

Respiratory pathophysiologic responses

The effect of local hyperthermia on allergen-induced nasal congestion and mediator release

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Background: Local hyperthermia reduces mast cell degranulation, the severity of acute lung injury, and exercise-induced asthma and decreases symptoms of rhinitis. We have investigated the effect of local hyperthermia on mast cell degranulation and symptom generation in allergic rhinitis to assess its effect and mechanism of action within the nose.

Methods: In a randomized, double-blind, placebo-controlled, crossover study, 10 subjects with rhinitis were treated for 30 minutes with local hyperthermia or placebo, which was followed 30 minutes later by nasal allergen challenge. During the first two visits nasal lavages were performed to assess vascular leakage and mediator release. During the last two visits nasal airway resistance, the number of sneezes, and mucus secretion were monitored.

Results: Local hyperthermia significantly reduced both nasal airway resistance ($p < 0.05$) and vascular leakage ($p < 0.02$) but had no significant effect on the number of sneezes, on mucus secretion, or on tryptase release.

Conclusion: Local hyperthermia reduces allergen-provoked nasal blockage and vascular leakage but has no effect on sneezing, rhinorrhea, or tryptase release. Nasal blockage occurs predominantly via newly formed lipid mediators and kinins, whereas sneezing and rhinorrhea occur predominantly via preformed mediators. We propose that local hyperthermia inhibits newly formed mediator production or release or reduces the sensitivity of the vasculature to inflammatory mediators in general. Further investigation into the mechanisms and potential uses of local hyperthermia is warranted. (*J ALLERGY CLIN IMMUNOL* 1993;92:850-6.)

Key words: Rhinitis, vascular leakage, nasal blockage, local hyperthermia, tryptase

Allergic rhinitis is common in westernized communities, with a cumulative prevalence of 5% to 20%.¹ Although the symptoms of nasal itch, sneezing, and rhinorrhea that characterize this condition respond well to treatment with H₁-antihistamines, nasal congestion does not.² This sensation of a limitation to airflow arises from engorgement of the venous sinusoids within the turbinates and can be related to the local generation and release of oxidative products of arachidonic acid and kinins from activated mast cells, basophils, and eosinophils within the nasal mucosa.^{3, 4} Thus treatments such as corticosteroids

Abbreviations used

AU: Arbitrary units
NAR: Nasal airway resistance

and immunotherapy, which reduce mucosal inflammation and cell activation, improve nasal airflow.^{5, 6} In nasal allergen challenge studies both cyclooxygenase and 5-lipoxygenase inhibitors limited induced nasal blockage.^{7, 8}

Clinical studies with local hyperthermia have suggested an improvement in nasal patency in patients with allergic rhinitis.^{9, 10} Diverse anti-inflammatory effects have been suggested in that local hyperthermia reduced morbidity and mortality rates in a model of acute lung injury,¹¹ the severity of exercise-induced asthma,¹² and the symptoms of the common cold¹³⁻¹⁵ and perennial^{15, 16} and seasonal¹⁷ allergic rhinitis.

The mechanism of action of local hyperthermia in rhinitis is incompletely understood, and its

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range of actions is poorly documented. There is, however, experimental *in vitro* evidence to suggest that hyperthermia may reduce inflammation by modifying cell activation. Increasing ambient temperature has been shown to inhibit IgE-dependent mast cell degranulation and leukocyte histamine release,¹⁸⁻²¹ to enhance the antiinflammatory effects of interferon- γ ,²²⁻²⁵ to reduce mononuclear cell production of interleukins-1 and -2 and granulocyte-macrophage colony-stimulating factor, and to reduce natural killer activity of mononuclear cells.²⁶ In addition, hyperthermia has been demonstrated to reduce the synthesis of cellular proteins, including proinflammatory mediators and enzymes, in favor of cell production of heat shock proteins,²⁷ a heterogeneous group of intracellular proteins that may have important protective antiinflammatory actions.^{28, 29}

Thus to understand further the mechanism of action of hyperthermia *in vivo* and to assess its potential as a treatment modality for allergic rhinitis, we have investigated its effect on the nasal response to allergen insufflation by objectively monitoring nasal patency and indices of cell activation and microvasculature permeability before and after challenge.

METHODS

Subjects

Eight male and two female subjects with rhinitis aged 21 to 23 years, took part in the study. All gave a history of seasonal allergic rhinitis and had a positive skin response (wheal >3 mm in diameter) to skin prick testing with mixed grass pollens (Bencard, Brentford, England). The study was undertaken between November and March when all were free of symptoms, and no subject was taking any medication that might affect the nasal response to challenge. Written informed consent was obtained from each subject, and the study was approved by the Southampton University and Hospitals Ethical Subcommittee.

Study design

After a positive nasal allergen challenge screening test, subjects entered the placebo-controlled, randomized, double-blind, crossover phase of the study for which one machine was set to deliver air at 31° C as the placebo treatment (a temperature chosen to leave the temperature of the anterior nasal mucosa unchanged¹³), and the other at 42° C as the active treatment. During each visit, the subjects inhaled the temperature-conditioned humid air for 30 minutes, followed by a 30-minute interval to allow the airways to recover to normal temperature, before they underwent the nasal allergen challenge. Subjects attended the laboratory on four occasions, with an interval of at least 2 weeks between visits, to undergo single-dose nasal provocation studies with the same concentration of allergen

after pretreatment with local hyperthermia or placebo. During the first pair of visits subjects underwent nasal lavage before and after the allergen challenge to monitor mediator and protein changes, whereas during the last pair of visits, the influence of allergen challenge on sneezing, rhinorrhea, and nasal obstruction was monitored objectively. The studies were performed at the same time of day for each subject, and the order in which the machines were used within each pair of visits was decided by allocation of randomly generated numbers.

Local hyperthermia

Local hyperthermia was delivered with a Virotherm (patent pending), a device designed to expel fully saturated air at a rate of 40 L/min through a ventilated face mask as described previously.¹² Subjects inhaled the heated humidified air by nasal tidal breathing through a face mask. A thermostat was preset to deliver the air at specific temperatures. Two machines were used, one regulated to 42° C and the other to 31° C. The temperature of the hot humid air leaving the two machines was verified at the point of air entry into the face mask by an independent observer.¹²

The machines were identical in appearance and were coded to render the study double-blind. Blinding was effected by the subjects using the machines 2 weeks apart to make recall of the relative temperatures difficult and by not informing subjects of whether the hotter or cooler temperature was expected to have beneficial effects.

Nasal allergen challenge

Challenge was undertaken bilaterally with mixed grass pollen solution (Bencard, Brentford, England) at a concentration of 10⁻² wt/vol with a handheld pump spray delivering 0.13 ml per actuation (total challenge volume = 0.26 ml). The allergen challenge solution was made up fresh each day from a stored concentrate with 0.9% NaCl as the diluent.

Nasal lavage

On the 2 challenge days involving nasal lavage, four baseline nasal lavages were carried out immediately before nasal allergen challenge to achieve stable low levels for the protein and mediator assays. Repeat lavages were performed at 2, 5, 10, 15, 20, and 30 minutes after challenge. Lavage was undertaken by introducing 2.5 ml of warmed 0.9% saline solution into each nostril and then collecting the fluid expelled into a funnel after a dwell time of 10 seconds.³⁰ The recovered fluid was drained through a 0.9 mm sieve and centrifuged at 1500 rpm for 6 minutes at 4° C to remove mucus, cells, and cell debris. The supernatant was aliquotted and stored at -70° C for protein and mediator assays.

Nasal monitoring

On the nonlavage challenge days nasal airway resistance (NAR) was measured before and at 2, 5, 10, 15,

20, and 30 minutes after challenge by active posterior rhinomanometry with a Mercury NR 6 rhinomanometer on-line to a BBC microprocessor (Mercury Electronics, Glasgow, Scotland), which automatically computed inspiratory and expiratory nasal resistance, as the mean of four separate breath cycles.³⁰ The number of sneezes and the amount of anterior mucus production (as determined by weighing preweighed dry tissues after gentle nose blowing)³⁰ were also monitored before and after challenge at the same time points as for NAR measurements.

Tryptase assay

Tryptase levels were measured by means of a radioimmunoassay with the tryptase-specific monoclonal antibody AA1³¹ coupled to cyanogen bromide-activated agarose (Pharmacia, Uppsala, Sweden). Duplicate samples of nasal lavage fluid concentrated six times by lyophilization (100 μ l) or tryptase standards (2 to 50 μ g/ml in 100 μ l) were incubated with AA5-agarose (2.5 mg in 100 μ l) for 16 hours at room temperature. After a washing stage with 0.9% saline solution, 25 μ l of iodine 125-labeled anti-tryptase monoclonal antibody (Pharmacia) was added for 16 hours, tubes were washed again, and samples were counted in a gamma counter.

Protein assays

Total protein concentrations were measured in 200 μ l replicates of nasal lavage fluid by the method of Bradford³² with Coomassie Blue G-250 reagent (Pierce Chemical Co., Rockford, Ill.) as indicator. Absorbance read at 595 nm was compared with a standard curve constructed with bovine serum albumin (Sigma, Poole, England) to give absolute protein concentrations.

Albumin levels were measured by rocket immunoelectrophoresis.³³ Duplicate 3 μ l aliquots of nasal lavage fluid were introduced into wells cut in agarose gel and subjected to electrophoresis (4 V/cm) for 16 hours into a "window" of agarose containing 1.18 μ l/ml of rabbit anti-human albumin antibody (Nordic, Tilburg, Netherlands). Immunoprecipitates were stained with Coomassie Blue. Albumin levels were quantified by comparison with standard curves constructed with human albumin (Sigma).

Statistical analysis

Both the maximal increase (peak NAR expressed as a percentage increase over baseline NAR) and the total increase (the area under the NAR-time curve, calculated by trapezoid integration) in NAR measurements were calculated and compared between groups. Similarly, both maximal and total nasal lavage albumin, total protein, and tryptase levels were calculated and compared. Comparisons between active and placebo groups were carried out with Wilcoxon's signed-rank test. The null hypothesis was rejected at $p < 0.05$. Results are expressed as medians and ranges.

The Spearman rank correlation test was used to investigate the relationship between tryptase levels and the response to allergen challenge in terms of protein

leakage, sneezing, mucus production, and nasal blockage.

RESULTS

All 10 subjects completed each of the two pairs of visits, and on the placebo days they demonstrated a significant response to nasal allergen challenge, with a median peak increase in NAR of 264% (40% to 593%) ($p < 0.05$), and nasal lavage total protein, albumin, and tryptase levels demonstrated a median peak increase of 197% (120% to 718%), 346% (133% to 829%), and 360% (-3% to $\infty\%$), respectively ($p < 0.05$). The treatment was well tolerated, and no extranasal adverse effects were reported.

Allergen-induced nasal blockage and nasal symptoms

There was no significant difference in the baseline NAR values on the active and placebo challenge days with median values of 0.31 $\text{Pa} \cdot \text{cm}^{-3} \cdot \text{sec}$ (0.16 to 0.52 $\text{Pa} \cdot \text{cm}^{-3} \cdot \text{sec}$) and 0.27 $\text{Pa} \cdot \text{cm}^{-3} \cdot \text{sec}$ (0.21 to 1.0 $\text{Pa} \cdot \text{cm}^{-3} \cdot \text{sec}$), respectively. The median peak increase in nasal resistance after active pretreatment (92% [13% to 421%]) was significantly less than that after placebo pretreatment (264% [40% to 593%], $p < 0.05$). There was a similar reduction in the total NAR, though this just failed to reach statistical significance; the total area under the curve of NAR was 1.4 arbitrary units (AU) for the active group and 11.7 AU for the placebo group ($p < 0.06$). There were no significant differences in either the number of sneezes recorded ($p > 0.3$) or the weight of mucus produced on the two occasions ($p > 0.2$) (Fig. 1).

Nasal lavage

Volumes of nasal lavage fluid sufficient for full analysis were recovered from 9 of the 10 subjects who took part in the study.

Microvascular leakage. The lavage albumin and protein levels were 17.5 $\mu\text{g} \cdot \text{ml}^{-1}$ (8 to 38 $\mu\text{g} \cdot \text{ml}^{-1}$) and 39 $\mu\text{g} \cdot \text{ml}^{-1}$ (20 to 74 $\mu\text{g} \cdot \text{ml}^{-1}$) before the challenge on the active pretreatment day and 17.5 $\mu\text{g} \cdot \text{ml}^{-1}$ (5 to 56 $\mu\text{g} \cdot \text{ml}^{-1}$) and 40 $\mu\text{g} \cdot \text{ml}^{-1}$ (10 to 101 $\mu\text{g} \cdot \text{ml}^{-1}$) on the placebo pretreatment day. Prior treatment with local hyperthermia resulted in a significant reduction in the challenge-related increase in microvascular permeability: the median areas under the nasal lavage albumin response-time curves (Fig. 2) for active versus placebo treatments were 160.8 AU (-101 to 2320 AU) and 1222 AU (535 to 3221 AU), respectively ($p < 0.01$), and the median areas under the nasal lavage total protein response-

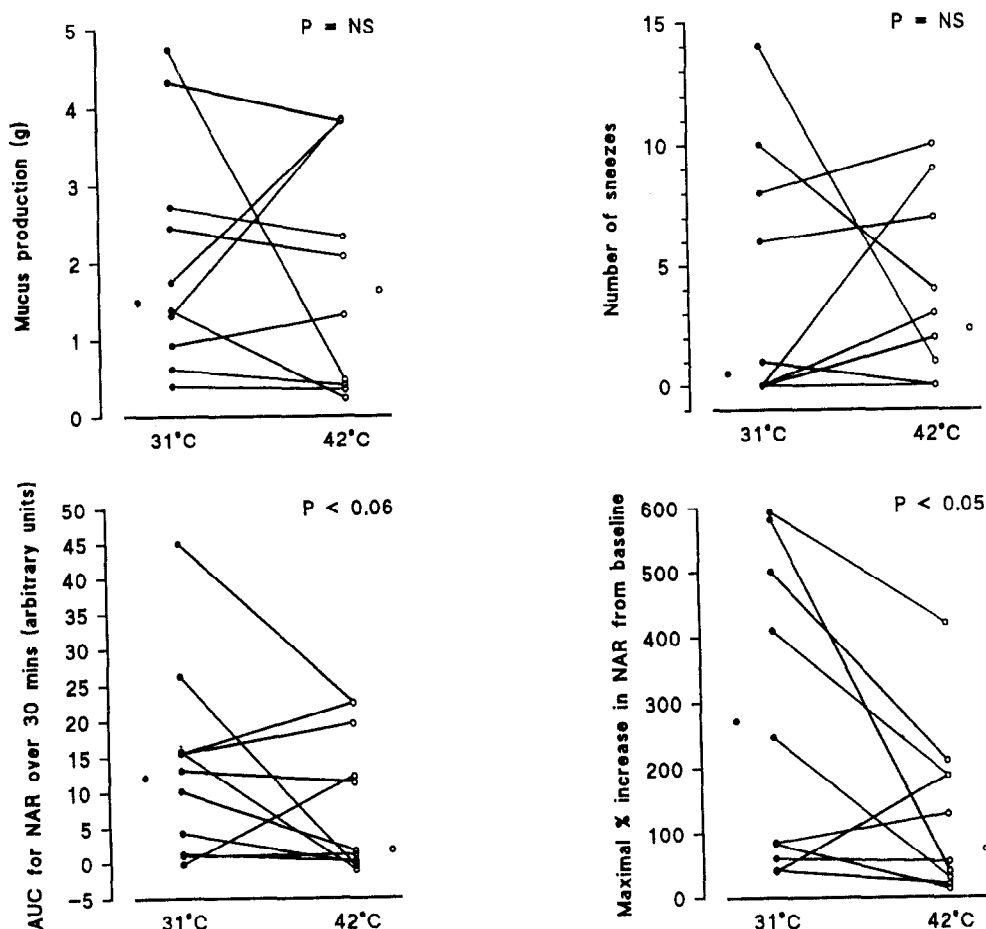


FIG. 1. The effect of local hyperthermia treatment at 42°C (open circles) and placebo treatment at 31°C (filled circles) on the response to nasal allergen challenge performed 1 hour after treatment. Plots in clockwise order from top left represent allergen-induced mucus production, sneezing, and peak and total NAR responses. Points joined by lines represent individual paired responses, and those alongside represent the median for all 10 subjects. Statistical analysis was done with the Wilcoxon signed-rank test.

time curves (Fig. 2) for active versus placebo treatments were 407.5 AU (−701 to 1328 AU) and 841.5 AU (535 to 2430 AU), respectively ($p < 0.02$).

Mast cell tryptase release. Lavage tryptase levels increased from 0.48 ng · ml^{−1} (0 to 0.88 ng · ml^{−1}) to 2.04 ng · ml^{−1} (0.27 to 13.59 ng · ml^{−1}) on the active treatment day ($p < 0.01$) and from 0.33 ng · ml^{−1} (0 to 0.72 ng · ml^{−1}) to 1.57 ng · ml^{−1} (0.32 to 5.73 ng · ml^{−1}) on the placebo treatment day ($p < 0.01$). The median area under the nasal lavage tryptase response-time curves (Fig. 2) for active versus placebo treatments was 12.5 AU (−1.6 to 268 AU) and 17.4 AU (−2.8 to 103 AU), respectively ($p > 0.4$), and there was no significant difference between the active and placebo treatments in terms of the peak tryptase level ($p > 0.2$). Prior treatment with local hyperthermia thus had no significant effect on allergen challenge-induced mast cell tryptase release.

The levels of tryptase identified in lavage correlated very strongly with both albumin leakage ($r = 0.9$, $p < 0.001$) and total protein leakage ($r = 0.817$, $p < 0.01$) for the active treatment, whereas the correlations were slightly less strong for the placebo treatment ($r = 0.77$, $p < 0.02$ for albumin and $r = 0.703$, $p < 0.05$ for total protein). There were no significant correlations between any measures of nasal blockage, sneezing or mucus production, and tryptase release.

DISCUSSION

This study demonstrates that prior treatment with local hyperthermia significantly inhibits the nasal response to local allergen challenge, reducing nasal congestion and vascular leakage but having no demonstrable effect on sneezing, mucus production, or mast cell tryptase release.

Although several previous studies have reported subjective improvements in nasal symp-

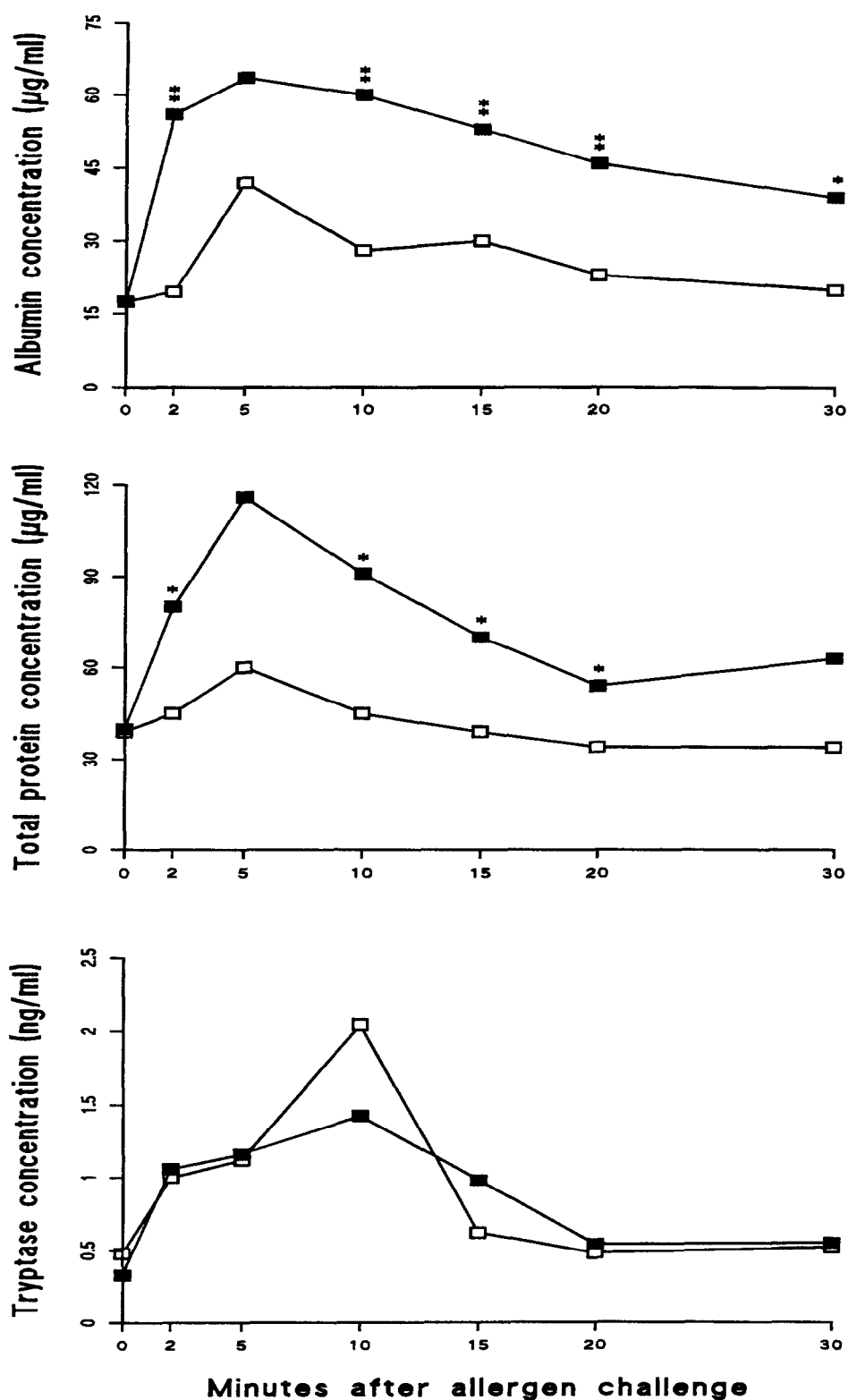


FIG. 2. The effect of local hyperthermia treatment at 42° C (*open squares*) and placebo treatment at 31° C (*filled squares*) on the response to nasal allergen challenge performed 1 hour after treatment. Plots from the top represent the nasal lavage levels (median values for nine subjects) of albumin, total protein, and tryptase in the 30 minutes after nasal allergen challenge. Statistical analysis was done with the Wilcoxon signed-rank test. For the paired analyses *p* values are designated as: ***p* < 0.01 and **p* < 0.05. The total areas under the albumin and total protein time curves were significantly lower for the active treatment compared with the placebo treatment (*p* < 0.02). There were no significant differences for the tryptase levels.

toms¹⁵⁻¹⁷ and in nasal blockage^{9, 10} with local hyperthermia pretreatment in rhinitis, there have been no previous objective investigations of the nasal effects of this intervention. Using controlled allergen challenge and objective monitoring of nasal airflow by active posterior rhinomanometry, we have been able to clearly demonstrate a protective effect of local hyperthermia on the nasal obstructive response to allergen exposure in rhinitis. In addition, this study indicates that this mode of therapy limits allergen-induced transvascular plasma exudation.

Both nasal obstruction and plasma protein extravasation reflect vascular events within the nose; the former is related to engorgement of venous sinusoids within the turbinates, and the latter to alterations in the interendothelial pores of the fenestrated superficial capillary network within the nasal mucosa. In contrast to the protective effect on these vascular events, local hyperthermia had no protective effect on the neurally mediated sneezing and rhinorrhea. We have previously proposed that because of its protective effect against exercise-induced asthma, local hyperthermia may inhibit mast cell degranulation.¹² It has been possible to directly investigate this in the present study by measuring tryptase levels in lavage fluid. Tryptase is a four-chain neutral protease unique to the mast cell granule and is thus a specific marker for mast cell activation. Other mast cell-associated mediators such as histamine, prostaglandin D₂, and leukotriene (LT) C₄ do not confer the same level of specificity, because they have other potential cell sources.⁴ Tryptase levels increased significantly after challenge (Fig. 2), confirming results of a previous study by Schwartz et al.,³⁴ but neither the peak levels after challenge nor the areas under the curve of the time-response plot were significantly altered by the hyperthermia pretreatment. This study thus demonstrates that hyperthermia 30 minutes before allergen exposure does not inhibit mast cell degranulation. Indirect evidence for this is also apparent from the failure to influence allergen-induced sneezing and rhinorrhea in the present study and also the reported beneficial effect in the common cold¹³⁻¹⁵ in which mast cell degranulation has not been found to be implicated.³⁵

Thus the nasal protective effects of local hyperthermia may be related either to a general effect on the nasal vasculature, rendering it unresponsive to vasoactive mediators of allergic inflammation, or, more likely, to the interference of this mode of treatment with the generation of nonpreformed mediators such as prostaglandin D₂, leu-

kotrienes C₄ and D₄, and the kinins, kallidin and bradykinin. All of these mediators have been shown to be generated during the immediate allergic response within the nose^{4, 36} and have predominantly vascular actions; they have no effect on nasal itch or sneezing.^{4, 37} Of these mediators, kinins have also been demonstrated to be present in elevated levels in the common cold,³⁵ and interference with either these or the tissue or plasma pathway of kinin generation would allow modification of this form of rhinitis.

In the present study the finding of a less powerful correlation between changes in lavage tryptase and changes in both albumin and total protein levels in lavage on the placebo rather than the active treatment day could be indirect evidence for an effect of hyperthermia on kinin generation. Tryptase is a marker of mast cell activation, and the immediate changes in albumin and protein levels can be related to the release of preformed mast cell mediators. The secondary generation of mediators, such as kinins and leukotrienes, will add to the response, particularly because bradykinin is more potent than histamine in inducing plasma protein leakage.³⁸ These additional contributory mediators, which may be derived from different sources, would thus diminish the correlation between a pure mast cell marker and the end organ response.

Clearly, further studies into the mechanism and extent of the effects of local hyperthermia on nasal mucosal inflammation are warranted. This study suggests, however, that this mode of treatment may offer a potential nonpharmacologic adjunct to the treatment of rhinitis.

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