Inflammopharmacology. 1998; 6: 159–178.

GB West Memorial Lecture

INFLUENCE OF ATRIAL NATRIURETIC PEPTIDE, BRAIN NATRIURETIC PEPTIDE AND URODILATIN ON THE HISTAMINE-INDUCED BRONCHOCONSTRICTION IN THE CONSCIOUS GUINEA PIG

M. HERMEL*
Institute of Pharmacology and Toxicology at the RWTH Aachen, Wendlingweg, 52049 Aachen, Germany
*Address for correspondence: Herforder Str. 23, 50737 Köln, Germany

ABSTRACT

Hermel M. Influence of atrial natriuretic peptide, brain natriuretic peptide and urodilatin on the histamine-induced bronchoconstriction in the conscious guinea pig. Inflammopharmacology. 1998;6: 159–178.

The influence of human atrial natriuretic peptide (ANP) and of two related peptides, human brain natriuretic peptide (BNP) and urodilatin (URO) on the bronchoconstriction induced by inhalation of histamine in conscious, non-anaesthetized guinea pigs was tested.

Changes in lung function were registered using two independent methods, one operating in a closed body-plethysmographic system, the other in an open system based on the time lag of air flow curves. The peptides were infused (0.25 ml/min) into the jugular vein for a period from 10 min before until 15 min after the histamine inhalation.

ANP displayed virtually no effect on the bronchoconstriction. URO showed some inibition at 1280 ng kg^{-1} min^{-1}, but not at lower doses. BNP (640 ng kg^{-1} min^{-1}) inhibited the bronchoconstriction markedly for the total registration period.

It can be concluded from these results that BNP exerts bronchoprotective effects in the conscious guinea pig, which are superior to those of ANP or URO.

Keywords: Natriuretic peptides, lung function, guinea pig, histamine bronchoconstriction

INTRODUCTION

In recent years, a possible role in the physiological regulation as well as in the pharmacological modulation of the airways has been attributed to a novel family of endogenous peptide hormones, the atrial natriuretic peptides.

De Bold et al. [1] postulated the existence of a natriuretic factor from the rat heart atrium (ANF) involved in the blood volume homeostasis. This finding led to the isolation of a new family of peptides from rat and human heart atria, capable of evoking smooth muscle relaxation and natriuresis [2–5].

This work is a short version in English of the doctoral thesis submitted to the Medical Faculty of the RWTH Aachen

Atrial natriuretic peptides were later found to influence the physiological regulation of the circulatory system at many levels. They were shown to reduce the circulating blood volume by enhancing natriuresis and diuresis [6–8], relax vascular smooth muscle directly [9], lower the cardiac output [10] and inhibit renine, aldosterone and vasopressin secretion [11–13]. All these effects lead to a reduction in blood pressure and circulating blood volume [11].

The brain natriuretic peptide (BNP), another new member of the atrial peptide family, was originally isolated from porcine brain [14,15]. However, the cardiac atrium of various species has since been identified as the main site of synthesis, storage and secretion of the peptide, which, like the ANP, seems to play a role as circulating hormone [14–24]. A number of effects similar to those of the ANP have been found in animals and humans [14,15,25–28] but species differences in the biological actions have also been postulated [29].

Urodilatin was originally isolated from human urine by Schulz-Knappe et al. [30]. It is an ANP variant extended by 4 N-terminal residues (ANP_{95-126}). Although it has not been found in human plasma, some of its systemic pharmacological effects were found to be stronger than those of ANP_{99-126} [31,32].

Dose-dependent relaxing effects of several variants of ANP on isolated trachea and pulmonary vasculature, first reported by O'Donnell et al. [33], were later confirmed by several authors [34–37]. The activity on human tissue preparations seemed to depend upon the bronchoconstrictory agent [38,39].

An in-vivo protective effect against a bronchoconstrictive agent was also shown for sheep and guinea pigs, but could not be demonstrated in dogs and rats [36,40–44]. In rabbits, an indirect effect of ANP on the airways has been postulated [45,46], and evidence for central nervous modulation of the tracheal tone of anaesthetized cats by ANP has also been reported [47].

In humans, intravenously applied ANP relaxed the basal tone of normal and asthmatic airways. It also displayed a protective potency against bronchoconstriction evoked by histamine or distilled water inhalation in asthmatic patients [48–52]. Asthmatic airways were also dilated and protected against histamine and methacholine by higher doses of inhaled ANP [53–56].

The other members of the ANP family also displayed actions on airways. In contrast to ANP, urodilatin infusions protected rats against stimulated bronchoconstriction. This peptide was also able to lower the resting tone of human asthmatic airways [45,57–59].

Effects of BNP on airways remained unknown until relaxing and protective effects of this peptide against histamine preconstriction of guinea pig tracheal muscle preparations were reported by Takagi and Araki [60]. A comparison of the effective doses (EC_{50}) of ANP [61] and BNP makes a superior relaxing action of BNP probable [60].

Results of tests of ANP on histamine bronchoconstriction in guinea pigs are not uniform [36,41], and no reports about actions of urodilatin on guinea pigs airways or in-vivo respiratory effects of BNP have come to our knowledge so far.

Here we report the effects of three different peptides (ANP, BNP and urodilatin) on histamine-induced bronchoconstriction in the conscious guinea pig.

MATERIALS AND METHODS

Methods

One single apparatus was constructed to permit the use of two different methods for non-invasive lung function assessment in conscious guinea pigs (Figure 1).

The animal is placed in the body chamber of a two chamber plethysmograph (Plexi, PVC), with its head in the head chamber, and its neck being held by an adjustable restrainer. The air-tightness of the seal between the mouth and the body chamber is provided by a pressure controlled inflatable rubber cuff and a small amount of silicone grease. A supply of humidified fresh air is provided via the mouth chamber before and between the measurements.

The plethysmograph was set up in a way to permit the employment of both methods almost simultaneously with a minimum of manipulations.

Interrupter method

The body plethysmographic (or interrupter) method was originally designed by Hutson et al. [62]. It allows the measurement of changes in the specific airway conductance. This method makes use of a plethysmograph, a pneumotachograph (P_{nc}, Fleisch mod. 0000, Switzerland) and a shutter (S) as part of a tubing circuit, which can be separated from the atmosphere (valves V_a and V_b) when the fresh air supply (valve V_c) is cut off. Once the air-tightness of the system has been checked, the air flow (V) oscillating through the circuit can be registered by Pn_c.

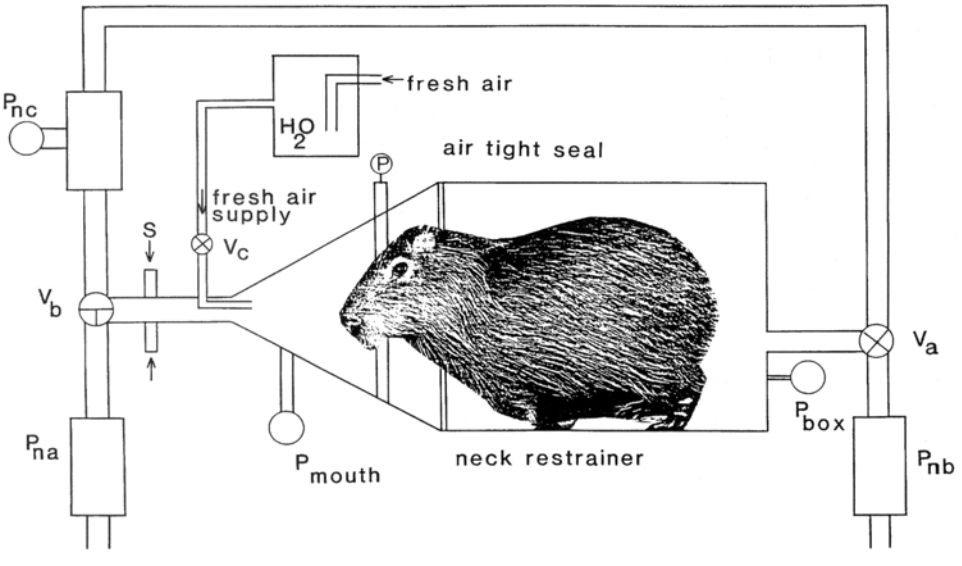

Figure 1. Construction of the apparatus

At the end of an expiration, the shutter (S) is closed to interrupt the airflow. During the following inspiration, pressure changes in the mouth and the body chamber are recorded via differential pressure transducers. The shutter, the air outlet and the fresh air supply are then reopened.

The airway resistance (R_{aw}), thoracic gas volume (V_{aw}) and the specific airway conductance (sG_{aw}) are calculated according to the equations given by Hutson et al. [62].

The absolute values of sG_{aw} are in the range given by Hutson et al. [62]. Since the dead volume of the mouth chamber cannot be measured and must be estimated individually for each animal, changes in the specific airway conductance are given instead of its absolute values.

Phase lag method

The method for non-invasive lung function assessment in the conscious guinea pig, originally described by Pennock et al. [63], was modified. It allows measurements in a constant pressure open system and does not depend on air-tightness of the plethysmograph.

The plethysmograph chambers are connected to the atmosphere via the pneumotachographs Pn_a and Pn_b (the valves V_a and V_b are set appropriately). When the fresh air supply (V_c) is stopped, the flow of air in and out of both chambers can be registered by the pneumotachographs with the animal breathing spontaneously. The delay between the pressure curve of the body and the mouth chamber is determined, and can be expressed as a phase lag. Assuming a sinusoidal curve, Pennock et al. [63] have derived the following equation for this phase lag:

$$\tan \theta = \omega \, C_a \, R_{aw} \tag{1}$$

where θ = phase shift between mouth and box flow curves (°), $\omega = 2 \pi f$, f = breath frequency (1/s), C_a = alveolar compliance (ml/cmH_2O), and R_{aw} = airway resistance (cmH_2O ml^{-1} s).

For an isothermal expansion, C_a equals the thoracic gas volume divided by ambient pressure:

$$C_a = \frac{V_{aw}}{(P_{atm} - P_s)\, u} \quad (ml/cmH_2O) \tag{2}$$

where P_{atm} = atmospheric pressure (mmHg), P_s = 47mmHg = saturation pressure of air with water at body temperature, u = 13.6 cmH_2O/mmHg, V_{aw} = thoracic gas volume.

It is thus possible to determine the specific airway resistance (sR_{aw}):

$$sR_{aw} = R_{aw} \, V_{aw} = (P_{atm} - P_s)\, u \, \frac{\tan \theta}{2 \pi f} \quad (cmH_2Os) \tag{3}$$

Further parameters, e.g. respiratory frequency, volume of expiration, maximal expiratory air flow and times of inspiration end expiration, can also be determined. Each single measurement is a mean of 10 breath periods.

The differential pressure transducers were obtained from Validyne Engineering Corp. (DP 45-28, 528A3S4D; MP 45-18-871; MP 45-14-871; Northridge, CA, USA), and other equipment (carrier frequency bridge amplifiers CFBA 677, recorder output module ROM 670, Linearcorder Mark VIII WR 3500 / WA 3503 and the pneumotachograph power supply) from Hugo Sachs Elektronik KG (March, Germany).

The data was recorded and processed using a specially designed computer programme and could be independently recorded on the paper chart recorder.

Animals

Male Hartley-Dunkin guinea pigs of 400–760 g body weight (Hagemann, Extertal, Germany) were used throughout the study. They were kept in an animal house with artificial light for 12 h, a constant temperature of 22–24°C, and a relative humidity of 50%, and fed standard guinea-pig food MS-206, Eggersmann GmbH, Rinteln-Gochsheim, Germany.

Study design

In a first series of experiments to measure the variability of the bronchoconstrictor response depending on inhalation time, the following schedule was applied: after 2–8 baseline measurements (BL), nebulized histamine was administered, and measurements were taken between 1 and 16 min after the end of challenge. The animal was then taken out of the plethysmograph and later placed in the apparatus again for a 60-min measurement. The 13, 15, 16, 60 min and the baseline measurements were a mean of 6 (interrupter method) or 4 (phase lag method = 40 breath periods) single recordings each, the other measurement a mean of at least 2 and 1 recordings, respectively. A histamine concentration of 0.5 mg/ml saline was used for each challenge, the challenge time varying between 15 and 60 s.

Since the plasma half-lives of atrial peptides are very short in most species – e.g. 0.5–1 min in mice and rats [64–68] and 2–5 min in humans [69–72] – the natriuretic peptides had to be administered by intravenous infusion. Under general anaesthesia using ketamine–HCl, a specially designed polyethylene catheter was inserted into the internal jugular vein and conducted through a subcutaneous tunnel out of the back of the animal. To prevent infections, doxycycline (4 mg per animal, Vibravenös, Pfizer GmbH, Karlsruhe, Germany) was injected intraperitoneally every other day until the end of the experiment. A recovery period of at least 48 h was maintained before the first experiment. The dead space volume of the catheter was approximately 0.2 ml.

In these experiments, the following measurement schedule was applied: first, two baseline measurements were performed (15 and 10 min before histamine administration). Then the infusion of saline or test solution was started. Two minutes before

histamine challenge, another measurement was taken to assess possible changes of the baseline lung function value induced by the infusion. Nebulized histamine was applied (time 0 min) for a constant exposure time of 30 s. Another 30 s were allowed for fresh air supply. Then, measurements at 1, 3, 5, 7, 11 and 15 min were performed, the infusion was stopped and the animal taken out of the apparatus. Another measurement was taken 2 h after histamine administration. The baseline, 15-min and 2-h measurements were again composed of 6 and 4, the other of at least 2 and 1 measurements in the interrupter and phase lag methods, respectively.

Between consecutive experiments, a minimum period of 48 h was allowed. The histamine evoked bronchoconstriction which showed considerable variability between the animals. To ensure a comparable response, the individual histamine dose was assessed using the following schedule: with the challenge time kept constant (30 s), the histamine concentration in saline was doubled until a sufficient response (rise in sR_{aw} by 80% or a fall in sG_{aw} by 30%) was obtained. Histamine concentrations of 0.5 mg/ml, 1 mg/ml, 2 mg/ml and 4 mg/ml were used.

Atrial natriuretic peptide ($hANP_{99-126}$), brain natriuretic peptide (hBNP) and urodilatin ($hANP_{95-126}$) (Bissendorf-Peptide, Hannover, Germany) were given by intravenous infusion in doses of 320, 640 and 1280 $ng\,min^{-1}\,kg^{-1}$ body weight. The peptides were stored at −20°C and diluted in saline immediately before use. The infusion was started 10 min before histamine challenge to ensure constant blood levels. The infusion rate of 0.25 ml/min was kept constant throughout the experiments.

For the bronchial challenge, histamine dihydrochloride (E. Merck, Darmstadt, Germany) was dissolved in saline and nebulized directly into the mouth chamber using an ultrasonic nebulizer (DeVilbiss GmbH, Dietzenbach, Germany; Mod. 35 B, 1.35 MHz, with a mean droplet diameter of 3 μm).

Statistics

Values are given as mean ± SEM. Statistical analysis was performed by the Student's *t*-test for paired or unpaired values, where appropriate [73]. Differences were considered significant if $p < 0.05$.

RESULTS

The mean baseline value of the specific airway resistance obtained with the phase lag method was found to be 2.96 ± 0.05 cmH_2Os ($n = 220$). Baseline values obtained over a period of 8 days showed on average a maximum deviation of $3.04 \pm 3.26\%$ ($n = 25$) in the body plethysmographic method and of $3.41 \pm 0.26\%$ ($n = 7$) in the phase lag method. Inhalation of saline caused no significant deviation.

Histamine-induced bronchoconstriction

Both methods recorded a significant dose-dependent bronchoconstriction caused by nebulized histamine. A dose-dependent response of the bronchi to both the histamine concentration and the inhalation time could be observed. An increase of inhalation time (15, 30 and 60 s) displayed a marked increase of the airway response (Figure 2).

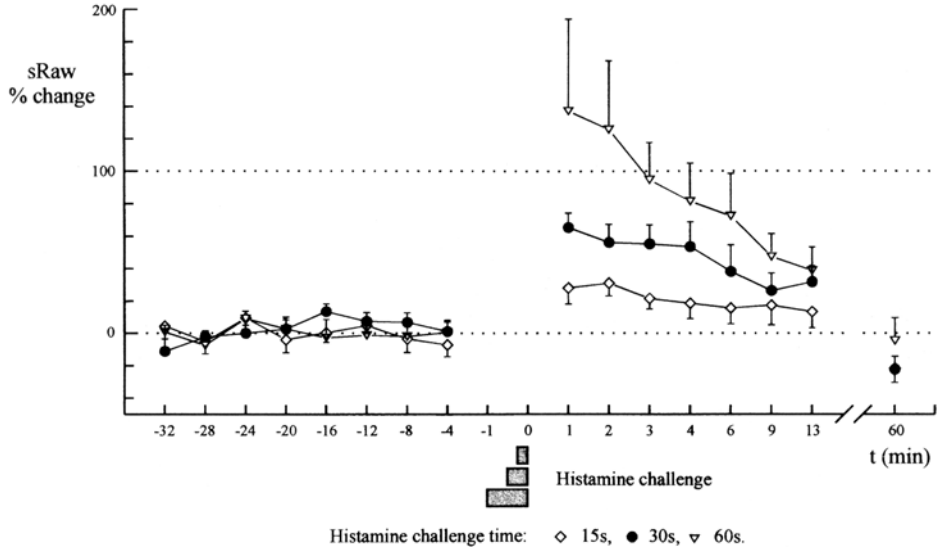

Figure 2. Dose dependency of the histamine-induced bronchoconstriction: variation of inhalation time (0.5 mg/ml, 15 s, 30 s, 60 s, mean ± SEM, $n = 7$). sRaw = specific airway resistance, phase lag method

The animals displayed marked differences in their susceptibility to inhaled histamine (Figure 3). The histamine concentration necessary to evoke a response of comparable intensity was therefore assessed individually.

The bronchoconstriction provoked using the depicted protocol proved to be significant ($p < 0.0001$ with both methods). The lung function values 2 min before (–2 min, after 8 min saline infusion) and 2 h after challenge did not differ significantly from baseline.

To ensure reproducibility of the bronchial reaction, a separate control group of 6 animals was investigated (Figure 4). All animals reacted to histamine with a marked bronchoconstriction. When the histamine challenge was repeated 48 h later, values obtained by both methods were found to be almost identical. Thus, the experimental protocol was shown to be reliable.

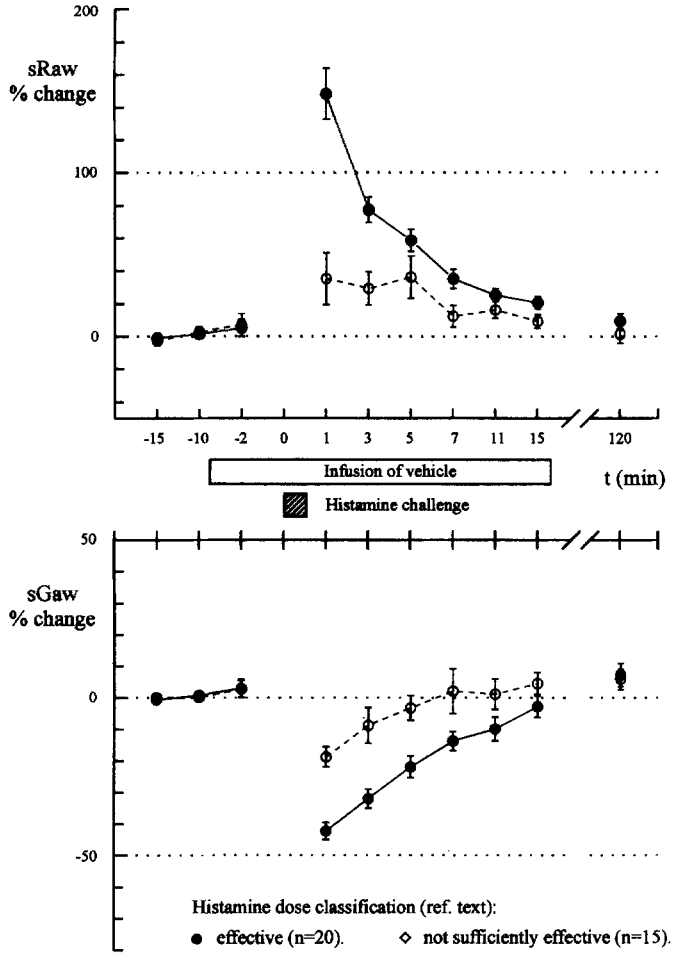

Figure 3. Histamine-induced bronchoconstriction. Investigations on the variability of bronchial responses during sequential assessment of the effective histamine dose. sRaw = specific airway resistance, phase lag method. sGaw = specific airway conductance, interrupter method

Effects of atrial peptides

Atrial natriuretic peptide did not display marked bronchoprotective potency. Although preliminary experiments applying a low infusion rate of 320 ng min^{-1} kg^{-1} showed some protective tendency ($n = 2$, $p < 0.05$ by one method), higher doses of the peptide failed to elicit an effect. When ANP was infused at a rate of 640 ng min^{-1} kg^{-1}, the intensity of the bronchial reaction was found to be reduced with both methods but no significance was demonstrated ($n = 4$). Finally, experiments using the highest dose of

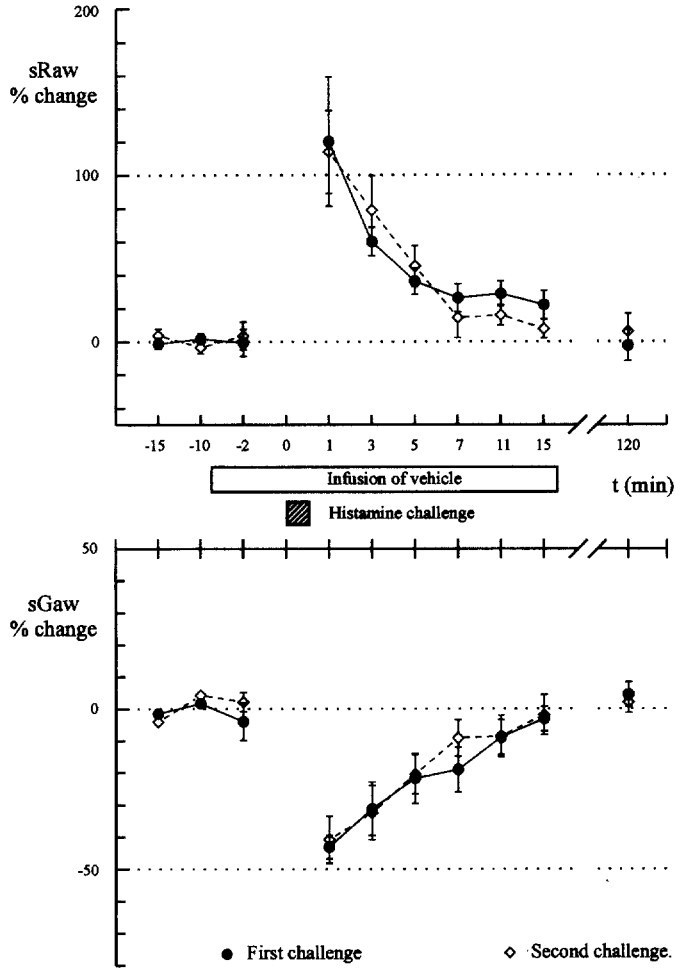

Figure 4. Reproducibility of histamine-induced bronchoconstriction. Repeated histamine challenge after 48 h (mean ± SEM, $n = 6$). sRaw = specific airway resistance, phase lag method. sGaw = specific airway conductance, interrupter method

1280 ng min^{-1} kg^{-1} showed no protective effect of the peptide ($n = 5$). Thus, atrial natriuretic peptide failed to display a marked inhibition of histamine-induced bronchoconstriction in conscious guinea pigs.

Urodilatin, when infused at a rate of 640 ng kg^{-1} min^{-1} displayed some reduction of bronchial response in the body plethysmographic method and the difference remained visible over 7 min. The phase lag method, however, showed a comparable tendency only 1 min after challenge but later the measurements did not differ from control values. The effects were not significant ($n = 5$).

Experiments using lower and higher infusion rates have also been attempted. Preliminary results showed only a marginal effect of the peptide when given at 320 $ng\,kg^{-1}\,min^{-1}$, but the highest dose of 1280 $ng\,kg^{-1}\,min^{-1}$ resulted in a more marked protective effect, which even reached significance with the phase lag method ($p<0.05$), although such doses could be tested in only 2 animals.

Amongst the three peptides, brain natriuretic peptide showed the most marked bronchoprotective potency. Under an infusion rate of 320 $ng\,kg^{-1}\,min^{-1}$ the phase lag method displayed a reduction in the evoked bronchoconstriction between the 5-min and 11-min values which reached significance at 7 min ($p<0.05$). However, the interrupter method did not display an effect (Figure 5).

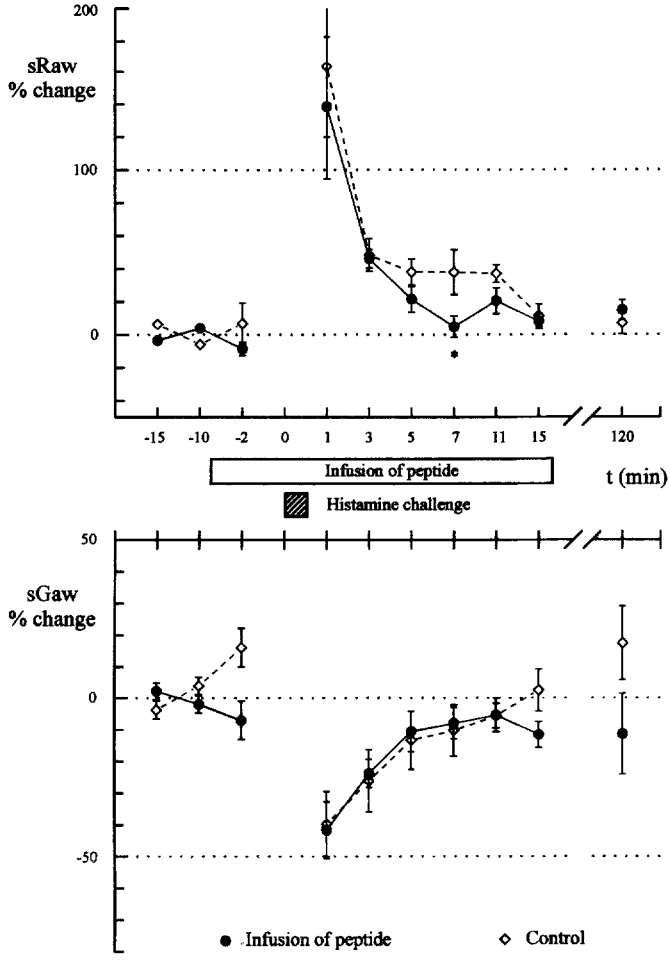

Figure 5. Effects of brain natriuretic peptide on histamine-induced bronchoconstriction. (Dose: 320 $ng\,kg^{-1}\,min^{-1}$ iv, mean ± SEM, $n=4$). sRaw = specific airway resistance, phase lag method. sGaw = specific airway conductance, interrupter method

When infused with a rate of 640 ng min^{-1} kg^{-1}, BNP inhibited the histamine-induced bronchoconstriction throughout the whole experiment (Figure 6). In the body plethysmographic method, the effect continued for 11 min and proved to be significant ($p < 0.001$). The phase lag method displayed a similar significant ($p < 0.01$) inhibition.

The inhibition of the airway response was also visible in experiments applying a 1280 ng min^{-1} kg^{-1} infusion rate (Figure 7). With the body plethysmographic method, a maximum fall in sG_{aw} by $45.4 \pm 7.51\%$ under saline infusion was reduced to $18.8 \pm 2.01\%$. The phase lag method displayed an initial increase in sR_{aw} by $77.4 \pm 23.9\%$ vs. $190 \pm 25.7\%$ control. The effect was significant with both methods ($p < 0.05$).

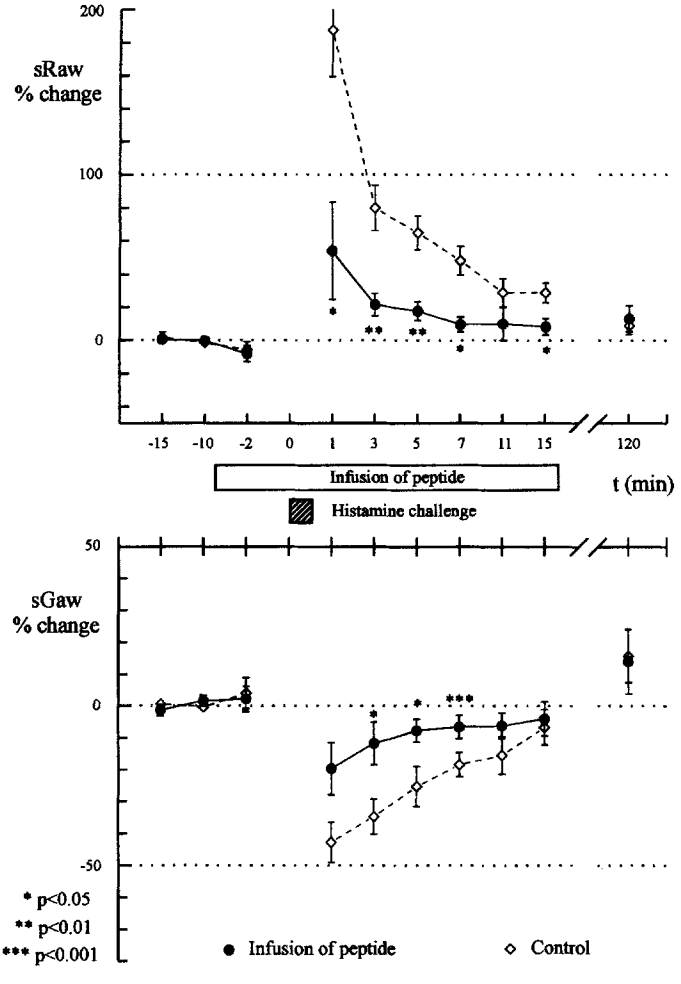

Figure 6. Effects of brain natriuretic peptide on histamine-induced bronchoconstriction. (Dose: 640 ng kg^{-1} min^{-1} iv, mean $\pm$ SEM, $n = 7$). sRaw = specific airway resistance, phase lag method. sGaw = specific airway conductance, interrupter method

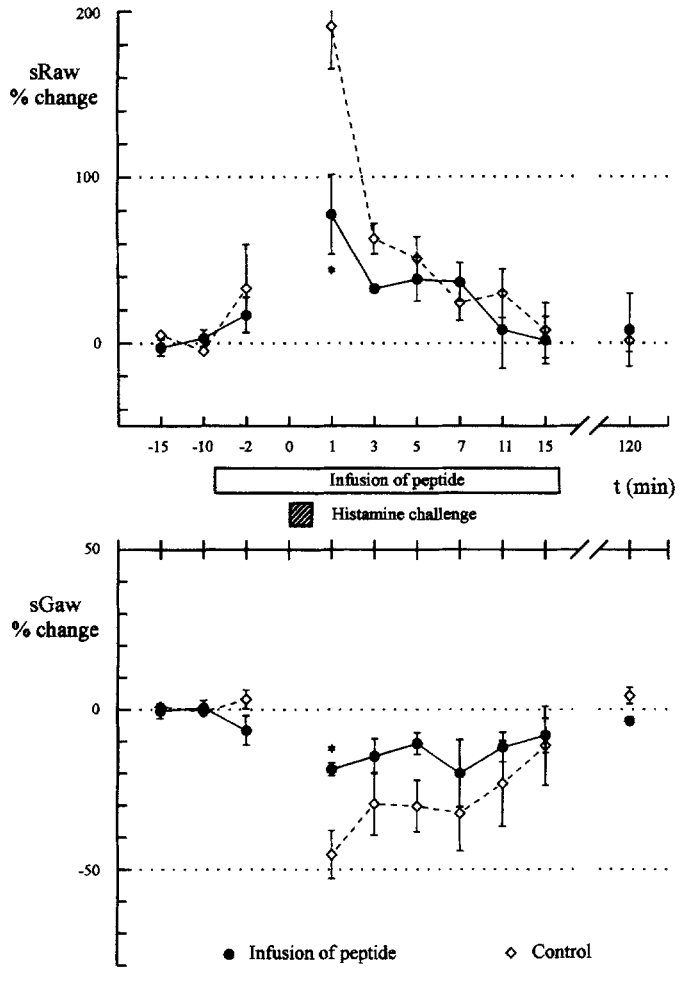

Figure 7. Effects of brain natriuretic peptide on histamine-induced bronchoconstriction. (Dose: 1280 ng kg^{-1} min^{-1} iv, mean $\pm$ SEM, $n = 4$). sRaw = specific airway resistance, phase lag method. sGaw = specific airway conductance, interrupter method

Thus, brain natriuretic peptide showed a marked potency in inhibiting histamine-evoked bronchoconstriction in conscious guinea pigs.in the conscious guinea pig. The spontaneous bronchial tone of the animals was not altered significantly by infusion of any of the peptides over 8 min.

DISCUSSION

Considerations of method

Normal values for lung function parameters in the guinea pig found in the literature are not uniform. On the one hand, considerable methodological differences, like bypassing the nasal/upper airways (which are reported to contribute 45–90% to the total respiratory resistance [74,75]), are found in many studies. On the other hand, the parameters measured (lung or airway resistance (R_L, R_{aw}) and specific airway parameters (sR_{aw}, sG_{aw})) can hardly be converted from one to the other without the use of estimated factors like the lung/airway resistance ratio [76] or functional residual capacity (FRC), as the FRC value is still unclear [77–81] and was additionally found to differ in conscious and anaesthetized animals [82].

Thus, if the effect of anaesthesia on the FRC is taken into account [82], the value estimated by the phase lag method in our system corresponds to the seemingly lower values of specific airway resistance given by Pennock et al. [63] or calculated from data of Agrawal [83], Amdur and Mead [74], Stein et al. [84] and Mead [85].

Further, considerably higher airway resistance values were found in guinea pigs in body plethysmographic studies of Hutson et al. [62] and in direct airway resistance measurements [75]. Therefore, our results fit into the range found in previous studies even if the possible influence of consciousness is not considered.

Histamine-evoked bronchoconstriction

Compared with intravenous injection, application of histamine as an aerosol offers the advantage of milder systemic effects and better intraindividual reproducibility [86]. However, the interindividual variability of the bronchial response seems to be higher [86], and the effective histamine dose (ED_{50}) cannot be estimated. The interindividual variability of the bronchial response found in our study is consistent with previous reports [83,86]. To prevent histamine tachyphylaxis, a sufficient recovery time between consecutive experiments should be given [87–89].

Effects of ANP

O'Donnell et al. had already by 1985 reported dose-related relaxing effects of atriopeptin III, II and I – shorter ANP variants – on isolated guinea pig trachea and pulmonary vessels [33]. The potency of ANP to relax the resting tone of the guinea pig trachea in vitro was later confirmed [34,35,90].

The peptide was also shown to inhibit the constricting actions of histamine, serotonin, methacholine, carbachol, LTD_4, arachidonic acid, specific antigen and perfusion with K^+-solution on isolated guinea pig tracheal muscle preparations [36,61,91–94].

Finally, in-vivo effects of ANP on the airways of anaesthetized guinea pigs have been

described. Intravenous ANP or anaritide ($ANP_{102-126}$) displayed a protective action against bronchoconstriction provoked by serotonin, carbachol or LTD_4, but not by arachidonic acid. Anaritide was further demonstrated to relax LTD_4-preconstricted airways with only a marginal effect on the resting tone [36,41,42].

Englebach and colleagues [41] found only a marginal effect of $ANP_{(102-126)}$ against histamine-induced bronchoconstriction whereas a dose-related protective action of ANP was reported by Potvin and Varma [36]. In our study, no marked effect of the peptide was found.

The dosage and structures of the peptides tested in our study were similar to those of both the previous investigations and are, therefore, not likely to be the main reason for these differences. However, other methodological dissimilarities have probably contributed to the discrepancy of the results.

In both previous investigations of ANP in intact guinea pigs, nasal airways were bypassed by tracheotomy and cannulation, thus neglecting any contributions of these airways to both histamine and ANP actions, and providing another possible explanation for differences between results [36,41,74,75].

Furthermore, Englebach et al. [41] as well as Potvin and Varma [36] used anaesthetized and ventilated animals. As differences in lung parameter assessment and histamine sensitivity under anaesthesia have been demonstrated [75,82], the potential influence of anaesthesia can hardly be estimated.

Finally, according to the data of Douglas et al. [86], the method of application of histamine may be of importance for distribution of its actions over the respiratory system. Further, data from in-vitro studies implies possible differential effects of ANP on various levels of the respiratory system [34,92]. As intravenous histamine was applied by Englebach et al. [41] and Potvin and Varma [36], whereas aerosolized challenge was used in our study, a different spectrum of actions may have been provoked.

The differences between the results of Englebach et al. [41] and Potvin and Varma [36] may have been caused partly by the utilization of pulmonary inflation pressure measurements and bolus injections of the peptide with possibly higher ANP peak levels, and partly by genetic and age differences of the animals [95,96].

Methodological differences may therefore have contributed to discrepancies between the results, but the main reasons remain obscure.

Effects of urodilatin

Intravenous administration of urodilatin in humans produces stronger and longer effects on the cardiovascular and especially renal functions than $ANP_{(99-126)}$ [31,32].

Urodilatin has not been detected in blood plasma so far. Its plasma $t_{\frac{1}{2}}$ after intravenous bolus injection is 2–3 min in men [31] similar to that of ANP. Although the turnover rate of urodilatin seems to be reduced due to its additional 4 N-terminal residues [97], the stronger action of this peptide is more likely to be the result of higher receptor affinity [98] or activation [32] than of higher degradation stability.

The effects of urodilatin on the airway have not been extensively investigated. The

peptide was shown to relax preconstricted guinea pig trachea in vitro [94]. In vivo, protective effects against acetylcholine-evoked bronchoconstriction in rats were shown for urodilatin, but not for ANP [44]. Dose-dependent relaxing action on the basal tone of both central and peripheral human asthmatic airways has also been reported for urodilatin [57–59,99]. Urodilatin might therefore exert bronchial effects superior to those of ANP_{99-126}.

The urodilatin dose applied in this study was considerably higher than the effective dose reported for rats and humans (40–80 $ng\,kg^{-1}\,min^{-1}$) [44,57–59,99]. However, in guinea pigs, much higher doses of ANP are necessary to evoke bronchial effects than in humans [36,41,48,50]. The effectiveness of atrial peptides appears therefore to be species specific.

Effects of BNP

A similar range of effects was reported for ANP and the brain natriuretic peptide [27]. BNP seems to act on the same receptor systems and also to increase the cGMP levels [27,100–102]. Nothing was known about the action of the peptide on airways until recently when dose-dependent relaxing effects of BNP on isolated guinea pig tracheal preparations were described [60]. The EC_{50} of BNP was lower than that of ANP or CNP – another member of the ANP family capable of relaxing guinea pig tracheal smooth muscle – thus implying that the actions of BNP in the respiratory system might be superior to those of other atrial peptides [60,61,103].

To our knowledge, there are no other reports on in-vivo effects of BNP in the respiratory system. Our assumption that BNP might exert a more powerful action on the airway tone than the other peptides is consistent with the in-vitro results.

The plasma half-life of BNP was found to be longer than that of the ANP in rats and humans [27,28,104,105]. Not only enzymic cleavage but also removal from circulation by receptor-mediated endocytosis (mainly via the C-type 'clearance' receptor) were postulated as degradation mechanisms for atrial peptides [106–109]. Since the affinity of BNP to the C-receptor subtype was found to be only 7% of the ANP affinity [102,105], a possible higher effectiveness of BNP could be due to a longer plasma half-life and different receptor binding affinities [100].

Effects of atrial peptides on the spontaneous airway tone

No effect on the spontaneous tone of guinea pig airways was found for any of the peptides. However, as the resting airway tension is much lower in guinea pigs than in humans and dogs, probably due to lower vagal activity [110,111], a possible bronchodilating action might be difficult to demonstrate.

Conclusion

Brain natriuretic peptide exerts protective effects against histamine-induced bronchoconstriction in the conscious guinea pig, which might be superior to those of atrial natriuretic peptide and urodilatin.

Acknowledgements

I would like to thank Prof. Dr. med. Wolfgang Schmutzler for his helpful suggestions, Dr. med. Stefan Bent and Dr. med. Joachim Forsting for introduction to the method, and Dr. med. Karl-Wilhelm von Eickstedt for providing the materials.

REFERENCES

1. De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci. 1981;28:89–94.
2. Currie MG, Geller DM, Cole BR et al. Bioactive cardiac substances: potent vasorelaxant activity in mammalian atria. Science. 1983;221:71–3.
3. Kangawa K, Matsuo H. Purification and complete amino acid sequence of α-human atrial natriuretic polypeptide (α-hANP). Biochem Biophys Res Commun. 1984;118(1):131–9.
4. Flynn TG, de Bold ML, de Bold AJ. The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. Biochem Biophys Res Commun. 1983;117(3):859–65.
5. Seidah NG, Lazure C, Chretien M et al. Amino acid sequence of homologous rat atrial peptides: natriuretic activity of native and synthetic forms. Proc Natl Acad Sci USA. 1984;81:2640–4.
6. Harris PJ, Thomas D, Morgan TO. Atrial natriuretic peptide inhibits angiotensin-stimulated proximal tubular sodium and water reabsorption. Nature. 1987;326:697–8.
7. Rocha AS, Kudo LH. Atrial peptide and cGMP effects on NaCl transport in innermedullary collecting duct. Am J Physiol. 1990;259:F258–F68.
8. Cogan MG. Atrial natriuretic peptide can increase renal solute excretion primarily by raising glomerular filtration. Am J Physiol. 1986;250:F710–F14.
9. Winquist RJ, Faison EP, Nutt RF. Vasodilator profile of synthetic atrial natriuretic factor. Eur J Pharmacol. 1984;102:169–73.
10. Sasaki A, Kida O, Kangawa K, Matsuo H, Tanaka K. Involvement of sympathetic nerves in cardiosuppressive effects of human atrial natriuretic peptide in anesthetized rats. Eur J Pharmacol. 1986;120:345–9.
11. Oelkers W, Kleiner S, Bähr W. Effects of incremental infusions of atrial natriuretic factor on aldosterone, renin and blood pressure in humans. Hypertension. 1988;12:462–7.
12. Matsuoka H, Ishii M, Hirata Y et al. Evidence for lack of a role of cGMP in effect of α-hANP on aldosterone inhibition. Am J Physiol. 1987;252 (Endocrinol Metab. 15):E643–E7.
13. Samson WK. Atrial natriuretic factor inhibits dehydration- and hemorrhage-induced vasopressin release. Neuroendocrinology. 1985;40:277–9.
14. Sudoh T, Kangawa K, Minamino N, Matsuo H. A new natriuretic peptide in porcine brain. Nature. 1988;332(3):78–81.
15. Sudoh T, Minamino N, Kangawa K, Matsuo H. Brain natriuretic peptide-32: N-terminal six amino acid extended form of brain natriuretic peptide identified in porcine brain. Biochem Biophys Res Commun. 1988;155(2):726–32.
16. Aburaya M, Hino J, Minamino N, Kangawa K, Matsuo H. Isolation and identification of rat brain natriuretic peptides in cardiac atrium. Biochem Biophys Res Commun. 1989;163(1):226–32.
17. Dagnino L, Drouin J, Nemer M. Differential expression of natriuretic peptide genes in cardiac and extracardiac tissues. Mol Endocrinol. 1991;5:1292–300.
18. Hino J, Tateyama H, Minamino N, Kangawa K, Matsuo H. Isolation and identification of human brain natriuretic peptides in cardiac atrium. Biochem Biophys Res Commun. 1990;167(2):693–700.

19. Kambayashi Y, Nakao K, Mukoyama M et al. Isolation and sequence determination of human brain natriuretic peptide in human atrium. FEBS Lett. 1990;259:341–5.
20. Minamino N, Aburaya M, Ueda S, Kangawa K, Matsuo H. The presence of brain natriuretic peptide of 12,000 Daltons in porcine heart. Biochem Biophys Res Commun. 1988;155(2);740–6.
21. Minamino N, Kangawa, K, Matsuo H. Isolation and identification of a high molecular weight brain natriuretic peptide in porcine cardiac atrium. Biochem Biophys Res Commun. 1988;157(1):402–9.
22. Rosenzweig A, Seidman CE. Atrial natriuretic factor and related peptide hormones. Annu Rev Biochem. 1991;60:229–55.
23. Saito Y, Nakao K, Itoh H et al. Brain natriuretic peptide is a novel cardiac hormone. Biochem Biophys Res Commun. 1989;158(2):360–8.
24. Togashi K, Hirata Y, Ando K, Takei Y, Kawakami M, Marumo F. Brain natriuretic peptide-like immunoreactivity is present in human plasma. FEBS Lett. 1989;250(2):235–7.
25. Jennings DB, Flynn TG. The disulfide bonded ring of iso-rANP, unlike that of rANP, has potent cardiovascular activity. Can J Physiol Pharmacol. 1990;68:131–6.
26. Jennings DB, McKirdy JC, Ohtake PJ, Sarda IR, Flynn TG, Hira G. Infusion of iso-rANP (1-45) or (17-45) increases plasma immunoreactive ANP and lowers plasma renin activity and aldosterone. Can J Physiol Pharmacol. 1990;68:1292–7.
27. McGregor A, Richards M, Espiner E, Yandle T, Ikram H. Brain natriuretic peptide administered to man: actions and metabolism. J Clin Endocrinol Metab. 1990;70:1103-7.
28. Holmes SJ, Espiner EA, Richards AM, Yandle TG, Frampton C. Renal, endocrine, and hemodynamic effects of human brain natriuretic peptide in normal man. J Clin Endocrinol Metab. 1993;76:91–6.
29. Kambayashi Y, Nakao K, Kimura H et al. Biological characterisation of human brain natriuretic peptide (BNP) and rat BNP: species-specific actions of BNP. Biochem Biophys Res Commun. 1990;173(2):599–605.
30. Schulz-Knappe P, Forssmann K, Herbst F, Hock D, Pipkorn R, Forssmann WG. Isolation and structural analysis of 'urodilatin', a new peptide of the cardiodilatin-(ANP)-family, extracted from human urine. Klin Wochenschr. 1990;66:752–9.
31. Saxenhofer H, Raselli A, Weidmann P et al. Urodilatin, a natriuretic factor from kidneys, can modify renal and cardiovascular function in men. Am J Physiol. 1990;259:F832–8.
32. Kentsch M, Ludwig D, Drummer C, Gerzer R, Müller-Esch G. Haemodynamic and renal effects of urodilatin bolus injections in patients with congestive heart failure. Eur J Clin Invest. 1992;22:662–9.
33. O'Donnell M, Garippa R, Welton AF. Relaxant activity of atriopeptins in isolated guinea pig airway and vascular smooth muscle. Peptides. 1985;6(4):597–601.
34. Chou J, Kubota E, Sata T, Said SI. Comparative relaxant activities of atrial natriuretic peptides (ANPs) and vasoactive intestinal peptide (VIP) on smooth muscle structures in lung. Federation Proc. 1986;45(1):553.
35. Watanabe H, Suzuki K, Takagi K, Satake T. Mechanics of atrial natriuretic polypeptide and sodium nitroprusside-induced relaxation in guinea pig tracheal smooth muscle. Arzneim-Forsch/Drug-Res. 1990;40(II,nr.7):771–6.
36. Potvin W, Varma DR. Bronchodilator activity of atrial natriuretic peptide in guinea pigs. Can J Physiol Pharmacol. 1988;67:1213–18.
37. Ishii K, Murad F. ANP relaxes bovine tracheal smooth muscle and increases cGMP. Am J Physiol. 1989;256 (Cell Physiol. 25):C495–500.
38. Hulks G, Crabb KG, McGrath JC, Thomson NC. In vitro effects of atrial natriuretic factor and sodium nitroprusside on bronchomotor tone in human bronchial smooth muscle (Abstract). Am Rev Resp Dis. 1991;143:A344.
39. Labat C, Norel X, Beneviste J, Brink C. Vasorelaxant effects of atrial peptide II on isolated human pulmonary muscle preparations. Eur J Pharmacol. 1988;150:397–400.
40. Banerjee MR, Newman JH. Acute effects of atrial natriuretic peptide on lung mechanics and hemodynamics in awake sheep. J Appl Physiol. 1990;69(2):728–33.
41. Englebach IM, Lappe RW, Hand JM. Bronchoprotective and bronchodilator activity of anaritide (human atrial natriuretic factor [102–126]) infusion in the anesthetized guinea pig. Pulmon Pharmacol. 1988;1:119–23.
42. Hand JM, Englebach IM, Lappe RW. Anaritide [human atrial natriuretic factor (102–126)] as a bronchodilator in the anesthetized guinea pig. Am Rev Respir Dis. 1988;137:A28.
43. Amyot R, Lesiège D, Michoud MC, Larochelle P, Hamet P. Effect of atrial natriuretic factor on airway sensitivity to histamine in anesthetized dogs. Eur Respir J. 1989;2(Suppl. 5):301s.

44. Flüge T, Hoymann H, Hohlfeld J, Heinrich U, Fabel H, Wagner TOF. Protective effect of urodilatin (URO) or ANP against an acetylcholine (ACH)-induced bronchoconstriction in the rat in vivo. Naunyn-Schmiedeberg's Arch Pharmacol. 1992;345 Suppl. 2:R51.
45. Robichaud A, Michoud MC, Saunier C, Duvivier C, Peslin R, du Souich P. Effect of atrial natriuretic peptide on bronchial tone in anesthetized rabbits. Peptides. 1993;14:1325–30.
46. Robichaud A, Saunier C, Michoud MC, du Souich P. Muscarinic effect of atrial natriuretic peptide on rabbit airways. Br J Pharmacol. 1993;110:804–8.
47. Haxhiu MA, Deal EC, van Lunteren E, Cherniack NS. Central effects of somatostatin and atrial natriuretic peptide on tracheal tone. J Appl Physiol. 1993;75:2353–9.
48. Hulks G, Jardine AG, Connell JMC, Thomson NC. Bronchodilator effect of atrial natriuretic peptide on asthma. Br Med J. 1989;299:1081–2.
49. Hulks G, Jardine AG, Connell JMC, Thomson NC. Effect of atrial natriuretic factor on bronchomotor tone in the normal human airway. Clin Sci. 1990;79:51–5.
50. Hulks G, Jardine AG, Connell JMC, Thomson NC. Influence of elevated plasma levels of atrial natriuretic factor on bronchial reactivity in asthma. Am Rev Respir Dis. 1991;143:778–82.
51. McAlpine LG, Hulks G. Thomson NC. Effect of atrial natriuretic peptide given by intravenous infusion on bronchoconstriction induced by ultrasonically nebulised distilled water (FOG). Am Rev Respir Dis. 1992;146:912–15.
52. Angus RM, McCallum MJA, Hulks J, Thomson NC. Bronchodilator, cardiovascular and cyclic guanylyl monophosphate response to high-dose infused atrial natriuretic peptide in asthma. Am Rev Respir Dis. 1993;147:1122–5.
53. Hulks G, Thomson NC. High dose inhaled atrial natriuretic factor is a bronchodilator in asthmatic subjects (Abstract). Eur Respir J. 1991;4 (Suppl. 14):607s.
54. Hulks G, Thomson NC. Inhaled atrial natriuretic factor inhibits histamine induced bronchonstriction (Abstract). Eur Respir J. 1991;4 (Suppl. 14):606s–7s.
55. Hulks G, Thomson NC. Inhaled atrial natriuretic peptide in asthmatic airways. Br Med J. 1992:304:1156.
56. Angus RM, McCallum MJA, Thomson NC. Effect of inhaled atrial natriuretic peptide on methacholine induced bronchoconstriction in asthma. Clin Exp Allergy. 1994;24:784–8.
57. Flüge T, Wagner TOF, Fabel H, von Kortzfleisch V, von Eickstedt K-W. Dose-dependent bronchodilation of urodilatin in asthmatic subjects in vivo. Naunyn-Schmiedeberg's Arch Pharmacol. 1992;345 Suppl. 2:R50.
58. Flüge T, Fabel H, Wagner TOF, Schneider B, Forssmann WG. Bronchodilating effect of natriuretic and vasorelaxant peptides compared to salbutamol in asthmatics. Regul Pept. 1995;59(3):357–70.
59. Flüge T, Fabel H, Wagner TOF, Schneider B, Forssmann WG. Urodilatin (Ularitide, INN): a potent bronchodilator in asthmatic subjects. Eur J Clin Invest. 1995;25:728–36.
60. Takagi K, Araki N. Relaxant effect of brain natriuretic peptide on guinea-pig tracheal smooth muscle. Clin Exp Pharmacol Physiol. 19993;20:239–43.
61. Watanabe H, Furui H, Yamaki K, Suzuki R, Takagi T, Satake T. Atrial natriuretic polypeptide causes dose-dependent relaxant effect on guinea pig tracheal smooth muscle. Am Rev Respir Dis. 1988;137:102.
62. Hutson PA, Church MK, Clay TP, Miller P, Holgate ST. Early and late-phase bronchoconstriction after allergen challenge of nonanesthetized guinea pigs. Am Rev Respir Dis. 1988;137:548–57.
63. Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. A noninvasive technique for measurement of changes in specific airway resistance. J Appl Physiol Resp Environ Exercise Physiol. 1979;46(2):399–406.
64. Gros C, Souque A, Schwartz JC. Degradation of atrial natriuretic factor in mouse blood in vitro and in vivo: role of enkephalinase (EC 3.4.24.11). Neuropeptides. 1990;17:1–5.
65. Katsube N, Schwartz D, Needleman P. Atriopeptin turnover: quantitative relationship between in vivo changes in plasma levels and atrial content. J Pharmacol Exp Ther. 1986;239:474–9.
66. Luft F, Lang RE, Aronoff GR et al. Atriopeptin III kinetics and pharmacodynamics in normal and anephric rats. J Pharmacol Exp Ther. 1986;236:416–18.
67. Murthy KK, Thibault G, Garcia R, Gutkowska J, Genest J, Cantin M. Degradation of atrial natriuretic factor in the rat. Biochem J. 1986;240:461–9.
68. Murthy KK, Thibault G, Schiffrin EL et al. Disappearance of atrial natriuretic factor from circulation in the rat. Peptides. 1986;7:241–6.
69. Gnädinger MP, Lang RE, Hasler L, Uehlinger DE, Shaw S, Weidmann P. Plasma kinetics of synthetic alpha-human atrial natriuretic peptide in man. Miner Electrolyte Metab. 1986;12:371–4.

70. Jüppner H, Brabant O, Kapteine U, Kirschner M, Klein H, Hesch RD. Direct radioimmunoassay for human atrial natriuretic peptide (hANP) and its clinical evaluation. Biochem Biophys Res Commun. 1986;139:1215–23.
71. Nakao K, Sugawara A, Morii N et al. The pharmacokinetics of alpha-human atrial natriuretic peptide in healthy subjects. Eur J Clin Pharmacol. 1986;31:101–3.
72. Yandle TG, Richards AM, Nichollis MG, Cuneo R, Espiner EA, Livesey JH. Metabolic clearance rate and plasma half life of alpha-human atrial natriuretic peptide in man. Life Sci. 1986;38:1827–33.
73. Sachs L. Angewandte Statistik. Anwendung statistischer Methoden. Berlin, Heidelberg: Springer Verlag; 1992;401–13.
74. Amdur MO, Mead J. Mechanics of respiration in unanesthetized guinea pigs. Am J Physiol. 1985;192(2):364–8.
75. Holroyde MC, Norris AA. The effect of ozone on reactivity of upper and lower airways in guinea pigs. Br J Pharmacol. 1988;94:938–46.
76. Cotes JE. Lung Function: Assessment and Application in Medicine, 4th edn. London: Blackwell Scientific Publications; 1979:81.
77. Tenney SM, Remmers JE. Comparative quantitative morphology of the mammalian lung diffusing area. Nature. 1963;197:54–7.
78. Forrest JB, Weibel ER. Morphometric estimation of pulmonary diffusion capacity. VII. The normal guinea pig lung. Respir Physiol. 1975;24(2);191–202.
79. Baumal R, Hooi C, McAvoy D, Broder I. Time course of morphometric changes after acute allergic bronchoconstriction in the guinea pig. Int Arch Allergy Appl Immunol. 1983;71(2):131–6.
80. Takezawa J, Miller FJ, O'Neil JJ. Single breath diffusing capacity and lung volume in small laboratory animals. J Appl Physiol. 1980;48(6):1052–9.
81. Pare PD, Michoud MC, Boucher RC, Hogg JC. Pulmonary effects of acute and chronic antigen exposure of immunized guinea pigs. J Appl Physiol. 1979;46:346–53.
82. Lamm WJE, Hildebrandt JR, Hildebrandt J, Lai YL. End-expiratory volume in the rat: effects of consciousness and body position. J Appl Physiol. 1982;53(5):1071–9.
83. Agrawal KP. Specific airway conductance in guinea pigs: normal values and histamine induced fall. Resp Physiol. 1981;43:23–30.
84. Stein M, Schiavi RC, Ottenberg P, Hamilton C. The mechanical properties of the lungs in experimental asthma in the guinea pig. J Allergy. 1961;32(1):8–16.
85. Mead J. Control of respiratory frequency. J Appl Physiol. 1960;15:325–36.
86. Douglas JS, Dennis MW, Ridgway P, Bouhuys A. Airway dilatation and constriction in spontaneously breathing guinea pigs. J Pharmacol Exp Ther. 1972;180(1):98–109.
87. Manning PJ, Jones GL, O'Byrne PM. Tachyphylaxis to inhaled histamine in asthmatic subjects. J Appl Physiol. 1987;63:1572–7.
88. Manning PJ, O'Byrne PM. Histamine bronchoconstriction reduces airway responsiveness in asthmatic subjects. Am Rev Resp Dis. 1988;137:1323–5.
89. Connolly MJ, Hendrick DJ. Refractory period following histamine provoked bronchospasm in asthmatic subjects. Thorax. 1985;40:216–17.
90. Candenas M-L, Naline E, Puybasset L, Devillier P, Advenier C. Effect of atrial natriuretic peptide and of atriopeptins on the human isolated bronchus. Comparison with the guinea-pig isolated trachea. Pulmon Pharmacol. 1991;4:120–5.
91. Abdallah A, Burnell J, Pelton T, Berman J Bronchodilator activity of synthetic atrial natriuretic factor (rANF-27);comparison with aminophylline and salbutamol. Fed Proc. 1986;M45(1):203.
92. Hamel R, Ford-Hutchinson AW. Relaxant profile of synthetic atrial natriuretic factor on guinea-pig pulmonary tissues. Eur J Pharmacol. 1986;121:151–5.
93. Watanabe H, Takagi K, Satake T. Relaxant effects of atrial natriuretic polypeptide on guinea pig tracheal smooth muscle. Prog Biochem Pharmacol. 1988;23:136–41.
94. Duft S, Marxen P, Deeb M et al. Urodilatin and atrial natriuretic factor relax preconstricted isolated perfused guinea pig trachea and increase cyclic GMP. Naunyn-Schmiedeberg's Arch Pharmacol. 1992;345(Suppl. 2):R49.
95. Grunstein MM, Tanaka DT, Knight MS. Maturation of histamine reactivity in the isolated rabbit airway (abstract). Am Rev Respir Dis. 1986;133:A113.
96. Fleisch JH, Maling AM, Brodie BB. Evidence for the existence of alpha-adrenergic receptors in the mammalian trachea. Am J Physiol. 1970:218:596–9.
97. Gagelmann M, Hock D, Forssmann W-G. Urodilatin (CCD/ANP-95-126) is not biologically inactivated by a peptidase from dog kidney cortex membranes in contrast to atrial natriuretic peptide/cardiodilatin (alpha-hANP/CDD-99-126). FEBS Lett. 1988;233:249–54.

98. Heim J-M, Kiefersauer S, Fülle H-J, Gerzer R. Urodilatin and β-ANF: binding properties and activation of particulate guanylate cyclase. Biochem Biophys Res Commun. 1989;163(1):37–41.
99. Flüge T, Fabel H, Wagner TOF, Forssmann WG. Localization of the bronchodilator effect induced by type A natriuretic peptide in asthmatic subjects. Clin Invest. 1994;72:772–4.
100. Suga S-I, Nakao K, Hosoda K et al. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide and C-type natriuretic peptide. Endocrinology. 1992;130:229–39.
101. Zhou H-L, Fiscus RR. Brain natriuretic peptide (BNP) causes endothelium-independent relaxation and elevation of cyclic GMP in rat thoracic aorta. Neuropeptides. 1989;14:161–9.
102. Nakao K, Mukoyama M, Hosoda K et al. Biosynthesis, secretion, and receptor selectivity of human brain natriuretic peptide. Can J Physiol Pharmacol. 1991;69:1500–6.
103. Takagi K, Araki N, Suzuki K. Relaxant effect of C-type natriuretic peptide on guinea pig tracheal smooth muscle. Arzneim-Forsch./Drug Res. 1992;42(II):1329–31.
104. Kita T, Kida O, Yokota N et al. Effects of brain natriuretic peptide-45, a circulating form of rat brain natriuretic peptide, in spontaneously hypertensive rats. Eur J Pharmacol. 1991;202:73–9.
105. Mukoyama M, Nakao K, Hosoda K et al. 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. 1991;87:1402–12.
106. Maack T, Suzuki M, Almeida FA et al. Physiological role of silent receptors of atrial natriuretic factor. Science. 1987;238:675–8.
107. Fuller F, Porter JG, Arfsten AE et al. Atrial natriuretic peptide clearance receptor. Complete sequence and functional expression of cDNA clones. J Biol Chem. 1988;263:9395–401.
108. Porter JG, Wang Y, Schwartz K et al. Characterization of the atrial natriuretic peptide clearance receptor using a vaccinia virus expression vector. J Biol Chem. 1988;263(35):18827–33.
109. Almeida FA, Suzuki M, Scarborough RM, Lewicki JA, Maack T. Clearance function of type C receptors of atrial natriuretic factor in rats. Am J Physiol. 1989;256:R469–75.
110. Michoud M-C, Fraser RS, Pare PD, Hogg JC. Effect of indomethacin and atropine in experimental asthma in conscious guinea pigs. J Appl Physiol. 1976;40(6):889–94.
111. Mills JE, Widdicombe JG. Role of the vagus nerves in anaphylaxis and histamine-induced bronchoconstriction in guinea pigs. Br J Pharmacol. 1976;39:724–31.

Manuscript received 3 March 1998.
Accepted for publication 5 March 1998.