Muscarinic effect of atrial natriuretic peptide on rabbit airways

*†A. Robichaud, ‡C. Saunier, †M.C. Michoud & *†P. du Souich

*Dépt. de pharmacologie, Faculté de Médecine, Université de Montréal, †Hôtel-Dieu de Montréal, Québec, Canada and ‡INSERM U14, Vandoeuvre-Les-Nancy, France

1 The aim of the present work was to investigate under which circumstances atrial natriuretic peptide (ANP) modulates airway resistance.

2 Of the six groups of rabbits ($n = 5$) studied, three received an infusion of ANP ($80 \text{ ng min}^{-1} \text{ kg}^{-1}$ i.v.) for a period of 100 min, while the other three were infused with the vehicle. Before receiving the infusion of ANP or the vehicle, the animals were pretreated with atropine (0.5 mg kg^{-1} i.v.), propranolol (2 mg kg^{-1} i.v.) or not pretreated. After 75 min of infusion of ANP, bronchoconstriction was induced by inhalation of histamine. Respiratory resistance (Rrs) was measured before and 3, 5, 10, 15 and 20 min post-histamine challenge.

3 Following 75 min of ANP infusion, plasma ANP concentration increased from 153 ± 52 (mean $\pm$ s.e.mean) to $1441 \pm 203 \text{ pg ml}^{-1}$ ($P < 0.05$) without affecting baseline Rrs. Control Rrs values ($12.5\text{--}20.4 \text{ cmH}_2\text{O l}^{-1} \text{ s}$) were significantly increased following the inhalation of histamine ($P < 0.001$). By themselves, atropine, propranolol or ANP did not modify the histamine-induced increase in Rrs. However, when the animals were pretreated with atropine, ANP infusion significantly reduced the increase in Rrs induced by histamine (30 ± 2 vs $51 \pm 6 \text{ cmH}_2\text{O l}^{-1} \text{ s}$; $P < 0.05$).

4 These data suggest that ANP has an indirect modulating effect on the airway smooth muscle and will decrease Rrs when muscarinic receptors are blocked.

Keywords: Atrial natriuretic peptide; respiratory resistance; airway smooth muscle; rabbit; histamine; atropine; propranolol

Introduction

There is growing evidence that the atrial natriuretic peptide (ANP), first known for its role in the regulation of vascular capacitance and of intravascular blood volume (Goetz, 1988; Cantin & Genest, 1986), also has extravascular effects. In addition to the cardiovascular system and the kidney, the central and autonomic nervous systems, the lungs, the adrenal glands, the digestive and reproductive system and lymph nodes have been suggested to be target organs for ANP (Vollmar, 1990; Gutkowska & Nemer, 1989).

In the lung, ANP has been demonstrated to be a powerful relaxant of guinea-pig and bovine isolated tracheal smooth muscle (O'Donnell *et al.*, 1985; Hamel & Ford-Hutchinson, 1986; Watanabe *et al.*, 1988; Potvin & Varma, 1989; Ishii & Murad, 1989). However, it has also been shown that ANP elicits no effect on rabbit and human airway smooth muscle *in vitro*, despite the fact that it can induce a bronchodilatation *in vivo* (Labat *et al.*, 1988; Candenas *et al.*, 1991; Hulks *et al.*, 1989; Chanez *et al.*, 1990; Robichaud *et al.*, 1993).

It has been suggested that the vaso- and bronchodilating actions of ANP are mediated by the guanosine 3':5'-cyclic monophosphate (cyclic GMP) intracellular messenger that induces the relaxation of smooth muscle (Hamet *et al.*, 1984; Winquist *et al.*, 1984; Cornwell & Lincoln, 1989; Watanabe *et al.*, 1990). However, there is growing evidence suggesting that these effects may be mediated by the autonomic nervous system (Kuchel *et al.*, 1987; Debinski *et al.*, 1990; Menard, 1991). Supporting a neuromodulatory function of ANP is the fact that it has been identified in peripheral parasympathetic (sensory nodose ganglia) and sympathetic (superior and inferior cervical, superior mesenteric and celiac ganglia (Debinski *et al.*, 1986; 1987a). In addition, several studies suggest that ANP stimulates vagal activity leading to a fall in arterial pressure and to a decrease in renal sympathetic nerve activity (Ackermann *et al.*, 1984; Thoren *et al.*, 1986; Volpe *et al.*, 1987; Imaizumi *et al.*, 1987; Schultz *et al.*, 1988; Zeuzem *et al.*, 1990).

To further understand the contradictory results concerning the modulatory effect of ANP on respiratory resistance, we investigated the action of ANP on airway smooth muscle tone during histamine-induced bronchoconstriction in rabbits pretreated with parasympathetic or sympathetic blockers.

Methods

Animal preparation

Experiments were conducted on 30 male, New Zealand rabbits (2.0–3.3 kg; CEGAV, St Mars d'Egrenne, France), divided into 6 groups of 5 animals each. The animals were anaesthetized with sodium thiopentone (Rhône-Poulenc, Paris, France) with an initial dose of $14\text{--}20 \text{ mg kg}^{-1}$ i.v. Additional anaesthetic was added as needed during the experiment. A tracheotomy was performed, but the animals breathed spontaneously. The left jugular vein and carotid artery were catheterized (PE 60, Clay Adams, Parsippany, NJ, U.S.A.) to permit the infusion of ANP and the determination of arterial blood pressure. Blood pressure was assessed by use of a Bell and Howell transducer (0–750 mmHg, type 4-327 I) coupled to a chart recorder (Brush 2400 Gould).

Experimental protocol

Three groups received an infusion of ANP ($80 \text{ ng min}^{-1} \text{ kg}^{-1}$ i.v.) (ANP [99–126]; IAF Biochem, Montreal, Canada), and three groups received an infusion of the vehicle, i.e. a solution of saline 0.9%:glucose 5% (50:50 v/v) containing 0.2% heat inactivated (50°C , 30 min) bovine serum albumin. Immediately before the beginning of the infusion of ANP or its vehicle, the animals were pretreated with atropine 0.5 mg kg^{-1} i.v. (groups AT-ANP or AT, respectively), ($\pm$)-propranolol 2 mg kg^{-1} i.v. (groups PR-ANP or PR, respectively), or not pretreated (groups ANP or sham, respectively) (Szarek *et al.*, 1986).

After surgical preparation of the animals, ANP was in-

¹ Author for correspondence.

fused for 75 min, in order to raise ANP plasma concentrations to a steady-state level (Marleau *et al.*, 1989). Following this, bronchoconstriction was induced by inhalation of a nebulized solution of histamine (1%) for 3 min, (Aerosoliseur Gauchard, Paris, France; flow rate: 0.12 ml min^{-1}); a dose producing approximately 50% response in rabbits (Berend *et al.*, 1986; Quan *et al.*, 1986). The infusion of ANP was continued during the entire experiment, resulting in a total infusion time of 100 min. Respiratory resistance, ventilation and respiratory frequency were measured before and 75 min after the beginning of ANP or vehicle infusion as well as 3, 5, 10, 15 and 20 min post-histamine inhalation. Measurements of blood gases and mean arterial blood pressure and blood sampling for plasma ANP determinations were made before and 75 min after the beginning of ANP infusion and 20 min post-histamine challenge.

Respiratory parameters

Respiratory resistance was measured by the forced oscillation method (Peslin, 1986) as described previously (Robichaud *et al.*, 1993). Briefly, sinusoidal pressure oscillations (amplitude $2 \text{ cmH}_2\text{O}$ peak to peak, frequency 20 Hz) were applied to the airway opening. The applied pressure and airway flow signal, measured with a Celesco[®] LCVR transducer (Canoga Park, CA, U.S.A.; range $\pm 2 \text{ cmH}_2\text{O}$) and a Fleish n°0 pneumotachograph (Metabo, Epalinges, Switzerland), respectively, were recorded for a period of 6.4 s at a sample frequency of 160 Hz by an Apple 2 microcomputer. Respiratory resistance was computed on a cycle-per-cycle basis from the pressure signal in phase with flow. Ventilation and respiratory frequency were obtained from the flow signal.

Analytical method

Plasma ANP was determined by radioimmunoassay (RIA) after extraction on Sep-pak cartridges according to the method described by Larose *et al.* (1985).

Statistical analysis

Results are presented as mean $\pm$ s.e.mean. Statistically significant differences between group means were calculated using a two-way analysis of variance for repeated measures combined with multiple procedures (Tuckey B) for each significant interaction (Winer, 1971). The alpha error was fixed at 5%.

Results

Arterial blood gases

In the sham group, arterial oxygen pressure (P_{aO_2}) and arterial partial pressure of CO_2 (P_{aCO_2}) were respectively 81.2 ± 1.6 and $27.1 \pm 0.7 \text{ mmHg}$ during the control period, 88.4 ± 3.4 and $25.0 \pm 1.1 \text{ mmHg}$ after 75 min of vehicle infusion, and 87.8 ± 3.5 and $21.2 \pm 1.8 \text{ mmHg}$ 20 min following histamine inhalation. Arterial pH of the sham animals was 7.43 ± 0.03 and 7.45 ± 0.02 at baseline and following 75 min of vehicle infusion, respectively. It increased significantly to 7.51 ± 0.02 after the inhalation of histamine. Mean values of P_{aO_2} , P_{aCO_2} , and pH were comparable between experimental groups.

Plasma ANP

Mean values of plasma ANP concentrations determined in the six experimental groups studied are shown in Figure 1. During the control period, baseline ANP plasma concentrations were similar in all groups. Compared to the baseline, the infusion of the vehicle, the inhalation of histamine, and parasympathetic (atropine) or sympathetic (propranolol)

blockade did not affect the levels of endogenous ANP. In the groups receiving the infusion of ANP (i.e. ANP, AT-ANP, PR-ANP), plasma ANP concentrations increased approximately ten fold; the concentrations generated by the infusion of ANP were comparable between the groups.

Mean arterial pressure

In the control period, mean arterial pressure was comparable between groups (Figure 2). Compared to baseline values, the infusion of the vehicle did not affect mean arterial pressure, whether histamine was present or not. The infusion of ANP

Figure 1 Atrial natriuretic peptide (ANP) plasma concentrations were determined before (baseline), 75 min after the beginning of the infusion of ANP (during ANP), and 20 min post-histamine challenge (post-histamine). *Significant difference from baseline at $P < 0.05$. (○) Sham = group receiving an infusion of the vehicle (saline 0.9%: glucose 5% [50:50 v/v] + inactivated bovine serum albumin 0.2%) ($n = 5$); (□) AT = group pretreated with atropine (0.5 mg kg^{-1} i.v.) + infusion of the vehicle ($n = 5$); (△) PR = group pretreated with propranolol (2 mg kg^{-1} i.v.) + infusion of the vehicle ($n = 5$); (▲) ANP = group receiving an infusion of ANP ($80 \text{ ng min}^{-1} \text{ kg}^{-1}$ i.v.) ($n = 5$); (◆) AT-ANP = group pretreated with atropine + infusion of ANP ($n = 5$); (●) PR-ANP = group pretreated with propranolol + infusion of ANP ($n = 5$).

Figure 2 Mean arterial pressure (MAP) was determined before (baseline), 75 min after the beginning of the infusion of atrial natriuretic peptide (ANP) (during ANP), and 20 min post-histamine challenge (post-histamine). *Significant difference from baseline at $P < 0.05$. (□) Sham = group receiving an infusion of the vehicle (saline 0.9%: glucose 5% 50:50 v/v] + inactivated bovine serum albumin 0.2%) ($n = 5$); (▨) AT = group pretreated with atropine (0.5 mg kg^{-1} i.v.) + infusion of the vehicle ($n = 5$); (▩) PR = group pretreated with propranolol (2 mg kg^{-1} i.v.) + infusion of the vehicle ($n = 5$); (■) ANP = group receiving an infusion of ANP ($80 \text{ ng min}^{-1} \text{ kg}^{-1}$ i.v.) ($n = 5$); (▤) AT-ANP = group pretreated with atropine + infusion of ANP ($n = 5$); (▥) PR-ANP = group pretreated with propranolol + infusion of ANP ($n = 5$).

Table 1 Respiratory parameters ($\dot{V}_E$ is ventilation, f is respiratory frequency) were determined prior to (baseline), 75 min after the beginning of the infusion of atrial natriuretic peptide (ANP) (during ANP), and 20 min post-histamine challenge (post-histamine)

Parameters	Groups	Baseline	During ANP	Post-histamine
$\dot{V}_E$ (l min ⁻¹)	Sham	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
	ANP	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
	AT	1.3 ± 0.1	1.2 ± 0.2	1.3 ± 0.3
	AT-ANP	1.1 ± 0.1	1.5 ± 0.3	1.3 ± 0.4
	PR	1.1 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
	PR-ANP	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.1
f (breaths per min)	Sham	72 ± 7	65 ± 7	68 ± 13
	ANP	66 ± 10	80 ± 15	77 ± 8
	AT	89 ± 5	65 ± 9	79 ± 13
	AT-PR	63 ± 5	82 ± 11	83 ± 16
	PR	93 ± 10	76 ± 5	75 ± 4
	PR-ANP	88 ± 16	75 ± 3	86 ± 5

Sham = group receiving an infusion of the vehicle (saline 0.9%: glucose 5% [50:50 v/v] + inactivated bovine serum albumin 0.2%) ($n = 5$); AT = group pretreated with atropine (0.5 mg kg⁻¹ i.v.) + infusion of the vehicle ($n = 5$); PR = group pretreated with propranolol (2 mg kg⁻¹ i.v.) + infusion of the vehicle ($n = 5$); ANP = group receiving an infusion of ANP (80 ng min⁻¹ kg⁻¹ i.v.) ($n = 5$); AT-ANP = group pretreated with atropine + infusion of ANP ($n = 5$); PR-ANP = group pretreated with propranolol + infusion of ANP ($n = 5$).

Figure 3 Mean respiratory resistance (Rrs) values during control ($t = 0$) and atrial natriuretic peptide (ANP) infusion periods ($t = 75$) and after histamine challenge (a) in the sham, ANP, AT and AT-ANP groups; (b) in the sham, ANP, PR and PR-ANP groups. *Significant difference at $P < 0.05$ compared to sham and ANP groups. (○) Sham = group receiving an infusion of the vehicle (saline 0.9%: glucose 5% [50:50 v/v] + inactivated bovine serum albumin 0.2%) ($n = 5$); (□) AT = group pretreated with atropine (0.5 mg kg⁻¹ i.v.) + infusion of the vehicle ($n = 5$); (Δ) PR = group pretreated with propranolol (2 mg kg⁻¹ i.v.) + infusion of the vehicle ($n = 5$); (▲) ANP = group receiving an infusion of ANP (80 ng min⁻¹ kg⁻¹ i.v.) ($n = 5$); (◆) AT-ANP = group pretreated with atropine + infusion of ANP ($n = 5$); (●) PR-ANP = group pretreated with propranolol + infusion of ANP ($n = 5$).

by itself also did not modify the mean arterial pressure; however, following histamine inhalation, blood pressure was significantly lower than baseline values. Atropine did not alter mean arterial pressure, but the combination of atropine and ANP infusion (i.e. AT-ANP group) produced a significant drop in blood pressure, a decrease that was slightly potentiated by the inhalation of histamine. Sympathetic blockade with propranolol did not significantly modify mean arterial pressure of animals receiving the vehicle. In animals receiving the infusion of ANP and pretreated with propranolol (i.e. group PR-ANP), mean arterial pressure remained stable during the infusion period. However, following the inhalation of histamine a significant fall in blood pressure was noted.

Ventilatory parameters

Mean values for ventilation and respiratory frequency of the different groups are shown in Table 1. These parameters were not affected by the infusion of ANP or its vehicle, or by the inhalation of histamine. The administration of parasympathetic or sympathetic blockers did not alter these respiratory characteristics of the animals.

Respiratory resistance

During the control period ($t = 0$), as well as after 75 min of ANP infusion, respiratory resistance remained stable in each group studied (Figure 3). In the sham group, the values of respiratory resistance increased significantly following the inhalation of histamine (from 16.9 ± 1.3 to 40.6 ± 2.7 cmH₂O l⁻¹ s) ($P < 0.001$). The infusion of ANP tended to increase the respiratory resistance, although the difference was not statistically significant.

Parasympathetic blockade

Compared to the sham group, the histamine-induced increase in respiratory resistance was not modified by atropine (i.e. AT group) (Figure 3a). However, in the animals pretreated with atropine and receiving an infusion of ANP (i.e. AT-ANP group), the increase in respiratory resistance induced by histamine was significantly lower 3 and 5 min after the challenge, compared to animals receiving only the infusion of ANP or of the vehicle.

Sympathetic blockade

Compared to the sham group, the administration of propranolol induced a slight, but not significant, increase in res-

piratory resistance following the inhalation of histamine (Figure 3b). The animals receiving the infusion of ANP (i.e. PR-ANP group) tended to show a decrease in the degree of histamine-induced bronchoconstriction; however, this did not reach statistical significance.

Discussion

Histamine is known to induce bronchoconstriction both by a direct effect on H_1 receptors and by cholinergic reflex activation (Shore *et al.*, 1983; Sheppard *et al.*, 1984). Since atropine did not influence the degree of histamine-induced bronchoconstriction under the present experimental conditions, we might assume that the cholinergic component of the action of histamine did not contribute much to the increase in respiratory resistance.

It has been demonstrated that rabbit airway smooth muscle is devoid of sympathetic innervation; however, catecholamine-containing cells have been identified in the ganglia of the bronchi (Mann, 1971), although their role in modulating bronchial tone does not appear important. In the present study, blockade of sympathetic β -adrenoceptors with propranolol tended to increase histamine-induced bronchoconstriction beyond the level observed in the sham group. Although it did not reach statistical significance, this action of propranolol is probably attributable to an increased cholinergic outflow as a result of withdrawal of the sympathetic activity. Alternatively, by treating the animals with a β -blocking drug, the α -adrenoceptors, being left unopposed, can induce a contraction of the airway smooth muscle (Kneussl & Richardson, 1978; Simonsson *et al.*, 1972), that is potentiated by the administration of histamine (Kneussl & Richardson, 1978). Therefore, it appears that the sympathetic nervous system may indirectly modulate rabbit airways and may partially blunt the response to histamine.

Following histamine inhalation, ANP tended to potentiate the bronchoconstriction. Since respiratory resistance was significantly decreased when ANP was infused to atropine-pretreated rabbits, we can assume that ANP elicits a parasympathomimetic effect in the respiratory system, as has already been described for the cardiovascular system. In addition, ANP appears to increase cholinergic activation by histamine, since atropine reduces ANP-induced changes in respiratory resistance below the values observed following the inhalation of histamine alone.

Several studies have demonstrated a direct sympatho-inhibitory effect of ANP *in vitro* as well as *in vivo* (Drewett *et*

al., 1988; 1989; Zukowska-Grojec *et al.*, 1986; Debinski *et al.*, 1987b; Bergey *et al.*, 1989; Floras, 1990). Therefore, in addition to a direct parasympathomimetic effect, we may speculate that ANP enhanced cholinergic outflow through sympathetic inhibition and induced a small bronchoconstriction that is reduced by atropine.

A slight decrease in the degree of histamine-induced bronchoconstriction (although not statistically significant) was also noted when ANP was infused in the presence of propranolol. Since, ANP exerts sympatho-inhibitory effects in the lung, it is possible that it exerts an α -adrenoceptor inhibition; this may explain why the combination of ANP and propranolol decreases histamine-induced increase in respiratory resistance. Supporting such speculation, Haass *et al.* (1985) have shown in vagotomized pithed rats that ANP can block blood pressure responses to α -adrenoceptor agonists.

In another study, we found that ANP decreased respiratory resistance following histamine inhalation (Robichaud *et al.*, 1993). The discrepancy with the results of the present investigation may be explained by differences in the experimental conditions. In the other study, the animals had a higher ventilation and tidal volume immediately before the histamine challenge, as well as a higher level of histamine-induced bronchoconstriction. Since lung irritant receptors associated with vagal reflex activation are more active in rabbits breathing more deeply (Mills *et al.*, 1969), it is possible that histamine triggered a more important cholinergic reflex activation in the previous study. Another difference between the two investigations, is the reduction in mean arterial pressure after ANP infusion. As a consequence, we may speculate that homeostatic reactions (activation of sympathetic, renin-angiotensin systems, etc.) were more important in the present study and that they may have changed bronchial reactivity to histamine in the presence of ANP, either directly or indirectly.

In summary, it appears that ANP may influence airway smooth muscle tone by affecting the pulmonary autonomic nervous system, and that the effect of ANP on airway smooth muscle tone depends upon the baseline conditions of the animal.

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