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Compared effects of natriuretic peptides on ovalbumin-induced asthmatic model

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Abstract

We compared the effects of natriuretic peptides on antigen-induced bronchoconstriction and airway microvascular leakage in sensitized guinea pigs. Anesthetized male guinea pigs, ventilated via a tracheal cannula, were placed in a plethysmograph to measure pulmonary mechanics for 10 min after challenge with 1 mg/kg of ovalbumin, and then Evans blue dye was extravasated into airway tissue in order to indicate and evaluate microvascular leakage. Three separate intravenous pretreatments using atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) significantly inhibited the ovalbumin-induced bronchoconstriction and microvascular leakage in a dose-dependent manner. These inhibitory effects were mimicked by 8-bromoguanosine 3′,5′-cyclic monophosphate. We showed that the rank order of inhibitory potencies, which were mediated by cyclic guanosine 3′,5′-monophosphate, was BNP ≥ ANP > CNP. These results gave us some clues for the clinical application of the natriuretic peptides.

Keywords: Natriuretic peptide; Airway microvascular leakage; Bronchoconstriction; 8-BrcGMP (8-bromoguanosine 3′,5′-cyclic monophosphate); Leukotriene; Evans blue dye

1. Introduction

Since the members of the natriuretic peptide family, namely atrial natriuretic peptide (ANP) (De Bold et al., 1981; Oikawa et al., 1984), brain natriuretic peptide (BNP) (Sudoh et al., 1988) and C-type natriuretic peptide (CNP) (Sudoh et al., 1990) were identified and their amino acid sequences determined, we have been interested in the relaxant effects of these natriuretic peptides on tracheal smooth muscle. Most of the biological activities of the natriuretic peptides were thought to be mediated by intracellular accumulation of cyclic guanosine 3′,5′-monophosphate (cGMP) through two receptor subtypes related to the production of cGMP, so-called natriuretic peptide receptors A and B (Schulz et al., 1989), also called guanylate cyclase A and B (Chinkers et al., 1989). We showed relaxant potencies of these three natriuretic peptides in isolated guinea-pig tracheal smooth muscle without changes in the levels of cyclic AMP, but with an increase in tissue cGMP levels in vitro (Watanabe et al., 1988; Takagi et al., 1992; Takagi and Araki, 1993), which were in agreement with those found in previous studies of ANP in the isolated guinea-pig tracheal smooth muscle (Hamel and Ford-Hutchinson, 1986) and in bovine tracheal smooth muscle with increased cGMP in vitro (Ishii and Murad, 1989).

The major molecular form of circulating ANP is a 28 amino acid residue (α-ANP) with a ring structure. ANP has a significant role in maintaining cardiovascular homeostasis by means of potent vasodilator, diuretic, and natriuretic actions (De Bold, 1985). Because of its secretion from the cardiac atria, ANP has received attention for its cardiovascular effects. However, autoradiography has identified ANP in the Type II alveolar epithelial cells of rat lung (Ishii et al., 1989), and the natriuretic peptide A receptor is expressed in various tissues including lung (Suga et al., 1992a), suggesting the possibility that lung tissue is a target organ. Furthermore, the significant bronchodilator effect of α-human ANP on human asthma, when given intravenously (Chanez et al., 1990) and by inhalation in high doses (Hulks and Thomson, 1994), indicated the possibility of its useful therapeutic applica-

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tion, while the in vivo effects of the other natriuretic peptides did not receive sufficient attention. Soon after its discovery in the porcine brain (Sudoh et al., 1988), BNP was found to be secreted mainly from the left ventricle (Saito et al., 1989). The structure of BNP differs among species, and the predominant circulating form of human BNP is a peptide with 32 amino acid residues (Kanbayashi et al., 1990). In addition to its high sequence homology to ANP, its cardiovascular potency was found to be similar to that of ANP (Mukoyama et al., 1991). Also, BNP showed an equally potent binding affinity for the natriuretic peptide A receptor (James and Burnstock, 1991; Suga et al., 1992a), suggesting that BNP might have an important effect on airway smooth muscle in the same manner as does ANP. On the other hand, CNP was found in highest concentration in the central nervous system (Sudoh et al., 1990), and was detected in only small quantities in other organs including the heart and lung (Komatsu et al., 1991). However, the natriuretic peptide B receptor was selectively activated by CNP (Koller et al., 1991; Suga et al., 1992a), and was distributed, not only in the central nervous system, but also in peripheral tissues, including the lung (Suga et al., 1992b). This led us to expect that CNP might be locally synthesized in pulmonary tissues and have some action on airway patency.

In spite of these facts, there have been only a few studies comparing bronchodilator effects in vivo for these peptides. Furthermore, considering the complicated pathophysiological conditions of asthma, one of the more difficult factors complicating asthmatic conditions is airway microvascular leakage followed by airway inflammation and plasma exudation into the airway (Persson, 1986). Barnes et al. (1990) also suggested that, in the management of asthma therapy, the reduction of microvascular leakage with plasma exudation into the airway may be a more valuable strategy than the bronchodilator effect. Our approach was aimed at comparing the effects of these peptides on both bronchoconstriction and airway microvascular leakage in sensitized guinea pigs, and also the clinical possibilities of these peptides in the asthmatic condition were examined for the first time in this study.

2. Material and methods

2.1. Animal preparation

Male Hartley guinea pigs (approximately 250 g, Japan SLC, Shizuoka, Japan) were used throughout the experiments. For sensitizing the animals, 0.5 ml of saline containing 0.1 mg of ovalbumin and 2 mg of aluminum hydroxide were first injected intraperitoneally. Then intraperitoneal injections of 0.1 mg of ovalbumin dissolved in 0.5 ml of 0.9% saline were carried out once a week. Unsensitized animals were similarly treated with three intraperitoneal injections of 0.5 ml saline (with 2 mg of

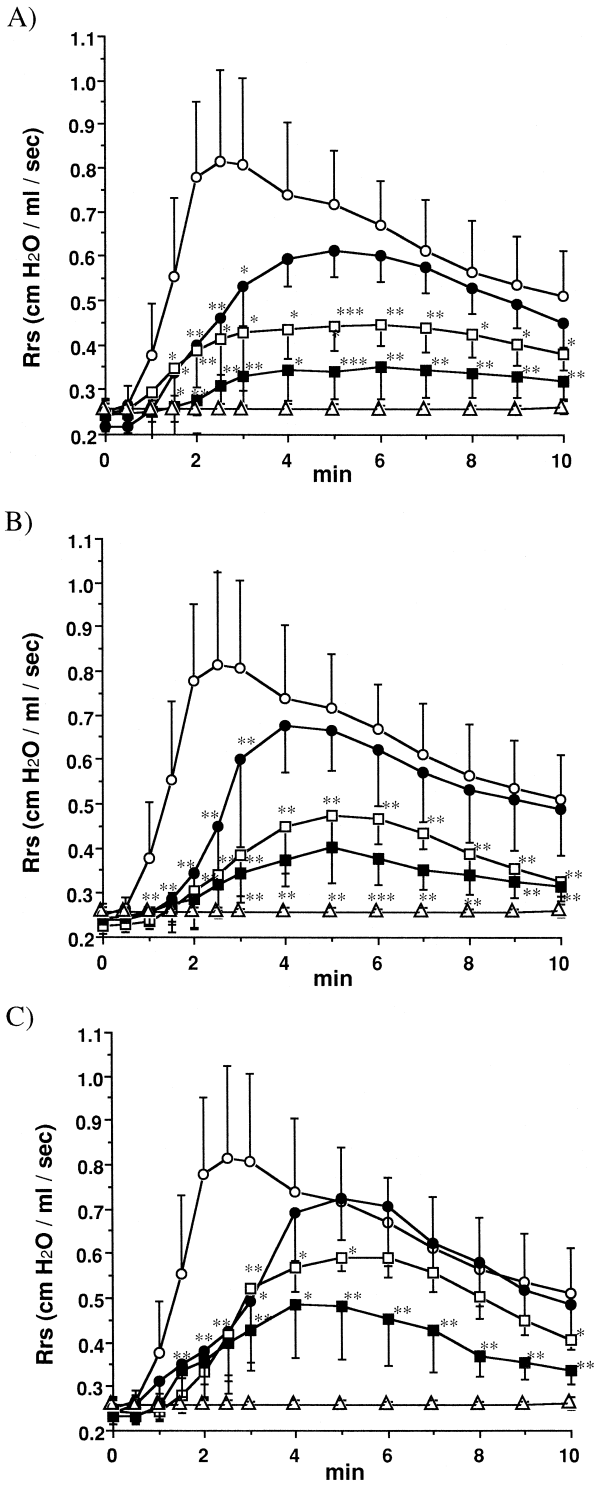

Fig. 1. Time-course of the inhibitory effects of the natriuretic peptides on the antigen-induced increases in respiratory resistance. The ovalbumin control (-○-) shows a significant increase in respiratory resistance compared with the basal control group (-△-). Each concentration of ANP (A) (-●-, 30 μg/kg; -□-, 100 μg/kg; -■-, 300 μg/kg), BNP (B) (-●-, 30 μg/kg; -□-, 100 μg/kg; -■-, 300 μg/kg) or CNP (C) (-●-, 300 μg/kg; -□-, 1000 μg/kg; -■-, 3000 μg/kg) had inhibitory, dose-dependent effects on the ovalbumin-induced bronchoconstriction. Data are shown as means ± S.D. of six experiments. Statistical significance: $* P < 0.05$, $** P < 0.01$ and $*** P < 0.001$ compared with the ovalbumin control group.

aluminum hydroxide only the first time). Three weeks later, guinea pigs, which then weighed 350–450 g, were anesthetized with intraperitoneal injections of pentobarbital sodium (50 mg/kg). We ensured an appropriate level of anesthesia as evidenced by the disappearance of the corneal reflex and of the withdrawal response to paw pinching. A tracheal cannula (7 mm length and 2.0 mm internal diameter) was inserted through a tracheostomy and connected to a constant-volume respirator (Model 683; Harvard Apparatus, South Natick, MA, USA) for keeping mechanical ventilation at a tidal volume of 10 ml/kg and a frequency of 60 breath/min. Then, a jugular vein and a carotid artery were cannulated for application of the drugs and measurement of systemic blood pressure, respectively. The animals were placed in a plethysmograph box (Model PLYAN; Buxco, USA). Signals from the plethysmograph box and catheters were sensed by differential pressure transducers, and monitored by a pulmonary mechanics analyzer (Buxco Model 6). All signals were recorded on a multichannel visicorder (Linearcorder F WR3701; Graphtec, Tokyo, Japan). Respiratory resistance and dynamic compliance were calculated using a Buxco program 1 (Aoki et al., 1991). This study was performed in accordance with the guidelines for animal experimentation set by Nagoya University School of Medicine.

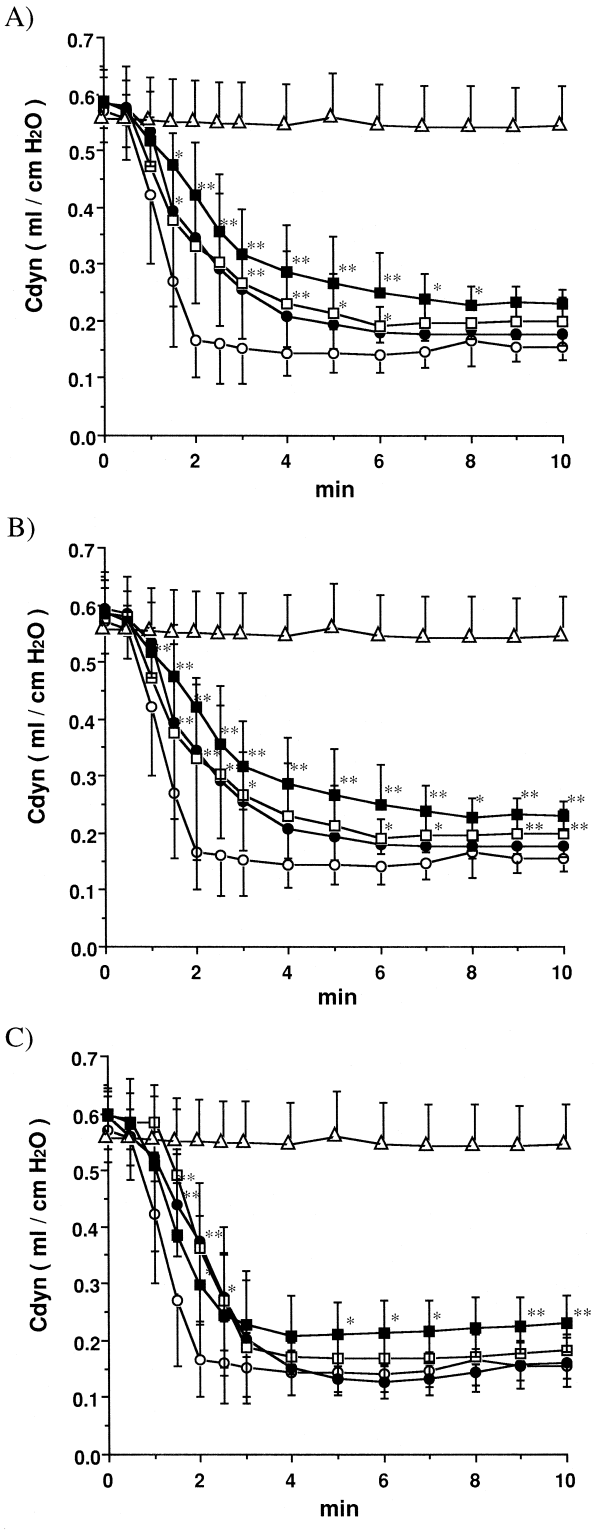

Fig. 2. Time-course of the inhibitory effects of the natriuretic peptides on the antigen-induced increase in dynamic compliance. The ovalbumin control (-○-) shows a significant reduction of dynamic compliance compared with the basal control group (-△-). Each concentration of ANP (A) (-●-, 30 μg/kg; -□-, 100 μg/kg; -■-, 300 μg/kg), BNP (B) (-●-, 30 μg/kg; -□-, 100 μg/kg; -■-, 300 μg/kg) or CNP (C) (-●-, 300 μg/kg; -□-, 1000 μg/kg; -■-, 3000 μg/kg) had dose-dependent inhibitory effects on the ovalbumin-induced bronchoconstriction. Data are shown as means ± S.D. of six experiments. Statistical significance: $* P < 0.05$, $** P < 0.01$ and $*** P < 0.001$ compared with the ovalbumin control group.

2.2. Protocol

We examined the influence of Evans blue dye and that of each natriuretic peptide without antigen challenge. We also examined the changes in bronchoconstriction, microvascular leakage and mean blood pressure with injections of the following agents: vehicle (0.9% saline, 1 mg/kg) as the basal control ($n = 6$), ANP (30 μg/kg i.v., $n = 4$), BNP (30 μg/kg i.v., $n = 4$), CNP (300 μg/kg i.v., $n = 4$), and 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP) (10 mg/kg i.v., $n = 4$).

The effects of natriuretic peptides on antigen-induced bronchoconstriction and microvascular leakage were studied in 60 sensitized animals in the first set of experiments. The animals were randomly divided into 10 groups (six animals per group): (1) ovalbumin (1 mg/kg i.v.) alone as the ovalbumin control group; (2)–(4) groups injected with ANP (30 μg/kg, 100 μg/kg and 300 μg/kg, respectively); (5)–(7) groups injected with BNP (30 μg/kg, 100 μg/kg and 300 μg/kg, respectively); (8)–(10) groups injected with CNP (300 μg/kg, 1000 μg/kg and 3000 μg/kg, respectively). Additionally, to find how the increase of cGMP contributed to the effects of the natriuretic peptides, we examined the effect of 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP), a cGMP ana-

Table 1
Effects of the natriuretic peptides on microvascular leakage and systemic hypotension without ovalbumin challenge

	Evans blue dye concentration (ng/mg of wet tissue)				Mean blood pressure	
	n	Trachea	Main bronchi	Intrapulmonary airway	Pre-injection (mmHg)	Post-injection (mmHg)
Vehicle	6	10.45 ± 3.63	10.03 ± 3.80	9.37 ± 1.69	49.27 ± 8.47	52.33 ± 7.71
Ovalbumin-control	6	94.71 ± 7.04[b]	97.67 ± 6.66[b]	95.47 ± 5.21[b]	49.31 ± 6.47	41.67 ± 1.29[c]
ANP (30 μg/kg)	4	28.77 ± 4.82[b]	29.06 ± 0.96[b]	27.79 ± 3.62[b]	53.31 ± 4.67	41.55 ± 4.03[e]
BNP (30 μg/kg)	4	20.11 ± 8.76[a]	27.59 ± 2.00[b]	22.63 ± 10.0[a]	48.89 ± 4.32	38.27 ± 0.69[d]
CNP (300 μg/kg)	4	13.08 ± 4.82	17.08 ± 7.00	13.91 ± 5.76	51.64 ± 2.89	36.92 ± 0.38[e]
8-BrcGMP (10 mg/kg)	4	13.50 ± 3.00	13.88 ± 2.87	8.34 ± 1.00	54.54 ± 4.68	47.08 ± 1.08[a]

ANP = atrial natriuretic peptide; BNP = brain natriuretic peptides; CNP = c-type natriuretic peptides; 8-BrcGMP = 8-bromoguanosine 3′,5′-cyclic monophosphate.
ANP and BNP induced significant increases in microvascular leakage in all three segments, but CNP and 8-BrcGMP did not. Intravenous injection of each natriuretic peptide and 8-BrcGMP caused a transient decrease of systemic blood pressure, while Evans blue dye caused no significant change.
Results are means ± S.D. *n* = number of guinea pigs per group. Statistical significance: [a]$P < 0.05$ and [b]$P < 0.001$ compared with the vehicle as the basal control (0.9% saline with 20 mg/ml Evans blue dye).
For mean blood pressure, significant differences relative to pre-injection are shown as: [c]$P < 0.05$; [d]$P < 0.01$; [c]$P < 0.001$.

logue, (10 mg/kg i.v., $n = 6$) against antigen-induced bronchoconstriction and microvascular leakage in sensitized guinea pigs.

In the second set of experiments, 30 unsensitized animals were studied in order to ascertain how the inhibitory effect of ANP was related to the leukotriene-mediated bronchoconstriction and microvascular leakage. We divided the animals randomly into five groups (six per group): (1) vehicle (0.9% saline, 1 mg/kg i.v.) as the basal control; (2) leukotriene D_4 (2 μg/kg i.v.) alone; (3) leukotriene D_4 (2 μg/kg i.v.) and ANP (100 μg/kg i.v.); (4) leukotriene D_4 (2 μg/kg i.v.) and ANP (300 μg/kg i.v.); (5) leukotriene D_4 (2 μg/kg i.v.) and ANP (1000 μg/kg i.v.).

2.2.1. Measurement of pulmonary mechanics and mean blood pressure in antigen-induced bronchoconstriction

For the purpose of enhancing the contribution of endogenous leukotrienes and avoiding effects of endogenous thromboxanes, prostaglandins and histamine (Ueno et al., 1982; Leitch et al., 1983), pretreatment with intravenous administration of 5 mg/kg indomethacin and 1 mg/kg mepyramine maleate was done at 3 and 2 min, respectively, before the challenge with 1 mg/kg ovalbumin solution containing 20 mg/kg Evans blue dye. Baseline recordings of pulmonary mechanics (respiratory resistance and dynamic compliance) and mean blood pressure were obtained, and each dose of the natriuretic peptides or 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP) (10 mg/kg) was injected intravenously at 1 min before challenge with the antigen. Then, we recorded the values for respiratory resistance, dynamic compliance and mean blood pressure for 10 min.

2.2.2. Quantitative analysis of airway microvascular leakage

This microvascular leakage was quantified by the extravasation of Evans blue dye into airway interstitial tissue as an index of airway vascular permeability, since it correlates with the extravasation of radiolabeled albumin in guinea-pig airways (Rogers et al., 1989). We used a technique that allowed measurement of both bronchocon-

Table 2
Effects of ANP, BNP or CNP on ovalbumin-induced microvascular leakage at each airway level

	n	Trachea		Main bronchi		Intrapulmonary airway	
		(ng/mg tissue)	(% inhibition)	(ng/mg tissue)	(% inhibition)	(ng/mg tissue)	(% inhibition)
ovalbumin-control	6	94.71 ± 7.04		97.67 ± 6.66		95.47 ± 5.21	
ANP (30 μg/kg)	6	55.37 ± 22.22[b]	41.5	83.81 ± 16.75	14.2	90.09 ± 26.89	5.6
ANP (100 μg/kg)	6	36.17 ± 17.24[c]	61.8	50.12 ± 8.32[c]	48.7	68.04 ± 9.59[c]	28.7
ANP (300 μg/kg)	6	33.27 ± 9.73[c]	64.8	56.38 ± 10.65[c]	42.3	67.48 ± 17.02[b]	29.3
BNP (30 μg/kg)	6	56.50 ± 15.43[c]	40.3	62.88 ± 10.53[c]	35.6	74.69 ± 13.11[c]	21.7
BNP (100 μg/kg)	6	43.92 ± 10.05[c]	53.6	54.74 ± 15.41[c]	44.0	57.00 ± 6.59[c]	40.3
BNP (300 μg/kg)	6	36.79 ± 7.04[c]	61.2	51.46 ± 10.65[c]	47.3	54.74 ± 13.45[c]	42.7
CNP (300 μg/kg)	6	60.20 ± 11.55[c]	36.4	75.50 ± 7.69[a]	22.7	90.73 ± 6.86	7.1
CNP (1000 μg/kg)	6	51.48 ± 11.51[c]	45.6	65.48 ± 9.09[b]	33.0	90.51 ± 8.41	5.2
CNP (3000 μg/kg)	6	42.52 ± 12.78[c]	55.1	67.48 ± 16.83[b]	30.9	68.40 ± 9.85[b]	28.4

Abbreviations as in Table 1. Results are means ± S.D. The values are expressed as ng of Evans blue dye per mg of wet tissue. *n* = number of guinea pigs per group.
Significance of differences from the ovalbumin-control is shown as: [a]$P < 0.05$; [b]$P < 0.01$; and [c]$P < 0.001$.

striction and airway microvascular leakage in the same animal. This technique had the advantage of giving the relationship between airway constriction and microvascular permeability. After measuring the antigen-induced bronchoconstriction, we immediately began the following stage. The animal was exsanguinated, and the chest cavity was opened. In order to eliminate extra Evans blue dye in its pulmonary circulation, we perfused 50 ml of 0.9% saline at a pressure of 120 mmHg from the pulmonary artery into the left atrium. The airway and lung were then removed, and the extraneous connective tissues, vasculature and parenchyma were gently scraped off with a blunt scalpel until only bronchial tissue remained. The airways were divided into three portions: trachea, main bronchi, and the intrapulmonary airways. Each tissue piece was weighed wet, and soaked in 2 ml of formamide at 37°C for 24 h to extract the Evans blue dye. We determined the amount of Evans blue dye extracted by measuring the optical density at 600 nm with a spectrophotometer (EIA plate reader, ELNX 96, Metertech, USA). Evans blue dye extravasation was calculated by interpolation on a standard curve constructed with five known dye concentrations in the range of 0.5–10 μg/ml, and the weight was expressed in ng per mg of wet tissue.

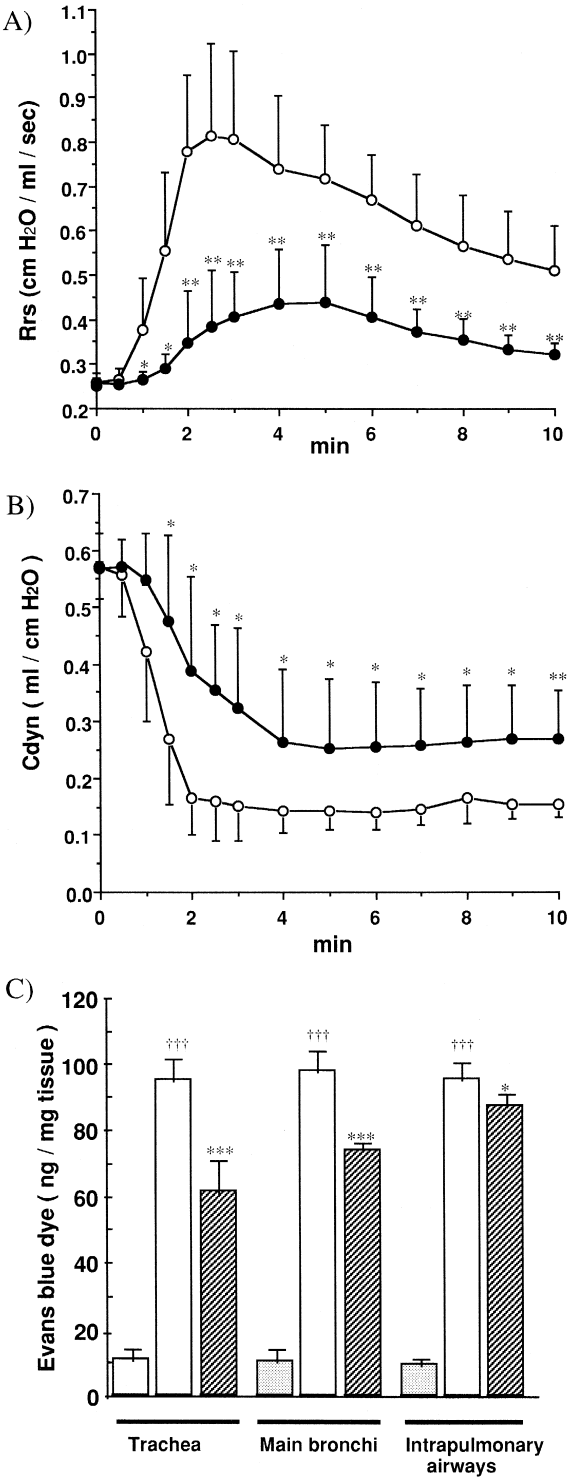

Fig. 3. (A) Effects of 8-BrcGMP against the ovalbumin-induced increase in respiratory resistance. Pretreatment with 8-BrcGMP (-●-, 10 mg/kg) resulted in significant inhibition of the antigen-induced increase in respiratory resistance. Significantly different from the ovalbumin control (-○-): $*P < 0.05$ and $**P < 0.01$. (B) Effects of 8-BrcGMP on the antigen-induced decrease in dynamic compliance. Pre-administration of 8-BrcGMP (-●-, 10 mg/kg) significantly inhibited the effect on the reduction of dynamic compliance. Data are shown as means ± S.D. of six experiments. Significantly different from the ovalbumin control (-○-): $*P < 0.05$ and $**P < 0.01$. (C) Effects of 8-BrcGMP, a cGMP analogue, on microvascular leakage induced by ovalbumin challenge at different airway levels in sensitized guinea pigs. Columns (dotted rectangle) and (rectangle) represent the basal control group (0.9% saline) and the ovalbumin control group, respectively. Intravenous preinjection of 8-BrcGMP (diagonally-striped rectangle, 10 mg/kg) significantly reduced microvascular permeability in our system. Each value is shown as means ± S.D. of six experiments. Statistical significance: $*P < 0.05$, $***P < 0.001$ compared with the ovalbumin control group; $^{\dagger\dagger\dagger}P < 0.001$ compared with the basal control group.

2.3. Drugs and chemicals

The following drugs and chemicals were used: mammalian C-type natriuretic peptide (CNP-(1–22)) and peptide leukotriene D_4 were kindly donated by Suntory (Osaka, Japan) and by Ono Pharmaceutical (Osaka, Japan), respectively. Human atrial natriuretic peptide (hANP-(1–28)) and human brain natriuretic peptide (hBNP-(1–32)) were purchased from Peptide Institute (Osaka, Japan); 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP) from Sigma (St. Louis, MO, USA); chicken ovalbumin, indomethacin, formamide and Evans blue from Waco Pure Chemical (Osaka, Japan); aluminum hydroxide (Al(OH)3) from Katayama Chemical (Osaka, Japan); and mepyramine maleate from Cosmobio. (Tokyo, Japan). Indomethacin was dissolved in a 7% sodium bicarbonate solution (Maylon, Ohtuka Pharmaceutical, Tokyo, Japan) and other agents were dissolved in 0.9% NaCl.

2.4. Statistical analysis

All data are expressed as means ± S.D. Statistical comparisons were made using either the unpaired Student's *t*-test (two-tailed) or a two-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. The mean blood pressure was calculated from recorded traces as diastolic blood pressure + 0.33(systolic blood pressure − diastolic blood pressure). *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Comparison of inhibitory effects on antigen-induced bronchoconstriction

We ascertained in a preliminary study that Evans blue dye alone had no influence on respiratory resistance and dynamic compliance. ANP (30 μg/kg i.v., $n = 4$), BNP (30 μg/kg i.v., $n = 4$), CNP (300 μg/kg i.v., $n = 4$), and 8-bromoguanosine 3′,5′-cyclic monophosphate (10 mg/kg i.v., $n = 4$) also led to no significant increase of respiratory resistance and decrease of dynamic compliance in the group not challenged by antigen; (data not shown). The administration of 1 mg/kg ovalbumin caused significant bronchoconstriction, which reached a maximum within 3 min after injection. The effects of these natriuretic peptides on changes of respiratory resistance, which are reflected mainly as the index of central airway constriction, are shown in Fig. 1A–C. The effects on changes in dynamic compliance, which are reflected mainly as the index of peripheral airway constriction, are shown in Fig. 2A–C. Intravenous pretreatment with ANP, BNP or CNP significantly reduced the ovalbumin-induced bronchoconstriction in a dose-dependent manner.

3.2. Comparison of inhibitory effects on microvascular leakage

In a preliminary study, we verified that ANP (30 μg/kg) and BNP (30 μg/kg) significantly increased mi-

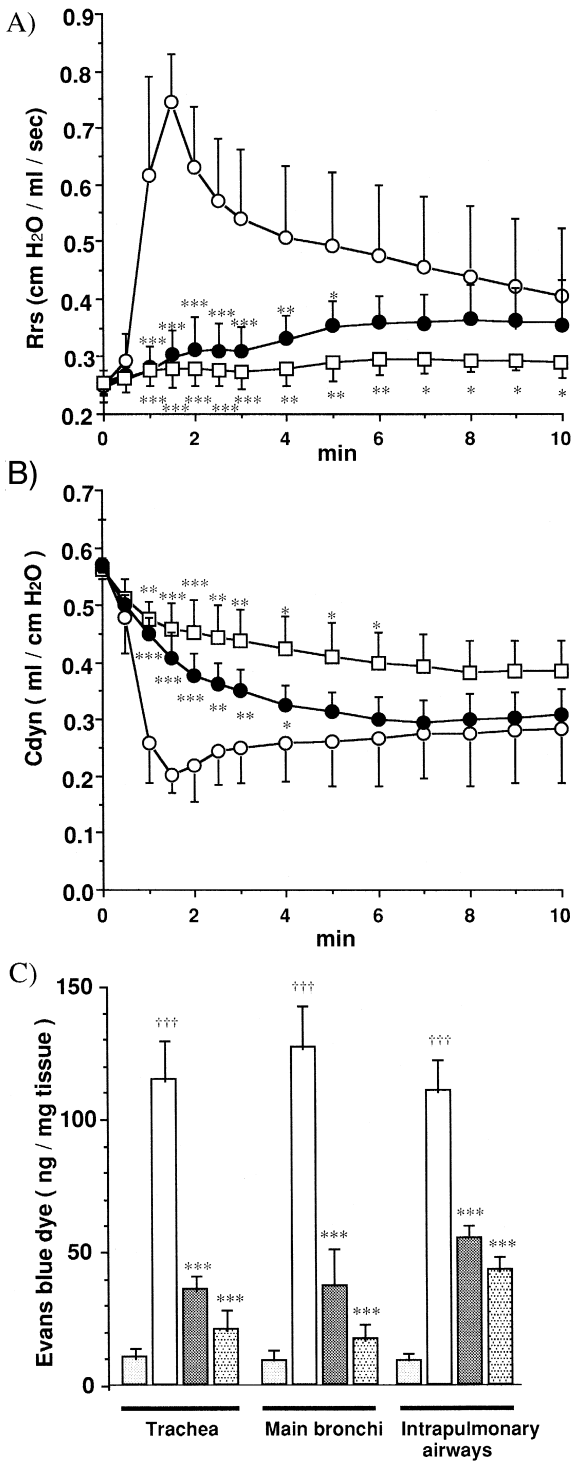

Fig. 4. (A) Reduction by ANP of leukotriene D_4-induced increase of respiratory resistance in unsensitized guinea pigs. Pretreatment with ANP (-●-, 30 μg/kg; -□-, 100 μg/kg) caused a significant inhibition of the leukotriene D_4-induced increase in respiratory resistance (-○- as the leukotriene D_4 control, 2 μg/kg) in a dose-dependent manner. Significantly different from the leukotriene D_4 control: $*\ P < 0.05$, $**\ P < 0.01$ and $***\ P < 0.001$. (B) Reduction by ANP of the leukotriene D_4-induced decrease of dynamic compliance in unsensitized guinea pigs. In the earlier half of the phase, pretreatment with ANP (-●-, 30 μg/kg; -□-, 100 μg/kg) shows significantly inhibited the leukotriene-induced reduction of dynamic compliance (-○- as the leukotriene D_4 control, 2 μg/kg) in a dose-dependent manner. Significantly different from the leukotriene D_4 control: $*\ P < 0.05$, $**\ P < 0.01$ and $***\ P < 0.001$. (C) Reduction by ANP of the leukotriene D_4-induced increase in microvascular leakage at different airway levels in unsensitized guinea pigs. Columns (dotted rectangle) and (rectangle) represent the basal control group (0.9% saline) and the leukotriene D_4 control group, respectively. The other columns (rectangle with intersecting diagonal lines and rectangle with patterned dots) represent ANP (30 μg/kg and ANP 100 μg/kg, respectively). Each value is shown as means ± S.D. of six experiments. Statistical significance: $***\ P < 0.001$ compared with the leukotriene D_4 control group; $^{\dagger\dagger\dagger}P < 0.001$ compared with basal control group.

crovascular leakage when compared with the basal control levels if the animals were not challenged with antigen, while CNP (300 μg/kg) and 8-bromoguanosine 3′,5′-cyclic monophosphate (10 mg/kg) did not. Intravenous administration of ovalbumin produced significant increases of extravasated Evans blue dye in the trachea, main bronchi, and intrapulmonary airways, respectively ($P < 0.001$) (Table 1).

Intravenous pretreatment with ANP, BNP and CNP significantly inhibited ovalbumin-induced plasma leakage at all three airway levels in a dose-dependent manner (Table 2). These results showed an interesting tendency in that all three natriuretic peptides acted more potently in the central airway than in the peripheral airway.

3.3. The inhibitory effects of 8-BrcGMP on antigen-induced airway changes

Intravenous pretreatment with 10 mg/kg of 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP) significantly inhibited the antigen-induced increase in respiratory resistance (Fig. 3A) and the decrease in dynamic compliance (Fig. 3B). Moreover, 8-BrcGMP also mimicked the significant inhibition of the ovalbumin-induced microvascular leakage in the same fashion as did the three natriuretic peptides (Fig. 3C).

3.4. The inhibitory effect of ANP on the leukotriene D_4-induced airway changes

Injection of 2 μg/kg leukotriene D_4 caused a significant increase in respiratory resistance which peaked at 1.5 min after injection (Fig. 4A), and a significant decrease in dynamic compliance, peaking at the same time (Fig. 4B). Both concentrations of ANP (30 and 100 μg/kg) completely reduced these leukotriene D_4-induced airway changes, especially in the earlier phase. Pretreatment with the same doses of ANP significantly decreased the extravasation of Evans blue dye caused by leukotriene D_4 in all airway segments ($P < 0.001$) (Fig. 4C).

3.5. Changes in mean blood pressure

Each natriuretic peptide and 8-bromoguanosine 3′,5′-cyclic monophosphate caused a significant decrease in mean blood pressure immediately after injection (Table 1). The fall in mean blood pressure was maximal within 1 min, followed by the recovery phase.

4. Discussion

We compared the effects of natriuretic peptides on antigen-induced bronchoconstriction and airway microvascular leakage in actively sensitized guinea pigs in this study. We demonstrated how the potent bronchodilator effects of these natriuretic peptides resulted in significant inhibition of antigen-induced bronchoconstriction as shown in the asthmatic phase. On the other hand, preliminary tests without ovalbumin challenge, as a normal non-asthmatic phase, assured us that there were no statistically significant bronchodilator effects due to these natriuretic peptides. These results were in agreement with those of previous in vitro studies done by us and other investigators showing the bronchodilator effects of these natriuretic peptides. We determined the effect of 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP), a membrane-permeable and more stable cGMP analogue, on sensitized guinea-pig airways to further investigate the mechanism of inhibitory effects against antigen-induced bronchoconstriction. This exogenously applied cGMP analogue elicited a significant reduction in bronchoconstriction, which mimicked the antigen-induced results in these experiments. The baseline pulmonary mechanics did not clearly reveal the amount of the increased baseline airway microvascular leakage in this experiment. However, in earlier antigen-induced asthmatic responses, the first factor may have been the reducing effects on bronchoconstriction rather than on increased baseline microvascular leakage. These facts suggest that the potent bronchodilator effects of these natriuretic peptides mainly result from a direct action on smooth airway muscle, mediated by cGMP.

We noticed that ANP and BNP increased baseline airway microvascular leakage which was at first unexpected, while 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP) did not in preliminary tests without antigen challenge as in a normal non-asthmatic phase. The increase in basal microvascular leakage caused by administration of ANP or BNP alone was much less than after antigen challenge. Considering that 8-BrcGMP did not influence baseline microvascular leakage, these effects on increased basal leakage may not have resulted from receptor-mediated functional changes caused by cGMP elevation but from some non-specific action by intravenous administration of the peptide itself. These facts suggest that increased changes in basal microvascular permeability are not necessarily physiological, and may prove to be negligible when one discusses the inhibitory mechanism of these peptides on antigen-induced airway microvascular leakage. On the other hand, these natriuretic peptides, as shown in Table 1, cause a significant drop in systemic mean blood pressure. The reduction of systemic mean blood pressure may be mainly due to cGMP-mediated direct vasodilator effects of natriuretic peptides on vascular smooth muscle (Winquist et al., 1984). Microvascular leakage may occur mainly in capillary vessels which have no vascular smooth muscle. These findings suggest that there may be another distinct mechanism involved in the inhibitory effect on microvascular leakage.

We ascertained that these natriuretic peptides showed significant potent inhibitory effects on antigen-induced microvascular leakage which were mimicked by 8-

bromoguanosine 3′,5′-cyclic monophosphate in this study. Our results were in agreement with those of Lofton et al. (1990), who showed the anti-edematic action of ANP in aortic endothelial cells, using 8-bromoguanosine 3′,5′-cyclic monophosphate. Additionally, several previous investigators reported that ANP inhibited increases in endothelial monolayer permeability and played a role in the prevention of pulmonary edema chemically induced by arachidonic acid, paraquat, oxygen-free radicals and other substances (Inomata et al., 1987; Lofton et al., 1991). These actions were thought to be receptor-mediated, possibly involving cGMP. On the other hand, two specific receptors subtypes related to the production of cGMP, natriuretic peptide A and B receptors, have been found to be localized on the endothelial cells of pulmonary vessels (Mantyh et al., 1986; Suga et al., 1992a). These facts suggest that the inhibitory mechanism of natriuretic peptides on airway microvascular permeability may be mainly mediated by cGMP in pulmonary capillary endothelial cells. The mechanism of cGMP action in decreased microvascular permeability is still unknown. However, since intracellular calcium is known to be of importance in the maintenance of the cytoskeleton and gap junctions, it is possible that stimulation of cGMP accumulation in endothelial cells by ANP may cause a reduction of free cytoplasmic calcium. This would result in the stabilization of the cytoskeleton and of the endothelial gap junctions between the cells (Lofton et al., 1990).

With the aid of the binding assay, the Northern blot technique and the cGMP production method, Suga et al. (1992a) elucidated the binding affinities of the natriuretic peptides for natriuretic peptide A and B receptors with their potencies for cGMP production in aortic smooth muscle cells. The rank orders of potencies for cGMP production via natriuretic peptide receptor binding were ANP > BNP ≫ CNP and CNP > ANP > BNP, respectively. The authors also indicated that the natriuretic peptide A receptor was expressed in a number of tissues including lung tissue, to some extent overlapping the tissue distribution of the natriuretic peptide B receptors. Although both receptors were present in lung tissue, there has been little published concerning their distribution in airway segments. We indicated that the rank order of inhibitory potencies of both bronchoconstriction and microvascular permeability in vivo was BNP ≥ ANP > CNP in this study. These rankings, which did not contradict our in vitro studies, suggested that the natriuretic peptide A receptor was more closely associated with inhibitory effects than was the natriuretic B peptide receptor. Moreover, in comparison with percent inhibition (Table 1), all natriuretic peptides showed a tendency to act more effectively in the central airway than in the peripheral airway. This tendency also appeared in the bronchoconstriction values (respiratory resistance and dynamic compliance). These phenomena might result from the distribution of the two receptor subtypes in the central and peripheral airways, and suggested that these receptors might be expressed more potently in the central airway. We anticipated that these results would provide some clues for clarifying the distribution of receptors in the lung.

In this study, we used intravenous administration of indomethacin and mepyramine maleate as pretreatment before antigen challenge to enhance the contribution of endogenous leukotrienes and avoid the influence of endogenous thromboxanes, prostaglandins and histamine (Ueno et al., 1982; Leitch et al., 1983). We became convinced that every natriuretic peptide had a significant reductive effect on antigen-induced microvascular leakage at all airway levels. Previous studies had shown that the peptide leukotrienes, 5-lipoxygenase products of arachidonic acid metabolism, had potent long-lasting bronchoconstricting effects and acted as major mediators of allergic conditions in human asthma (Dahlen et al., 1983). Leukotrienes were also one of the major mediators accounting for changes in airway microvascular permeability not only in human asthma, but also in sensitized guinea pigs (Hui et al., 1989). Antigen challenge released leukotriene C_4 and leukotriene D_4 from passively sensitized human lung fragments (Lewis et al., 1980). Leukotriene D_4 was generated by immunological challenge of sensitized guinea-pig lung tissue (Morris et al., 1980). Additionally, it was recently reported that ONO-1078 (4-oxo-8-[4-(4-phenylbutoxy)-benzoyiamino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate), a potent selective leukotriene receptor antagonist, inhibited the antigen-induced late-phase asthmatic response in allergic guinea-pigs (Nakagawa et al., 1993). These results led us to think that protection from leukotrienes was an indispensable factor in the therapeutic strategy for asthma, and to investigate the inhibitory effects on leukotriene-induced bronchoconstriction and microvascular leakage. As we had expected, ANP significantly inhibited both leukotriene D_4-induced bronchoconstriction and airway microvascular leakage in the same fashion than as it did during the antigen-induced phase.

Unfortunately, the factor that most interferes with therapeutic application of exogenous natriuretic peptides is their rapid in vivo metabolism. Rapid clearance due to rapid metabolism required high injection doses in this experiment. In addition to the high binding affinities of clearance receptors for natriuretic peptides (Suga et al., 1992a), active enzymatic degradation by the enzyme, neutral endopeptidase-24.11(NEP, EC 3.4.24.11), is widely believed to play a physiological role in metabolizing ANP. Recent studies showed that neutral endopeptidase-24.11 inhibitor enzymes prolong the half-life and enhance the biological actions of exogenously given ANP in vivo (Trapani et al., 1989). These studies also demonstrated how SCH 42354 *N*-[2(*S*)-(mercaptomethyl)-3-(2-methylphenyl)-1-oxopropyl]-L-methionine, a neutral endopeptidase-24.11 inhibitor, increased the potency of ANP in isolated pulmonary vessels and isolated perfused whole rat lungs

(Thompson and Morice, 1995). The above results suggested that the natriuretic peptides were also metabolized by the action of the enzyme neutral endopeptidase-24.11. It is not known how the rapid metabolism caused by the effects of clearance receptors and the enzyme neutral endopeptidase-24.11 influences the inhibitory effects of the natriuretic peptides under asthmatic conditions. However, we believe that the solution to these problems will shed light on the next stage of therapeutic application of natriuretic peptides.

In conclusion, this study was the first to compare the effects of three natriuretic peptides on antigen-induced microvascular leakage and bronchoconstriction. These results should prompt further experimental and clinical studies to evaluate the potential use of natriuretic peptides for bronchial asthma in humans.

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References

Aoki, S., Tsukagoshi, H., Kurosawa, M., 1991. Changes in respiratory resistance and dynamic compliance induced by histamine in the guinea pig (abstract). Jpn. J. Thorac. Dis. 29, 1246.

Barnes, P.J., Boschetto, P., Rogers, D.F., Belvisi, M., Roberts, N., Chung, K.F., Evans, T.W., 1990. Effects of treatment on airway microvascular leakage. Eur. Respir. J. 3, 663S–671S.

Chanez, P., Mann, C., Bousquet, J., Chabrier, P.E., Godard, P., Braquet, P., Michel, F.B., 1990. Atrial natriuretic factor (ANF) is a potent bronchodilator in asthma. J. Allergy Clin. Immunol. 86, 321–324.

Chinkers, M.D., Garbers, D.L., Chang, M.S., Lowe, D.G., Chin, H., Goeddel, D.V., Schulz, S., 1989. A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. Nature 338, 78–83.

Dahlen, S.E., Hedqvis, P., Hammatström, S., 1983. Contractile activities of several cysteine-containing leukotrienes in the guinea-pig lung strip. Eur. J. Pharmacol. 86, 207–215.

De Bold, A.J., 1985. Atrial natriuretic factor: a hormone produced by the heart. Science (Washington, DC) 230, 767–770.

De Bold, A.J., Borenstein, H.B., Veress, A.T., Sonnenberg, H., 1981. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci. 28, 89–94.

Hamel, R., Ford-Hutchinson, A.W., 1986. Relaxant profile of synthetic atrial natriuretic factor on guinea-pig pulmonary tissues. Eur. J. Pharmacol. 121, 151–155.

Hui, K.P., Lothwall, J., Rogers, D.F., Chung, K.F., Barnes, P.J., 1989. Airway microvascular leak and changes in lung resistance and compliance induced by aerosolized allergen challenge in sensitized guinea-pigs in vivo. Thorax 40, 895P (abstract).

Hulks, G., Thomson, N.C., 1994. High dose inhaled atrial natriuretic peptide is a bronchodilator in asthmatic subjects. Eur. Respir. J. 7, 1593–1597.

Inomata, N., Ohnuma, N., Furuya, M., Hayashi, Y., Kanai, Y., Ishihara, T., Noguchi, T., Matsuo, H., 1987. Alpha-human atrial natriuretic peptide (α-hANP) prevents pulmonary edema induced by arachidonic acid treatment in isolated perfused lung from guinea pig. Jpn. J. Pharmacol. 44, 211–214.

Ishii, K., Murad, F., 1989. ANP relaxes bovine tracheal smooth muscle and increases cGMP. Am. J. Physiol. 256, C495–C500.

Ishii, Y., Watanabe, T., Watanabe, M., Hasegawa, S., Uchiyama, Y., 1989. Effects of atrial natriuretic peptide on Type II epithelial cells of the rat lung. J. Anat. 166, 85–95.

James, S., Burnstock, G., 1991. Atrial and brain natriuretic peptides share binding sites on cultured cells from the rat trachea. Cell Tissue Res. 265, 555–565.

Kanbayashi, Y., Nakao, K., Mukoyama, M., Saito, Y., Ogawa, Y., Shiono, S., Inoue, K., Yoshida, N., Imura, H., 1990. Isolation and sequence determination of human brain natriuretic peptide in human atrium. FEBS Lett. 259, 341–345.

Koller, K.J., Lowe, D.G., Bennett, G.L., Minamino, N., Kangawa, K., Matsuo, H., Goeddel, D.V., 1991. Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). Science 252, 120–123.

Komatsu, Y., Nakao, K., Suga, S., Ogawa, Y., Mukoyama, M., Arai, H., Shirakami, G., Hosoda, K., Nakagawa, O., Hama, N., Kishimoto, I., Imura, H., 1991. C-type natriuretic peptide (CNP) in rats and humans. Endocrinology 129, 1104–1106.

Leitch, A.G., Austen, K.F., Corey, E.J., Drazen, J.M., 1983. Effects of indomethacin on the guinea-pig pulmonary response to intravenous leukotrienes C_4 and D_4. Clin. Sci. 65, 281–287.

Lewis, R.A., Austen, K.F., Drazen, J.M., Clark, D.A., Marfat, A., Corey, E.J., 1980. Slow reacting substances of anaphylaxis: identification of leukotrienes C-1 and D from human and rat sources. Proc. Natl. Acad. Sci. USA 77, 3710–3714.

Lofton, C.E., Newman, W.H., Currie, M.G., 1990. Atrial natriuretic peptide regulation of endothelial permeability is mediated by cGMP. Biochem. Biophys. Res. Commun. 172, 793–799.

Lofton, C.E., Baron, D.A., Heffner, J.E., Currie, M.G., Newman, W.H., 1991. Atrial natriuretic peptide inhibits oxidant-induced increases in endothelial permeability. J. Mol. Cell Cardiol. 23, 919–927.

Mantyh, C.R., Kruger, L., Brecha, N.C., Mantyh, P.W., 1986. Localization of specific binding sites for atrial natriuretic factor in peripheral tissues of the guinea pig, rat, and human. Hypertension 8, 712–721.

Morris, H.R., Taylor, G.W., Piper, P.J., Tippins, J.R., 1980. Structure of slow-reacting substance of anaphylaxis from guinea-pig lung. Nature 285, 104–106.

Mukoyama, M., Nakao, K., Hosoda, K., Suga, S., Saito, Y., Ogawa, Y., Shirakami, G., Jougasaki, M., Obata, K., Yasue, H., Kambayashi, Y., Inouye, K., Imura, H., 1991. Brain natriuretic peptide as a novel cardiac hormone in humans: evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J. Clin. Invest. 87, 1402–1412.

Nakagawa, N., Obata, T., Kobayashi, T., Okada, Y., Nambu, F., Terawaki, T., Furuya, T., Muryobayashi, K., Sawada, M., Aishita, H., 1993. Effect of a peptide leukotriene receptor antagonist, ONO-1078, on guinea-pig models of asthma. Eur. J. Pharmacol. 235, 211–214.

Oikawa, S., Imai, M., Ueno, A., Tanaka, S., Noguchi, T., Nakazato, H., Kanagawa, K., Fukuda, A., Matsuo, H., 1984. Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide. Nature 309, 724–726.

Persson, C.G.A., 1986. Role of plasma exudation in asthmatic airways. Lancet i, 1126–1128.

Rogers, D.F., Boschetto, P., Barnes, P.J., 1989. Plasma exudation: correlation between Evans blue dye and radiolabelled albumin in guinea pig airways in vivo. J. Pharmacol. Methods 21, 309–315.

Saito, Y., Nakao, K., Itoh, H., Yamada, T., Mukoyama, M., Arai, H., Hosoda, K., Shirakami, G., Suga, S., Minamino, N., Kangawa, K., Matsuo, H., Imura, H., 1989. Brain natriuretic peptide is a novel cardiac hormone. Biochem. Biophy. Res. Commun. 158, 360–368.

Schulz, S., Singh, S., Bellet, R.A., Singh, G., Tubb, D.J., Chin, H., Garbers, D.L., 1989. The primary structure of a plasma membrane guanylate cyclase demonstrates diversity within this new receptor family. Cell 58, 1155–1162.

Sudoh, T., Kangawa, K., Minamino, N., Matsuo, H., 1988. A new natriuretic peptide in porcine brain. Nature 332, 78–81.

Sudoh, T., Minamino, N., Kangawa, K., Matsuo, H., 1990. C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. Biochem. Biophy. Res. Commun. 168, 863–870.

Suga, S., Nakao, K., Hosoda, K., Mukoyama, M., Ogawa, Y., Shirakami, G., Arai, H., Saito, Y., Kambayashi, Y., Inouye, K., Imura, H., 1992a. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. Endocrinology 130, 229–239.

Suga, S., Nakao, K., Kishimoto, I., Hosoda, K., Mukoyama, M., Arai, H., Shirakami, G., Ogawa, Y., Komatsu, Y., Nakagawa, O., Hara, N., Imura, H., 1992b. Phenotype-related alteration in expression of natriuretic peptide receptors in aortic smooth muscle cells. Circ. Res. 71, 34–39.

Takagi, K., Araki, N., 1993. Relaxant effects of brain natriuretic peptide on guinea-pig tracheal smooth muscle. Clin. Exp. Phamacol. Physiol. 20, 239–243.

Takagi, K., Araki, N., Suzuki, K., 1992. Relaxant effects of C-type natriuretic peptide on guinea-pig tracheal smooth muscle. Arzneim.-Forsch. 42, 1329–1332.

Thompson, J.S., Morice, A.H., 1995. Neutral endopeptidase inhibition increases the potency of ANP in isolated rat pulmonary resistance vessels and isolated blood perfused lungs. Pulm. Pharmacol. 8, 143–147.

Trapani, A.J., Smits, G.J., McGraw, D.E., Spear, K.L., Koepke, J.P., Olins, G.M., Blaine, E.H., 1989. Thiorphan, an inhibitor of endopeptidase 24.11, potentiates the natriuretic activity of atrial natriuretic peptide. J. Cardiovasc. Pharmacol. 14, 419–424.

Ueno, A., Tanaka, K., Katori, M., 1982. Possible involvement of thromboxane in bronchoconstrictive and hypertensive effects of LTC_4 and LTD_4 in guinea pigs. Prostaglandins 23, 865–880.

Watanabe, H., Takagi, K., Satake, T., 1988. Relaxant effects of atrial natriuretic peptide on guinea pig tracheal smooth muscle. Prog. Biochem. Pharmacol. 23, 136–141.

Winquist, R.J., Faison, E.P., Waldman, S.A., Schwartz, K., Murad, F., Rapoport, R.M., 1984. Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. Proc. Natl. Acad. Sci. USA 81, 7661–7664.