

Effects of Intranasal Administration of Endothelin-1 to Allergic and Nonallergic Individuals

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Endothelin-1 (ET-1), a 21 amino acid peptide, and its receptors are distributed in the mammalian respiratory tract. To examine the responses of human upper airways to ET-1, we investigated the effects of intranasal administration of ET-1 to nine symptomatic allergic and nine nonallergic volunteers. Paper discs were used to administer ET-1 or diluent to one side of the nasal mucosa, and to collect secretions from the ipsilateral (challenged) and contralateral (opposite) nostrils. ET-1 (0.3–10 μ g), but not diluent, induced dose-dependent bilateral increases in secretion weights, lysozyme secretion, symptoms of rhinorrhea and itch, and sneezing in both populations. ET-1 did not induce albumin secretion, histamine release, or symptoms of nasal congestion. Compared with the nonallergic subjects, allergic individuals sneezed more and had significantly higher bilateral secretion weights, contralateral lysozyme secretion, and symptoms of rhinorrhea following ET-1 provocation. In summary, ET-1 induced symptoms relevant to inflammatory upper airway diseases in allergic and nonallergic subjects. However, responses of allergic subjects were more pronounced, particularly with respect to symptoms associated with neural reflex responses, such as sneezing and contralateral secretion. Therefore, allergic inflammation enhances responsiveness of the nasal mucosa to ET-1. **Riccio MM, Reynolds CJ, Hay DWP, Proud D. Effects of intranasal administration of endothelin-1 to allergic and nonallergic individuals.**

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In 1988 endothelin-1 (ET-1), a 21 amino acid peptide with potent vasoconstrictor activity, was isolated and purified from cultured porcine endothelial cells (1). Screening of a genomic DNA library subsequently revealed two additional genes that encode two structurally related isoforms, ET-2 and ET-3, which together with ET-1 constitute the endothelin family of vasoconstrictor peptides (2). Although the endothelins were first described as potent constrictors of vascular smooth muscle, it soon became apparent that these peptides, particularly ET-1, can induce an array of effects in other cell and target tissues. In the respiratory tract, for example, the endothelins are potent contractile agonists of airway smooth muscle from several species, including humans (3–6). They are also capable of affecting mucus secretion (7, 8), stimulating smooth muscle proliferation (9), and modulating neurotransmission (10, 11). These effects, together with the observations that levels of immunoreactive ET-1 are increased in bronchoalveolar lavage fluids from subjects with asthma (12, 13), which can be attenuated by inhaled corticosteroid treatment (13, 14), and that enhanced expression of ET-1 is seen in airway epithelium of subjects with asthma (15), have led to the suggestion that endothelins may play a role in the pathophysiology of inflammatory airway diseases (15–17).

Few studies, however, have investigated the involvement of en-

dothelins in the pathogenesis of inflammatory diseases of the upper airways. In this regard, immunoreactive-ET has been detected in human nasal mucosal explants (18, 19), particularly in the epithelium, submucosal glands, and endothelial cells of the nasal vasculature. Moreover, application of ET-1 to nasal mucosal tissue *in vitro* induced serous and mucus cell secretion (18) and prostanoid release (20), while ET-1 given intravenously to anesthetized rabbits caused a pronounced vasoconstriction of the surface blood vessels located on the nasal inferior turbinates (19). To further examine the potential role of ET-1 in upper airway physiology and pathophysiology, we have examined the effects of intranasal provocation with this peptide in human allergic and nonallergic volunteers *in vivo*.

METHODS

Materials

The following were purchased: ET-1 (Peninsula Laboratories, Belmont, CA); physiologic saline (Kendall McGraw Laboratories, Irvine, CA); allergen extracts for skin testing (Greer Laboratories, Lenoir, NC); white filter cards (#190005; Shandon, Pittsburgh, PA); sheep antibody to human albumin and horseradish peroxidase-conjugated sheep antibody to human lysozyme (The Binding Site, Inc., San Diego, CA); rabbit antibody to human lysozyme and horseradish peroxidase-conjugated sheep antibody to human albumin (Dako Corp., Carpinteria, CA); 2,2'-azino-di-[3ethylbenzthiazoline sulphononic acid] (ABTS) and human serum albumin (Sigma, St. Louis, MO); and lysozyme (Calbiochem, San Diego, CA). Stock solutions of ET-1 (1 mg/ml) were made up in 0.9% sterile physiologic saline and stored as 100 μ l aliquots at -80° C with a fresh aliquot used for each experiment. The diluent (saline) solution was treated in the same manner. The solutions were both pH 6.5 and were within the normal osmolarity range of the nasal mucosa at 329 and 337 mosM/L for the ET-1 and diluent solutions, respectively.

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Subjects

Nasal ET-1 challenge was performed in nine nonallergic volunteers aged 23 to 53 yr (mean age = 33.0 ± 3.1), and nine allergic subjects, aged 27 to 39 yr (mean age = 32.7 ± 1.2). Nonallergic subjects (five male) were defined as individuals with no previous history of allergic symptoms as well as negative intradermal skin tests to a range of allergens. The allergic group consisted of nine individuals, three (one male) of whom were seasonal allergic subjects and six (three male) of whom were perennial allergic individuals. We selected allergic subjects who were symptomatic at the time of the study to assess the contribution that allergic inflammation may play in the nasal responses to ET-1. To ensure that all subjects were symptomatic, seasonal subjects were challenged during the grass (May–June) or ragweed (September) seasons while perennial subjects, who had year-round symptoms, were challenged between May and October. Visual confirmation of the presence of inflammation (e.g., mucosal reddening, turbinate engorgement, rhinorrhea) was performed, and all allergic subjects affirmed that they were symptomatic. Nonallergic subjects were challenged during May–October.

Subjects refrained from taking medications during the course of the study. No subject had taken astemizole in the previous 3 mo, while glucocorticosteroids were stopped 1 mo before challenge. Decongestants were discontinued 24 h before challenge, while antihistamines were withheld for 2–5 d before investigation, depending on the characteristics of the antihistamine. The Institutional Review Board of the Johns Hopkins Bayview Medical Center gave approval for the study, and each subject gave written informed consent before entering the study.

Skin Testing

Epicutaneous skin prick tests were performed using the following common allergens: ragweed, mixed grasses (Timothy, Orchard, Bermuda), *D. pteronyssinus*, *D. farinae*, cat, dog, mixed trees (oak, beech, maple, elm), mold 1 (*Aspergillus*, *Penicillin*, *Mucor fusarium*), and mold 2 (*Alternaria*, *Helminthosporium*, *Cladosporium*, *Botrytis*). In addition, a negative control subject with the extract vehicle and a positive control subject with 0.01% histamine were obtained. If the epicutaneous skin puncture yielded negative results in the nonallergic subjects, intradermal skin testing with a 1:100 dilution of original extract was performed by injecting 0.05 ml of the diluted extract into the dermis to confirm nonatopic status. A positive reaction was defined as a wheal that was > 5 mm larger than the wheal induced by the vehicle and > 10 mm of erythema 15 min after application.

Nasal Endothelin Challenge Protocol

Nasal challenges were performed and nasal secretions collected using filter paper discs punched from filter cards with an 8-mm hole punch (21). Each disc used for collection of secretions was sealed in 1.5 ml microtubes and weighed on a Mettler Analytical Balance AE240. Each disc could hold approximately 50 μ l of solution.

A diagram illustrating the protocol used for the ET-challenge is summarized in Figure 1. Subjects visited the laboratory on two separate occasions, separated by at least 24 h, when they were challenged with ET-1 (0.3–10 μ g) or 0.9% physiologic saline (diluent) in a double-blind, randomized, crossover study. To create a uniform baseline level of secretion and to clear the nose of any residual mucus, three nasal lavages were performed before challenge at each visit. Each lavage used 10 ml of 0.9% physiologic saline solution at 37°C, which was instilled 5 ml into each nostril and then expelled into a collecting basin and discarded.

Five minutes after performing the nasal lavages, the challenge protocol began with the application of a disc containing 50 μ l of saline to the left nasal septal mucosa using a surgical headlight, a speculum, and duck bill forceps. The disc was left in place for 1 min. After removing the challenge disc and waiting 30 s, a preweighed collection disc was placed at the same spot on the challenged (ipsilateral) side, while an additional disc was placed at the corresponding location on the opposite (contralateral) side of the septal mucosa for 30 s. Discs were then removed, resealed in their original microtube, and reweighed. These discs represented the secretions recovered 2 min after the saline challenge. To fully recover all evoked nasal secretions, a second collection of nasal secretions was made 4 min and 30 s later by applying preweighed collection discs to both sides of the nasal mucosa for 30 s, which were then removed, resealed, and reweighed. These discs reflected the secretions

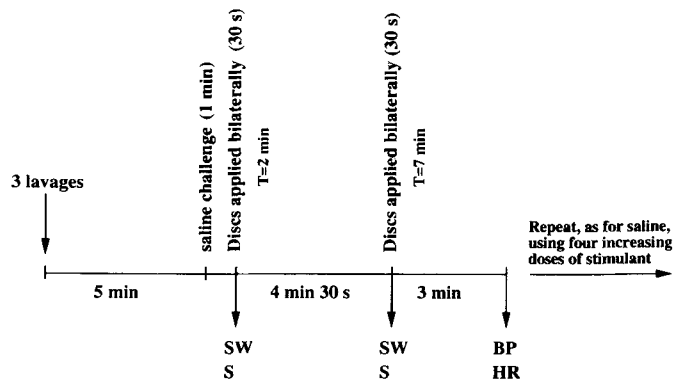


Figure 1. Schematic diagram of the nasal disc challenge protocol. Subjects made two visits separated by at least 24 h, where they received three nasal lavages and an initial baseline saline challenge followed by either four increasing doses of ET-1 or four challenges with repeated diluent (50 μ l saline), depending on the randomization protocol. SW = secretion weight; S = symptom recording; BP = blood pressure; HR = heart rate, T = time point.

recovered 7 min after the initial challenge. The subjects were then allowed to rest for 3 min.

Depending on the study schedule (Figure 1), the subjects then received discs containing 50 μ l of either increasing doses of ET-1 (final doses: 0.3, 1, 3, 10 μ g) or repeated applications of diluent (50 μ l saline), each of which was applied at 10-min intervals. These time intervals were chosen based on preliminary studies that indicated that nasal secretory responses to the highest dose of endothelin (10 μ g) had returned to baseline levels by 10 min. Collections of nasal secretions using preweighed discs were made at 2 and 7 min after each challenge dose of ET-1 or diluent, in a manner identical to that described for the baseline saline challenge. Increases in secretion weights were calculated by subtracting the precollection weight from the postcollection weight of each microtube containing the discs. For each side of the nose, data are presented as the sum of secretions collected at 2 min and 7 min after each challenge.

Throughout both study days, subjects maintained a symptom score record for symptoms of rhinorrhea, nasal congestion, itch, and pain on a 4-point scale from zero to three (zero equals no symptoms, three equals severe symptoms) in the ipsilateral and contralateral nostrils. In addition, the number of sneezes was counted. These symptom scores were also taken at 2 and 7 min postchallenge and summed separately for each side of the nose. Blood pressure and heart rate measurements were taken after each challenge and were not significantly affected by either ET-1 or diluent (data not shown).

Disc Elution and Measurement of Mediators

At the end of each experiment, 250 μ l of phosphate-buffered saline containing 40 mM EDTA was added to each microtube containing collection discs, and the tubes were incubated at 4°C for 30 min. For any given challenge stimulus, the eluates from discs used to collect secretions from a given nostril at 2 and 7 min postchallenge were pooled, aliquoted, and stored at -80° C.

Human serum albumin levels from the samples were assayed by non-competitive ELISA. A 1:900 dilution of sheep antibody to human albumin (primary antibody) was immobilized on 96-well microtiter plates overnight. Plates were then washed and nonspecific binding sites were blocked by incubation with 1% sheep serum for 30 min at room temperature. After washing the plate, standard albumin along with the appropriate samples were added to the plate and incubated at 37°C for 90 min. After washing, the plates were incubated for a further 90 min with a 1:1,000 dilution of sheep anti-human albumin antibody (secondary antibody), which was conjugated to horseradish peroxidase. Plates were then washed and the reaction developed using 1 mM 2,2'-azino-di-[3ethyl-benzthiazoline sulfonic acid] as a substrate, and the absorbance read at 410 nm. The assay standard curve for albumin ranged from 1 to 256 ng/ml.

Lysozyme was assayed in an identical manner to albumin by substituting a 1:400 dilution of sheep antibody to human lysozyme as the primary antibody and a 1:800 dilution of anti-human lysozyme antibody for the secondary antibody. The assay standard curve for lysozyme ranged from 1 to 128 ng/ml. Histamine release was determined using a radioenzymatic assay as described previously (22).

Statistics

Values of all parameters collected at both time points (2 and 7 min) following each dose of ET-1 or diluent were summed and expressed as mean $\pm$ SEM. Nonparametric methods were used for statistical analysis because the data obtained were not normally distributed. The responses of allergic and nonallergic subjects to the baseline saline challenge for each study day were compared by Kruskal-Wallis. To determine if the response to ET-1 or repeated diluent were significantly different to their appropriate baseline saline challenge, responses were compared by Friedman ANOVA for both the ipsilateral and contralateral nostrils. For comparisons across groups, area under the curve (AUC) values were calculated for each response parameter by subtracting the responses to the baseline saline challenge from each respective dose of ET-1 or repeated diluent and summing the resulting values. The AUC values therefore represent the overall increases above baseline for responses to ET-1 or diluent. AUC values between allergic and nonallergic subjects or between ET-1 and repeated diluent were compared by the Mann Whitney *U* test and are quoted in the results text where appropriate. For all comparisons, a *p* value < 0.05 was considered significant.

RESULTS

Nasal Responses to Repeated Diluent Challenge

Repeated application of diluent (saline) to the nasal mucosa did not increase secretion weights, lysozyme secretion, or symptoms of rhinorrhea, nasal congestion, itch, or pain above baseline saline responses in either group (Table). Repetitive diluent application did not increase the number of sneezes in allergic (baseline = 0.1 $\pm$ 0.1; AUC[diluent] = 1.7 $\pm$ 1.2) or nonallergic (baseline = 0; AUC[diluent] = 0) subjects. Repeated diluent did, however, induce a modest but significant ($p < 0.05$) increase in albumin levels in the contralateral nostril and also increased ipsilateral albumin levels of allergic subjects (Table 1), although this failed to achieve significance ($p = 0.07$).

Nasal Responses to Increasing Doses of ET-1

Secretion Weights. The effects of intranasal application of ET-1 (0.3–10 μ g) on secretion weights are illustrated in Figure 2. Ipsilateral and contralateral secretion weights after the baseline saline challenge were not significantly different between allergic and nonallergic subjects. Increasing doses of ET-1 induced a dose-related increase in ipsilateral ($p < 0.001$) and contralateral ($p < 0.05$) secretion weights in both allergic and nonallergic subjects when compared with their appropriate initial baseline saline responses. There was no significant difference between the responses induced on the ipsilateral side compared with the contralateral side after ET-1 challenge in the allergic group. Nonallergic subjects, however, had significantly lower secretion weights in the contralateral compared with the ipsilateral nostril ($p < 0.05$). Moreover, contralateral ($p < 0.05$) and ipsilateral ($p < 0.05$) secretion weights were significantly higher in the allergic compared with the nonallergic group.

Mediators. To compare the relative contribution of vascular and serous glandular components to nasal secretion weights, we determined levels of lysozyme (from serous glands) and albumin (from vascular leakage) present in the nasal secretions. Moreover, to assess whether mast cell activation could contribute to the responses observed with ET-1 challenge, histamine release was determined.

Ipsilateral and contralateral responses after the baseline saline challenge were not significantly different between allergic and nonallergic subjects for lysozyme, albumin, or histamine levels.

The effects of intranasal application of ET-1 (0.3–10 μ g) on lysozyme secretion is illustrated in Figure 3. ET-1 caused a significant dose-dependent increase in lysozyme secretion both ipsilaterally ($p < 0.001$) and contralaterally ($p < 0.001$) compared with the initial saline responses in nonallergic subjects, although ipsilateral lysozyme secretion was significantly higher than contralateral secretion ($p < 0.05$) in this group. ET-1 also evoked significant increases in lysozyme secretion ipsilaterally ($p < 0.05$) and contralaterally ($p < 0.05$) in the allergic group, which were not different between nostrils. In addition, allergic subjects had significantly increased lysozyme secretion in the contralateral ($p <$

TABLE 1
THE EFFECT OF REPEATED DILUENT CHALLENGE ON NASAL PARAMETERS

	Ipsilateral		Contralateral	
	Baseline	AUC (diluent)	Baseline	AUC (diluent)
Secretion weights, mg				
Allergic	10.2 $\pm$ 3.0	-4.1 $\pm$ -2.3	10.1 $\pm$ 3.4	-8.3 $\pm$ -2.9
Nonallergic	6.1 $\pm$ 2.5	-5.9 $\pm$ -5.9	5.9 $\pm$ 2.4	-3.0 $\pm$ -4.4
Lysozyme, μ g/ml				
Allergic	3.7 $\pm$ 2.6	-9.6 $\pm$ 10.4	1.1 $\pm$ 0.4	2.2 $\pm$ 1.9
Nonallergic	2.4 $\pm$ 1.7	-5.7 $\pm$ 7.0	0.8 $\pm$ 0.3	2.7 $\pm$ 1.3
Albumin, μ g/ml				
Allergic	6.3 $\pm$ 1.9	60.1 $\pm$ 31.9	6.1 $\pm$ 2.0	55.0 $\pm$ 28.4*
Nonallergic	12.6 $\pm$ 5.2	-4.5 $\pm$ -0.4	11.3 $\pm$ 4.1	23.0 $\pm$ 7.6
Rhinorrhea score				
Allergic	0.8 $\pm$ 0.3	2.1 $\pm$ 1.4	0.8 $\pm$ 0.3	0.7 $\pm$ 0.8
Nonallergic	0	0.3 $\pm$ 0.2	0	0.2 $\pm$ 0.2
Nasal congestion score				
Allergic	1.7 $\pm$ 0.6	1.6 $\pm$ 1.1	1.0 $\pm$ 0.5	3.2 $\pm$ 1.3
Nonallergic	0	0.8 $\pm$ 0.7	0.2 $\pm$ 0.2	1.3 $\pm$ 1.0
Itch score				
Allergic	0.4 $\pm$ 0.3	1.2 $\pm$ 0.6	0.4 $\pm$ 0.3	0.8 $\pm$ 0.4
Nonallergic	0	0.2 $\pm$ 0.2	0	0

Data represent mean $\pm$ SEM of nine allergic and nine nonallergic subjects. Baseline responses are those elicited by the initial saline challenge. AUC values represent the overall response above the baseline saline response for repeated diluent (see METHODS).

* $p < 0.05$ in comparison to baseline (Friedman ANOVA).

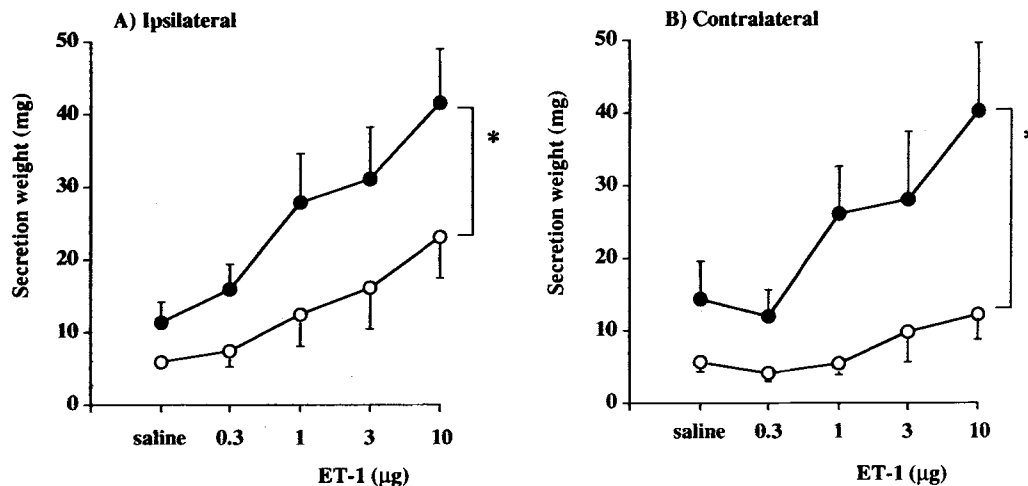


Figure 2. The effect of increasing doses of ET-1 on secretion weights of allergic (closed circles) and nonallergic (open circles) subjects in (A) the ipsilateral nostril and (B) the contralateral nostril. Data represent the mean and SEM of nine allergic and nine nonallergic subjects. ET-1 induced significant increases in secretion weights for both groups in both nostrils (see text). Allergic subjects had significantly higher secretion weights than nonallergic subjects (* $p < 0.05$). Ipsilateral secretion weights were significantly elevated in the ipsilateral compared with contralateral nostril of nonallergic subjects ($p < 0.05$).

0.05) nostril compared with nonallergic individuals, while ipsilateral lysozyme secretions were not different between the two populations.

The effects of ET-1 (0.3–10 μ g) on levels of albumin are represented in Figure 4. ET-1 caused a modest but significant increase in ipsilateral albumin levels in the allergic subjects ($p < 0.05$) compared with the baseline saline responses. Comparisons between the responses evoked by ET-1 and repeated diluent challenges (described previously) in the allergic subjects and the nonallergic subjects revealed that these results were not different.

ET-1 (0.3–10 μ g) did not induce significant histamine release compared with the initial baseline saline responses for allergic subjects in the ipsilateral (baseline = 0.5 ± 0.1 mg/ml; AUC

(ET-1) = 1.2 ± 0.6 mg/ml) or contralateral (baseline = 0.8 ± 0.3 mg/ml; AUC (ET-1) = 0.1 ± 0.3 mg/ml) nostrils. In the nonallergic subjects there was also no significant histamine release in the ipsilateral (baseline = 0.4 ± 0.1 mg/ml; AUC (ET-1) = 0.2 ± 0.3 mg/ml) or contralateral (baseline = 0.5 ± 0.2 mg/ml; AUC (ET-1) = 0.6 ± 1.0 mg/ml) nostrils.

Symptom scores. The effects of intranasal application of ET-1 (0.3–10 μ g) on symptom scores for rhinorrhea are illustrated in Figure 5. Baseline symptoms of rhinorrhea induced by the initial saline challenge were significantly higher in allergic subjects than nonallergic individuals both ipsilaterally ($p < 0.05$) and contralaterally ($p < 0.05$). In nonallergic individuals, ET-1 induced significant increases in ipsilateral ($p < 0.005$) and contralateral

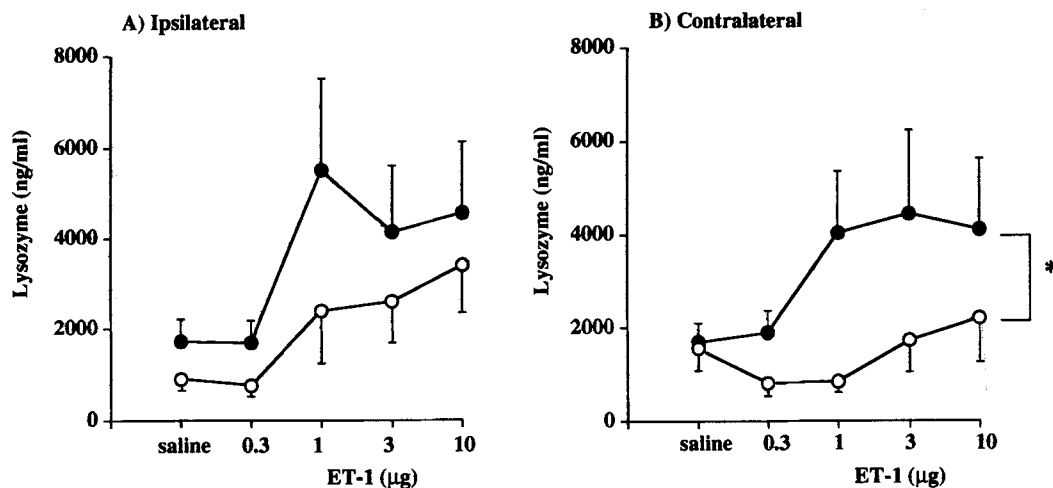


Figure 3. The effect of increasing doses of ET-1 on lysozyme secretion of allergic (closed circles) and nonallergic (open circles) subjects in (A) the ipsilateral nostril and (B) the contralateral nostril. Data represent the mean and SEM of nine allergic and nine nonallergic subjects. ET-1 induced significant increases in lysozyme for both groups in both nostrils (see text). Allergic subjects had significantly higher contralateral lysozyme secretion than nonallergic subjects (* $p < 0.05$). Ipsilateral lysozyme secretion was significantly elevated in the ipsilateral compared with contralateral nostril of nonallergic subjects ($p < 0.05$).

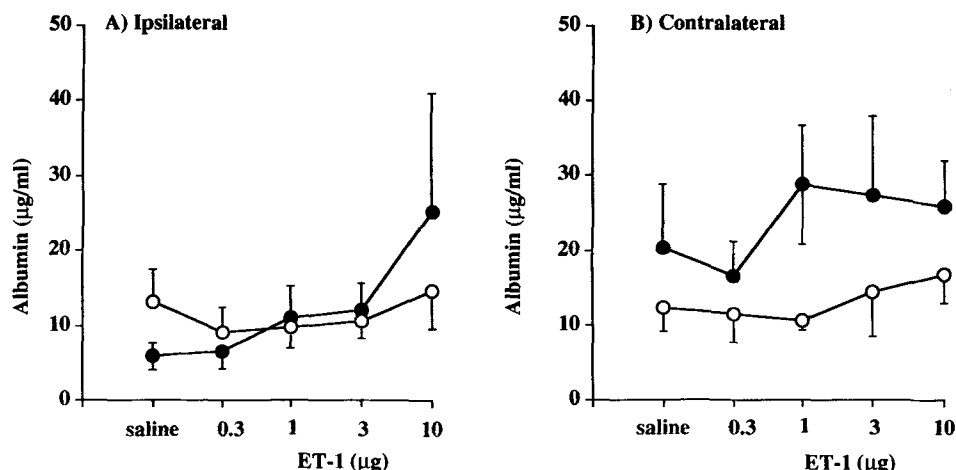


Figure 4. The effect of increasing doses of ET-1 on albumin secretion of allergic (closed circles) and nonallergic (open circles) subjects in (A) the ipsilateral nostril and (B) the contralateral nostril. Data represent the mean and SEM of nine allergic and nine nonallergic subjects.

($p < 0.05$) symptoms of rhinorrhea compared with the initial saline challenge, although there was significantly less rhinorrhea in the contralateral compared with the ipsilateral nostril ($p < 0.05$). ET-1 induced similar significant increases in symptoms of rhinorrhea ipsilaterally ($p < 0.005$) and contralaterally ($p < 0.005$) compared with the initial baseline saline challenge in allergic subjects. Allergic subjects, however, had significantly higher symptoms of rhinorrhea in the contralateral nostril ($p < 0.005$) than nonallergic subjects, while ipsilateral rhinorrhea symptoms were not different between the two groups.

Baseline symptoms of nasal congestion induced by the initial saline challenge were not different between allergic and nonallergic subjects in either nostril. Compared with the initial saline challenge, application of ET-1 (0.3–10 µg) to the nasal mucosa induced significant increases in symptoms of nasal congestion

in the ipsilateral nostril for both allergic (baseline = 1.33 ± 0.58 ; AUC (ET-1) = 4.56 ± 1.83 , $p < 0.05$) and nonallergic (baseline = 0.2 ± 0.2 ; AUC (ET-1) = 1.9 ± 0.8 ; $p < 0.005$) subjects, which were not significantly different between the two populations. Although there was a trend for an increase in contralateral symptoms of nasal congestion in the contralateral nostrils of allergic (baseline = 1.6 ± 0.4 ; AUC (ET-1) = 3.7 ± 1.8 ; $p = 0.08$) and nonallergic (baseline = 0.4 ± 0.3 ; AUC (ET-1) = 1.9 ± 1.4 ; $p = 0.07$) subjects after ET-1 provocation, this did not achieve significance. There were no differences for either group in symptoms of nasal congestion in the ipsilateral compared with the contralateral nostril.

Baseline symptoms of itch induced by the initial saline challenge were not different between allergic and nonallergic subjects in the ipsilateral (allergic = 0.4 ± 0.3 ; nonallergic baseline =

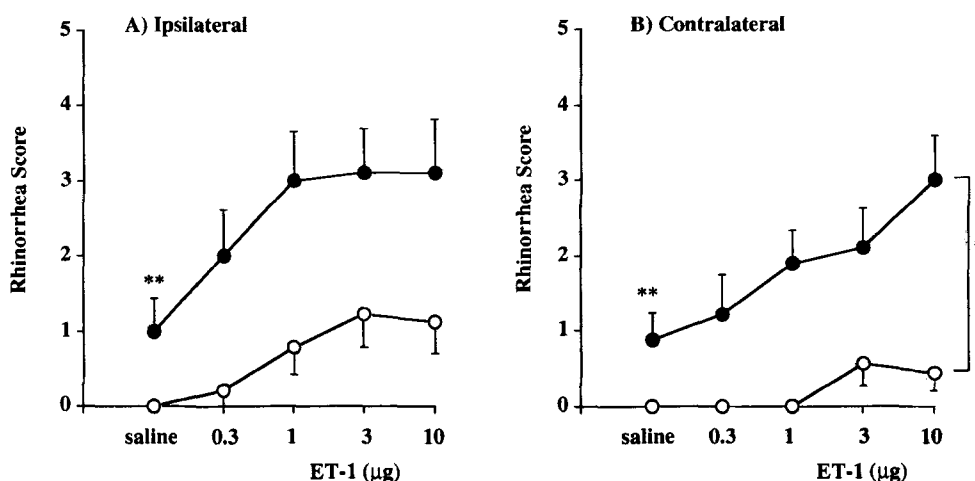


Figure 5. The effect of increasing doses of ET-1 on symptoms of rhinorrhea in allergic (closed circles) and nonallergic (open circles) subjects in (A) the ipsilateral nostril and (B) the contralateral nostril. Data represent the mean and SEM of nine allergic and nine nonallergic subjects. Symptoms of rhinorrhea after the baseline saline challenge were significantly higher in both nostrils of allergic subjects compared with nonallergic subjects (** $p < 0.05$). ET-1 induced significant increases in symptoms of rhinorrhea for both groups in both nostrils (see text). Allergic subjects had significantly higher contralateral symptoms of rhinorrhea than nonallergic subjects (* $p < 0.05$). Ipsilateral symptoms of rhinorrhea were significantly elevated in the contralateral compared with ipsilateral nostrils of nonallergic subjects ($p < 0.005$).

0.1 ± 0.1) and contralateral (allergic = 0.4 ± 0.3 ; nonallergic = 0) nostrils. Intranasal administration of ET-1 ($0.3\text{--}10\text{ }\mu\text{g}$) induced significant increases in symptoms of itch in the ipsilateral nostril of allergic (AUC [ET-1] = 2.9 ± 0.9 ; $p < 0.005$) and nonallergic (AUC [ET-1] = 2.6 ± 1.4 ; $p < 0.05$) subjects compared with baseline saline values. Ipsilateral symptoms of itch were not significantly different between allergic and nonallergic subjects. There was no effect of ET-1 on sensations of itch in the contralateral nostril of either allergic (AUC [ET-1] = 1.2 ± 0.5) or nonallergic (AUC [ET-1] = 0.6 ± 0.4) individuals.

Baseline numbers of sneezes induced by the initial saline challenge were not different between allergic and nonallergic subjects in either nostril (Figure 6). ET-1 ($0.3\text{--}10\text{ }\mu\text{g}$) caused a marked increase in the number of sneezes compared with baseline in both allergic ($p < 0.05$) and nonallergic ($p < 0.05$) subjects (Figure 6). Allergic subjects, however, had a significantly higher number of sneezes following ET-1 challenge than nonallergic subjects ($p < 0.05$).

DISCUSSION

The present study has investigated the responses of the human nasal mucosa to localized challenge with ET-1 in an attempt to understand the role this peptide may play in nasal physiology and to determine if the nasal response induced by this peptide can be modulated by allergic inflammation. To date there have been few studies examining the responses of the nasal mucosa to ET-1, and to our knowledge this is the first *in vivo* human study performed to directly assess this. By using paper disks to administer challenges and collect secretions, and by documenting the resulting symptoms, we have demonstrated that ET-1 can induce multiple nasal responses, including rhinorrhea, ipsilateral and contralateral glandular secretion, sneezing, and itching. Another interesting finding was the significant difference between allergic and nonallergic individuals in the effects of ET-1 on some of the parameters investigated, suggesting that allergic inflammation may alter nasal responsiveness to ET-1.

In both nonallergic and allergic individuals, ET-1 caused a significant increase in ipsilateral and contralateral secretion weights. Assessment of the components of the increased bilateral secretions after ET-1 challenge revealed enhanced levels of lysozyme, a serous glandular marker. This finding is in agreement with data obtained in cultured human nasal explants *in vitro* (18). With regard to the increased lysozyme secretion in the ipsilateral nostril, it is not known whether the effects of ET-1 are direct or indirect via the release of secondary mediators. However, ET-1 binding sites are present on serous glands in nasal turbinate explants (18), which would suggest a possible direct mechanism of action of ET-1 to cause glandular secretion. Moreover, ET-1 and ET-2, but not ET-3, caused glandular secretion from these same nasal explants *in vitro* (18), implying receptor-specific requirements for glandular secretion. Several ET receptor subtypes exist (23), although the one(s) responsible for mediating the ET-1-induced nasal responses is not known; assessment requires studies using clinically available subtype-specific receptor antagonists.

In addition to a significant increase in ipsilateral serous glandular secretion, ET-1 also induced an increase in contralateral secretion weights and lysozyme levels in allergic and nonallergic subjects. The presence of a contralateral glandular response is usually considered to be indicative of reflex glandular secretion induced by the activation of sensory neural pathways in the ipsilateral nostril (21, 24–27). In comparison to the secretory responses evoked by ET-1 in the ipsilateral nostril, nonallergic subjects had much lower secretion weights and lysozyme levels in the contralateral nostril, whereas allergic subjects had compara-

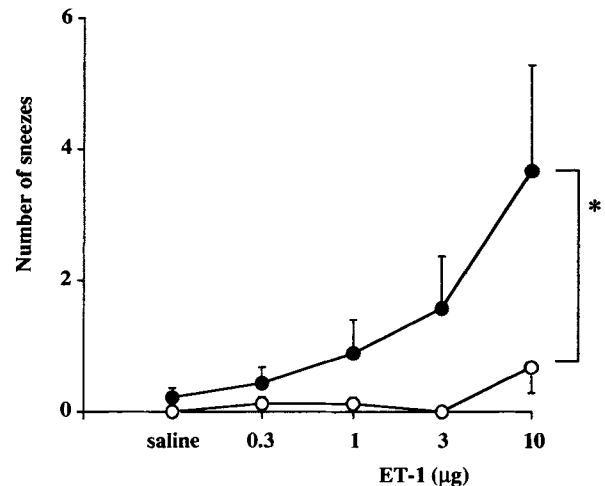


Figure 6. The effect of increasing doses of ET-1 on numbers of sneezes in allergic (closed circles) and nonallergic (open circles) subjects. Data represent the mean and SEM of nine allergic and nine nonallergic subjects. ET-1 induced significant increases in sneezing in allergic and nonallergic subjects compared with baseline (see text), although the number of sneezes evoked by ET-1 provocation was higher in allergic subjects (* $p < 0.05$).

ble responses in both. The fact that contralateral secretion weights were much lower than ipsilateral secretion weights in nonallergic subjects, whereas bilateral secretion weights were similar in allergic subjects, suggests that allergic inflammation may enhance the ability of ET-1 to induce reflex glandular secretion. This, together with the fact that allergic subjects sneezed more in response to ET-1 than nonallergic individuals, suggests that afferent neural pathways can be stimulated by ET-1 and that the responsiveness of these pathways may be enhanced by allergic inflammation. In common with the ability of allergic inflammation to alter nasal neural responsiveness to ET-1, neural responsiveness to bradykinin challenge is also enhanced in symptomatic allergic subjects using the same disc challenge method (28).

Whether ET-1 is acting directly on sensory nerve terminals in the nasal mucosa is not known because, to date, no studies have definitively addressed this issue. However, there have been reports of interactions between ET-1 and nerves. In cultured rat sensory neurons, for example, ET-1 potentiated capsaicin-induced calcitonin gene-related peptide (CGRP) release (29). ET receptors, mostly of the ET_B subtype, were localized to cell bodies, processes, and varicosities of cholinergic and adrenergic intramural autonomic nerves in primary cultures of guinea pig tracheal smooth muscle, and stimulation of these neuronal ET_B receptors elicited contraction of adjacent smooth muscle cells (30). Moreover, ET receptors have been localized to airway parasympathetic ganglia (10), and ET-3 can enhance the cholinergic contractile responses of rabbit bronchi induced by electrical field stimulation (11).

We have investigated histamine levels following ET-1 challenge to determine if ET-1 could induce nasal responses via histamine released as a result of mast cell degranulation (31). However, histamine levels were unchanged following ET-1 challenge. ET-1 has also been demonstrated to induce the release of Prostaglandin D₂ (PGD₂) from cultured human nasal explants (20) and from human lower airways (6), although in the latter case, ET-1 failed to evoke histamine release or peptidoleukotriene production (6). These data indicate that ET-1 may not cause mast cell activation and that the PGD₂ most likely came from other cellular sources,

possible macrophages or the microvasculature (6). The lack of histamine release in the lower human airways (6) is consistent with the present data and supports the hypothesis that histamine release from mast cell activation is not a critical mediator in the actions of ET-1 in human airways.

In an attempt to explore the responses of the nasal vasculature to ET-1, we examined the effects of ET-1 on symptoms of nasal congestion and albumin secretion. ET-1 caused a significant increase in nasal congestion in both allergic and nonallergic groups. Since ET-1 has potent vasoconstrictor effects, it would be expected that it would decrease nasal congestion by a vasoconstrictor action on the venous sinusoids where its binding sites are present (18). The reason ET-1 induced nasal congestion is not immediately apparent but may reflect the ability of endothelins to induce vasodilation under certain conditions. In this regard, it was recently shown that in isolated perfused rat lung preparations where tone had been increased, ET-3 caused vasodilation (32).

There was a modest but significant increase in albumin levels after repeated diluent (saline) challenges and by ET-1 exposure, although there were no differences between the two treatments. The levels of albumin are, however, very low and may reflect a minor irritant effect of repeated application of dry disks to the nasal mucosa. The fact that ET-1 did not induce significantly more albumin release than repeated diluent challenge suggests that ET-1 does not influence vascular permeability, which is in contrast to studies in animals. ET-1, for example, increased microvascular permeability in several tissues in the rat, including bronchi, via an ET_A receptor mechanism (33). Moreover, in perfused lung preparations from rats and guinea pigs, ET-1 elicits enhanced microvascular permeability and pulmonary edema (34–37). However, it was hypothesized that this was due to a hydrostatic mechanism rather than increasing vascular permeability *per se* (34, 35). The difference between our results and these other studies may reflect species differences in airway responses to this mediator.

In summary, ET-1 induces symptoms relevant to inflammatory upper airway diseases, such as glandular secretion and sneezing, in both allergic and nonallergic subjects, although these responses were increased in the allergic subjects. These observations therefore provide preliminary *in vivo* evidence for a role of allergic inflammation in enhancing the responsiveness of the nasal mucosa to ET-1.

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