Evidence for mast cell activation during exercise-induced bronchoconstriction

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ABSTRACT: Controversy remains about the causative mediators in the bronchoconstrictive response to exercise in asthma. This study examined whether mast cell activation is a feature of exercise-induced bronchoconstriction by measuring urinary metabolites of mast cell mediators.

Twelve nonsmoking subjects with mild asthma and a history of exercise-induced bronchoconstriction exercised on a stationary bicycle ergometer for 5 min at 80% maximum work load. Pulmonary function was monitored and urine was collected before and 30 and 90 min after the provocation. The urinary concentrations of the mast cell markers $9\alpha,11\beta$ -prostaglandin (PG) F_2 and *N*-methylhistamine, as well as leukotriene E_4 (LTE $_4$) were determined by immunoassay.

Seven of the 12 subjects (responders) experienced bronchoconstriction (>15% fall in the forced expiratory volume in one second) following exercise, whereas the pulmonary function of the remaining five subjects (nonresponders) remained stable. The urinary excretion (mean $\pm$ SE) of $9\alpha,11\beta$ -PGF $_2$ in the responders increased significantly compared with the nonresponders at 30 (77.1 $\pm$ 14.4 versus 37.2 $\pm$ 5.6; $p<0.05$) and 90 min (79.3 $\pm$ 8.6 versus 40.4 $\pm$ 8.5, $p<0.05$) after exercise challenge. The urinary excretion of *N*-methylhistamine and LTE $_4$ was not significantly different between the two groups at 30 or 90 min after exercise.

The findings represent the first documentation of increased urinary levels of $9\alpha,11\beta$ -prostaglandin F_2 in adults following exercise challenge and provides clear evidence for mast cell activation during exercise-induced bronchoconstriction in asthmatics.

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Exercise-induced bronchoconstriction (EIB) is thought to occur in 70–80% of asthmatics, most commonly amongst those with moderate-to-severe airway hyperresponsiveness [1]. It is characterized by transient airflow obstruction resulting in a $\geq 15\%$ decrease in forced expiratory volume in one second (FEV $_1$) following 5–8 min of exercise. The fall in FEV $_1$ reaches a maximum approximately 10 min after exercise and gradually normalizes over the next hour [2]. The precise pathophysiology of EIB remains unclear, although it is widely accepted that during exercise the upper airways are unable to adequately warm and humidify the increased volumes of inspired air. This results in airway cooling and increased airway fluid osmolality in the lower airways [3, 4]. One hypothesis suggests that hyperosmolar triggering of mast cells and possibly other inflammatory cells results in the release of bronchoconstricting mediators, *e.g.* cysteinyl-leukotrienes (cys-LTs), histamine and prostaglandin (PG) D_2 [5].

Previous indications for the participation of mast cell mediators in the pathogenesis of EIB are based on pharmacological data. For example, disodium cromoglycate (DSCG), a drug which stabilizes mast cell membranes, is effective in blunting exercise-induced asthma [6, 7]. Pre-treatment with a number of H_1 receptor antagonists has

been shown to attenuate EIB [8–10]; however, the degree of protection afforded by this class of drugs has been modest. More recently, studies employing leukotriene receptor antagonists [11, 12] and biosynthesis inhibitors [13] have implicated leukotrienes in the airway bronchoconstrictor response to exercise.

The direct approach of measuring mast cell mediator release in response to exercise challenge has yielded ambiguous results. Some studies have reported an elevation in plasma and whole blood histamine concentrations following EIB [14, 15], while other studies have failed to verify those findings [16, 17]. Apart from the methodological problems encountered when sampling plasma histamine [18] and its short half-life in the circulation, approximately 1 min [19], it has been suggested that elevations in plasma histamine mainly reflect the basophilia which normally accompanies exercise [20]. Increases in the levels of tryptase and PGD $_2$, both specific mast cell markers, have been detected in nasal lavage following nasal provocation with cold dry air [21], but not after exercise [22, 23].

The purpose of this study was to provide evidence to establish mast cell activation as a feature of EIB. To this end, a combination of mast cell markers, $9\alpha,11\beta$ -PGF $_2$

and *N*-methylhistamine, was measured before and after exercise challenge in a group of asthmatics with a history of EIB. The major cyclooxygenase product of mast cells PGD₂, is metabolized in the lung, *via* the action of the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzyme 11-ketoreductase to 9 α , 11 β -PGF₂, and is excreted intact into the urine [24]. Since PGD₂ production in humans is restricted almost exclusively to the mast cell [25], PGD₂ and its metabolites may be employed as mast cell markers. In some reports macrophages [26] and platelets [27] have been ascribed the ability to synthesize PGD₂; however, the levels reported are not of a comparable magnitude to those produced by mast cells.

Measurement of urinary metabolites of histamine overcomes the aforementioned problems with sampling plasma histamine. Thus, histamine is catabolized by *N*-methyltransferase to form *N*-methylhistamine, which is further broken down to form the principal urinary metabolite 1-methyl-4-imidazoleacetic acid. Because of kinetic considerations this study measured *N*-methylhistamine, the earliest urinary metabolite of histamine to appear, which is a reliable marker of systemic histamine production [28]. The observation that histamine from skin mast cells is catabolized *via* an alternative pathway makes urinary *N*-methylhistamine particularly useful in studies of pulmonary reactions.

With the exception of one study in children [29], it has not previously been possible to demonstrate increased levels of leukotriene (LT)E₄ in urine after exercise [30, 31]. Owing to the impressive pharmacological data implicating cys-LTs in EIB [11–13] and the abundant production of these compounds by the mast cell [32], urinary LTE₄, which reflects changes in whole-body production of cys-LTs [33], was also measured after exercise.

Methods

Subjects

Twelve nonsmoking subjects with mild asthma and a history of EIB participated in the study (table 1). The

study was approved by the local Ethics Committee and informed consent was given by each subject. All subjects had stable asthma, controlled by inhaled β_2 -agonists only. Ten subjects were atopic, as demonstrated by at least one positive skin-prick test to a set of 10 common allergens including house dust mite, birch and grass pollen and a selection of animal dander. The exercise challenges were performed out of the pollen season for sensitive individuals. Subjects were not permitted to use their β_2 -agonists for at least 8 h before the exercise challenge and were instructed to avoid caffeine-containing drinks and strenuous exercise on the study day.

Study design

Each subject was studied at the same time in the morning. Immediately before exercise, baseline lung function was measured with an MDI Compact Vitalograph (Förbandsmaterial AB, Stockholm, Sweden) and the best of three FEV₁ measurements was considered as the baseline value. Subjects exercised on a stationary bicycle ergometer (Seca Cardiotest 100, Hamburg, Germany), for 5 min at 80% maximum work load, which had been established at an earlier screening session. Subjects wore noseclips and breathed dry air (0.005% humidity) at room temperature (20°C) through a one-way mouthpiece from a 300 L Douglas bag, constantly fed with compressed air (AGA AB, Lidingö, Sweden). Pulmonary function was monitored as FEV₁ at 0, 1, 3, 5, 8 and 10 min after exercise, and thereafter at 10 min intervals for up to 90 min.

Subjects emptied their bladder 5 min before exercise and urine was collected again at 30 and 90 min after the end of the challenge. Urinary output was recorded at each urine collection and subjects were encouraged to drink a glass of water every hour. Creatinine was measured in all urine samples by a commercially available colorimetric assay (Sigma Chemical Company, St. Louis, MO, USA), using an alkaline picrate method, in order to correct for diuresis. Therefore, the urinary excretion of 9 α , 11 β -PGF₂ and LTE₄ was expressed as ng·mmol creatinine⁻¹. Urine samples were stored, without the addition of any preservatives, at -20°C until analysis.

Table 1. – Subject characteristics

	Subject No.	Age yrs	Sex	FEV ₁ % pred	Workload % max	PD ₂₀ histamine μ g	Atopy
Responders	1	29	F	95	70	250	+
	2	22	M	83	80	900	-
	3	22	F	123	85	270	-
	4	28	M	84	80	150	+
	5	30	M	85	85	1350	+
	6	24	M	97	75	120	+
	7	25	M	98	70	115	+
Mean $\pm$ SEM		25.7 $\pm$ 1.2		95 $\pm$ 5.3	77.9 $\pm$ 2.4	287 (109–759)*	
Nonresponders	8	25	F	100	70	1350	+
	9	23	F	105	85	>2000	+
	10	36	M	85	80	120	+
	11	25	M	97	85	250	+
	12	19	F	103	85	700	+
Mean $\pm$ SEM		25.6 $\pm$ 2.8		98 $\pm$ 3.5	81 $\pm$ 2.9	562 (173–1820)*	

F: female; M: male; FEV₁: forced expiratory volume in one second; PD₂₀: provocative dose of histamine causing a 20% fall in FEV₁. *: Geometric mean and range.

Histamine provocation

At least 1 week before exercise challenge, a bronchial challenge with histamine (histamine diphosphate was prepared by the hospital pharmacy) was performed as described previously [34]. In brief, histamine was inhaled from a jet nebulizer equipped with a dosimeter (Spira Electro 2; Respiratory Care Center, Hämeenlinna, Finland). Pulmonary function was monitored on a vitalograph as FEV₁. The dose of histamine was increased by approximately half a log order of magnitude every 2 min until FEV₁ had decreased by 20% or more.

Enzyme immunoassay

Enzyme immunoassay analyses of (EIA) 9 α ,11 β -PGF₂ and LTE₄ were performed in unextracted urine samples with polyclonal antisera and acetylcholinesterase-linked tracers (Cayman Chemical, Ann Arbor, MI, USA; Cascade Biochem, Reading, UK). The concentration of each sample was determined from a standard curve ranging from 7.8–1000 pg·mL⁻¹. The precision of the EIA for 9 α ,11 β -PGF₂ [35] and LTE₄ [36] is 9.7% and 17.6%, respectively. Crossreactivity of the 9 α ,11 β -PGF₂ antibody against an array of related compounds has been tested previously [34] and was: PGD₂, 1.7%; PGF_{2 α} , 1.7%; 8-epi-PGF_{2 α} , <0.01%.

Radioimmunoassay

Measurements of urinary *N*-methylhistamine were performed with a commercial double-antibody radioimmunoassay (Pharmacia AB, Uppsala, Sweden). The concentration of *N*-methylhistamine in the sample was determined from a standard curve ranging from 0–10 μ g·L⁻¹. Samples exceeding these concentrations were subsequently diluted to ensure that they fell within the standard curve. The detection limit for the assay was 0.1 μ g·L⁻¹, as stated by the manufacturer, and the crossreactivity of the

antiserum with closely related compounds was as follows: histamine, 5.6%; serotonin, 0.003%; histidine, 0.0005%. The precision of the radioimmunoassay for *N*-methylhistamine was 8.8%. Concentrations of *N*-methylhistamine are expressed as μ g·mmol creatinine⁻¹.

Statistical analysis

Calculations of geometric mean values of the provocative dose causing a 20% fall in FEV₁ (PD₂₀) were performed on log-transformed raw data. Exercise bronchoconstriction was determined as the maximal percentage change in FEV₁ from baseline. All data are presented as mean $\pm$ SE unless otherwise stated. Differences in the excretion of urinary mediators were compared by an unpaired Student's *t*-test and considered significant if the *p*-value was <0.05. The statistical calculations were performed with the use of a validated statistical software package for personal computers (Sigma Suite™; Jandel Scientific, Sausalito, CA, USA).

Results

Seven of the 12 subjects experienced a decrease of $\geq$ 15% in their FEV₁ values following 5 min of exercise at 80% of maximum workload (responders). The mean $\pm$ SE maximal fall in FEV₁ in the responder group was 23.0 $\pm$ 8%, with the peak bronchoconstriction occurring 15 min after completion of exercise (fig. 1). In contrast, the FEV₁ values for five of the subjects (nonresponders) remained stable after exercise, with a maximal drop of not $\geq$ 4 $\pm$ 0.9% from the pre-exercise baseline (fig. 1). The subject characteristics of the responder and nonresponder groups did not differ significantly from each other (table 1) although PD₂₀ values for histamine were slightly higher amongst the responder group.

The mean urinary levels (ng·mmol creatinine⁻¹) of 9 α ,11 β -PGF₂, *N*-methylhistamine and LTE₄ before exercise did not differ between the responder and nonresponder

Fig. 1. — Mean ($\pm$ SEM) time course of forced expiratory volume in one second (FEV₁) values (% change from baseline) for the responders (●) and nonresponders (○) following exercise challenge. Pulmonary function was measured before exercise, at 1, 3, 5, 8 and 10 min after exercise and thereafter at 10 min intervals for up to 90 min.

Fig. 2. — Mean ($\pm$ SEM) urinary excretion of 9 α ,11 β -prostaglandin (PG)F₂ in the responder (▨) and nonresponder (□) groups after 5 min of exercise. *: *p*<0.05, significant increase in levels of 9 α ,11 β -PGF₂ in the responder group at 30 and 90 min compared with the nonresponders at the same time points. The concentration of 9 α ,11 β -PGF₂ in the responder group at 90 min was also significantly increased (*p*<0.05) above baseline levels.

Fig. 3. – Mean (+SEM) urinary excretion of *N*-methylhistamine in the responder (▨) and nonresponder (□) groups before and 30 and 90 min after exercise.

groups, as assessed by an unpaired *t*-test. Mean urinary excretion of $9\alpha,11\beta$ -PGF₂ in the responder group increased significantly compared with the nonresponders at 30 min (37.2 ± 5.6 versus 77.1 ± 14.4 ; $p < 0.05$) and 90 min (40.4 ± 8.5 versus 79.3 ± 8.6 ; $p < 0.05$) after completion of the exercise challenge (fig. 2). The concentration of $9\alpha,11\beta$ -PGF₂ in the responder group at 90 min was also significantly increased above the prechallenge level in this particular group (58.3 ± 7.0 versus 79.3 ± 8.6 ; $p < 0.05$). There was an increase in levels of *N*-methylhistamine after 90 min but it failed to reach statistical significance (fig. 3). In the case of urinary LTE₄, the excretion was not significantly different between the two groups at 30 or 90 min after exercise (fig. 4). There was, however, a propensity for a decrease from baseline values of urinary LTE₄ in the nonresponder group after exercise.

Discussion

The role of the mast cell and its spasmogenic mediators in exercise-induced bronchoconstriction has attracted considerable attention; however, the literature to date concerning the detection of mast cell-derived mediators in EIB has been equivocal. The results of the present investigation provide novel direct evidence that the mast cell is activated during EIB. In association with EIB in a group of asthmatics there was a significant postexercise increase in the urinary level of the PGD₂ metabolite, $9\alpha,11\beta$ -PGF₂. In contrast, the level of $9\alpha,11\beta$ -PGF₂ was unaltered in the group that did not experience EIB. These findings represent the first documentation of increased urinary levels of the PGD₂ metabolite in adults following EIB. They are in excellent agreement with the results of NAGAKURA *et al.* [37], who recently found an increase of similar magnitude in urinary $9\alpha,11\beta$ -PGF₂ in children following exercise, when samples were analysed by gas chromatography mass spectrometry (GC/MS) [37]. Release of a mast cell marker during EIB corroborates evidence of an increased percentage of degranulating mast cells in bronchial biopsies after EIB [38].

Previously, several groups have utilized histamine as a marker for mast cell activation in EIB, without producing conclusive results [14–17]. This confusion is probably due

Fig. 4. – Mean (+SEM) urinary excretion of leukotriene (LT)E₄ in the responder (▨) and nonresponder (□) groups before and 30 and 90 min after exercise.

to the technical difficulties with measuring small changes in circulating concentrations of histamine following exercise. Since the basophil is another potential source of histamine, it has been suggested that the increases in plasma or whole-blood histamine can be attributed to the basophilia which ensues exercise [20, 39]. However, NAGAKURA *et al.* [40] demonstrated a rise in histamine that was restricted to those who had experienced EIB, despite the fact that both EIB-positive and EIB-negative asthmatics had similar postexercise basophilia. Measurements of increased mast cell-associated neutrophil chemotactic factor (NCF) following exercise [15, 41] lend further support to the hypothesis that mast cells are activated during EIB.

No increase in the urinary excretion of *N*-methylhistamine could be demonstrated following EIB, although a trend towards increased levels of this metabolite was observed 90 min after exercise. The present findings confirm previous negative data from studies in which *N*-methylhistamine [42] and 1-methyl-4-imidazoleacetic acid [43], the major urinary metabolite of histamine, were determined following EIB. In a recent study conducted in our laboratory where atopic asthmatics were challenged with allergen, it was observed that in all cases where *N*-methylhistamine levels were elevated above prechallenge level, the concomitant relative increases in $9\alpha,11\beta$ -PGF₂ were greater [44]. The 30% increase in levels of $9\alpha,11\beta$ -PGF₂ following exercise is very much lower than the approximate 200–300% increase seen after allergen challenge [35]. It therefore appears that $9\alpha,11\beta$ -PGF₂ is more sensitive than *N*-methylhistamine for monitoring mild and transient episodes of mast cell activation. In addition, in contrast to the rapid turnover of plasma histamine [19], *N*-methylhistamine has a comparatively long half-life. Following intrabronchial administration of ¹⁴C-histamine to a group of asthmatics, excretion of labelled *N*-methylhistamine was complete within 3 h, with the majority of the metabolite being detected in the urine during the first hour after administration, while excretion of 1-methyl-4-imidazoleacetic acid peaked after 6 h [28]. Since urine was collected between 30 and 90 min after the challenge the peak excretion of *N*-methylhistamine may have been missed.

Exercise-induced bronchoconstriction was not associated with an increased excretion of urinary LTE₄, thereby

confirming previous studies which have failed to detect increased LTE₄ concentrations in urine [30, 31] or bronchoalveolar lavage fluid [23] following exercise. Metabolism of leukotrienes in the lung is very rapid, with inhaled leukotrienes recovered in the urine within 1 h of inhalation [45]. It is therefore reasonable to expect that with urine collections at 30 and 90 min after exercise, increased generation of leukotrienes could have been detected. It may be that local increases in leukotriene production in the lung are not of a sufficient magnitude to alter urinary concentrations of LTE₄. It is noteworthy that during the review of this manuscript REISS *et al.* [46] have published a report in which they demonstrated a small but significant two-fold increase in urinary LTE₄ in a group of 13 asthmatics after exercise challenge. Previous studies, including our own, have included 6–9 subjects and it may be that the expanded number of subjects was a contributory factor in obtaining an increase in urinary LTE₄ after an exercise challenge. As evidenced by a strong body of pharmacological data from studies with the cys-LT receptor antagonists [11, 13], there is no doubt that the cys-LTs are major mediators of EIB.

Exercise is a much less vigorous challenge than allergen provocation, where mediators can be elevated in the blood and urine for several hours. The difficulty encountered with demonstration of various mediators following exercise-induced bronchoconstriction presumably relates to the inferior strength and short duration of the stimulus. Therefore, the findings of increased urinary levels of $9\alpha,11\beta$ -prostaglandin F₂ provide strong new evidence for mast cell activation during exercise-induced bronchoconstriction. The findings also support the usefulness and sensitivity of $9\alpha,11\beta$ -prostaglandin F₂ as a mast cell marker.

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