

Atrial natriuretic peptide gene transfer by means of intranasal administration attenuates airway reactivity in a mouse model of allergic sensitization

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Atrial natriuretic peptide (ANP) is a bronchodilator; however, the short half-life of ANP in vivo limited its therapeutic utility to treat asthma. The efficacy of intranasally administered plasmid DNA-expressing ANP (pANP; amino acid 99-126; Acc. No. XM131840) on the prevention of allergen-induced airway hyperresponsiveness (AHR) was examined in this study by using a mouse model of asthma. Ovalbumin-sensitized mice were treated with pANP versus control plasmids, and AHR was monitored after ovalbumin challenge for 5 weeks on 10-day intervals starting 4 days after gene transfer. Mice administered pANP demonstrated significantly less AHR for 20 days after treatment. The results demonstrate that pANP gene transfer protects against AHR and might be useful in the treatment of asthma. (J Allergy Clin Immunol 2002;110:879-82.)

Key words: Atrial natriuretic peptide, gene therapy, allergic asthma

Allergic disease, particularly asthma, is a debilitating disease with high prevalence, affecting more than 155 million people in the developed world. Asthma is one of the most common chronic diseases and is rapidly increasing by 20% per decade, particularly in children. Short-acting and long-acting bronchodilators are considered the first line of therapy for asthma; however, their effects only last for hours, requiring frequent dosing.

Atrial natriuretic peptide (ANP) is a 28-amino-acid hormone secreted by the cardiac atria and lung tissue.¹ ANP has vasodilator, natriuretic, and diuretic properties.¹ ANP infused at high concentrations reduces airway resistance in healthy human subjects.² In patients with asthma, ANP produces a significant bronchodilator response.³ Inhaled ANP attenuates histamine- and methacholine-induced bronchoconstriction.^{3,4} However, the amount necessary for bronchodilation and its short half-life⁵ limit

Abbreviations used

AHR: Airway hyperresponsiveness
ANP: Atrial natriuretic peptide
OVA: Ovalbumin
pANP: Plasmid DNA-expressing ANP

its use for long-term modulation of airway hyperresponsiveness (AHR).

It was reasoned that it might be possible to achieve a prolonged modulation of airway reactivity by using ANP gene transfer. ANP cDNA cloned in a mammalian expression vector was intranasally administered for expression in the murine lung, and the modulation of methacholine-induced airway reactivity was examined to test this possibility in a mouse model of asthma. The results demonstrate that ANP gene transfer provides effective protection against allergen-induced airway reactivity.

METHODS

Animals

Six-week-old female BALB/c mice from Jackson Laboratory were maintained in pathogen-free conditions in accordance with animal research committee regulations. The investigation was approved by the Animal Care Committee of the University of South Florida and the James A. Haley Veterans Hospital, Tampa, Fla.

Construction of ANP expression vector

Total RNA was isolated from murine hearts by using Trizol reagent (Life Technology), according to the manufacturer's protocol. The cDNA sequence for ANP amino acids 99 to 126 from ANP prohormone was amplified by using RT-PCR. The following primers were used: forward, 5'-GAC GGC AAG CTT ACT ATG GGC AGC CCC TGG GAC CC-3', reverse, 5'-ACC CCC CTC GAG TTA TTA TCT TCG TAG GCT CCG-3'. A translation initiation codon was inserted in the forward primer so that ANP (amino acids 99-126) had an additional amino acid, methionine, as the first amino acid apart from its known amino acid sequence. The PCR product was cloned in pVAX vector (Invitrogen) at the *Hind*III and *Xho*I sites. The cloned ANP sequence was verified by means of DNA sequencing, and its expression was checked in A549 human epithelial cells and in the murine lung.

Estimation of ANP by means of ELISA

The expression of ANP was measured with a commercial ELISA kit (SPI-BIO), according to the manufacturer's instruction. This ELISA was devised to measure rat ANP, which has 100% cross-reactivity with mouse ANP.

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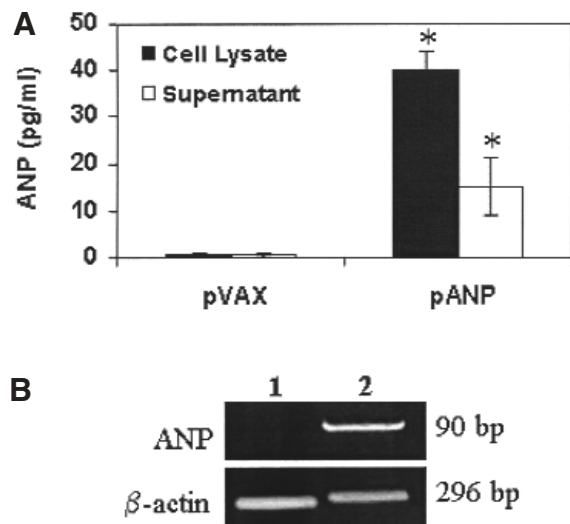


FIG 1. A, Expression of ANP (amino acids 99-126) in the human type II alveolar epithelial cell line A549. A549 cells were either transfected with pANP or pVAX vector control. Expression of ANP was detected in cell supernatant and lysate from pANP-transfected cells alone but not pVAX control cells. * $P < .05$ compared with pVAX control cells. **B**, Expression of ANP in murine lung tissue. Mice were administered intranasally with either pANP or pVAX, as previously described. Three days after the last DNA administration, ANP expression in lung tissue was checked by means of RT-PCR. Mice receiving pANP (lane 2) exhibited ANP expression, which was not present in control mice receiving empty pVAX plasmid (lane 1).

Allergen sensitization, intranasal gene transfer, and pulmonary function

BALB/c mice ($n = 6$) were sensitized once with an intraperitoneal injection of 10 μ g of ovalbumin (OVA) precipitated with 1 mg of alum on day 0. Mice were intranasally administered either plasmid DNA-expressing ANP (pANP) or vector control (pVAX; 10 μ g of plasmid + 50 μ g of lipofectamine in PBS) 3 times a day on days 15, 18, and 21. Animals were challenged with OVA (50 μ g in PBS) 3 days after the last intranasal DNA delivery, and 24 hours later, AHR was measured by using a whole-body plethysmograph (Buxco), as previously described.⁶ AHR was measured at 10-day intervals up to day 56. Mice were challenged with OVA 24 hours before each AHR measurement.

RT-PCR analysis

Total RNA was isolated from murine lung and spleen tissue with Trizol reagent (Life Technology), and RT-PCR was performed by using ANP-specific primers, as described previously. The resultant PCR products were analyzed by means of electrophoresis on 1.5% agarose gel, and the products were visualized by means of staining with ethidium bromide.

Measurement of OVA-specific IgE

OVA-specific IgE was measured to monitor the degree of sensitization. Microtiter plates were coated overnight at 4°C with 100 μ L of OVA (5 μ g/mL). Sera obtained at day 21 from sensitized and nonsensitized mice ($n = 4$) were incubated to the antigen-coated wells, and bound IgE was detected with biotinylated anti-mouse IgE (02112D, Pharmingen). Biotin anti-mouse IgE (02122D) reacts specifically with the mouse IgE of Igh^a and Igh^b haplotypes and does not react with other IgG isotypes. Diluted streptavidin-peroxidase conjugate was added, the bound enzyme was detected with TMB, and the absorbance was read at 450 nm.

Statistical analysis

Pairs of groups were compared by using the Student *t* test. Differences between groups were considered significant at a *P* value of less than .05. Values for all measurements are expressed as the mean $\pm$ SD.

RESULTS

Expression of ANP in human type II alveolar A549 cells and murine lung tissue

ANP was amplified by using PCR, the product was cloned into pVAX vector, and either pANP or empty plasmid vector pVAX were transfected to the human alveolar epithelial A549 cells to determine the expression of ANP (Fig 1). Cells and supernatants were collected 48 hours after transfection, and ANP concentrations were measured by means of ELISA. Cells transfected with pANP produced ANP in both supernatant and cell extracts (Fig 1, A), whereas the cells transfected with pVAX did not. There was more ANP in the cell lysate than in the supernatant (Fig 1, A). These results demonstrate that ANP peptide is expressed and secreted into the culture medium from the human A549 cells.

RT-PCR was performed to confirm the expression of ANP in murine lung and spleen tissue. ANP-specific transcript was observed in the lung tissue of mice receiving pANP construct and not from the mice receiving the empty plasmid vector (Fig 1, B). No expression of ANP was observed from the spleen tissue (data not shown).

ANP gene transfer decreases airway reactivity

The effect of pANP gene transfer was examined in an OVA-sensitized BALB/c mouse model. AHR was measured in BALB/c mice sensitized with OVA and administered with pANP or control vector plasmid before OVA challenge. The control mice received empty vector. The experimental protocol is outlined in Fig 2, A. OVA-specific IgE was measured in the serum to determine the degree of sensitization achieved after OVA injection. Mice that received OVA with alum had significantly ($P < .01$) higher OVA-specific IgE titers than the control group of mice that received PBS (Fig 2, B). Mice administered pANP showed significantly less AHR ($P < .05$) than did control animals to inhaled methacholine (Fig 2, C). These results suggest that in OVA-sensitized animals ANP gene transfer induces decreases in AHR.

ANP gene transfer induces long-term protection

Intranasal gene transfer induces long-term overexpression of cloned genes in the lungs of mice.⁷ Mice were challenged at 10-day intervals up to 56 days with OVA and then tested for AHR with a plethysmograph to determine the duration of the protective effect exerted by pANP gene transfer (Fig 2, A). The bronchoprotective effect of pANP gene transfer lasted for greater than 25 days during which the AHR of pANP-treated mice was significantly lower ($P < .05$) than that of the mice that received the control plasmid (Fig 3). Although there was

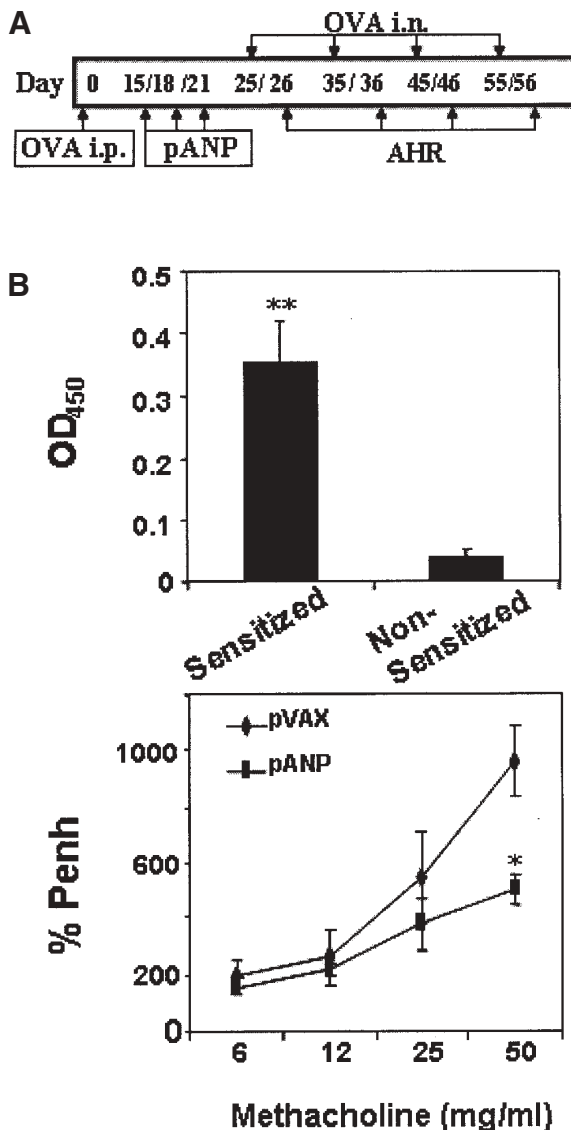


FIG 2. Modulation of AHR by pANP gene transfer. Mice ($n = 6$) were sensitized and treated with either pANP or pVAX, as previously described. **A**, Experimental protocol. **B**, Estimation of degree of sensitization after OVA injection. Mice ($n = 4$) were injected intraperitoneally either with OVA and alum or PBS, and on day 21, their sera were analyzed for OVA-specific IgE. Mice receiving OVA and alum exhibited higher titers ($P < .01$) of OVA-specific IgE than those receiving the PBS control. The experiments were repeated twice, and data from a representative experiment are shown.

a decrease in AHR on day 56, the differences between values were not significant. Thus the effect of ANP gene transfer is long lasting.

DISCUSSION

The present study demonstrates, for the first time, that intranasal gene transfer of ANP protects OVA-sensitized and OVA-challenged BALB/c mice from AHR

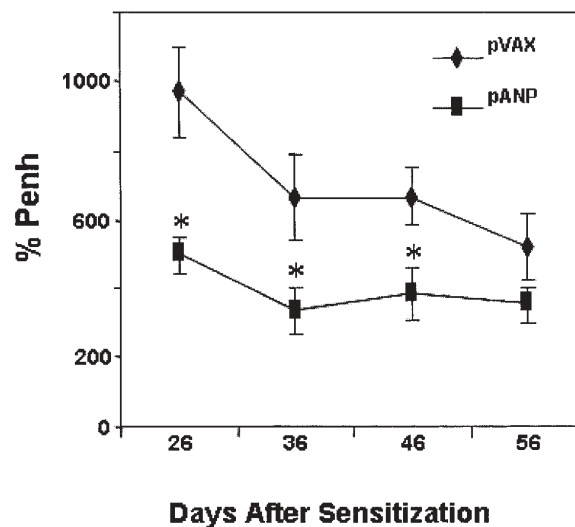


FIG 3. Long-term effect on AHR after ANP gene transfer. Enhanced pause (in percentage) was measured at 50 mg/mL methacholine. * $P < .05$ compared with pVAX control cells. The experiment was repeated twice, and data from a representative experiment are shown.

development, and the protection lasts for 25 days after pANP administration.

ANP is a bronchodilator, although its mechanism of action has not been defined at present. ANP-specific receptors are present in lung tissue,⁷ but cells bearing these receptors are not yet completely characterized. ANP protein is expressed in type II lung epithelial cells transfected with pANP, which is consistent with the identification of ANP in type II alveolar epithelial cells from rat lung tissue.⁸ RT-PCR analysis also confirmed the expression of ANP in lung tissue but not in spleen tissue, which could be due to the local expression of ANP in lung tissue after intranasal gene delivery.

Plasma ANP concentrations are significantly increased during severe asthma exacerbations, suggesting that ANP might have an important and bronchoprotective effect.⁹ Ohbayashi et al¹⁰ demonstrated that intravenous ANP had an inhibitory effect on OVA-induced bronchoconstriction in guinea pigs. However, they used intravenous indomethacin and mepyramine to enhance the contribution of endogenous leukotrienes and avoid the influence of endogenous thromboxanes, prostaglandins, and histamine as pretreatment before antigen challenge.

A major drawback of using ANP in asthma therapy is that it is rapidly metabolized in vivo.¹¹ Therefore a high exogenous dose of ANP is required for effective therapy. Intranasal gene transfer with as little as 90 μ g of pANP per mouse not only protected BALB/c mice from development of AHR but also conferred protection lasting for 25 days when challenged repeatedly with the OVA. The mechanism of the bronchodilatory effect of pANP is unclear. One possibility is that the ANP cDNA used in this study might contain unmethylated CpG motifs, which might induce the reduction in AHR through their immunomodulatory T_H1-promoting effects. However,

this is unlikely because the control mice receiving the pVAX plasmid without the ANP sequence exhibited no reduction in AHR. This is consistent with our previous observation.⁷ Also, no CpG or canonical CpG sequences were found in the ANP cDNA. Alternatively, because ANP elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle,¹² it is likely that pANP might act through the induction of guanyl cyclase and cyclic guanosine monophosphate, leading to a decrease of intracellular Ca⁺⁺. The precise mechanism underlying ANP-induced reduction of lung AHR remains to be investigated.

In conclusion, ANP gene therapy in mice is protective against allergen-induced bronchoconstriction.

REFERENCES

1. Needleman P, Greenwald JE. Atriopeptin: a cardiac hormone intimately involved in fluid, electrolyte, and blood-pressure homeostasis. *N Engl J Med* 1986;314:828-34.
2. Hulks G, Jardine AG, Connell JM, Thomson NC. Effect of atrial natriuretic factor on bronchomotor tone in the normal human airway. *Clin Sci* 1990;79:51-5.
3. Hulks G, Thomson NC. Inhaled atrial natriuretic peptide and asthmatic airways. *BMJ* 1992;304:1156.
4. Angus RM, McCallum MJ, Thomson NC. Effect of inhaled atrial natriuretic peptide on methacholine induced bronchoconstriction in asthma. *Clin Exp Allergy* 1994;24:784-8.
5. Vesely DL. Atrial natriuretic peptides in pathophysiological diseases. *Cardiovasc Res* 2001;51:647-58.
6. Matsuse H, Behera AK, Kumar M, Rabb H, Lockey RF, Mohapatra SS. Recurrent respiratory syncytial virus infections in allergen-sensitized mice lead to persistent airway inflammation and hyperresponsiveness. *J Immunol* 2000;164:6583-92.
7. Kumar M, Behera AK, Matsuse H, Lockey RF, Mohapatra SS. Intranasal IFN-gamma gene transfer protects BALB/c mice against respiratory syncytial virus infection. *Vaccine* 1999;18:558-67.
8. Chanez P, Mann C, Bousquet J, Chabrier PE, Godard P, Braquet P, et al. Atrial natriuretic factor (ANF) is a potent bronchodilator in asthma. *J Allergy Clin Immunol* 1990;86:321-4.
9. Ishii Y, Watanabe T, Watanabe M, Hasegawa S, Uchiyama Y. Effects of atrial natriuretic peptide on type II alveolar epithelial cells of the rat lung. Autoradiographic and morphometric studies. *J Anat* 1989;166:85-95.
10. Boiteau R, Thomas P, Perrin GD, Tenaillon A, Viossat I. Increase in atrial natriuretic factor (ANF) in acute severe asthma (ASA) [abstract]. *Am Rev Respir Dis* 1988;137:A484.
11. Ohbayashi H, Suito H, Takagi K. Compared effects of natriuretic peptides on ovalbumin-induced asthmatic model. *Eur J Pharmacol* 1998;346:55-64.
12. Winquist RJ, Faison EP, Waldman SA, Schwartz K, Murad F, Rapoport RM. ANP elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. *Proc Natl Acad Sci U S A* 1984;81:7661-4.

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