

Bronchoprotective effects of atrial natriuretic peptide against propranolol-induced bronchoconstriction after allergic reaction in guinea pigs

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Summary

Objective To study the effects of intravenous atrial natriuretic peptide (ANP) on antigen-induced bronchoconstriction, propranolol-induced bronchoconstriction (PIB) after antigen challenge, and histamine-induced bronchoconstriction in guinea pigs.

Methods Allergic bronchoconstriction was evoked by inhalation of ovalbumin (OA) and PIB was caused when 10 mg/mL of propranolol was inhaled 20 min after OA challenge in passively sensitized and artificially ventilated guinea pigs. 25, 50, 100 and 200 µg/mL of histamine were inhaled for 20 s at 5-min intervals in non-sensitized guinea pigs.

Results Pretreatment with ANP in doses of 0.1 and 1.0 nmol/kg injected intravenously 15 min after antigen challenge reduced PIB in a dose-dependent manner, and 5 min before antigen challenge significantly attenuated PIB but not antigen-induced bronchoconstriction. Intravenous ANP significantly reduced bronchial responses to increasing concentrations of inhaled histamine in a dose-dependent manner.

Conclusion These results suggest that ANP possesses protective effects against propranolol-induced and histamine-induced bronchoconstriction, albeit by a non-specific mechanism in guinea pig *in vivo*.

Keywords: allergen induced bronchoconstriction, atrial natriuretic peptide, guinea-pig airway, propranolol induced bronchoconstriction

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Introduction

Atrial natriuretic peptide (ANP) dilates the trachea and bronchi of different species in addition to natriuresis, vasorelaxation and hypotension [1]. Many studies have previously demonstrated that ANP can act as a bronchodilator and protect against bronchoconstriction induced by a number of pharmacological stimuli, in several species including humans. ANP was found to produce concentration-dependent relaxation of spontaneous tone of the isolated guinea-pig trachea [2]. Hamel and Ford-Hutchinson [3] demonstrated that ANP was a more potent relaxant of intrinsic tonus or contractions induced by methacholine as compared with contractions induced by leukotriene D₄. The

mechanism of ANP-induced bronchodilation is not completely understood but may involve action on particulate guanyl cyclase to cause the increase of the intracellular second messenger cyclic guanosine 3',5'-monophosphate (cGMP) in bovine tracheal smooth muscle [4]. ANP possesses limited bronchodilator activity against increase in pulmonary inflation pressure induced by serotonin, histamine and carbachol in anaesthetized guinea pigs [5].

Propranolol, a non-selective β -adrenoceptor blocker, has been shown to cause bronchoconstriction in asthmatic patients when administered by inhalation but not in normal subjects [6,7]. It is well known that mast cells in the human lungs possess β -receptors on their surface and β -adrenoceptor agonists have been shown to inhibit anaphylactic release of mediators including histamine, slow-reacting substances of anaphylaxis (leukotrienes C₄, D₄ and E₄) and thromboxane A₂, however, the mechanism of

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propranolol-induced bronchoconstriction (PIB) remains unclear [8,9].

The aim of the present study was to investigate the effects of ANP on both PIB and allergen-induced bronchoconstriction, with its effects on histamine studied to examine whether this effect was specific.

Materials and methods

Animals

Male, albino, Hartley-strain guinea pigs weighing 380–450 g were used in this study.

Passive sensitization of animals

Guinea pig homocytotropic antiserum was obtained according to the method of Santives *et al.* [10]. Briefly, 500 µg of ovalbumin (OA) was emulsified in Freund's complete adjuvant and injected intradermally into each guinea pig at multiple sites. Boosting was carried out in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after boosting, pooled and kept frozen until use. The antibody titre of this serum was 1 : 12 800, 1 : 6400 and 1 : 512 at 4 h, 24 h and 7 days, respectively, as estimated by passive cutaneous anaphylaxis. Normal guinea pigs were passively sensitized with 1.0 mL of antiserum/kg given intraperitoneally (i.p.). Seven to eight days after passive sensitization guinea pigs were anaesthetized with sodium pentobarbital (75 mg/kg i.p.). They were placed in the supine position and a polyethylene tube (outside diameter 2.5 mm, inside diameter 2.1 mm) was inserted into the trachea and the left jugular vein was cannulated for administration of drugs. After surgery, each guinea pig was artificially ventilated with a small animal respiratory pump (Model 1680, Harvard Apparatus Co. Inc., South Natick, MA, USA) adjusted to a tidal volume of 10 mL/kg at a rate of 60 strokes min⁻¹. The change in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening; Pao [cm H₂O]), was measured using a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co. Ltd, Tokyo, Japan) by the modified method of Konzett and Rossler [11] as described by Jones *et al.* [12]. Since the change in Pao following inhalation of leukotriene C₄ (LTC₄) represented the average of the changes in pulmonary resistance (RL) and reciprocal dynamic lung compliance (1/Cdyn) [13], we used Pao as an overall index of bronchial response to bronchoactive agents.

Before surgery, passively sensitized animals were given diphenhydramine hydrochloride (60 mg/kg i.p.) to block the action of histamine simultaneously with sodium pentobarbital but not non-sensitized animals. The lungs were over-inflated with two times the tidal volume for two breaths by

clamping the outlet port of the respirator to standardize the volume history of the lung.

The aerosol was generated during a 20-s period by an ultrasonic nebulizer developed for small animals at our institution. The amount of aerosol was 15.2 mL/min, and 46.4% of the aerosol was deposited in the lung as measured by radio-aerosol technique [14].

Propranolol induced bronchoconstriction

Fifteen minutes after the preparation, when Pao had stabilized, passively sensitized guinea pigs were challenged with nebulized ovalbumin (OA) dissolved in physiological saline (1.0 mg/mL) under constant ventilation.

The OA aerosol was generated for 30 s with an ultrasonic nebulizer. Twenty minutes after the OA provocation, 10 mg/mL of propranolol was inhaled for 30 s in the passively sensitized animals.

Ovalbumin followed by ANP or saline followed by propranolol

ANP in a dose of 0.1 and 1.0 nmol/kg dissolved in physiological saline or saline was given intravenously 15 min after the challenge with OA, and then propranolol inhalation was performed 5 min later, 20 min after the OA challenge.

ANP or saline followed by ovalbumin followed by propranolol

ANP in a dose of 0.1 and 1.0 nmol/kg, or saline was administered intravenously 5 min before the OA challenge followed by propranolol inhalation.

In non-sensitized animals ANP or saline followed by histamine

ANP in a dose of 0.1 and 1.0 nmol/kg, or saline alone was given intravenously to non-sensitized guinea pigs 10 min after the surgery. Three minutes later, 25 µg/mL of histamine dissolved in physiological saline was inhaled for 20 s and Pao was measured for 5 min. The same procedure was repeated with aerosol challenge with 50, 100 and 200 µg/mL of histamine at 5 min intervals.

Statistical analysis

All data are shown as mean ± standard error of the mean. Statistical differences were determined by non-parametric ANOVA among three groups and Mann-Whitney's *U*-test between two groups. Time course curves for percentage increase in Pao from the baseline value after OA challenge and inhalation of propranolol were compared among

animals treated with ANP (0.1 and 1.0 nmol/kg) and saline, using two-way analysis of variance (ANOVA) for repeated measures. A *P*-value of 0.05 or less was considered to be significant.

Chemicals

The following chemicals were used: sodium pentobarbital (Abbot Laboratories, North Chicago, IL, USA); ovalbumin (Sigma, St Louis, MO, USA); diphenhydramine hydrochloride (Sigma); DL-propranolol hydrochloride (Sigma); histamine (Wako Pure Chemical Ind., Osaka, Japan); and atrial natriuretic peptide (human; synthetic) (Sigma).

Results

Ovalbumin followed by ANP or saline followed by propranolol

Time courses of percentage increase in Pao from the baseline value after inhalation of propranolol following OA challenge in the three groups are shown in Fig. 1. The peak values after OA challenge were 259.9 ± 20.9 or 235.6 ± 20.0 and 256.2 ± 34.1 with saline ($n=9$) or 0.1 ($n=7$) and 1.0 ($n=9$) nmol/kg of ANP, respectively, and the values did not differ from one another. Percentage increase in Pao immediately before propranolol inhalation (20 min after OA challenge) were 161.7 ± 16.7 or 164.6 ± 21.6 and $150.3 \pm 18.8\%$ in the groups given saline or 0.1 and 1.0 nmol/kg of ANP, respectively. These values were not significantly different. The time courses of percentage increase in Pao after propranolol inhalation were significantly different ($P < 0.05$). The maximum percentage increases in Pao after propranolol inhalation from pre-OA challenge values were 374.2 ± 52.3 or 296.4 ± 23.4 and $263.4 \pm 10.7\%$ in the respective group with saline or 0.1 and 1.0 nmol/kg of ANP. The value with 1.0 nmol/kg of ANP was significantly lower ($P < 0.02$) than that with saline.

ANP or saline followed by ovalbumin followed by propranolol

There was no significant difference among the Pao values before antigen challenge in the animals pretreated with saline ($n=9$) or 0.1 ($n=9$) and 1.0 ($n=8$) nmol/kg of ANP.

The peak values after OA challenge were 262.2 ± 31.2 or 205.9 ± 32.8 and $193.5 \pm 17.2\%$ with saline or 0.1 and 1.0 nmol/kg of ANP, respectively. The maximal increase in Pao was not significantly different among three groups (Fig. 2), although 0.1 and 1.0 nmol/kg of ANP group appeared to be a small decrease in the time courses of Pao after antigen challenge. The maximum percentage increases in Pao after propranolol inhalation were 471.2 ± 30.3 or

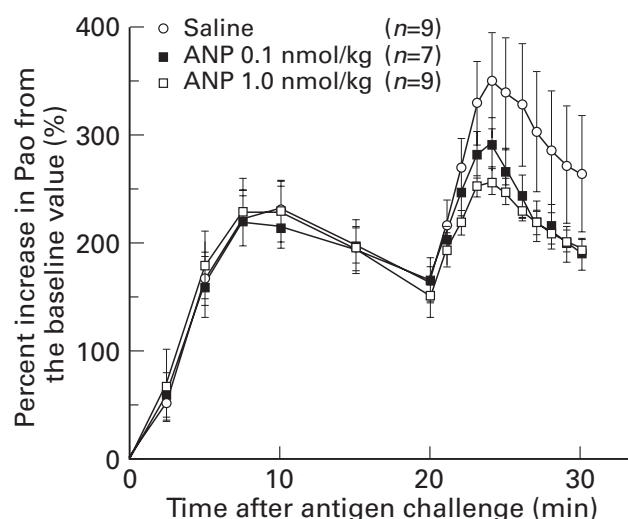


Fig. 1. Time course of percent increase in Pao from the pre-ovalbumin provocation value after inhalation of propranolol following ovalbumin challenge in passively sensitized guinea pigs treated intravenously with ANP (0.1 and 1.0 nmol kg⁻¹) and vehicle (saline) 5 min before the propranolol challenge. Each vertical bar represents SEM. The time courses of percentage increase in Pao after propranolol inhalation were significantly different among three groups ($P < 0.05$). Curves for antigen-induced bronchoconstriction (0–20 min after OA challenge) were significantly reduced by pretreatment with ANP ($P < 0.04$). The peak value after propranolol inhalation from the baseline value was significantly lower with 1.0 nmol/kg of ANP ($P < 0.01$) than with saline. * $P < 0.05$, ** $P < 0.01$ compared with the saline pretreatment group

325.2 ± 40.0 and $297.7 \pm 38.1\%$ in the respective group with saline or 0.1 and 1.0 nmol/kg of ANP.

These were significantly different from one another ($P < 0.01$), while the Pao values immediately before propranolol provocation did not differ significantly (Fig. 3).

In non-sensitized animals ANP or saline followed by histamine

The Pao values immediately before inhalation of histamine in non-sensitized guinea pigs intravenously administered with saline ($n=9$) or 0.1 ($n=8$) and 1.0 ($n=9$) nmol/kg of ANP were not significantly different.

Figure 4 shows dose–response curves of the percentage increase in Pao by inhaled histamine in anaesthetized and artificially ventilated guinea pigs treated with saline or 1.0 and 0.1 nmol/kg of intravenous ANP, respectively. Bronchial responsiveness to inhaled histamine was significantly reduced by pretreatment with intravenous ANP in a dose-dependent manner. Treatment with 1.0 nmol/kg of intravenous ANP significantly inhibited bronchial

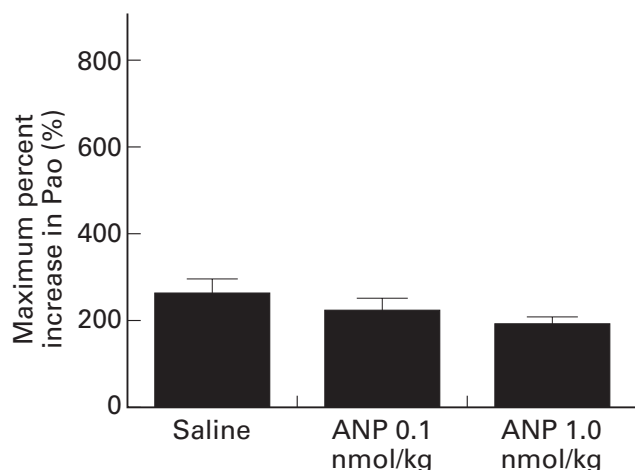


Fig. 2. Effect of intravenous ANP on antigen-induced bronchoconstriction in passively sensitized guinea-pigs. The peak value after antigen inhalation from the baseline value was not significantly reduced by pretreatment with intravenous ANP in a dose-dependent manner

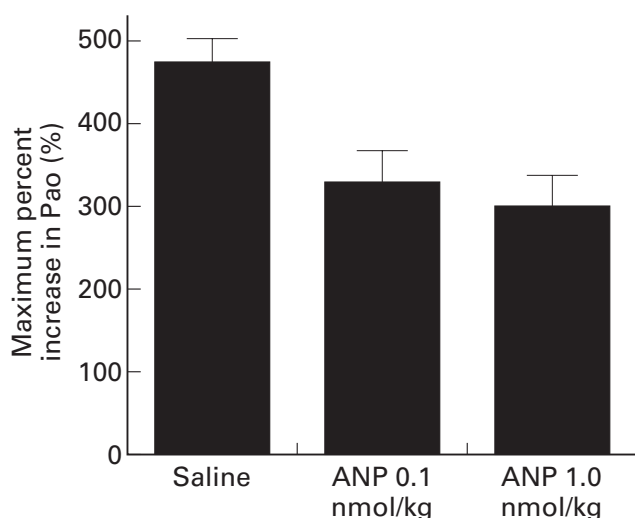


Fig. 3. PIB after antigen-challenge was reduced by ANP pretreated before OA challenge

responsiveness to histamine at concentrations of 100 and 200 $\mu\text{g/mL}$, compared with saline. ANP evoked a significantly greater attenuation than that evoked by saline (ANOVA $P < 0.02$).

Discussion

The present study showed that ANP significantly inhibited the bronchoconstriction induced by aerosolized propranolol administered 20 min after antigen inhalation.

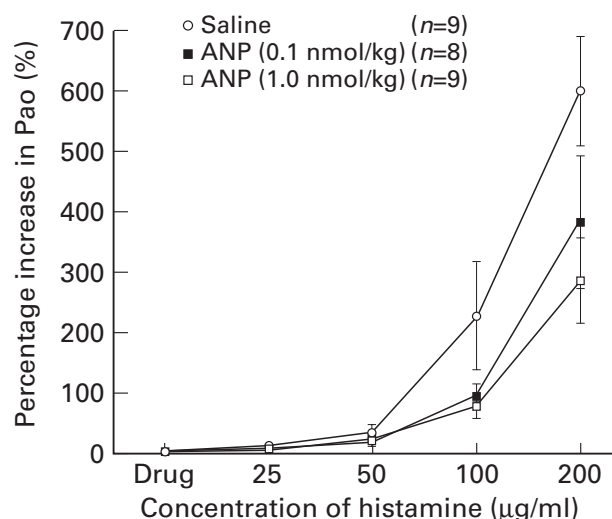


Fig. 4. Effect of intravenous ANP (0.1 and 1.0 nmol kg^{-1}) on aerosolized histamine-induced bronchoconstriction, compared with vehicle (saline) in anaesthetized and artificially ventilated guinea pigs. Values are mean $\pm$ SEM. ANP evoked a significantly greater attenuation than that evoked by saline (ANOVA $P < 0.02$)

Pretreatment with ANP before antigen challenge did not result in a decrease in the airway responses produced by antigen provocation, but not PIB. Further, ANP significantly reduced bronchial responsiveness to inhaled histamine.

ANP is a 28-amino acid hormone secreted by the cardiac atria and isolated lung tissue that has been found to have vasodilator, natriuretic and diuretic properties. ANP has received attention for its cardiovascular effects, however, autoradiography has identified ANP in the Type II alveolar epithelial cells of rat lung [15] and the natriuretic peptide A receptor is expressed in various tissues including lung [16], suggesting the possibility that lung tissue is a target organ. In human intravenous infusion of ANP has been shown to modify bronchial reactivity to inhaled histamine [17] and to ultrasonically nebulized distilled water [18]. It has been reported that plasma ANP concentrations are significantly increased during acute severe asthma attack compared with remission, suggesting that ANP may be important and bronchoprotective effect in asthma [19]. Thus the ability of ANP to inhibit non-specific bronchial hyperresponsiveness and also a more specific bronchial challenge in asthma is further evidence. In guinea pigs synthetic atrial natriuretic factor was a more potent relaxant of intrinsic tonus or contractions induced by methacholine as compared with contractions induced leukotriene D_4 on the trachea. Potvin and Varma *et al.* [5] reported that ANP was less potent and efficacious than isoprenaline in inhibiting the serotonin-, histamine- and carbachol-induced increase in pulmonary inflation pressure in anaesthetized guinea pigs, and ANP had limited bronchodilator activity. Intravenous propranolol

challenge of asthmatic patients leads to bronchoconstriction usually within 10 min [20]. The reaction can be severe, prolonged, and difficult to reverse. Songür *et al.* [21] recently reported that propranolol-induced bronchoconstriction developed within a few minutes when propranolol was inhaled 20 min after challenge with aerosolized antigen in passively sensitized and artificially ventilated guinea pigs, but not in non-sensitized animals, or during methacholine-induced bronchoconstriction.

Carpentiere *et al.* [22] have reported that a histamine H_1 -receptor antagonist attenuated airway responsiveness to inhaled propranolol, suggesting that histamine may be involved in PIB. However, they did not find any correlation between the degree of bronchodilation provoked by the histamine receptor antagonist and the magnitude of the decrease in responsiveness to propranolol. Although the effect of histamine in this reaction has been proposed, it is obvious that histamine alone can not explain the exact mechanism of PIB. β_2 -agonists have no effect of recovery from PIB in asthmatic subjects [23–25]. In animal models β_2 - and β_3 -agonists also did not reduce PIB [26]. In some of asthmatic subjects who caused bronchoconstriction by inhaled propranolol and in animal model, intravenous injection of aminophylline improved the PIB [26,27]. And β -adrenoceptor blocker-induced asthma is inhibited by pretreatment by inhaled anticholinergic drugs suggesting that cholinergic nerves may be involved in the bronchoconstrictor response to propranolol [28]. However, a previous study [26] revealed that pretreatment of the animals with atropine did not reduce the PIB. Therefore PIB has been proposed to be specific to bronchial asthma as well as bronchoconstriction induced by distilled water, cold and dry air, and so on. As mentioned above, some other mediators appear likely to be involved in PIB.

In our guinea pig model, antigen inhalation induced PIB as well as bronchial hyperresponsiveness [29]. It is unlikely that PIB resulted from the bronchial hyperresponsiveness because PIB does not correlate with bronchial hyperresponsiveness to methacholine in asthmatic patients and propranolol does not cause bronchoconstriction in normal subjects [26].

In humans ANP effectively protects the hyperreactive airway when given intravenously or by inhalation [30,31]. However, its duration of effect is short lasting and this would limit its use clinically unless the molecule was altered, or breakdown pathways for atrial natriuretic peptides were inhibited. In our passively sensitized model, we used diphenhydramine hydrochloride intraperitoneally to block the action of histamine in allergic reaction because the antihistamine drugs have been known to have little effect on bronchoconstriction in asthmatics. In ovalbumin-induced asthmatic guinea pigs model, Ohbayashi *et al.* [32] demonstrated that intravenous ANP had an inhibitory effect on

antigen-induced bronchoconstriction. However, their usage concentrations of ANP were 30, 100 and 300 $\mu\text{g/kg}$ and 10–100-fold higher than our usage. Also, in their study, intravenous administration of indomethacin as pretreatment before antigen challenge to avoid the influence of endogenous thromboxanes, prostaglandins. Therefore, our results may demonstrate that inhibitory concentrations of ANP on the PIB would have little effect on antigen-induced bronchoconstriction.

We believe this to be the first report on bronchoprotective effects of ANP against propranolol-induced bronchoconstriction after allergic reaction in guinea pigs *in vivo*. We found that it had considerable bronchoprotective properties. Also we recently observed that brain natriuretic peptide, one of the natriuretic peptide family, could inhibit PIB when this compound was intravenously administered 5 min before the inhalation of propranolol after allergic bronchoconstriction (unpublished data).

Our findings primarily suggest that ANP is an effective bronchoprotective agent against PIB and histamine-induced bronchoconstriction, albeit by acting as an apparently non-specific functional antagonist. Further studies are indicated to determine if this pathway can be exploited therapeutically in asthma.

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