

Ibuprofen augments bradykinin-induced glycoconjugate secretion by human nasal mucosa in vivo

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Bradykinin (BK) stimulates vascular permeability and glycoconjugate secretion in human nasal mucosa. Since some of the effects of BK may be mediated by autocrine generation of arachidonic acid metabolites, the influence of ibuprofen, a cyclooxygenase inhibitor, on BK-induced nasal secretion was studied. Six normal male subjects had nasal provocations with 0, 10, 100, and 1000 nmol of BK before and after treatment with 400 mg of ibuprofen. Secretions were collected by nasal lavage. Total protein (marker of protein secretion), glycoconjugate (mucous cell marker), lysozyme (serous cell marker), and albumin (marker of vascular permeability) were measured. Basal glycoconjugate secretion was higher after ibuprofen ($219 \pm 32 \mu\text{g/ml}$) than before ($81 \pm 56 \mu\text{g/ml}$; $p < 0.05$ by analysis of variance). BK stimulated significant, dose-dependent albumin, total protein, and glycoconjugate secretion. Lysozyme secretion was not stimulated. BK (1000 nmol) significantly increased total protein secretion, tenfold to twentyfold, and albumin secretion by 40-fold to 60-fold. Ibuprofen did not alter BK-induced total protein or albumin secretion. Glycoconjugate secretion after ibuprofen treatment was significantly higher than normal at 10 nmol ($p < 0.05$), 100 nmol ($p < 0.02$), and 1000 nmol of BK ($519 \mu\text{g/ml} \pm 74$ versus $213 \pm 15 \mu\text{g/ml}$; $p < 0.05$). Therefore, BK induces vascular permeability and exocytosis from glycoconjugate-containing cells but does not stimulate serous cells. Ibuprofen increases baseline secretion of glycoconjugate and enhances BK-induced glycoconjugate secretion. Ibuprofen does not alter BK-induced vascular permeability. (J ALLERGY CLIN IMMUNOL 1992;89:1032-9.)

Key words: Bradykinin, ibuprofen, albumin, glycoconjugate, mucus, lysozyme, mucosal secretion, rhinitis

BK is generated by human nasal mucosa during allergic, nonallergic, and infectious nasal inflammation.¹⁻⁵ BK induces vasodilation, vascular permeability, and the secretion of an albumin-rich fluid.⁶⁻¹¹ The

Abbreviations used

BK:	Bradykinin
OD:	Optical density
CV:	Coefficient of variation
ANOVA:	Analysis of variation
PGE ₂ , PGD ₂ :	Prostaglandins E ₂ and D ₂
15-HETE:	15-Hydroxyeicosatetraenoic acid
PAF:	Platelet-activating factor
LTC ₄ , LTD ₄ :	Leukotrienes C ₄ and D ₄
AA:	Arachidonic acid

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vascular permeability is likely due to the direct binding of BK to vascular B₂-receptors,^{9, 12} since ¹²⁵I-labeled BK binding sites are present on the vascular smooth muscle and endothelium of all vessels in human nasal mucosa.¹³ B₁-receptors have not been identified by human nasal provocation studies⁹ or competitive li-

gand binding studies in sheep turbinate¹² or human or guinea pig lung.¹⁴

In contrast to the vascular responses to BK, glandular secretion is less well studied. BK stimulates glycoconjugate secretion *in vitro* even though ¹²⁵I-labeled BK binding sites are not found on glands or the epithelium.¹³ This finding suggests an indirect route of stimulation. Since some of the effects of BK in respiratory mucosa may be due to autocrine release of PGE₂,^{15, 16} thromboxanes,¹⁷ peptidyl leukotrienes,¹⁸ 15-HETE,¹⁹ and PAF,²⁰ and since PAF,^{21, 22} LTC₄, LTD₄,²³⁻²⁶ and 15-HETE²⁷⁻²⁹ potentially stimulate mucous glycoconjugate release from respiratory tissues *in vitro* and *in vivo*,³⁰⁻³² it is possible that autocrine release of eicosanoids mediates BK-induced glandular secretion. If this finding is so, modulation of AA metabolism^{13, 22, 27, 33, 34} could alter the effects of BK on mucosal secretion.

The effects of ibuprofen on macromolecule secretion during BK nasal provocation were determined in normal subjects with the well-characterized nasal provocation technique according to the method of Raphael et al.³³⁻³⁸ Total protein (nonspecific measure of protein secretion), lysozyme (serous cell marker), glycoconjugate (marker of mucus-containing cells), and albumin (marker of vascular permeability) were measured with standard methods.³³⁻³⁸

METHODS

Subjects

Six normal men, aged 24 to 37 years, who gave informed consent, were studied with a protocol approved by the Royal Brompton Hospital Ethics Committee. None of the subjects had nasal symptoms, pathology, sensitivity to aspirin, or recent ingestion of nonsteroidal anti-inflammatory drugs.

Subjects had BK nasal provocation ("Normal" on Figs. 1 to 4), and then at least 3 days later, received ibuprofen (400 mg, by mouth) 2 and 6 hours before their second BK nasal provocation ("Ibuprofen" on Figs. 1 to 4). In preliminary studies, two BK nasal provocations in a separate group of normal subjects, separated by 3 days, had no effect on total protein, albumin, or glycoconjugate secretion (data not presented).

Sneezes were counted, and sensations of nasal and throat burning were noted. Rhinorrhea and the sensation of nasal congestion were not assessed since application of saline during the protocol could confound subject responses. Nasal patency was not measured.

Nasal provocation

An established technique of nasal provocation with saline lavage and vacuum-suction removal of the lavage fluid and nasal secretions was used.^{6, 33-37} Pliable 8F catheters with 2 to 3 mm holes, 3, 15, and 30 mm from the tip, were inserted 30 to 35 mm into each nostril and connected to a specimen trap and suction apparatus. Hand-actuated metered-dose in-

halers (Schering-Plough, Kenilworth, N.J.) were used to administer 5 to 10 ml of saline at 0.1 ml per spray during a 10-minute period to suspend, mobilize, and remove preexisting secretions. After a 10-minute waiting period, the washing was repeated with 5 ml of saline sprayed into each nostril in a 30- to 60-second span. These preliminary lavage fluids were discarded. The total time for the preliminary washing was approximately 20 to 25 minutes.

With the vacuum turned off, an initial challenge with 1 ml of saline was performed bilaterally by applying sequential doses of 0.1 ml of saline from a 1 ml syringe in a 30-second period onto the inferior turbinate and anterior inferior meatus and having the subject sniff. The vacuum was turned on 30 seconds later. After 10 minutes, 5 ml of saline was administered to each nostril, and the saline-induced secretions (0 nmol of BK) collected separately. Approximately 85% of the administered lavage fluid was recovered.

BK (1 mmol) was diluted with saline, and 1 ml aliquots containing 10, 100, and 1000 nmol were prepared. BK (10 nmol) was administered to one nostril in the same fashion as saline (above). After 10 minutes of incubation, both nostrils were separately lavaged with 5 ml of saline each as outlined above. Subsequent doses of 100 and 1000 nmol of BK were administered by the same protocol. The total procedure time was approximately 70 minutes.

Lavage fluids were vigorously shaken and then centrifuged (800 g for 5 minutes) to remove macroscopic mucous globules. Visual inspection of vigorously shaken samples revealed few macroscopic mucous globules in the present protocol. The resulting fluids were frozen for later analysis. Inclusion of macroscopic gel phase mucous globules can interfere with the colorimetric and turbidometric methods used for macromolecule quantification.

Total protein

The methods according to Baraniuk et al.⁶ and Lowry et al.³⁹ were scaled down for use in 96-well microtiter plates (NUNC, Copenhagen, Denmark). Bovine serum albumin was used as a standard. The OD (650 nm) was measured with a Dynatech (Alexandria, Va.) microtiter-plate reader. Sample protein concentrations were interpolated from standard curves. The CV was $\pm 2.4\%$.

Since BK was collected in the nasal secretions, the maximum possible contribution of BK to the total protein measurements was determined. If 100% of the administered 1000 nmol of BK was recovered in 5 ml of lavage fluid, the BK would account for 12.5 $\mu\text{g}/\text{ml}$. Total protein measurements for samples with 1000 nmol of BK were corrected for this amount.

Albumin

Human serum albumin was measured by a competitive ELISA.^{6, 33-37} The CV was $\pm 3.4\%$.

Glycoconjugate

An estimate of glycoconjugate mucopolysaccharide content was made with an hexose assay.^{6, 40, 41} In preliminary

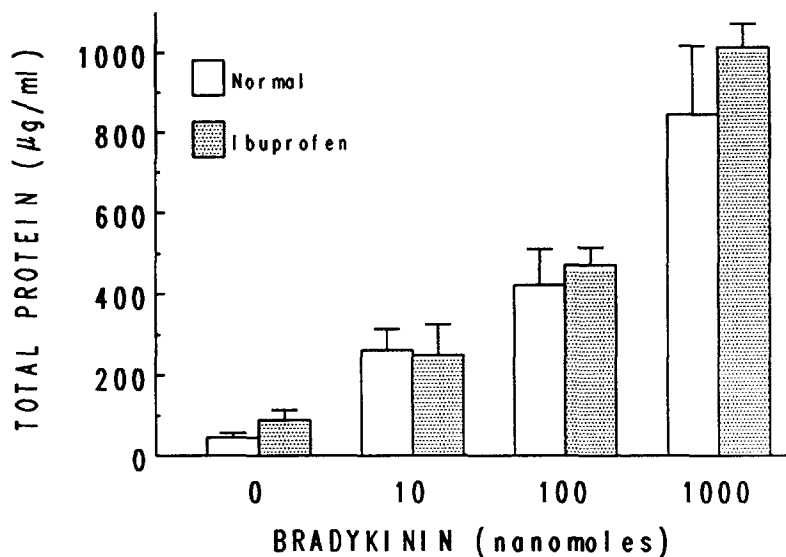


FIG. 1. Ibuprofen treatment had no significant effect on BK-induced total protein secretion. BK induced dose-dependent secretion before ibuprofen treatment (*open bars*) and after treatment (*hatched bars*).

studies, it was determined that all the hexose-containing material in lavage fluid was precipitated by ethanol or trichloroacetic acid and stained with alcian blue. Since alcian blue is a specific stain of goblet and mucous cells in human nasal mucosa, this infers that these cells were the origins of the lavage fluid glycoconjugates. Specific mucin and proteoglycan fractions produced by human nasal mucosa were not quantified.^{42, 43} Porcine stomach type II mucin (Sigma Chemical Co. Ltd., Poole, Dorset, England) in saline was used as a standard glycoconjugate. After hydrolysis in 0.2 N NaOH, samples were heated in orcinol-60% sulfuric acid to 80° C, and then the OD at 420 nm was measured. Sample concentrations were interpolated from standard curves. The CV was $\pm 2.0\%$. Since the concentrations of glycoconjugates are quoted in terms of micrograms of standard glycoconjugate per milliliter, these concentrations are not additive with total protein, lysozyme, or albumin concentrations. BK did not interfere with this assay.

To test if ibuprofen altered the yield of centrifugation-precipitable glycoconjugates, baseline samples from normal and ibuprofen-treated subjects were centrifuged (800 g for 5 minutes, as above), and the supernatants were carefully and completely aspirated. Original uncentrifuged samples, centrifuged supernatants, and centrifuge tube pellets, were hydrolyzed in NaOH (as above), and the orcinol assay was carried out.

Lysozyme

Standard solutions of lysozyme in pH 7.4 phosphate-buffered saline were prepared.^{6, 33} The reduction in OD of a suspension of *Micrococcus lysodieticus* cell walls (Sigma

Chemical Co. Ltd.) was measured at 450 nm. BK did not interfere with this assay. The CV was $\pm 3.6\%$.

Statistics

The mean $\pm$ standard error of the mean was determined for each parameter. Dose dependence of secretion was determined by ANOVA for repeated measurements. When significant dose dependence was found, differences from baseline values (0 nmol of BK) were compared for each dose by ANOVA, and the lowest dose producing a significant change is quoted in RESULTS. The effects of ibuprofen treatment were determined by comparing results before and after ibuprofen treatment by ANOVA. Significant differences caused by ibuprofen treatment are depicted in Figs. 1 to 4.

RESULTS

Symptoms

Nasal and throat burning were reported after 100 nmol (three subjects) and 1000 nmol of BK (five subjects). The same number of sneezes (0.7 ± 0.3 after 1000 nmol of BK) were recorded in the presence and absence of ibuprofen.

Total protein secretion

After 0 nmol of BK, normal subjects had 45 ± 11 $\mu\text{g/ml}$ of total protein in their nasal lavage fluid, whereas after ibuprofen, they had 91 ± 38 $\mu\text{g/ml}$ (Fig. 1). The difference was not significant ($p < 0.20$). BK induced significant dose-dependent

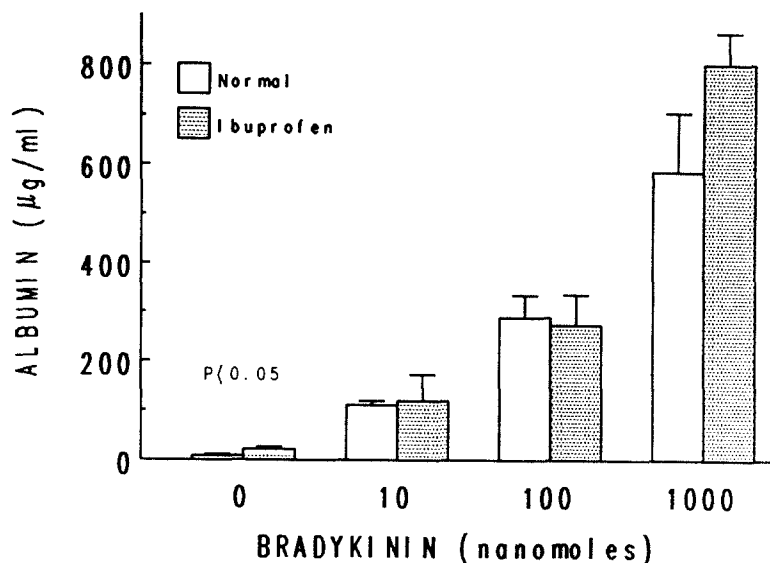


FIG. 2. Ibuprofen treatment had no significant effect on BK-induced albumin secretion. Ibuprofen treatment (hatched bars) induced significantly more albumin secretion after 0 nmol of BK compared with pretreatment levels (open bars) ($p < 0.05$). BK induced dose-dependent secretion of albumin both before and after ibuprofen treatment.

protein secretion. Secretion after 10 nmol of BK was significantly different from baseline ($p < 0.01$) both before and after ibuprofen treatment. After 1000 nmol of BK, total protein secretion reached 846 ± 168 $\mu\text{g/ml}$ in normal subjects and 1065 ± 75 $\mu\text{g/ml}$ after ibuprofen treatment ($p < 0.50$). Ibuprofen treatment did not significantly alter the total protein secreted in response to 10, 100, or 1000 nmol of topically applied BK.

Albumin secretion

After 0 nmol of BK, ibuprofen-treated subjects had significantly more albumin in their lavage fluid (22 ± 5 versus 9.0 ± 3.0 $\mu\text{g/ml}$; $p < 0.05$) (Fig. 2). BK induced significant dose-dependent albumin secretion before and after ibuprofen treatment. Secretion after 10 nmol of BK was significantly different from baseline before ($p < 0.01$) and after ($p < 0.05$) ibuprofen treatment. Ibuprofen treatment had no significant effect on the amounts of albumin secreted after 10, 100, or 1000 nmol of BK.

The ratio of albumin to total protein is a measure of vascular permeability.³⁶ The ratios rose from $20\% \pm 7\%$ (normal subjects before ibuprofen treatment) and $24\% \pm 2\%$ (ibuprofen-treated) under baseline conditions to $73\% \pm 7\%$ (normal subjects before ibuprofen treatment) and $76\% \pm 5\%$ (ibuprofen-treated) after 1000 nmol of BK. Therefore, treatment of normal subjects with ibuprofen marginally in-

creased their baseline albumin secretion but did not alter BK-induced albumin secretion.

Glycoconjugate secretion

After 0 nmol of BK, normal subjects secreted 81 ± 56 $\mu\text{g/ml}$ of glycoconjugate (Fig. 3), but ibuprofen-treated subjects secreted significantly more, 219 ± 32 $\mu\text{g/ml}$ ($p < 0.05$).

Centrifugation of baseline samples from normal (54 ± 29 $\mu\text{g/ml}$ after centrifugation versus 76 ± 11 $\mu\text{g/ml}$ if samples were uncentrifuged) and ibuprofen-treated (219 ± 52 $\mu\text{g/ml}$ after centrifugation versus 247 ± 19 $\mu\text{g/ml}$ if samples were uncentrifuged) subjects did not significantly reduce the amount of glycoconjugate in solution and did not yield detectable amounts of centrifugation-precipitable glycoconjugates. This finding indicates the absence of significant macroscopic mucous globules in these samples. Therefore, the increase in glycoconjugate secretion observed after ibuprofen treatment likely reflects a real increase in macromolecular secretion and not ibuprofen-induced dissociation of secreted mucous globules.

BK stimulated significant glycoconjugate secretion. Before ibuprofen treatment, normal subjects secreted significantly increased amounts of glycoconjugate only after 1000 nmol (247 ± 54 $\mu\text{g/ml}$; $p < 0.001$ compared with that of baseline). After ibuprofen treatment, however, BK induced significant glycoconjugate secretion with 100 nmol ($p < 0.05$ compared

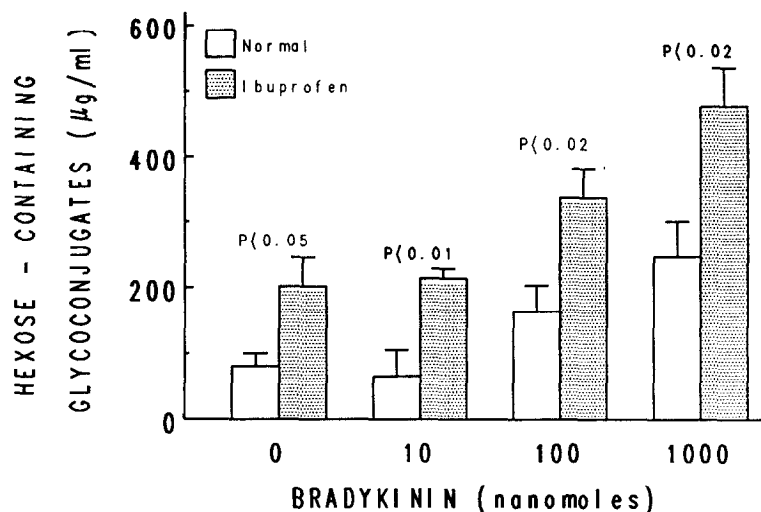


FIG. 3. Effect of ibuprofen treatment on BK-induced glycoconjugate secretion (*hatched bars*); significantly more glycoconjugate secretion after 0 nmol of BK compared with pretreatment levels (*open bars*) ($p < 0.05$). Significantly more BK-induced glycoconjugate secretion by ibuprofen-treated group after 10 nmol ($p < 0.01$), 100 nmol ($p < 0.02$), and 1000 nmol of BK ($p < 0.02$) compared with levels of pretreatment group.

with that of baseline). Significantly more glycoconjugate secretion was induced after ibuprofen treatment by 10 ($213 \pm 15 \mu\text{g/ml}$; $p < 0.01$), 100 ($332 \pm 45 \mu\text{g/ml}$; $p < 0.02$), and 1000 nmol of BK ($519 \pm 74 \mu\text{g/ml}$; $p < 0.05$) compared with pretreatment values (Fig. 3). Therefore, treatment of normal subjects with ibuprofen led to increased basal glycoconjugate secretion and increased BK-induced glycoconjugate secretion.

The glycoconjugate to total protein ratio, a measure of the relative contribution of glycoconjugates to the total amount of secretion, was 1.8 ± 0.8 before ibuprofen treatment and 2.2 ± 0.6 for ibuprofen-treated subjects after 0 nmol of BK. After 1000 nmol of BK, the ratio was 0.29 ± 0.06 before ibuprofen treatment and 0.46 ± 0.03 after ibuprofen treatment. The difference was significant ($p < 0.05$). The decrease in the glycoconjugate to total protein ratio was consistent with the increasing contribution of albumin secretion after BK provocation.

Lysozyme secretion

In baseline secretions, $9 \pm 3 \mu\text{g/ml}$ of lysozyme was secreted before ibuprofen treatment and $14 \pm 5 \mu\text{g/ml}$ after ibuprofen treatment (Fig. 4). BK did not induce lysozyme secretion before ibuprofen treatment. After ibuprofen treatment, 10 nmol of BK induced the highest concentration of secreted lysozyme ($35 \pm 9 \mu\text{g/ml}$), but this amount was not significantly different from the baseline value ($p < 0.10$). However, this amount was higher than that secreted

before ibuprofen treatment ($9 \pm 3 \mu\text{g/ml}$; $p < 0.05$). BK and ibuprofen did not have consistent, dose-dependent effects on serous-cell lysozyme secretion.

DISCUSSION

BK induced dose-dependent total protein and albumin secretion in normal male subjects, both before and after ibuprofen treatment. These and other results^{2, 6-11} indicate that the major effect of BK in human nasal mucosa is B_2 -receptor-mediated vasodilation and vascular permeability.^{9, 12, 13} Ibuprofen treatment had no effect on BK-induced vascular permeability. A similar lack of effect on vascular permeability has been observed in guinea pig airways.²⁰

BK stimulated dose-dependent glycoconjugate secretion before and after ibuprofen treatment. The dose of BK required to stimulate significant glycoconjugate secretion was tenfold higher than the dose required to increase vascular permeability. Therefore, BK appears to induce vascular permeability more readily than glycoconjugate secretion.

Although glycoconjugates are produced by submucosal mucous cells and epithelial goblet cells, submucosal serous cells produce lysozyme and other protein products.^{33, 38} In vitro, BK does not stimulate lysozyme exocytosis from human nasal mucosa (Peden D. Personal communication). This observation has now been confirmed for normal subjects in vivo. After ibuprofen treatment, however, there was a trend to higher lysozyme secretion in the baseline lavage

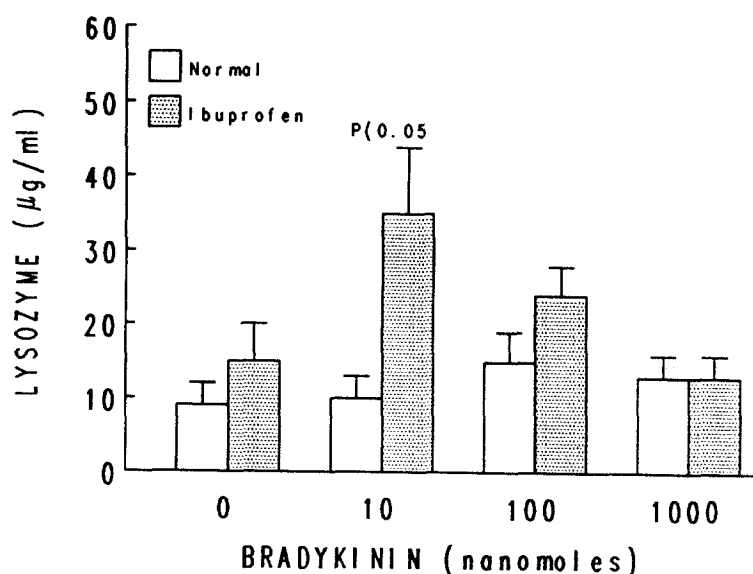


FIG. 4. Effect of ibuprofen treatment on BK-induced lysozyme secretion. BK did not stimulate lysozyme secretion. Ibuprofen treatment (hatched bars) induced significantly more lysozyme secretion after 10 nmol of BK ($p < 0.05$) compared with pretreatment levels (open bars); no other significant changes in lysozyme secretion.

and after the initial 10 nmol dose of BK. BK did not induce dose-dependent serous-cell exocytosis because subsequent doses did not produce any additional lysozyme secretion. It is possible that after ibuprofen treatment, BK induced contraction of glandular ducts and/or myoepithelial cells and caused the extrusion of lysozyme-containing mucus without serous-cell exocytosis. This observation is in contrast to the dose-dependent response of glycoconjugate-containing cells, suggesting that these cell populations may be regulated by different sets of mediators.

Autocrine generation of PAF and AA metabolism could account for some of the effects of BK in respiratory mucosa.¹⁵⁻²⁰ BK receptors may be linked to phospholipase A₂, phospholipase C, and other signal-transducing enzymes,^{44, 45} since administration of BK in various systems generates PGE₂,^{15, 16} thromboxanes,¹⁷ peptidyl leukotrienes,¹⁸ 15-HETE,¹⁹ and PAF.²⁰ LTC₄, LTD₄, and 15-HETE potentially stimulate mucous glycoconjugate release.²¹⁻³¹ Cyclooxygenase inhibition and shunting of AA to the lipoxygenase pathways could lead to increased generation of LTC₄ and LTD₄.⁴⁶ Increased production of prosecretory eicosanoids, or decreased production of inhibitory prostaglandins, could potentially explain the increased amounts of glycoconjugate secretion observed under basal conditions and after BK by ibuprofen-treated subjects, as well as similar effects in vitro.^{22, 27} However, ibuprofen has been found to decrease BK-induced glycoconjugate secretion from human nasal mu-

cosal explants in vitro.¹³ The explanation for this contrary effect in vitro is unclear but could be due to other physical properties of the drug⁴⁷ or differences between in vivo and in vitro conditions.

The inability of ibuprofen to alter vascular permeability suggests that the direct effects of BK alone were so potent that any additional contribution by lipid and other autocrine vasoactive agents could not be detected. Nasal provocations with LTC₄,⁴⁸ LTD₄,⁴⁹ PGD₂,⁵⁰ and PAF-acether⁵¹ induce vasodilation with increased nasal airway resistance but without pruritus, sneezing, or significant rhinorrhea in the doses used. However, the constituents of the secretions from these nasal provocations have not been biochemically analyzed, and therefore, the effects on glandular exocytosis and vascular permeability have not been evaluated. It will be important to analyze the macromolecules secreted after provocation with these agents to determine their relative effects on vascular and glandular processes in vivo.

BK-induced vasodilation, vascular permeability, and glycoconjugate secretion could be of pathologic importance during rhinitis, asthma, chronic bronchitis, aspirin sensitivity, viral infections, and other inflammatory states. The recent introduction of specific BK, PAF, and leukotriene antagonists will allow exploration of the specific effects of BK on vascular permeability and glycoconjugate secretion in pathologic settings and determine if BK generates autocrine mediator release in human nasal mucosa in vivo.

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REFERENCES

- Proud D, Kaplan AP. Kinin formation: mechanisms and role in inflammatory disorders. *Ann Rev Immunol* 1988;6:49-83.
- Proud D, Reynolds CJ, Lacapra S, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am Rev Respir Dis* 1988;137:613-6.
- Proud D, Togias A, Naclerio RM, Crush SA, Norman PA, Lichtenstein LM. Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J Clin Invest* 1983;72:1678-85.
- Naclerio RM, Proud D, Lichtenstein LM, et al. Kinins are generated during experimental rhinovirus colds. *J Infect Dis* 1987;157:133-42.
- Proud D, Naclerio RM, Gwaltney JM Jr, Hendley JO. Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis* 1990;161:120-3.
- Baraniuk JN, Brayton P, Kaliner MA, Barnes PJ. Perennial rhinitis subjects have altered vascular, glandular, and neural nasal responses to bradykinin [Abstract]. *Am Rev Respir Dis* 1991;143:617.
- Pongratic JA, Naclerio RM, Reynolds CJ, Proud D. A competitive kinin receptor antagonist, [D-Arg0, Hyp3, D-Phe7]-bradykinin, does not affect the response to nasal provocation with bradykinin. *Br J Clin Pharmacol* 1991;31:287-94.
- Holmberg K, Bake B, Pipkorn U. Vascular effects of topically applied bradykinin on the human nasal mucosa. *Eur J Pharmacol* 1990;175:35-41.
- Rajakulasingam K, Polosa R, Holgate S, Howarth P. Comparative nasal effects of bradykinin, kallidin, and [des-Arg9]-bradykinin in atopic rhinitic and normal volunteers. *J Physiol* 1991;437:557-87.
- Rajakulasingam K, Polosa R, Lau LCK, Holgate ST, Howarth PH. The nasal effects of bradykinin (B) and capsaicin (C): role of "C" sensory fibres [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:215.
- Erjafalt I, Persson CGA. Allergen, bradykinin and capsaicin increase outward but not inward macromolecule permeability of guinea pig tracheobronchial mucosa. *Clin Exp Allergy* 1991;21:217-24.
- Lyon RA, Weisshaar PS, Phipps RJ. Identification and characterization of B₂ bradykinin receptors in sheep nasal turbinate membranes. *Pulm Pharmacol* 1990;3:209-14.
- Baraniuk JN, Lundgren JD, Mizoguchi H, et al. Bradykinin receptor distribution in human nasal mucosa, and analysis of secretory responses in vitro and in vivo. *Am Rev Respir Dis* 1990;141:706-14.
- Mak JCW, Barnes PJ. Autoradiographic visualization of bradykinin receptors in human and guinea pig lung. *Eur Respir J* 1991;194:37-43.
- Aizawa H, Shigematsu N. Noninvasive superfusion of canine tracheal segment to study prostaglandin release in vivo. *Fukuoka Igaku Zasshi* 1989;80:95-101.
- Leikauf GD, Ueki IF, Nadel JA, Widdicombe JH. Bradykinin stimulates chloride secretion and prostaglandin E₂ release by canine tracheal epithelium. *Am J Physiol* 1985;248(1 Pt 2):F48-F55.
- Lau WA, Rechtman MP, Boura AL, King RG. Synergistic potentiation by captopril and propranolol of bradykinin-induced bronchoconstriction in the guinea pig. *Clin Exp Pharmacol Physiol* 1989;16:849-57.
- Leikauf GD, Ueki IF, Widdicombe JH, Nadel JA. Alteration of chloride secretion across canine tracheal epithelium by lip-oxygenase products of arachidonic acid. *Am J Physiol* 1986;250(1 Pt 2):F47-F53.
- Salari H, Chan-Yeung M. Release of 15-hydroxyeicosatetraenoic acid (15-HETE) and prostaglandin E₂ (PGE₂) by cultured human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1989;1:245-50.
- Rogers DF, Dijk S, Barnes PJ. Bradykinin-induced plasma extravasation in guinea pig airways: involvement of platelet-activating factor. *Br J Pharmacol* 1990;101:739-45.
- Goswami SK, Ohashi M, Stathas P, Marom Z. Platelet-activating factor stimulates secretion of respiratory glycoconjugate from human airways in culture. *J ALLERGY CLIN IMMUNOL* 1989;84:726-34.
- Lundgren JD, Kaliner M, Logun C, Shelhamer JH. Platelet-activating factor and tracheobronchial respiratory glycoconjugate release in feline and human explants: involvement of the lipooxygenase pathway. *Agents Actions* 1990;30:329-37.
- Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C₄ and D₄ increase the release of mucus from human airways in vitro. *Am Rev Respir Dis* 1982;126:449-51.
- Coles SJ, Neill KH, Reid LM. Effects of leukotrienes C₄ and D₄ on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins* 1983;25:155-70.
- Peatfield AC, Piper PJ, Richardson PS. The effect of leukotriene C₄ on mucus release into the cat trachea in vivo and in vitro. *Br J Pharmacol* 1982;77:391-6.
- Johnson HG, Chinn RA, Chow AW, Bach MK, Nadel JA. Leukotriene C₄ enhances mucus production from submucosal glands in canine trachea in vivo. *Int J Immunopharmacol* 1983;5:391-6.
- Marom Z, Shelhamer JH, Kaliner M. The effects of arachidonic acid, monohydroxyeicosatetraenoic acid, and prostaglandins on the release of mucous glycoproteins from human airways in vitro. *J Clin Invest* 1981;67:1695-1702.
- Marom Z, Shelhamer JH, Sun F, Kaliner M. Human airway monohydroxyeicosatetraenoic acid generation and mucus release. *J Clin Invest* 1983;72:122-7.
- Johnson HG, McNee ML, Sun FF. 15-hydroxyeicosatetraenoic acid is a potent inflammatory mediator and agonist of canine tracheal mucus secretion. *Am Rev Respir Dis* 1985;131:917-22.
- Lundgren JD, Shelhamer JH. Pathogenesis of airway mucus hypersecretion. *J ALLERGY CLIN IMMUNOL* 1990;85:399-417.
- Lundgren JD, Shelhamer JH, Kaliner MA. The role of eicosanoids in respiratory mucus hypersecretion. *Ann Allergy* 1985;55:5-12.
- Kowalski M, Wojciechowska B, Sliwiska-Kowalska M, et al. Biochemical analysis of nasal secretions induced by aspirin (ASA) challenges in patients with ASA sensitivity, nasal polyps, and chronic rhinosinusitis [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:216.
- Raphael GD, Jeney EV, Baraniuk JN, Kim I, Meredith SD, Kaliner MA. The pathophysiology of rhinitis: lactoferrin and lysozyme in nasal secretions. *J Clin Invest* 1989;84:1528-35.
- Meredith SD, Raphael GD, Baraniuk JN, Banks SM, Kaliner MA. The pathophysiology of rhinitis. III. The control of IgG secretion. *J ALLERGY CLIN IMMUNOL* 1989;84:920-30.
- Raphael GD, Druce HM, Baraniuk JN, Kaliner MA. Pathophysiology of rhinitis. I. Assessment of the sources of protein in methacholine-induced nasal secretions. *Am Rev Respir Dis* 1988;138:413-20.
- Raphael GD, Meredith SD, Baraniuk SD, Druce HM, Banks

- SM. Kaliner MA. The pathophysiology of rhinitis. II. Assessment of the sources of protein in histamine-induced nasal secretions. *Am Rev Respir Dis* 1989;139:791-800.
37. Raphael GD, Hupfisch-Raphael M, Kaliner MA. Gustatory rhinitis: a syndrome of food-induced rhinorrhea. *J ALLERGY CLIN IMMUNOL* 1989;83:110-5.
38. Raphael GD, Baraniuk JN, Kaliner MA. How and why the nose runs [Postgrad course]. *J ALLERGY CLIN IMMUNOL* 1991;87:457-67.
39. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:165-75.
40. Rogers DF, Barnes PJ. Opioid inhibition of neurally mediated mucus secretion in human bronchi. *Lancet* 1989;1:930-1.
41. Winzler RJ. Determination of serum glycoproteins. *Methods Biochem Anal* 1955;11:279-320.
42. Patow CA, Shelhamer J, Marom Z, et al. Production of mucous glycoproteins by nasal turbinates in vitro. *Otolaryngol* 1984;92:424-6.
43. Patow CA, Shelhamer J, Marom Z, Logun C, Kaliner M. Analysis of human nasal mucous glycoproteins. *Am J Otolaryngol* 1984;5:334-43.
44. Smith JJ, McCann JD, Welsh MJ. Bradykinin stimulates airway epithelial cell Cl⁻ secretion via two second messenger pathways. *Am J Physiol* 1990;258(6 Pt 1):L369-L77.
45. Birnbaumer L, Brown AM. G proteins and the mechanisms of action of hormones, neurotransmitters, and autocrine and paracrine regulatory factors. *Am Rev Respir Dis* 1990;141:S106-S14.
46. Juergens UR, Christiansen SC, Stevenson DD, Zuraw BL. Elevated thromboxane B₂ (TxB₂) and leukotriene B₄ (LTB₄) secretion from monocytes of aspirin (ASA) sensitive asthmatic patients [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:221.
47. Brooks PM, Day RO. Drug therapy: nonsteroidal antiinflammatory drugs—differences and similarities. *New Engl J Med* 1991;324:1716-25.
48. Bisgaard H, Olsson P, Bende M. Effect of leukotriene D₄ on nasal mucosal blood flow, nasal airway resistance, and nasal secretion in humans. *Clin Allergy* 1986;16:289-97.
49. Miadonna A, Tedeschi A, Leggieri E, et al. Behaviour and clinical relevance of histamine and leukotrienes C₄ and B₄ in grass pollen induced rhinitis. *Am Rev Respir Dis* 1987;136:357-62.
50. Howarth P, Walsh S, Robinson C. Prostaglandin D₂ a mediator of nasal blockage [Abstract]. *J ALLERGY CLIN IMMUNOL* 1990;85:223.
51. Miadonna A, Leggieri E, Tedeschi A, Bianco A, Lorini M. PAF-acether increases nasal airway resistances in man [Abstract]. *J ALLERGY CLIN IMMUNOL* 1990;85:223.