

Natriuretic peptides and genesis of asthma: An emerging paradigm?

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Exposure to allergens and infections contribute to early immune development. However, knowledge of the role of cellular metabolic, physiologic, and endocrinologic factors in controlling immune development and asthma is limited. Immune cells, including macrophages, dendritic cells, and T lymphocytes, express receptors for atrial natriuretic peptide (ANP) both in the fetal and neonatal lymphoid organs. ANP has garnered much attention for its cardiovascular effects, but its apparently significant role in the physiology and immunity of the lung has been underappreciated. Studies indicate that ANP also plays a significant role in shaping the early immune responses to environmental antigens. The C-terminal prohormone natriuretic peptide ANP (or NP₉₉₋₁₂₆), which possesses bronchodilatory properties, is involved in polarizing dendritic cells to produce a T_H2 response. Also, *de novo* overexpression of another pro-ANP peptide, NP₇₃₋₁₀₂, provides persistent bronchoprotection and induces significant anti-inflammatory activities in the lung epithelial cells. Thus natriuretic peptides appear to play a pivotal role in the genesis and control of asthma, and they might provide an important target to modulate allergen-induced immune responses in allergic patients. (J Allergy Clin Immunol 2004;114:520-6.)

Key words: Atrial natriuretic peptide, kaliuretic peptide, asthma, inflammation, bronchodilation, nuclear factor κ B

Development of the allergic immune response in genetically predisposed individuals appears to involve immune interactions, leading to polarization of allergen-induced T cells to T_H2 cells. These cells predominate in allergic subjects compared with in healthy nonallergic individuals. Evidence also suggests that in addition to T_H2 cells, natural killer (NK) T cells play an important role in

Abbreviations used

ANP: Atrial natriuretic peptide
cGMP: Cyclic guanosine monophosphate
ERK: Extracellular receptor kinase
KP: Kaliuretic peptide
MAPK: Mitogen-activated protein kinase
NF κ B: Nuclear factor κ B
NK: Natural killer
NO: Nitric oxide
NOS: Nitric oxide synthase
NP: Natriuretic peptide
NPR: Natriuretic peptide receptor

the pathogenesis of asthma, and as in the absence of NK cells, T_H2 cells alone do not induce asthma pathology.^{1,2} The main premise of this review is that the cellular endocrine milieu, particularly natriuretic peptide (NP) hormones, plays an important role in the pathogenesis of the allergic response of the mucosa of the respiratory, gastrointestinal, and urinary tracts and protects against the development of asthma. NP hormones with diuretic properties, first reported in 1981, have been extensively studied for their cardiovascular effects, but their role in regulating the development of local immunity and the physiology of the lung is underappreciated. Recent reports suggest that NP hormones are important players in both the pathogenesis of and protection against allergic diseases, including asthma.

NP SYSTEM

The 126-amino-acid atrial natriuretic peptide (ANP) prohormone gives rise to 4 peptides: long-acting NP (amino acids 1-30), vessel dilator (amino acids 31-67), kaliuretic peptide (KP; amino acids 79-98), and ANP (amino acids 99-126).³ NPs are produced in various mucosal tissues, in the central nervous system, and in the cardiovascular system and are released into the circulation. The common physiologic effects of these peptides include volume homeostasis, vasodilation, inhibition of aldosterone secretion, hypotension, and regulation of vascular tone.⁴ A comparison of functions of different NPs is shown in Table I.⁵⁻¹³ The C-terminal prohormone peptide ANP (NP₉₉₋₁₂₆) and a number of ANP-like

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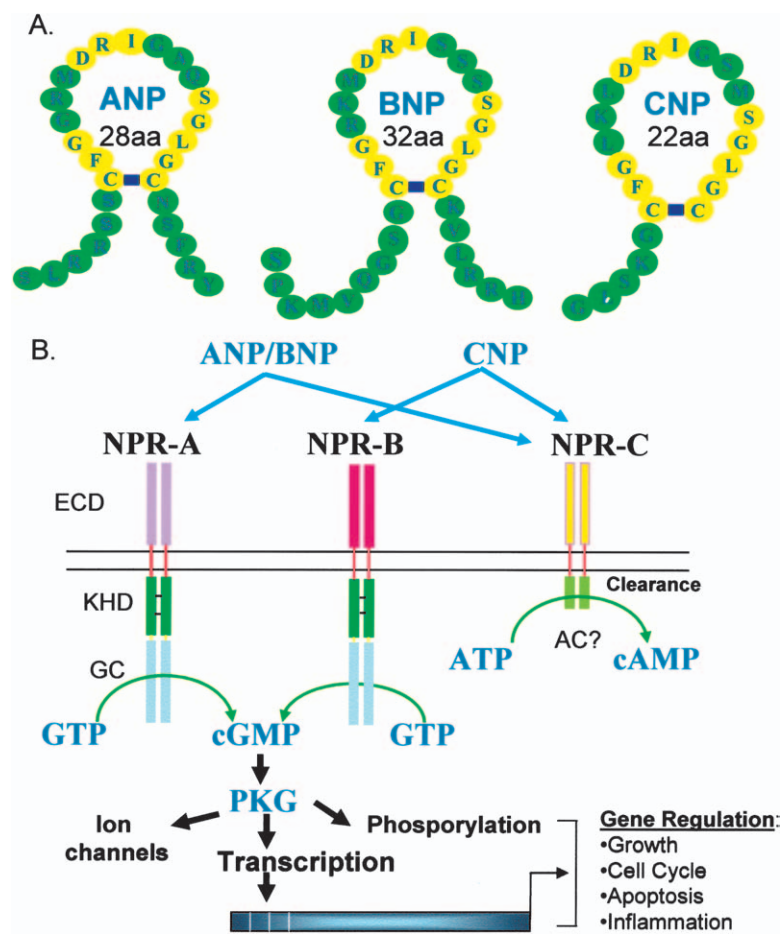


FIG 1. A, Schematic diagram of the ring structure of the 3 C-terminal ANP-like peptides: ANP, brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). **B,** Diagrammatic presentation of the receptors and the signal transduction pathways affecting various biologic functions. *ECD*, Extracellular domain; *KHD*, kinase homology domain; *GTP*, guanosine triphosphate; *ATP*, adenosine triphosphate; *cAMP*, cyclic adenosine monophosphate; *PKG*, protein kinase G.

TABLE I. Functional distinction among NPs

Features	LANP (aa 1-30)	VD (aa 31-67)	KP (aa 78-98)	ANP (aa 99-126)
Half-life <i>in vivo</i> (min) ⁸	28	107	21	2.5
Plasma circulation ⁹	+++	+++	++	++
Na excretion ⁹	++	++	—	++
K excretion ⁹	±	—	++	+
cGMP metabolism ¹⁰ involves	PDE	PDE	PDE	extruded from cell
Adremedullin release ⁷	—	—	—	+
Plasma aldosterone level ⁵	+ (3 h)	—	— (3 h)	+ (30 min)
PGE ₂ levels ^{5,6}	++	+	+++	—
Na ⁺ K ⁺ ATPase Inhibition ^{5,11}	++	+	+++	±
Urodilatin release and clearance ¹²	—	—	—	+
Circulating endothelin ¹³	+	+	— (early)	— (late)

LANP, Long-acting natriuretic peptide; aa, amino acids; VD, vessel dilator; PDE, phosphodiesterase; PGE₂, prostaglandin E₂.

peptides, including brain natriuretic peptide, the C-type natriuretic peptides,¹⁴ and urodilatin produced by renal cells,¹⁵ have been studied extensively. These peptides contain 2 cysteine residues that produce a characteristic ring-like folding, which is essential for activity (Fig 1, A).

Furthermore, a number of truncated peptides of ANP have been reported that exhibit activity that is antagonistic to that of ANP.¹⁶ The signaling mechanisms for ANP and brain natriuretic peptide, which involves natriuretic peptide receptor (NPR)—A and NPR-B is schematically

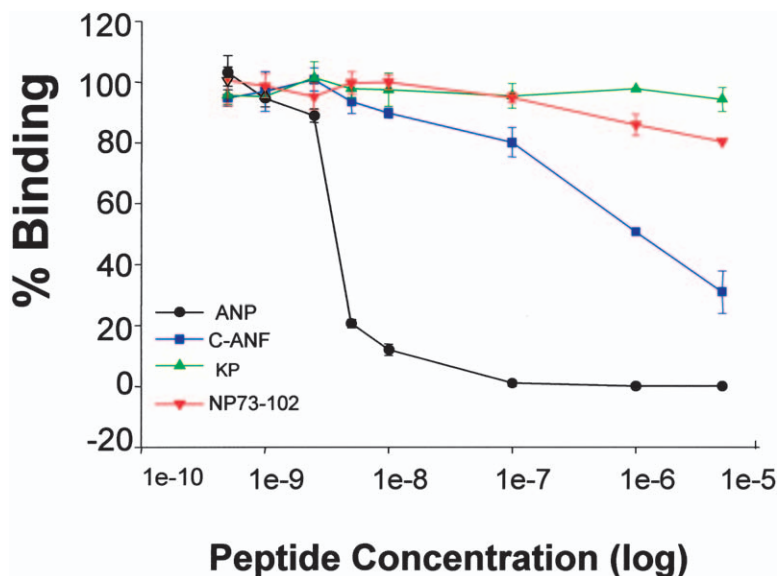


FIG 2. Evidence that NP₇₃₋₁₀₂ does not bind to NPR-A. Binding analysis was performed with HEK293GCA cells overexpressing NPR-A. C-ANF, c-type natriuretic factor (also, CNP).

shown in Fig 1, *B*, and reviewed previously.¹⁷ Furthermore, NPR-C also appears to signal phospholipase C activation and a decrease in adenylate cyclase activity.^{18,19} The mechanisms underlying ANP's growth-inhibitory effects might involve signaling through activation of mitogen-activated protein kinases (MAPKs).¹⁹ The potential effects might include inhibition of extracellular receptor kinase (ERK), activation of epidermal growth factor, cGMP-dependent protein kinase–induced uncoupling of Ras/Raf1 interaction, or induction of MKP-1, a MAPK phosphatase that inactivates signaling through a number of growth factors, such as endothelin, epidermal growth factor, and fibroblast growth factor.²⁰

In contrast to C-terminal ANP, the receptors for the N-terminal NPs are yet to be characterized, and hence the signaling mechanisms are unknown. The results also indicate that neither KP nor NP₇₃₋₁₀₂ bind to NPR-A (Fig 2). These results are similar to those found with long-acting NP and vessel dilator peptides.⁵ This is what would be expected because these peptide hormones are linear peptides, whereas binding to the ANP-A and ANP-B receptors requires a ring peptide, such as ANP. However, a negative feedback loop exists among the peptide hormones, which determines the levels of each peptide in the circulation and the ultimate outcome of response in a specific disease context.²¹ For instance, it has been suggested that the change in levels of ANP, KP, and ANP/KP might be important in controlling atrial fibrillation.⁶

NP AND LUNG DISEASE

Since the first report in 1986 of immunoreactive ANP in the lung,²² ANP has been localized in peripheral lung tissue, tracheal epithelial cells, respiratory epithelium, human fetal lung tissue, pulmonary veins, striated muscle

cells, and the superior vena cava, implicating the lung as a direct site of ANP synthesis. The ANP receptors are found in the lungs of human subjects, pigs and rats, and cattle.²³⁻²⁶ Lung parenchymal cells are also targets of ANP action.²⁷ The kinetics of lung ANP receptor have been defined in many species.²⁸ Lung tissue-specific expression of ANP is controlled by a number of factors, including glucocorticoids, thyroid hormone, hemodynamic overload, and hydration status.²⁹ In addition, the lung metabolizes pro-ANP quickly because lung homogenate degrades pro-ANP with neutral endopeptidases to a greater degree than kidney and liver homogenates.³⁰ Rapid clearance of ANP has been reported to occur as a result of its binding to clearance receptors on the pulmonary parenchyma.³¹

Thus although the amount of ANP detected in the lung is substantially lower than that in the atria, because of its relatively larger mass, the lung appears to be a significant source of ANP. These findings suggest that the lung, as a specific organ system, might possess its own ANP regulatory circuit. The ANP system in the lung plays an important role in the biologic activities of the lung, such as vasodilatation, bronchorelaxation, pulmonary permeability, and surfactant production and action.^{22,28} The biologic activities of pro-ANP in the lung render it as a pivotal control factor in a number of pulmonary diseases, including pulmonary hypertension, asthma, chronic obstructive pulmonary disease, pulmonary edema, obstructive sleep apnea, and lung carcinoma.²⁸

DEVELOPMENT OF THE IMMUNE SYSTEM AND THE PATHOGENESIS OF ASTHMA

The coexpression of receptors for NPs in various immune cells, such as macrophages, dendritic cells, and

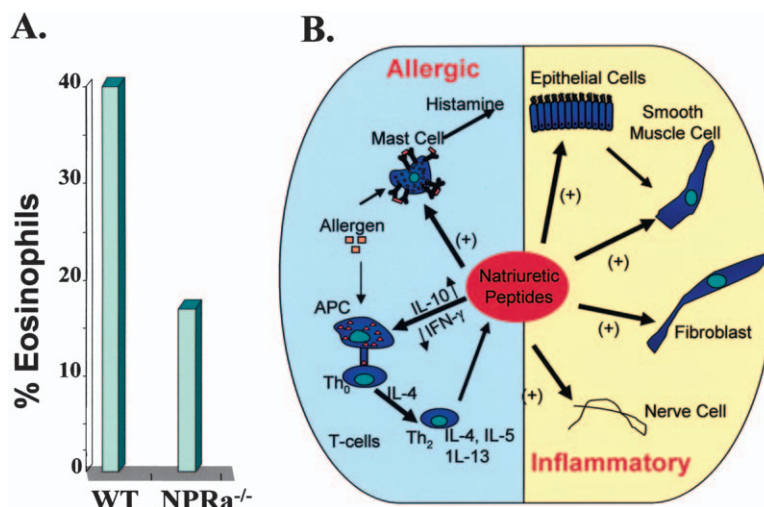


FIG 3. A, Analysis of eosinophilia in bronchoalveolar lavage fluid of NPR-A^{-/-} (*NPRa*^{-/-}) and wild-type (*WT*) DBA/2 mice sensitized and challenged with ovalbumin. The result of a representative of 2 experiments is shown. **B,** A schematic presentation of ANP-mediated augmentation of allergic inflammation by the action of ANP on both immune and nonimmune cells plays a role in both allergic (*left*) and inflammatory (*right*) processes. +, Activation by the natriuretic peptide ANP; APC, antigen-presenting cell.

T lymphocytes, and their differential regulation by immunomodulators suggest discrete functions of these peptides within the immune system.³² The ANP prohormone is detectable in the thymus, spleen, and lymph nodes of the pig, rat, and mouse, and ANP mRNA can be detected in the thymus and tonsils of the human, bird, and rat.^{33,34} However, the role of ANP in local immunity of the lung remains poorly understood. ANP acting through innate immune cells in the lung can modulate the immune response to allergens, which in turn augments allergic inflammation by acting on a number of cells in the lung, as depicted schematically in Fig 3. This hypothesis for the role of ANP cascade on the genesis of asthma is based on the following evidence. ANP receptors on rat thymocytes inhibit proliferation of thymocytes during thymic differentiation.³⁵ ANP stimulates migration of human neutrophils³⁶ and inhibits nitric oxide (NO) and TNF- α production by murine macrophages.^{37,38} GATA-3, the transcription factor involved in upregulation of IL-4 in T cells, also upregulates ANP prohormone synthesis, suggesting a possible autocrine loop.^{7,39} Also, ANP induces mast cells to release histamine, an important mediator of allergic inflammation.^{40,41} ANP acts on epithelial cells to produce TGF- β_1 , which is involved in the genesis of pulmonary fibrosis, a characteristic of remodeled lung. ANP enhances human NK T cells,⁴² which are crucial to the development of airway hyperreactivity.⁴³ ANP also activates cyclic guanosine monophosphate (cGMP) production in human lung fibroblasts⁴⁴ and increases ERK activity in human endothelial cells.⁴⁵ In LPS-activated dendritic cells, ANP induces decreased production of IL-12 and increased production of IL-10. ANP acts through its receptor on dendritic cells to polarize these cells toward a T_H2 phenotype, the hallmark of the allergic immune response.⁴⁴

In attempts to evaluate the role of ANP in allergic disease, NPR-A-deficient and wild-type mice were sensitized and challenged with ovalbumin, and pulmonary inflammation was examined. The NPR-A-deficient mice had significantly decreased eosinophilia compared with that seen in control animals (Fig 3, A). The inflammatory response on OVA challenge, as measured by means of infiltration of inflammatory cells to the lung and mucus production, was less in the mice lacking the NPR-A receptor (data not shown). These data suggest that the ANP system provides a crucial impetus for the propagation of the allergic response. Together, these findings suggest that ANP promotes allergic inflammation by acting on both immune and nonimmune cells, as schematically shown in Fig 3, B.

NP-MEDIATED IMMUNOMODULATION OF ALLERGIC DISEASE

ANP concentrations in the plasma are increased during severe asthma, suggesting that ANP might have bronchoprotective effects.⁴⁶ In human subjects the highest levels of ANP are found in the pulmonary arteries,⁴⁷ and ANP exerts a greater dilatory effect on these versus systemic arteries.^{48,49} ANP relaxes the pulmonary arteries in various animal species.^{48,50-52} These observations and the vasodilatory function of ANP led several investigators to examine the bronchoprotective role of ANP. In ovalbumin-induced asthmatic guinea pigs, intravenous ANP inhibits antigen-induced bronchoconstriction.⁵³ In human subjects ANP reverses airway hyperreactivity when administered intravenously or by means of inhalation^{54,55} and also modifies bronchial reactivity to inhaled histamine, propanolol,^{56,57} and nebulized water.⁵⁸

NF κ B-EGFP Expression in A549 cells

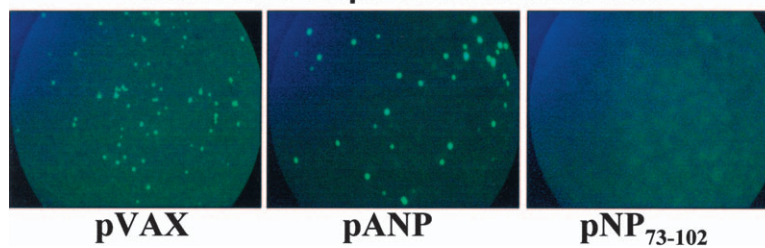


FIG 4. Comparison of NF κ B activation in A549 cells transfected with expression plasmid vector from Invitrogen (pVAX), pANP (pVAX expressing ANP), and pNP₇₃₋₁₀₂ (pVAX expressing NP₇₃₋₁₀₂). Cells transfected with NF κ B promoter fused to a reporter enhanced the green fluorescence protein (EGFP) gene. Cells were observed with a fluorescent microscope after 24 hours of transfection and photographed.

In randomized, double-blind, placebo-controlled studies, urodilatin, a peptide similar to ANP but modified by means of posttranslational processing within the kidney, was significantly superior in its dose-dependent bronchodilating properties compared with those of ANP, and it was as effective as salbutamol at all levels of the tracheobronchial tree.⁵² Urodilatin combined with albuterol improved lung function better than either alone.⁵² However, the effects of ANP and urodilatin *in vivo* were limited by the short half-life of these peptides compared with that of albuterol.

A significant development in this field is the recent demonstration that the *de novo* expression of a plasmid-encoded NP₇₃₋₁₀₂ leads to a significant reduction of airway reactivity lasting up to 3 weeks in a mouse model of ovalbumin-induced asthma.⁵⁹ These results suggest the potential utility of the NPs in the treatment of allergic diseases, including asthma. However, irrespective of whether such treatment can reverse established asthma, potential adverse effects of such treatment and the mechanism underlying the effectiveness of these peptides remain to be elucidated.

MECHANISMS OF NP-MEDIATED PROTECTION

ANP

Airway smooth muscle hypertrophy and hyperplasia are important determinants of airway remodeling and bronchial responsiveness in asthma. ANP dilates the trachea and bronchi of a number of species⁶⁰⁻⁶⁴; however, the precise mechanism is unclear. It is likely that it is similar to ANP-induced vasodilation of endothelium, which involves activation of guanylate cyclase,^{50,59,65} leading to the production of NO, which affects airway hyperreactivity both in animal models and in human subjects.^{66,67} The antiproliferative effects of ANP and NO in human airway smooth muscle cells, mediated through both cGMP-dependent and cGMP-independent mechanisms, has been reported.⁷ Thus ANP-induced NO, in addition to its direct effects on membrane-bound guanylate cyclase, might contribute to the mechanism of ANP's bronchoprotection. The effects of ANP on airway inflammation are less well studied. However, ANP

reduces the cytokine- and stress-stimulated activation of nuclear factor (NF) κ B in various cell types, decreasing proinflammatory cytokine production.⁶⁸⁻⁷⁰ It can reduce TNF- α -stimulated production of adhesion molecules in the endothelium⁶⁹ and attenuate TNF- α -induced actin polymerization through activation of MAPK phosphatase 1 and inhibition of p38 activity, leading to decreased permeability.⁷¹ It is unclear whether the effects of ANP on endothelial cells are relevant to airway inflammation because increased ANP production appears to increase the allergic diathesis.

NP₇₃₋₁₀₂

NP₇₃₋₁₀₂ produces bronchoprotective effects in mice; however, the precise mechanism of protection is not completely known. This peptide was overexpressed in A549 alveolar type II and normal human bronchial epithelial cells to get an insight into the mechanism, and results showed increases in the intracellular Ca⁺⁺ release and release of NO by activation of constitutive nitric oxide synthase (NOS).⁷² This is similar to ANP's effects on murine macrophages, which also results in increased intracellular calcium and inhibition of the inducible isoform of NOS.⁶⁷ Extracellular Ca⁺⁺ has been reported to affect eosinophil migration,⁶⁷ to affect IL-9 expression and mucus production,⁷³ and to represses inducible NOS in macrophages through destabilization of its mRNA.³⁷ Thus the increased production of NO by NHP₇₃₋₁₀₂ overexpression in airway epithelial cells might be attributed to the control of bronchial hyperreactivity. Also, NP₇₃₋₁₀₂ induces anti-inflammatory activity, as revealed by its ability to substantially decrease activation of NF κ B and ERK1/2, which play a central role in the upregulation of gene expression in the asthmatic lungs of numerous inflammatory molecules. A direct comparison of NF κ B activation with a reporter construct showed that NP₇₃₋₁₀₂ induces a significantly higher decrease of NF κ B activation compared with ANP (Fig 4), suggesting that the former might provide a better therapeutic value. Thus NO release and decreased activation of inflammatory signaling pathways, respectively, could be responsible for the bronchoprotective and anti-inflammatory activity of NP₇₃₋₁₀₂.

CONCLUSIONS

Tremendous progress has occurred in understanding the role of the NP hormones in both the basic and clinical arenas, particularly in relation to the cardiovascular system. A similar detailed knowledge of the NP system in the lung is not as advanced. Furthermore, the NP system appears to greatly affect lymphoid organs, such as the thymus, and the development of immunity and tolerance to diverse antigens. Studies on the role of NPs in the promotion the production of specific cytokines and chemokines that favor the development of allergic diseases, including asthma, in human subjects are in their infancy. The potential to modulate mucosal immunity or tolerance with appropriate antagonists suggests the need for intense investigation of this system in the lung. Understanding NP signaling in immune cells in lymphoid organs and in the lung in the context of asthma presents a challenge for the future.

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