\)g kg\(^{-1}\), i.v.); group 5 was pretreated with vehicle (saline, 1 ml kg\(^{-1}\), i.p.) and 24 h later received i.v. injections of the vehicle for APNEA/compound 48/80; and the animals in group 6, which were pretreated with dexamethasone (1 mg kg\(^{-1}\), i.p.), received injections of the vehicle for APNEA/compound 48/80. Blood was collected between 3 to 5 min after each injection of APNEA, compound 48/80, or vehicle by allowing it to drip into potassium EDTA-coated tubes. Plasma samples were prepared and stored at −30°C until assayed for histamine using a commercially available enzyme-linked immunosorbent assay test system (see Hannon et al., 1995, 2001).

At the end of blood sampling, pieces of thymus, skin, and skeletal muscle were removed for histological analysis and fixed in phospho-buffered formalin. Paraffin sections were prepared and stained with toluidine blue. The degree of mast cell degranulation was essentially intact mast cells with no cell body visible. One section was scored for each tissue from the different groups ranging between 1314 to 1641, 1017 to 1747, and 635 to 790 for the thymus, skin, and skeletal muscle samples, respectively.

Materials. Pentobarbitone sodium was obtained from Sanofi Sante Animale (Libourne, France). Compound 48/80 (condensation product of N-methyl-p-methoxyphenylethylamine with formaldehyde) and dexamethasone 21-phosphate (diosodium salt) were obtained from Sigma-Aldrich (Buchs, Switzerland). APNEA, CPA, CGS 21680, and 8-SPT were synthesized at Novartis Pharma AG (Basel, Switzerland). The adenosine receptor agonists were dissolved in 50% dimethyl sulfoxide in distilled water and diluted immediately before use in 0.9% w/v NaCl. 8-SPT (40 mg) was dissolved in 0.2 ml of 0.4 N NaOH and diluted with distilled water to 20 mg ml\(^{-1}\). Compound 48/80 and dexamethasone were made up in 0.9% w/v NaCl.

Data Presentation. Mean values (± S.E.M.) from individual experiments are presented. Details of the statistical analyses are given in the text or in the legends to the figures. A P value <0.05 was considered significant.

Results

Effect of Dexamethasone on the Cardiovascular Responses to APNEA, Compound 48/80, CPA, and CGS 21680. 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-SPT (40 mg kg\(^{-1}\), i.v., −5 min) induced falls in blood pressure at the two highest doses accompanied by small falls in heart rate (Fig. 1). The effect of dexamethasone given intraperitoneally at different doses and times of pretreatment on the cardiovascular response to APNEA are shown in Fig 1; the corresponding baseline mean arterial blood pressure (BP) and heart rate (HR) values prior to starting the APNEA injection sequences are shown in Table 1. Pretreatment with dexamethasone (1 mg kg\(^{-1}\)) for 24 h induced significant but limited blockade of the hypotensive response to APNEA. The doses of APNEA that reduced blood pressure by 30 mm Hg (ED\(_{30}\)) were 6.5 ± 2.2 (n = 5) and 17.7 ± 2.0 (n = 5) \(\mu\)g kg\(^{-1}\) for the animals given vehicle or dexamethasone, respectively (p < 0.05; Student’s t-test with Hommel-Hochberg correction). A dose of 0.3 mg kg\(^{-1}\) of dexamethasone was without effect (ED\(_{30}\) 6.3 ± 0.3 \(\mu\)g kg\(^{-1}\), n = 4), and no further blockade could be achieved by

![Fig. 1. Effects of dexamethasone on the cardiovascular responses to APNEA in anesthetized rats.](https://jpet.aspetjournals.org/doi/10.1124/jpet.1017.059263)
increasing the dose of dexamethasone from 1 to 3 mg kg\(^{-1}\) (ED\(_{30}\); 21.3 ± 6.0 \(\mu g\) kg\(^{-1}\), \(n = 4\)). A 3-h pretreatment with dexamethasone (1 mg kg\(^{-1}\)) was without significant effect (ED\(_{30}\); 9.8 ± 2.2 \(\mu g\) kg\(^{-1}\), \(n = 3\)). Moreover, although pretreatment with 1 mg kg\(^{-1}\) on 3 successive days resulted in significant (\(p < 0.05\)) suppression of responses to APNEA (ED\(_{30}\); 23.2 ± 2.3 \(\mu g\) kg\(^{-1}\), \(n = 4\)), the degree of blockade was not significantly greater than that seen at 24 h after a single dose.

Cardiovascular responses to the mast cell degranulating agent compound 48/80 (100–300 \(\mu g\) kg\(^{-1}\) i.v.) were qualitatively similar to those of APNEA (see Hannon et al., 1995; Fig. 2A). Dexamethasone, 1 mg kg\(^{-1}\) (24-h pretreatment) or 1 mg kg\(^{-1}\), given on 3 successive days, did not affect the blood pressure fall induced by compound 48/80. Similarly, neither the cardiovascular responses to the A\(_1\) adenosine receptor agonist CPA nor the A\(_2A\) adenosine receptor ligand CGS 21680 were affected by pretreatment with dexamethasone, 1 mg kg\(^{-1}\) for 24 h (Fig. 2, B and C); the corresponding baseline mean arterial BP and HR values prior to starting the agonist injection sequences are shown in Table 2.

### Plasma Histamine Concentrations and Mast Cell Degranulation Following APNEA and Compound 48/80: Effects of Dexamethasone

The plasma histamine concentrations increased dose dependently following intravenous injection of APNEA (10 and 30 \(\mu g\) kg\(^{-1}\) or compound 48/80 (100 and 300 \(\mu g\) kg\(^{-1}\)) (Table 3). Pretreatment with dexamethasone, 1 mg kg\(^{-1}\), i.p. 24 h prior to the experiment did not alter baseline plasma histamine concentrations per se. The increases in the plasma histamine concentrations induced by the 10 \(\mu g\) kg\(^{-1}\) dose of APNEA and the 100 \(\mu g\) kg\(^{-1}\) dose of compound 48/80 were significantly reduced in animals pretreated with dexamethasone (1 mg kg\(^{-1}\), −24 h) compared with vehicle-pretreated controls. In contrast, the response to the higher doses of APNEA and compound 48/80 were not significantly altered by dexamethasone pretreatment (Table 3).

APNEA induced mast cell degranulation in thymus but not in skin or skeletal muscle. In contrast, compound 48/80 induced substantial and significant degranulation of mast cells in all three tissues (Fig. 3). As shown in Fig. 3, pretreatment with dexamethasone (1 mg kg\(^{-1}\) i.p., −24 h) significantly reduced APNEA-induced degranulation of mast cells in the thymus and slightly, but significantly, inhibited degranulation in response to compound 48/80 in each of the tissues analyzed.

### Discussion

The hypotensive response to APNEA in the Sprague-Dawley rat pretreated with 8-SPT is a consequence of activation of adenosine A\(_3\) receptors (Fozard and Carruthers 1993;
that a 3-h pretreatment with 1 mg kg

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cells is well established and includes biochemical and pharmacological differences (Metcalfe et al., 1997). It would not be too unusual for this heterogeneity to extend to susceptibility to inhibition by dexamethasone. However, further studies would be needed to verify the fact. Finally, it bears emphasis that a 3-h pretreatment with 1 mg kg

There can be little doubt that suppression of the hypotensive response to APNEA by dexamethasone reflects down-regulation of the A3 receptor-mediated mast cell degranulation, which underlies the hypotensive response to APNEA in the presence of 8-SPT in the Sprague-Dawley rat (Fozard and Carruthers, 1993; Fozard and Hannon, 1994; Fozard et al., 1996). Thus, blockade was associated with a decrease in the plasma histamine concentrations and reduced mast cell degranulation in the thymus. Moreover, the fact that histamine levels associated with the lower but not the higher dose of APNEA were significantly affected is consistent with the observed limited blockade by dexamethasone of the hypotensive response to APNEA. The choice of tissues for histological analysis was based on the results from earlier experiments (Fozard et al., 1996) and represents those tissues where the most marked changes were evident following APNEA. The lack of effect of APNEA on mast cells in the skin was therefore unexpected considering our earlier finding. It probably reflects the fact that a lower dose of APNEA (30 μg kg

Thus, the lack of effect of dexamethasone on hypotensive responses to compound 48/80 is of particular interest. The finding is unlikely to reflect a mast cell-independent mechanism contributing to the fall in blood pressure, since, like APNEA, the effects of compound 48/80 have been shown to be largely a consequence of mast cell activation in this model (Hannon et al., 1995; Fozard et al., 1996). There is an apparent anomaly in that despite their being no effect on the hypotensive response, the histamine release induced by the lower dose of compound 48/80 was significantly inhibited. Moreover, a significant, albeit slight, reduction in the degranulation response in thymus, skin, and skeletal muscle is seen. The explanation may be related to the fact that the hypotensive response to compound 48/80 manifests a steep dose-response relationship; indeed, it is all-or-nothing between 30 and 100 μg kg

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NEA, the concentration of plasma histamine associated with a fall in blood pressure lies between 154 and 227 ng ml$^{-1}$, which is well below the 828 ng ml$^{-1}$ present after administration of compound 48/80 following treatment with dexamethasone. This provides further evidence that dexamethasone inhibition of mast cell degranulation is limited in scope.

Our findings indicate suppression, by dexamethasone, of a mechanism(s) common to both the A$_3$ receptor- and com-

Fig. 3. The degranulation status of mast cells in different tissues following pretreatment with vehicle (saline, 1 ml kg$^{-1}$, i.p., −24 h; left panels) or dexamethasone (1 mg kg$^{-1}$, i.p., −24 h; right panels) and subsequent administration of vehicle, APNEA (30 μg kg$^{-1}$ i.v.), or compound 48/80 (300 μg kg$^{-1}$ i.v.) to anesthetized rats. % cells, percentage of mast cells per tissue (means ± S.E.M. of sections from 4–5 rats) with a status defined according to the following scale: 0 = essentially intact mast cells with no, or only marginal, degranulation; 1 = mast cells showing unequivocal signs of degranulation; and 2 = degranulated mast cell with no cell body visible. *, $p < 0.05$, **, $p < 0.01$ that the value differs from that of vehicle/vehicle-treated animals; †, $p < 0.05$, ††, $p < 0.01$ that the value differs from that of dexamethasone-pretreated animals (Student’s unpaired t test; Mann-Whitney U test).
bound 48/80-induced mast cell mediator release. Both stimuli manifest their effects on mast cells via the trimeric GTP-binding protein, G, although there are fundamental differences in that APNEA involves coupling to Gi2,3 following A3 receptor activation (Fredholm et al., 2000), whereas compound 48/80 directly activates Gi3 (Aridor et al., 1993). Thus, the logical explanation for the effects would be that dexamethasone down-regulates the level and/or activity of the Gi protein(s). However, where such activity on G proteins has been sought, dexamethasone pretreatment generally had minimal effects (Gerwins and Fredholm, 1991; McLellan et al., 1992; Kalavantavanich and Schramm, 2000) or, in the RBL-2H3 cell line, even increased the expression of certain G protein subunits (Ramkumar et al., 1995).

The present findings stand in marked contrast to those from the RBL-2H3 cell line, where pretreatment with dexamethasone caused up-regulation of the response to A3 receptor activation associated with an increase in expression of both the adenosine A3 receptor and certain G protein subunits (Collado-Escobar et al., 1990a,b; Qian and McCloskey, 1993; Ramkumar et al., 1995). An explanation for this difference must remain speculative until further information is available. Clearly, however, it underlines the very real differences that may arise between results obtained with experiments in isolated cells and those generated in integrated organ systems.

Finally, our data may have relevance to the clinical situation in asthma, where the bronchoconstrictor response to adenosine (in the form of AMP) is mast cell-mediated (Fozard and Hannon, 2000; Meade et al., 2001). Treatment with glucocorticosteroids partially suppresses the responsiveness to AMP in asthmatics (Holgate et al., 2000; Van Schoor et al., 2000). Interestingly, in a study with mometasone, inhibition was incomplete and could not be further increased by increasing the dose (Holgate et al., 2000). The results of the present study would predict that suppression of a mast cell-mediated functional response would occur following glucocorticosteroid administration, but that the degree of inhibition would be limited. The nonspecific decrease in mast cell responsiveness identified in the present study may contribute to the suppression of adenosine-induced bronchoconstriction by glucocorticosteroids in asthma.

References

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