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Aminopeptidase activity in human nasal mucosa

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Background: Aminopeptidases activate bradykinin and degrade many inflammatory peptides.
Objective: The objective of this study was to identify the types of aminopeptidase activities in human nasal mucosa.
Methods: Human nasal mucosa was homogenized (n = 12), and cytoplasmic (S2) and membrane-rich (P2) fractions were obtained. Several aminopeptidase (Ap) activities were defined by (1) substrate specificity with leucine-enkephalin (leu-Ap) and alanine-nitroanilide (ala-Ap), (2) inhibitor studies with puromycin and bestatin, (3) enzyme activity histochemistry (zymography), (4) immunohistochemistry, and (5) gel electrophoresis. Human volunteers had methacholine, histamine, and allergen nasal provocations to determine the mechanisms controlling nasal aminopeptidase secretion in vivo.
Results: P2 was the largest reservoir of puromycin-resistant aminopeptidase activity (630 pmol leu-enk/min/mg protein). S2 contained 32 pmol leu-enk/min/mg activity, with 80% representing puromycin-resistant activity and 20% puromycin-sensitive aminopeptidase (PS-Ap). Ala-Ap was detected in both P2 and S2 fractions and was localized by zymography to epithelial and gland cells. Anti–rat brain–soluble PS-Ap IgG detected immunoreactive material in epithelium, glands, and endothelium. In nasal provocation studies, leu-AP correlated with glandular exocytosis but not vascular leak.
Conclusions: The predominant aminopeptidase in human nasal epithelial and submucosal gland cells was membrane-bound puromycin-resistant aminopeptidase. A novel soluble puromycin-resistant aminopeptidase and lower amounts of soluble PS-Ap were also detected. (J Allergy Clin Immunol 1998;102:741-50.)

Key words: *Enkephalin-degrading aminopeptidase, puromycin sensitive aminopeptidase, aminopeptidase M, glandular secretion, bradykinin, rhinitis*

Abbreviations used
ACE: Angiotensin-converting enzyme
Ala-Ap: Alanine aminopeptidase
Ala-MNA: L-alanine-4-methoxy-2-naphthylamide
Ala-NA: Alanine-*p*-nitroanilide
ApM: Aminopeptidase M
Leu-Ap: Leucine-enkephalin–degrading aminopeptidase
NEP: Neutral endopeptidase
PS-Ap: Puromycin-sensitive aminopeptidase

A wide range of peptidolytic activities has been described including aminopeptidases that digest peptides from their N-terminus, carboxypeptidases,[1] and neutral endopeptidase (NEP)[2] and angiotensin-converting enzyme (ACE)[3] that cleave internal peptide bonds. In airway mucosa, aminopeptidases may be critical for the generation of bradykinin but may also reduce the duration of action of bradykinin, neuropeptides released from nociceptive neurons (eg, substance P), and other proinflammatory peptides.[4-7]

Aminopeptidase activities have frequently been identified with the use of leucine-enkephalin (enkephalin–degrading aminopeptidase, leu-Ap) as a substrate.[4,8] The major enkephalin-degrading aminopeptidase activities were initially described on the basis of their arylamidase activity[8] but have subsequently been referred to as either aminopeptidase M (ApM, also known as aminopeptidase N, CD13) or the puromycin-sensitive aminopeptidase (PS-Ap).[9-12] ApM is an integral membrane protein that is relatively resistant to inhibition by puromycin with coefficients of inhibition (Ki) of 100 μmol/L to 1 mmol/L.[4,13] PS-Ap is predominantly a cytosolic enzyme but can be found associated with membranes. It is more sensitive to inhibition by puromycin (Ki approximately 1 μmol/L).[4,7] Both ApM and PS-AP are inhibited by bestatin. The cDNAs for rat kidney ApM[9] and human PSAp[14] have been cloned and their respective amino acid sequences deduced.

To begin defining the physiology of aminopeptidases in airways, we have used multiple assays to characterize aminopeptidase activity in soluble and membrane fractions of human nasal mucosa. Substrate specificity was assessed with the use of leucine-enkephalin (leu-Ap activity) and alanine-nitroanilide (ala-Ap activity). Inhibition of leu-Ap by puromycin defined PS-Ap and rela-

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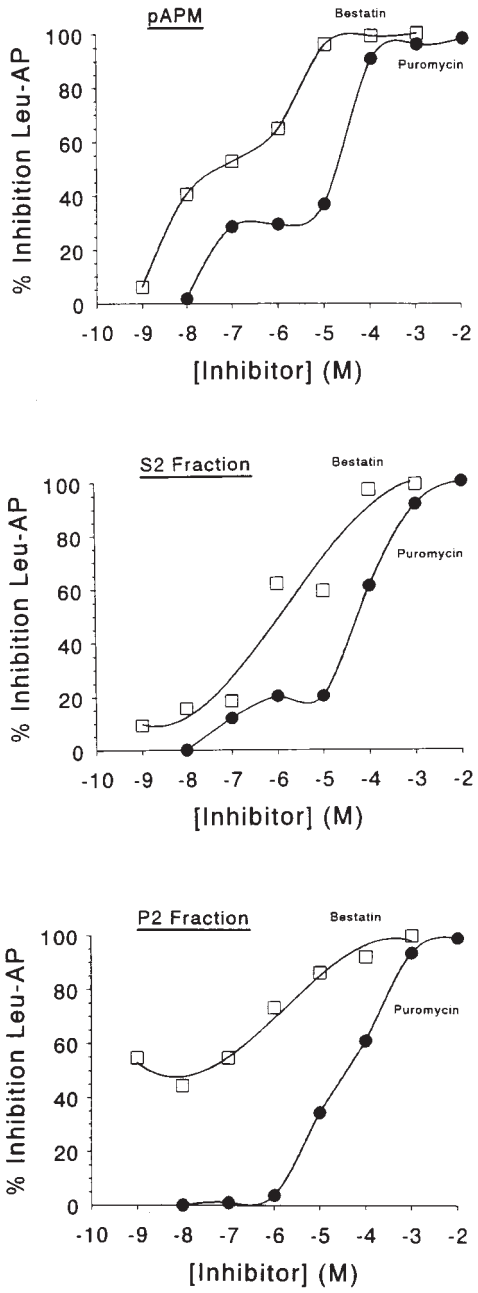

FIG 1. Inhibition of leucine-enkephalin–degrading aminopeptidase *(leu-AP)* by bestatin (*open circles*) or puromycin (*closed circles*) in human nasal mucosal S2 and P2 fractions.

tively resistant activities. Similar studies were performed with bestatin. Ala-Ap activities were localized on cryostat tissue sections by enzyme activity histochemistry (zymography), whereas PS-Ap–immunoreactive material was identified by immunohistochemistry. This study complements previous analyses of neutral endopeptidase (neprilysin, NEP, CD10, CALLA, gp100),[2] ACE,[3] and carboxypeptidase N (CPN)[1] that also modulate neurogenic and other peptide-mediated inflammatory events.[5]

METHODS

Human inferior turbinate processing

Human inferior nasal turbinates were obtained at the time of surgery from 12 patients with nasal airway obstruction as previously described.[1-3] Tissue was dissected from the conchal bones within 20 minutes of excision and immediately prepared for future use.

Six mucosal specimens were used for enzyme assays and were frozen in 2-methylbutane on dry ice for 20 seconds and then stored at 70° C.[1-3] Frozen turbinates were weighed (average wet weight 371 ± 60 mg, range 168 to 653 mg), finely dissected with razor blades, suspended in 10 volumes (volume/wet tissue weight) of 0.32 mol/L sucrose in 50 mmol/L tris HCl (pH 7.4), and homogenized with a Polytron homogenizer (STD Tissumizer, Tekmar, Cincinnati, Ohio) at 4° C at maximum speed for 30 seconds twice. The homogenates were centrifuged at 800*g* for 5 minutes at 4° C and supernatants were collected. The pellets were suspended again in the same buffer and recentrifuged at the same rate and the pellet discarded. Both supernatants were combined and designated S1. S1 was centrifuged at 100,000*g* for 60 minutes at 4° C and the resulting supernatant was collected (S2). The 100,000*g* pellet (membrane fraction) was washed and resuspended in 50 mmol/L tris HCl (pH 7.4) and was designated as P2.

Leu-Ap assay

Nasal extract samples (10 μL) were incubated in 40 μL of 10 mmol/L tris HCl buffer (pH 7.4) with 50 μL of [^{3}H]leuenkephalin (39.8 Ci/mmol, NENDupont, Wilmington, Del; 36.9 nmol/L final concentration) as a tracer and leucine-enkephalin (10 μmol/L final concentration; Peninsula Laboratories, Belmont, Calif) as substrate.[4,15-17] Phosphoramidon (1 μmol/L final concentration; Peninsula) and captopril (10 μmol/L final concentration; provided as a gift from Squibb, Princeton, NJ) were added to inhibit NEP and ACE activities, respectively. Incubations were stopped after 60 minutes by addition of 50 μL of 2N HCl. The metabolite [^{3}H]tyrosine was separated from nonmetabolized enkephalin by chromatography on a Porapack Q 100-120 mesh polystyrene bead column (Waters, Milford, Mass). Blanks were obtained from parallel incubations performed in the presence of 1 mmol/L puromycin (Calbiochem, La Jolla, Calif) or 10 μmol/L bestatin (Calbiochem). Porcine aminopeptidase M (Sigma Chemical Co, St Louis, Mo) was used as a standard for confirming assay conditions and inhibitor activities. Enkephalin-degrading aminopeptidase activity was expressed as picomoles of leuenkephalin metabolized per minute per milligram of protein for extracts, or as picomoles of leu-enkephalin metabolized per minute per milliliter for nasal lavage fluid samples.

Puromycin and bestatin inhibition studies

Puromycin and bestatin were added to the leu-Ap in concentrations of 10^{-9} to 10^{-2} mol/L and the Ki defined as the concentration reducing leu-Ap activity by 50%.

Alanine aminopeptidase (Ala-Ap) assay samples (25 μL) were added to cuvettes containing 1.65 mL of 10 mmol/L tris HCl buffer (pH 7.4), 1 μmol/L phosphoramidon, and 10 μmol/L captopril, mixed thoroughly, and incubated for 1 minute. L-alanine-4-nitroanilide (50 μL, ala-NA; Sigma; concentration 65 mmol/L) was added and the kinetics of nitroanilide formation measured by absorbance at 405 nm (A_{405}) over time (T, minutes).[18] The catalytic concentration of the samples (b in units per milliliters) were calculated as $b = 6566 \times A_{405}/T$. Puromycin (1 mmol/L) was added to negative control samples.

Substrate competition studies

The relative activities toward leuenkephalin and ala-NA were compared in each S2 and P2 sample by using competition assays.

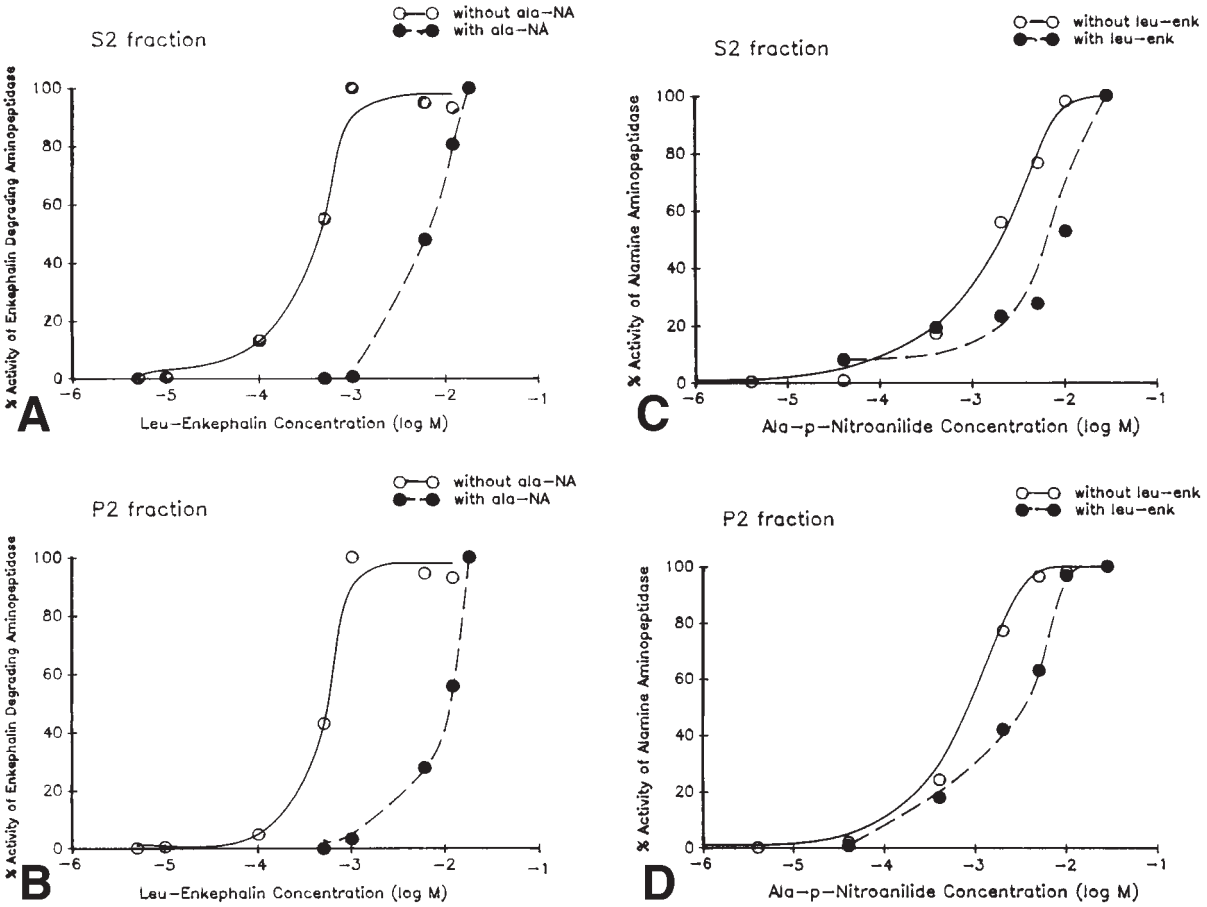

FIG 2. Substrate competition study in human nasal mucosal extracts. **A**, Leucine-enkephalin–degrading aminopeptidase *(leu-Ap)* activities in S2 fractions (*open circles*) were inhibited and shifted right by addition of alanine-*p*-nitroanilide *(ala-NA)* (*filled circles*). **B,** Leu-Ap activities in P2 fractions (*open circles*) were inhibited and shifted right by adding ala-NA (*filled circles*). **C,** Alanine aminopeptidase *(ala-Ap)* activity of S2 fractions (*open circles*) was inhibited and shifted right by adding leucine enkephalin *(leu-enk, filled circles).* **D,** Ala-Ap activities in P2 fractions (*open circles*) were inhibited and shifted right by adding leu-enk (*filled circles*).

The Km was determined for each substrate. In the first set of competition studies, leu-enkephalin was added at its Km concentration along with a wide range of ala-NA concentrations to determine the effect of leu-enkephalin on the ala-NA assay. Similarly, ala-NA was added at its Km to the leu-enkephalin assays to determine their degree of interaction.

Antibody to PS-Ap

Rat brain–soluble PS-Ap was purified as described.[11-13,16] A female goat was injected subcutaneously with this enzyme, then boosted with 200 μg of protein in incomplete Freund's adjuvant 2 and 6 weeks later. After phlebotomy, the IgG fraction was obtained by precipitation with 45% ammonium sulfate and dialyzed against PBS.

Native-PAGE and enzyme blot

Nasal extracts were treated with native sample buffer without SDS or mercaptoethanol.[1-3] Samples (10 μL) and buffer (negative control) were applied to 6% Tris-glycine polyacrylamide gels (Novex Experimental Technology, San Diego, Calif), electrophoresed, then stained for protein with Coomassie blue (Pharmacia LKB, Piscataway, NJ). Enzyme activity was detected by placing gels in 1.5 mmol/L L-alanyl-4-methoxy-2-naphthylamide (L-Ala-

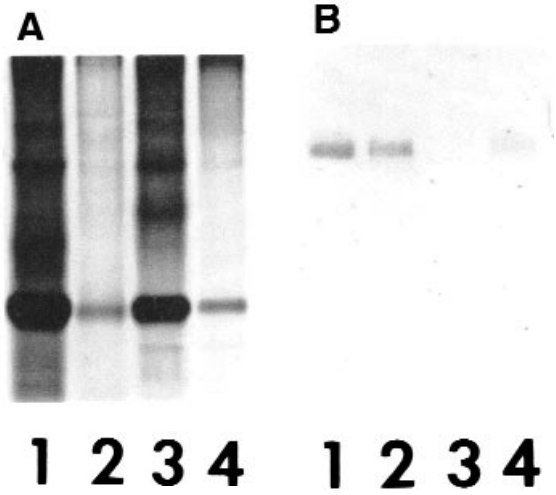

FIG 3. Native-PAGE and enzyme blot of human nasal mucosa extracts. *Lane 1,* Concentrated S2 fraction; *Lane 2,* concentrated P2 fraction; *Lane 3,* S2 fraction; *Lane 4,* P2 fraction. **A,** Native-PAGE gel was stained by Coomassie blue. **B,** Alanine-aminopeptidase activity zymogram.

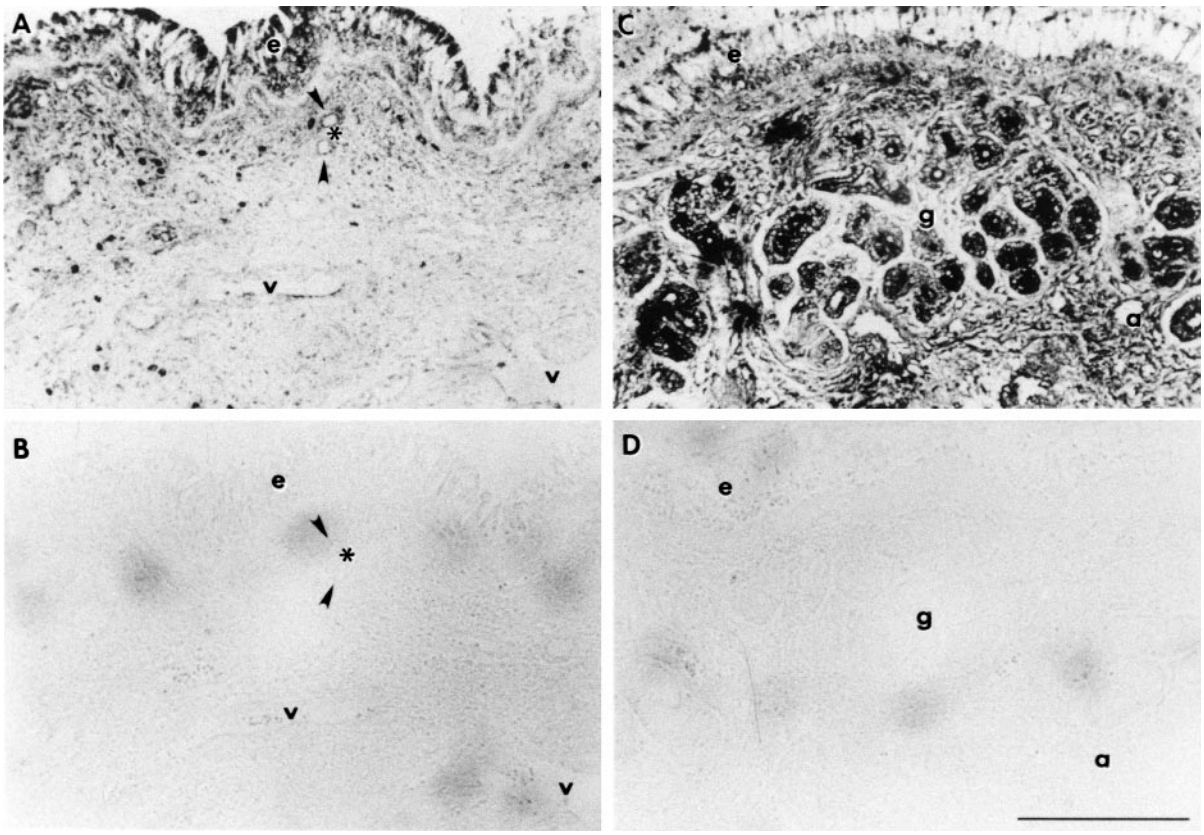

FIG 4. Puromycin-sensitive aminopeptidase (PS-Ap)-immunoreactive material in human nasal mucosa. **A,** PS-Ap–immunoreactive material (intensely stained black material) was present in glycocalyx and epithelial cells *(e)*. Goblet cells did not stain for PS-Ap. Endothelial surfaces of small veins (*arrow heads**) stained slightly. **B,** Nonimmune serum negative control. **C,** PS-Ap immunoreactivity was noted in inner lumen of gland ducts and glandular cells *(g)*. **D,** Negative control. Nuclear fast red counterstain. *Bars* represent 200 μm.

MNA, Sigma) substrate solution and observing for color development. Negative controls included staining in the absence of either substrate or enzyme.[19]

Western blot analysis

After electrophoresis, proteins were transferred from the native gel to nitrocellulose membranes by unilateral electrodiffusion blotting at 30 V for 120 minutes.[1-3] Membranes were blocked with 5% nonimmune rabbit serum, 0.25% BSA in tris buffer (0.9% NaCl, 50 mmol/L tris HCl, pH 7.4) at room temperature for 30 minutes, rinsed twice in tris buffer, then incubated at 4° C for 16 hours in PS-Ap polyclonal antibody diluted 1:500 in 0.25% BSA in tris buffer. After 3 10-minute washes in tris buffer, the membranes were incubated with phosphatase-labeled rabbit anti-goat IgG (Kirkegaard & Perry Laboratories, Gaithersburg, Md) diluted 1:2000 in 0.25% BSA in tris buffer at room temperature for 120 minutes. After washing 3 times in tris buffer, membranes were placed in BCIP/NBT phosphatase substrate (Kirkegaard & Perry) for 20 minutes at room temperature. The color reactions were terminated by washing in large volumes of distilled water.

For Western blots, samples were concentrated through Centricon 10 microconcentrators (Amicon, Danvers, Mass). S2 preparations were concentrated to approximately 20 mg/mL, P2 to approximately 5 mg/mL, and nasal lavages to approximately 50 mg/mL.

Indirect immunohistochemistry

Fresh tissues (n = 4) were fixed with 4% paraformaldehyde in PBS (pH 7.4) at 4° C for 4 hours and stored in PBS before being embedded in paraffin.[1-3] Six-micron-thick paraffin sections were cleared in xylene and graded alcohols, washed in water and PBS, and blocked in 5% BSA in PBS. Sections were incubated with goat polyclonal anti–rat brain–soluble PS-Ap (1:100 dilution in 1% BSA, PBS) for 20 hours at 4° C in a humidified chamber, washed in PBS, and incubated with colloidal gold–labeled rabbit anti-goat IgG (Biocell, Ted Pella, Redding, Calif; 1:100 dilution with 1% BSA in PBS) at room temperature for 60 minutes. After washing 3 times in PBS for 5 minutes each and 3 times in distilled water for 3 minutes each at room temperature, silver enhancing solution (IntenSE, Janssen) was added. The development of the stained slides was monitored under light microscopy. The slides were washed in distilled water and counterstained with nuclear fast red.

Enzyme histochemistry (zymogram)

Cryostat sections were cut from frozen tissues (n = 4) and allowed to dry at room temperature before fixation in 100% analytical grade acetone for 5 minutes at 20° C followed by brief drying in air. Aminopeptidase activity was demonstrated with the use of the simultaneous azo-coupling technique with L-ala-MNA (final con-

centration 1.5 mmol/L) and fast blue BB salt (Sigma Chemical Co, final concentration 1 mg/mL) in 1.8% dimethylformamide and 98.2% trismalate buffer (pH 6.5). Slides were incubated in this substrate for 15 minutes at 30° C, washed for 1 to 3 minutes in distilled water, fixed for 20 minutes in 4% aqueous formalin, rinsed briefly in distilled water, and mounted in glycerol gelatin.[18] The sites of L-ala-MNA catabolism were identified by red staining of the tissue.

Nasal challenges in vivo

A total of 18 adult subjects (9 male, 9 female) between the ages of 24 and 53 years were studied. Subjects were excluded if they had had an upper respiratory tract infection in the past 3 weeks, any nasal symptoms at the time of the study, or were taking any medications in the previous 48 hours. Atopic subjects (n = 14) with perennial (n = 3) or seasonal (n = 11) symptoms were defined by at least 1 positive allergy prick skin test (wheal >4 mm than glycerol negative control) from a panel of 16 relevant aeroallergens. Seasonally atopic subjects were studied out of their allergy season. Nonatopic subjects (n = 4) had no allergic symptoms and had negative skin test results.

Nasal challenges were performed as previously reported.[1-3,20] Nasal lavage fluid was collected through 8F rubber catheters. After the nasal cavity was washed with 4 4-mL saline prewashes, the challenge protocols were begun. Nasal challenge solutions prepared in normal saline were applied to one or both sides of the nasal cavity from a nebulizer bottle. Each 10-minute challenge period was followed by a 4-mL saline lavage. In the first protocol, 10 subjects were challenged sequentially with saline followed by 25 mg methacholine. In the second protocol, saline challenge was followed by challenge with 1 mg histamine. In the third protocol, subjects were sequentially challenged with 100 μg atropine and then 25 mg methacholine. Finally, 8 patients received an allergen challenges as previously described.[21] Baseline secretions were collected 10 minutes after saline challenge. Solutions of relevant allergen (75 PNU) were instilled, then lavages were collected every 10 minutes for the first hour and then every 20 minutes for the second hour.

Nasal lavage fluid assays

Leu-AP was assessed as described except that nasal lavage samples (10 μL) were incubated with 50 μL of [^{3}H]leuenkephalin (36.9 nmol/L final concentration) and leuenkephalin (1 μmol/L final concentration) in 10 mmol/L tris HCl (pH 7.4). Lactoferrin was measured by a modified noncompetitive ELISA with an assay range of 1 to 200 ng/mL.[20,21] Albumin was measured by a direct, competitive ELISA with an assay range of 1 to 100 μg/mL.[20,21] IgG was measured by a direct, noncompetitive ELISA with an assay range of 1 to 100 ng/mL.[20,21] Total protein was measured by the Lowry method with BSA as standard.[21,22]

RESULTS

Leu-Ap activity

LeuAp activity was detected in both the cytosolic (S2) fraction and membrane-rich (P2) fractions from each of 6 adult human nasal turbinates. The specific activity was significantly higher in P2 fractions (630.54 ± 98.48 pmol/min/mg protein, range 210.50 to 988.40) than S2 fractions (32.20 ± 6.96 pmol/min/mg protein, range 10.01 to 58.10) ($P < .01$).

Puromycin and bestatin inhibition studies

In all samples, the Ki values for puromycin were higher than for bestatin (Fig 1). Puromycin completely inhibited leu-Ap activity in all samples, but only at very high concentrations. In the S2 fraction, 2 components were discernible, 1 being a puromycin-sensitive fraction representing 20% of the total ED-Ap activity, and exhibiting a Ki of 0.1 μmol/L. This behavior was consistent with this fraction being soluble PS-Ap. The remaining 80% of the activity had a Ki of 37 μmol/L and thus represented a novel aminopeptidase activity. All of the activity in the P2 fraction exhibited a high Ki for puromycin of 30 μmol/L. Thus no PS-Ap was detected in the membrane fraction.

Bestatin inhibited all leu-Ap activity in S2 (Ki = 1 μmol/L) and P2 (Ki = 0.05 μmol/L).

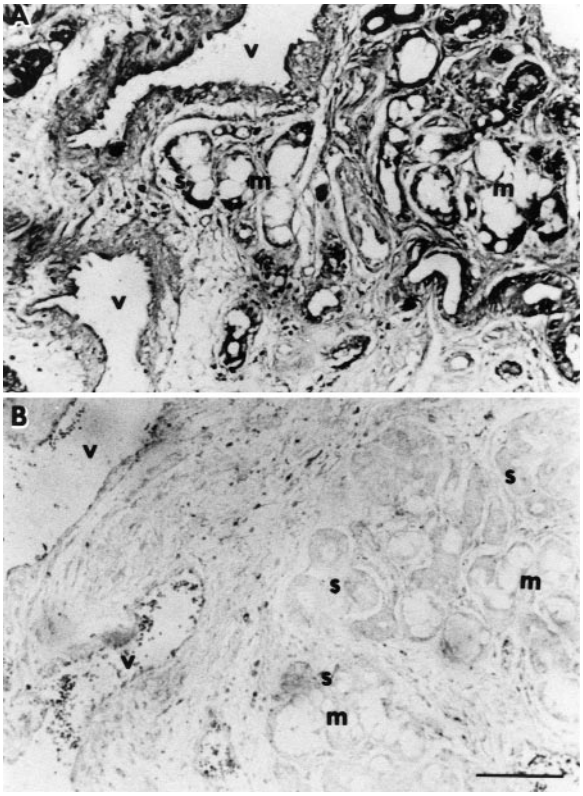

FIG 5. Puromycin-sensitive aminopeptidase (PS-Ap)-immunoreactive material in human nasal mucosa. **A,** PS-Ap–immunoreactive material (intensely stained black material) was present in glandular serous cells *(s)* but not in mucous cell vacuoles *(m)*. Endothelium of veins *(v)* was also stained. **B,** Nonimmune serum negative control. Nuclear fast red counterstain. *Bar* represents 50 μm.

Ala-Ap assay

Ala-Ap activity was detected in both S2 and P2 fractions. The activity in S2 fractions was 4.49 ± 0.57 mU/mg protein (range 3.00 to 6.71). P2 fractions had higher activities of 43.90 ± 3.44 mU/mg protein (range 32.27 to 57.02).

Substrate competition studies

The Km for leu-enkephalin was 0.4 mmol/L in S2 and 0.5 mmol/L in P2 (Fig 2). When ala-NA was the substrate, Km was 2 mmol/L with S2 and 1 mmol/L with P2. The addition of 2 mmol/L ala-NA to S2 samples shifted the leu-enkephalin degradation curve 20-fold to the right. In the P2 fraction, addition of 1 mmol/L ala-NA

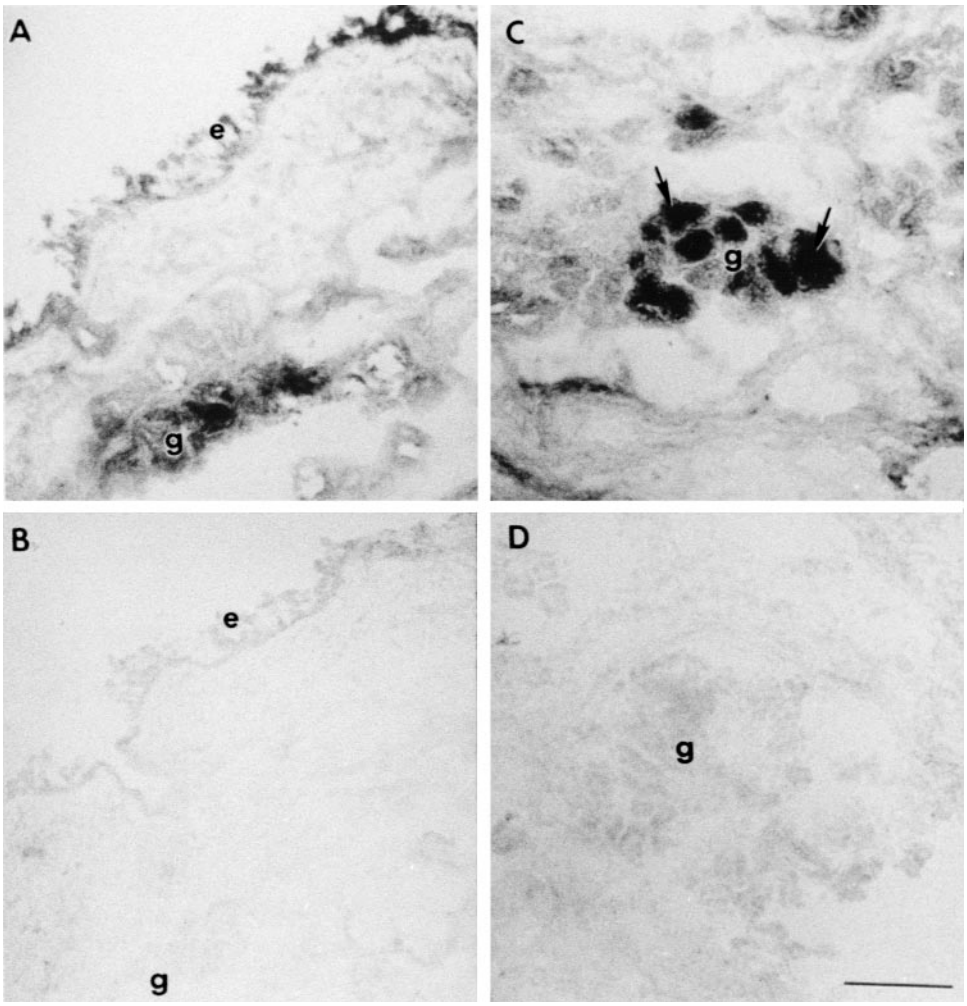

FIG 6. Zymogram of aminopeptidase M activity in human nasal mucosa. **A,** L-alanine-4-methoxy-2-naphthylamide–cleaving aminopeptidase was localized in epithelium *(e)* and submucosal glands *(g).* **B,** Inhibition control. Adjacent sections preincubated with 1 mmol/L puromycin showed no aminopeptidase activity. **C,** Aminopeptidase activity was most concentrated in central region of glandular acini. **D,** Inhibition control for **C**. *Bars* represent 100 μm.

increased Km by 22-fold. The addition of 0.4 mmol/L leu-enkephalin to S2 fractions shifted the Km for ala-NA to 8 mmol/L, a 4-fold increase. Similarly, the Km for ala-NA was increased 4-fold by leu-enkephalin in P2 fractions. These data indicate that leu-enkephalin and ala-NA measure the same aminopeptidase activities.

Native-PAGE and enzyme blot

Electrophoresis of the dilute and concentrated S2 (30 mg/mL) and P2 (7.5 mg/mL) fractions followed by L-ala-NMA azocoupling zymography demonstrated a single band in both preparations with the same mobility (Fig 3). The differences between P2 and S2 concentrations suggest a higher specific activity for aminopeptidase in the membrane-rich P2 fraction. PS-Ap may have had too low an activity to be detectable by these methods.

PS-Ap indirect immunohistochemistry

Soluble PS-Ap–immunoreactive material was localized to the epithelium, submucosal glands, and endothelium in human nasal turbinate mucosa (Figs 4 and 5). The most intense staining was concentrated in the outer layer of the epithelium in all the specimens. The immunoreactivity was identified in both the columnar and basal cells but not in the goblet cell vacuoles (Fig 4, *A*). In the submucosal glands, immunoreactivity was seen in both serous and mucous cells (Fig 4, *C*), but the serous cell immunoreactivity was more intense (Fig 5, *A*). Glandular staining was inconsistent between samples, suggesting variable expression or loss after exocytosis or other processes. Immunoreactivity was seen in both small (Fig 4, *A*) and large vein endothelial cells (Fig 5, *A*).

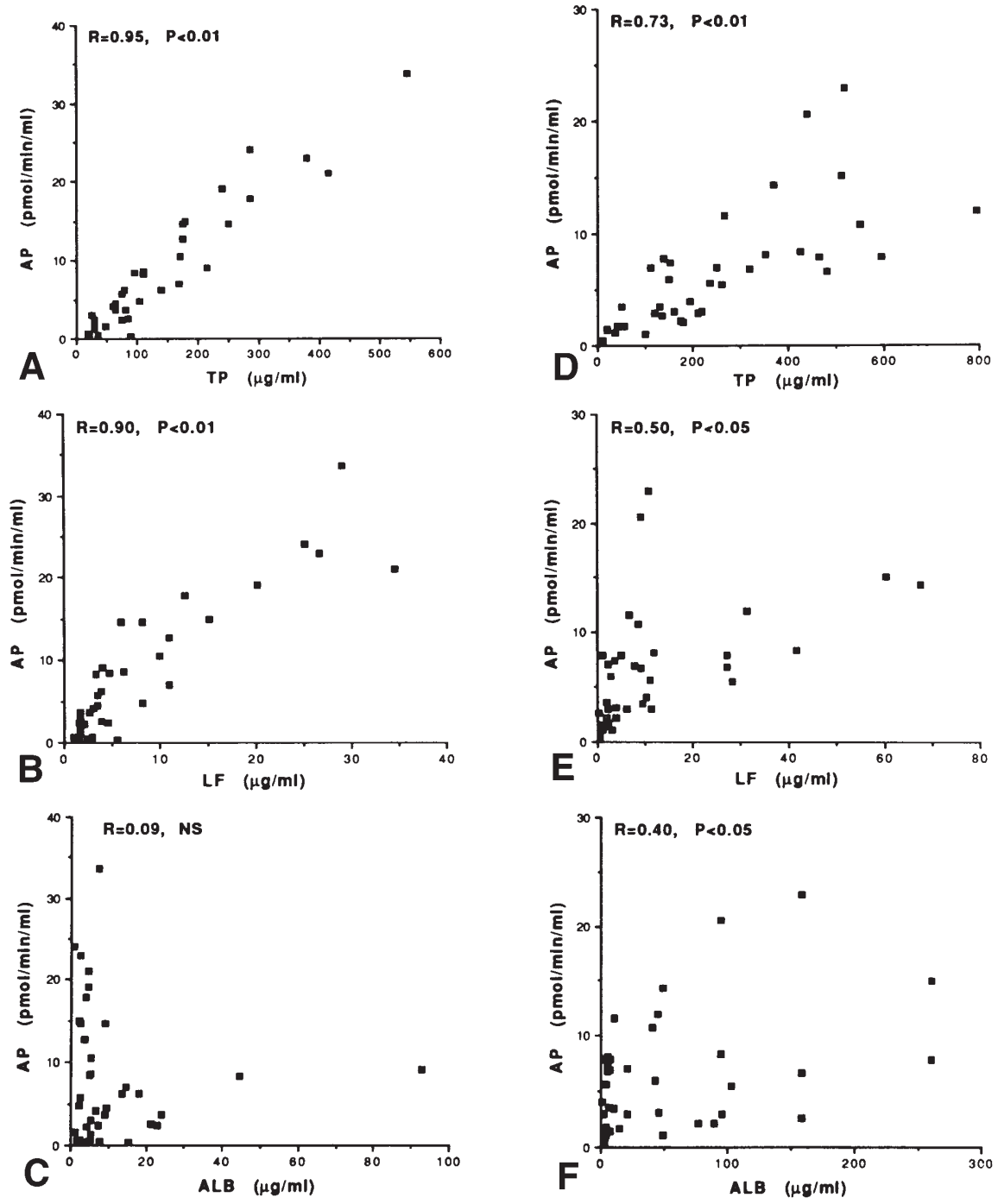

FIG 7. Methacholine and histamine-induced nasal secretions. Correlations between nasal lavage fluid concentrations of leucine-aminopeptidase *(leu-Ap)* activity and total protein *(TP)* (**A, D**), lactoferrin *(LF)* (**B, E**), and albumin *(ALB)* (**C, F**) are shown after methacholine (**A, B, C**) and histamine (**D, E, F**) provocations. Coefficients of variation (*r*) were determined by linear regression.

Enzyme histochemistry (zymogram)

Enzyme histochemistry was used to localize L-ala-MNA–cleaving aminopeptidase to the epithelium and submucosal glands (Fig 6, *A* and *C*). These L-ala-MNA histozymograms were inhibited by preincubation with 100 μmol/L puromycin for 10 minutes at 30° C (Fig 6, *B* and *D*). The glandular staining was most concentrated on the lumenal surface of acinar cells, making it difficult to distinguish serous cells from mucous cells. There was almost no L-ala-MNA activity in either small or large vessels.

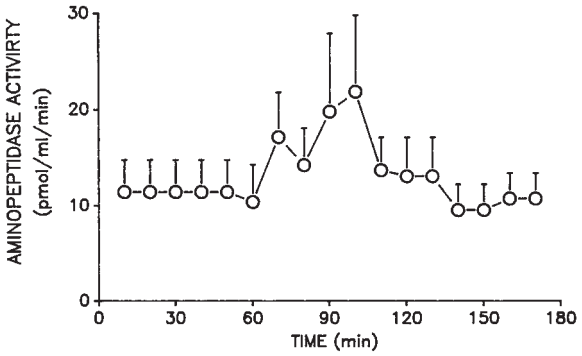

FIG 8. Bestatin-inhibitable leucine-enkephalin–degrading aminopeptidase *(leu-Ap)* activity in nasal lavage fluids after antigen challenge (mean ± SEM). Atopic subjects (n = 8) received serial saline nasal lavages for 50 minutes, then were challenged topically with allergen at 60 minutes. Lavages were then collected at 10-minute intervals between 70 and 120 minutes and at 20-minute intervals between 140 and 180 minutes.

Leu-AP activity in nasal lavage fluids

Leu-Ap activity after saline challenge was 3.02 ± 0.44 pmol/min/mL (range 0.49 to 7.88). Methacholine provocation increased leu-Ap activity to 15.81 ± 2.92 pmol/min/mL (range 2.33 to 33.66), indicating active secretion. Pretreatment with atropine blocked the methacholine-induced leu-Ap release (6.27 ± 1.85 pmol/min/mL, range 0.41 to 17.77). Histamine increased leu-Ap to 9.68 ± 1.73 pmol/min/mL (range 2.13 to 20.53). Methacholine stimulated glandular exocytosis and secretion, whereas histamine caused vascular leak with extravasation of plasma proteins plus stimulation of nociceptive nerves and recruitment of cholinergic reflexes that cause glandular exocytosis.[1-3,20,21] These secretory data suggest that cholinergic stimulation of glands by direct and reflex actions was the predominant mechanism regulating leu-Ap secretion in human nasal mucosa in vivo.

Correlation coefficients

To determine if leu-Ap secretion occurred in association with glandular and/or vascular proteins, leu-Ap activity in the secretions was compared with the total protein, lactoferrin (a specific marker of serous cell secretion), and albumin (a plasma protein marker reflecting vascular permeability) (Fig 7). After methacholine provocations (Fig 7, *a*, *b*, and *c*), leu-Ap was highly correlated with lactoferrin ($r = 0.90$, $P < .01$) and total protein ($r = .95$, $P < .01$), whereas leu-Ap and albumin secretion were not correlated ($r = 0.05$, not significant). After histamine provocation (Fig 7, *d*, *e*, and *f*), leu-Ap was correlated with total protein ($r = 0.73$, $P < .01$), lactoferrin ($r = 0.50$, $P < .05$) and albumin ($r = 0.40$, $P < .05$). These data are in agreement with other studies of histamine provocations that show parallel stimulation of both vascular permeability and cholinergic reflex-mediated glandular secretion.[1-3,20,21] Thus both methacholine and histamine provocation systems indicate that leu-Ap secretion correlated most closely with glandular secretion.

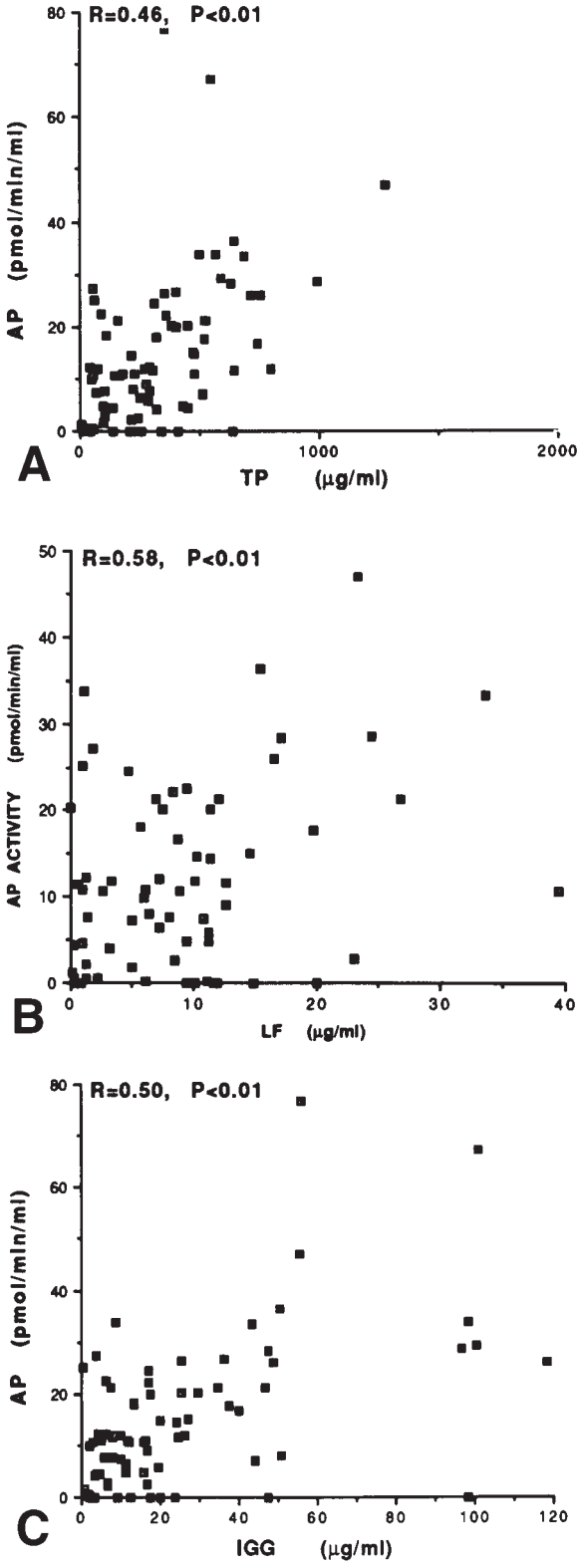

FIG 9. Allergen-induced nasal secretions. Data and coefficients of variation (*r*) determined by linear regression are shown for secretion of leucine-enkephalin–degrading aminopeptidase *(leu-Ap)* and **A,** total protein *(TP);* **B,** lactoferrin *(LF);* and **C,** IgG *(IGG)* concentrations.

Antigen challenge

Repeated saline challenges (10 to 60 minutes, Fig 8) had no effect on the leu-Ap content of nasal lavage fluids. Allergen given at 60 minutes induced sneezing, rhinorrhea, nasal pruritus, and congestion in all 8 subjects within 10 minutes of challenge. Nasal lavages were enriched with total protein, albumin, IgG, and lactoferrin. Leu-Ap increased 10 minutes after challenge (70 minutes), reached maximum concentration after 50 minutes (110-minute time point), and returned to baseline 90 minutes after allergen. Leu-Ap levels from the first hour after allergen (70 to 120 minutes) were significantly higher than levels from saline (10 to 60 minutes) and the second (130 to 180 minutes) lavage fluids ($P < .05$, analysis of variance for multiple comparisons with Bonferroni correction). There were no significant differences between individual time points because of the wide variances in leu-Ap values. Leu-Ap release between 0 and 120 minutes correlated with lactoferrin ($r = 0.58$, $P < .01$), IgG ($r = 0.50$, $P < .01$) and total protein ($r = 0.46$, $P < .01$) (Fig 9). These experiments indicate that leu-Ap secretion was increased during the immediate phase of an allergic reaction.

DISCUSSION

Identification of Ap activities in human nasal mucosa has been difficult because of the diverse assays, substrates, and inhibitors required. Despite these limitations, 3 aminopeptidase activities were discovered. The majority of aminopeptidase activity (approximately 80%) was found in the membrane-rich (P2) tissue fraction. This puromycin-resistant/bestatin-sensitive aminopeptidase probably represented epithelial, glandular, and other cell surface ApM activity.

The soluble S2 fraction contained the remaining approximately 20% of total aminopeptidase activity. Soluble aminopeptidase activities have also been detected in urine,[18,22] bile acid,[23] serum,[24] and nasal lavage fluid.[5] Approximately 20% of the S2 activity (4% of total extract aminopeptidase activity) can be attributed to PS-Ap. The PS-Ap–immunoreactive material was localized to epithelium, glands, and endothelium with the use of anti–rat brain–soluble PS-Ap. Release of this soluble enzyme from exocrine cells may have been under cholinergic control.

The bulk of the S2 activity (80% of S2, 16% of the total aminopeptidase activity) was a soluble puromycin-insensitive/bestatin sensitive aminopeptidase. This activity may correspond to the novel aminopeptidase recently described by Erbeznik and Hersh.[25] Their "Ap200"[25] had a Ki for puromycin of 27 mmol/L and 1.6 mmol/L for bestatin. Their 200-kd macromolecule may have been a dimer because high salt led to dissociation into one 100-kd electrophoretic band. This may correspond to the ala-Ap activity detected in epithelium and gland cells by zymography (Fig 3, *B*).

The nasal provocation experiments demonstrated that methacholine, antigen, and histamine could each induce aminopeptidase secretion that correlated with exocytosis of markers of glandular secretion but not markers of vascular permeability.[1-3,20,21] Because glandular exocytosis is regulated by cholinergic reflexes, parasympathetic pathways may regulate aminopeptidase activity in secreted mucus.

Benefits of secreting aminopeptidases into airway mucus may include their antiviral activity and capacity to generate bradykinin. Cell surface aminopeptidase acts as a binding site for coronaviruses.[26] It is conceivable that mucus aminopeptidase may bind to these virions to prevent epithelial cell binding, uptake, and infection. Virion destruction could be mediated by plasma proteins because aminopeptidases generate bradykinin from one of its precursors, lysyl-bradykinin (kallidin).[5,27,28] Bradykinin potently stimulates vascular permeability that causes flooding of the airway lumen by immunoglobulin-containing, complement-containing, and fibrin-containing plasma. Vasodilation, nociceptive sensory nerve activation that conveys sensations of burning pain, and arachidonic acid metabolism that promotes additional glandular exocytosis, also occur.[29] Bradykinin is elevated in secretions during the common cold[5] and after allergen provocation.[27] However, bradykinin antagonists have had little effect in the common cold, and inhibition of aminopeptidase with bestatin does not accentuate the vascular permeability induced by tobacco in the hamster cheek pouch model.[30] These findings suggest an alternative hypothesis: Secretion of glandular aminopeptidase along with kininogens of vascular and glandular origin into the airway mucus leads to bradykinin production that is only the consequence and marker of their parallel secretion rather than the primary cause of mucosal inflammation. These competing hypotheses require additional testing that should include human nasal provocations with aminopeptidase inhibitors such as bestatin.

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