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Impaired endothelium-dependent relaxation by adrenomedullin in monocrotaline-treated rat arteries

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Abstract

The effects of adrenomedullin were evaluated in isolated vascular rings from rats treated with monocrotaline (60 mg/kg, s.c.) causing pulmonary hypertension and right ventricular hypertrophy within 3 to 4 weeks. Sham animals (NaCl-treated rats) were used for comparison. The relaxing effects of adrenomedullin (10^{-8} M) and acetylcholine (10^{-6} M) were determined in thoracic aorta and pulmonary artery rings precontracted with phenylephrine (10^{-7} M). In sham animals, adrenomedullin caused significant vasorelaxation of aorta and pulmonary artery although of different amplitude ($24 \pm 3\%$ and $40 \pm 2\%$, respectively). A greater relaxation was observed in response to acetylcholine. Monocrotaline-treated rats exhibited a reduction in adrenomedullin relaxation in pulmonary artery (54 and 68% loss of effect, at 3 and 4 weeks, respectively, $P < 0.01$ vs. sham) and comparable reductions in acetylcholine responses. The decrease in adrenomedullin relaxing effect was less pronounced in aorta than in pulmonary artery, suggesting a distinct tissue sensitivity to monocrotaline. In contrast, the relaxing effect of acetylcholine on aorta was decreased at 4 weeks (36% reduction, $P < 0.01$ vs. sham). In this model, the adrenomedullin-induced relaxation of the pulmonary artery was impaired due to a severe endothelial dysfunction which may contribute partly to the evolving pathophysiological process.

Keywords: Pulmonary hypertension; Aorta; Pulmonary artery; Vasorelaxation; Heart failure; Nitric oxide (NO)

1. Introduction

The vascular endothelium contributes to the modulation of the arterial tone via a balance between relaxing and contracting released factors. Among the relaxing factors, the endothelium-derived nitric oxide (NO) plays an important role (Palmer et al., 1987). It has been shown that the release of NO can be induced by a variety of endogenous substances (Furchgott and Vanhoutte, 1989). Recently, adrenomedullin, a vasoactive peptide with potent hypotensive and natriuretic activities, has been described as an important regulating factor in the cardiorenal homeostasis (Kangawa et al., 1996). The prominent mechanism responsible for adrenomedullin-induced relaxation has been shown to be a stimulation of specific binding sites located on endothelial cells resulting in an increase in endothelial cAMP generation and enhanced NO release (Matsunaga et al., 1996; Yang et al., 1996). The expression of adrenomedullin mRNA in a variety of cells and tissues, including endothelial cells, vascular smooth muscle cells, and heart indicates that adrenomedullin may have a role not only as a circulating hormone but also as a local autocrine/paracrine regulator of vascular tone (Nishimura et al., 1997).

Several pathological conditions such as myocardial infarction, pulmonary hypertension or congestive heart failure are associated with an increase of adrenomedullin concentration both in plasma and in cardiac tissues. Although this increase has been observed in animal models and in patients (Nishikimi et al., 1995; Jougasaki et al., 1996; Kobayashi et al., 1996; Morimoto et al., 1999), the precise role of the endothelium-dependent relaxing effect of adrenomedullin in cardiovascular diseases has not been clearly established. Among the proposed molecular mechanisms of vascular endothelial defect described in experimental models of cardiac dysfunction, a dramatic reduction

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in gene expression of the constitutive NO synthase has been demonstrated. This reduced gene expression may be responsible for a reduced NO production reported in the pathology, thus contributing to a depressed endothelium-dependent vasodilator responses (Smith et al., 1996).

It has been described that rats treated with a single injection of monocrotaline develop a progressive pulmonary hypertension which induces right ventricular hypertrophy and overt signs of heart failure 4 weeks after injection (Wilson et al., 1992). A significant increase in adrenomedullin production occurring during this pathological process has been reported in right ventricle (Shimokubo et al., 1995). As in other models, alterations observed in the diseased animals include reduced expression of the constitutive NO synthase from the vascular tissue suggesting an impaired endothelium-mediated vasorelaxation in this model (Comini et al., 1996).

The main objective of the present study was to investigate the vasorelaxing efficacy of adrenomedullin in isolated thoracic aorta and pulmonary artery from rats. The second objective was to determine whether the vasorelaxing properties of adrenomedullin are modified in rats with pulmonary hypertension and right ventricular hypertrophy induced by monocrotaline. These experiments were designed in order to evaluate the importance of endothelial dysfunction in this model.

2. Materials and methods

All experiments were performed in compliance with the NIH guidelines for the use and care of experimental animals (publication no. 85-23).

2.1. Animal and tissue preparation

Right ventricular hypertrophy and failure was produced by a single injection of monocrotaline, a pyrrolizidine alkaloid. Pathological changes occurring in the lungs include capillary thrombosis and medial thickening of the pulmonary arteries leading to a rise in pulmonary arterial pressure and subsequent development of cardiac hypertrophy (Wilson et al., 1992). Eight-week-old male Wistar rats (Janvier, Le Genest, France) were used (275–300 g). To evaluate the effect of evolving pathology, the rats were maintained for 3 and 4 weeks after treatment with monocrotaline. Therefore, four distinct groups were identified which included two monocrotaline-treated groups (3 and 4 weeks) and their two respective sham-treated groups. Monocrotaline (60 mg/kg) dissolved into physiological saline (0.9% NaCl) was administered subcutaneously (1.5 ml/100 g body weight) as previously described (Miyauchi et al., 1993). Sham-treated rats received a subcutaneous injection of the same volume of 0.9% NaCl.

The rats were then anesthetized with sodium thiopental (50 mg/kg, i.p., Nesdonal, Rhône Mérieux, Lyon, France) 3 or 4 weeks after monocrotaline injection, and were exsanguinated. Whole blood was collected in EDTA-coated tubes and centrifuged at $4000 \times g$, 20 min at 4°C. Plasma samples were stored at −70°C for subsequent assays.

The heart, the pulmonary artery and the thoracic aorta were excised carefully and placed in cold Krebs solution. The heart and the right ventricle of each rat were weighed to determine the macroscopic changes induced by the pathology.

2.2. Vasorelaxation studies

After excision, the pulmonary artery was kept intact whereas the thoracic aorta was dissected free of connective tissue. The vessels were cut into rings (3 mm width) and mounted in 15 ml organ bath filled with Krebs–Henseleit (KH) solution oxygenated with a 95% O_2/5% CO_2 mixture with a temperature adjusted to 37°C. The composition of the KH solution was as follows (mM): NaCl (95), KCl (5), $CaCl_2$ (2.6), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $NaHCO_3$ (24.9), and glucose (10), pH 7.4. The tension was measured isometrically using a force transducer (EMKA Technologies, Paris, France) and printed on a chart recorder (RS-3400, Gould Instruments, Ballainvilliers, France). The preparations were allowed to equilibrate for at least 45 min, at a resting tension of 0.6 g and were precontracted with phenylephrine (10^{-7} M) as previously described (Saïag et al., 1996; Hussain and Marshall, 1997). The patency of the endothelium was evaluated by determination of the relaxation induced by acetylcholine. Test drugs were then applied at the maximum of contraction induced by phenylephrine (10^{-7} M).

Adrenomedullin was tested at a concentration (10^{-8} M) established in preliminary dose–responses experiments (from 10^{-10} to 10^{-7} M) using control rat arteries. This concentration was shown to produce a vasorelaxation by approximately 40% in rat preparations (Matsunaga et al., 1996; Wanstall and Crilley, 1996; Yang et al., 1996; Nishimura et al., 1997). Each ring was exposed to a single concentration of adrenomedullin only. The concentration of 10^{-8} M adrenomedullin caused a submaximal vasorelaxation in the pulmonary artery in agreement with the literature reports. The same concentration produced a less marked vasorelaxation in the aorta. Acetylcholine which has been shown to produce graded relaxations of aortic preparations was used at a concentration of 10^{-6} M in the present study (Furchgott and Zawadzki, 1980).

In some experiments, the endothelium was removed as previously described by brief repeated exposures of the vessel ring to a solution of saponin (0.05 mg/ml organ bath solution) (Saïag et al., 1996). In other experiments, inhibition of the NO synthase was produced by addition of L-NAME (10^{-4} M) in the organ bath 20 min prior to contraction (Saïag et al., 1996). Sodium nitroprusside (10^{-7} M) was also used to check the endothelium-independent relaxation on normal and pathological tissues.

2.3. Drugs and chemicals

Acetylcholine chloride, N^G-nitro-L-arginine methyl ester, HCl (L-NAME), monocrotaline, phenylephrine, HCl, sodium nitroprusside (Sigma, St. Louis, MO); human adrenomedullin 1-52 (Bachem Biochimie, Voisins-le-Bretonneux, France); saponin (Aldrich, St. Quentin-Fallavier, France). The plasma concentrations of endothelin-1 were determined using a radioimmunoassay kit (NEN Life Science Products, Le Blanc Mesnil, France).

2.4. Calculations and statistical analysis

In all experiments on isolated vessels, the relaxation was assessed as the percent decrease of the maximal contraction caused by phenylephrine. The rate–pressure product values cited in the text which represent an index of left ventricular work are defined as the product of heart rate by systolic arterial blood pressure measured in anesthetized rats (Landais et al., 1998).

All the data are expressed as means ± S.E.M. Comparisons between groups were performed using the analysis of variance (ANOVA) followed by the Sidak multiple comparison test as appropriate. A P value less than 0.05 represented the level for statistical significant difference.

3. Results

A total of thirty four rats have been used in this study. One rat from the 4-week monocrotaline-treated group died within the last week after treatment and was not included in the experimental protocol.

3.1. Development of monocrotaline-induced pulmonary hypertension

As summarized in Table 1, the most dramatic changes in heart and body weight induced by monocrotaline were observed 4 weeks after treatment. The body weight was slightly lower in monocrotaline-treated rats than in sham animals 3 weeks after a single injection of monocrotaline and became significantly reduced after 4 weeks ($P < 0.01$). After 4 weeks, the monocrotaline-treated rats exhibited a significant right ventricular hypertrophy as indicated by a marked increase in right ventricular weight (+79%, $P < 0.001$ vs. sham) and in right ventricular weight/heart weight ratio (+58%, $P < 0.001$). Although the heart weight did not change significantly after treatment, the heart weight/body weight ratio also tended to be higher in monocrotaline rats than in sham rats (+39% after 4 weeks, $P < 0.001$) because of the body weight loss after treatment. At necropsy, evidence of pulmonary hemorrhage, pleural and peritoneal effusions were observed mainly after 4 weeks of treatment by monocrotaline. A twofold increase in the weight of the pulmonary artery rings was also observed (7.2 ± 0.5 mg, $n = 9$ sham, to 15.4 ± 1.7 mg, $n = 8$ 4-week monocrotaline-treated; $P < 0.05$) whereas the aorta weight remained unchanged. The circulating plasma level of endothelin-1 rose from 39 ± 4 pg/ml ($n = 6$) in sham rats to 66 ± 15 ($n = 5$) and 81 ± 15 pg/ml ($n = 4$, $P < 0.05$), respectively, 3 and 4 weeks after monocrotaline-treatment.

3.2. Validation experiments

Preliminary studies were performed to determine the role of the vascular endothelium in the relaxing effect of adrenomedullin. As shown in Fig. 1, a reduction of the effects of adrenomedullin (10^{-8} M) and of acetylcholine (10^{-6} M) was observed after brief repeated exposures of the vessel rings to a saponin solution or by pretreatment with L-NAME 20 min prior to phenylephrine contraction ($P < 0.05$, Fig. 1). These results demonstrated that, similarly to acetylcholine, the adrenomedullin-induced relaxation was mediated through an endothelium-dependent mechanism. In order to check that saponin did not alter the intrinsic ability of vascular smooth muscle to relax in the loss of response to adrenomedullin and acetylcholine, preliminary experiments were performed. We observed that when saponin suppressed the relaxation induced by acetylcholine, the vasorelaxant response of the same vessel ring to sodium nitroprusside (10^{-7} M), a nitric oxide donor, was unchanged (data not shown).

An altered responsiveness to spasmogens has been reported in this model such as increased response to 5-HT and reduced response to endothelin (Wanstall and O'Donnell, 1990). Therefore, the lack of deleterious effect of

Table 1
Morphological changes observed in rats treated with monocrotaline

	3 Weeks		4 Weeks	
	Sham ($n = 8$)	Monocrotaline ($n = 8$)	Sham ($n = 8$)	Monocrotaline ($n = 8$)
Body weight (g)	432 ± 29	368 ± 16	464 ± 20	377 ± 10[b]
Heart weight (g)	1.30 ± 0.06	1.28 ± 0.11	1.27 ± 0.06	1.42 ± 0.07
Right ventricular weight (mg)	0.23 ± 0.03	0.34 ± 0.03[a]	0.24 ± 0.02	0.43 ± 0.03[c]
Heart weight/body weight (mg/g)	3.00 ± 0.02	3.45 ± 0.18	2.72 ± 0.05	3.78 ± 0.27[c]
Right ventricular weight/heart weight (mg/g)	0.17 ± 0.02	0.25 ± 0.01[b]	0.19 ± 0.02	0.30 ± 0.02[c]

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ vs. sham.

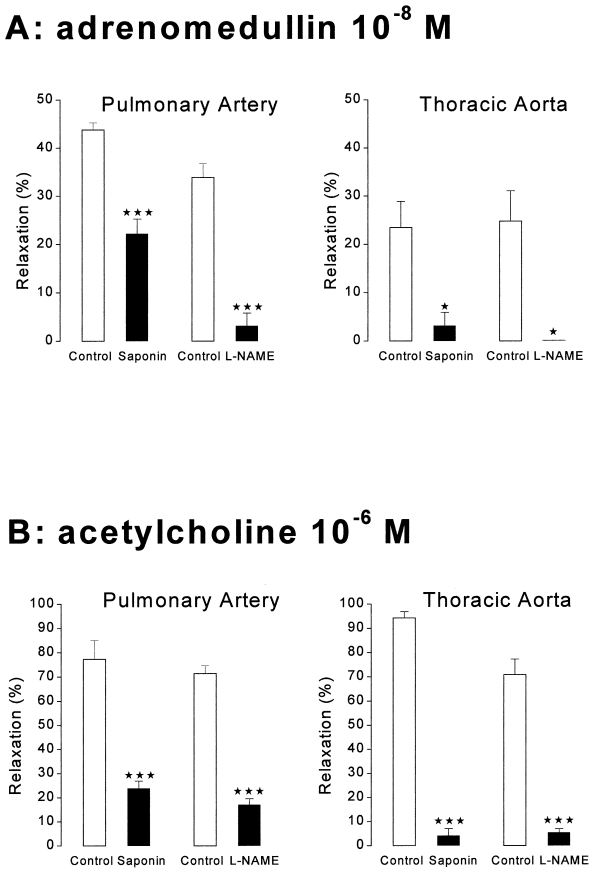

Fig. 1. Effect of saponin and L-NAME pretreatment on the vasorelaxing effect of adrenomedullin (panel A) and acetylcholine (panel B) in control rat arteries precontracted with phenylephrine 10^{-7} M ($n = 4$–5 animals). Saponin (0.05 mg/ml bath solution) or L-NAME (10^{-4} M) is applied prior to phenylephrine contraction. The results are expressed as a percentage of relaxation of the vessel ring precontracted with phenylephrine 10^{-7} M. $^{*}P < 0.05$; $^{***}P < 0.001$ vs. control.

monocrotaline on phenylephrine-induced contraction in arteries was checked by measuring the amplitude of response to phenylephrine in subsets of pulmonary artery and aorta rings. No significant change in the level of steady state phenylephrine contraction was observed in the 3- and 4-week monocrotaline-treated rats vs. sham-treated rats (data not shown).

Finally, application of 10^{-7} M sodium nitroprusside in phenylephrine-precontracted pulmonary artery rings demonstrated a selective loss of relaxing efficacy (from $87 \pm 2\%$ in sham rats to $49 \pm 6\%$ in 4-week monocrotaline-treated rats, $n = 10$, $P < 0.05$) whereas no change at all was observed in aortic rings ($94 \pm 1\%$ and $91 \pm 4\%$ in sham and monocrotaline rats, respectively, $n = 8$, NS).

3.3. Rat pulmonary artery

In phenylephrine-precontracted pulmonary artery rings, adrenomedullin and acetylcholine induced a mean vasorelaxation of $40 \pm 2\%$ ($n = 11$) and $70 \pm 3\%$ ($n = 11$), respectively. The percentage of relaxation induced by adrenomedullin was significantly attenuated 3 and 4 weeks after monocrotaline injection (53.9 and 67.5% loss of relaxing effect, respectively, $P < 0.05$ vs. sham animals). Similarly, the vasorelaxing response of rat pulmonary artery to acetylcholine was decreased by 41.1% and by 62.8%, 3 and 4 weeks after monocrotaline treatment, respectively (Fig. 2). The loss of vasorelaxing efficacy was almost identical with both vasodilator agents and was more severe 4 weeks after monocrotaline injection.

3.4. Rat thoracic aorta

In phenylephrine-precontracted rings of thoracic aorta, the mean vasorelaxing effects of adrenomedullin and acetylcholine were $24 \pm 3\%$ ($n = 12$) and $84 \pm 3\%$ ($n = 12$), respectively. Adrenomedullin exhibited clearly weaker relaxation in this tissue than in the pulmonary artery whereas acetylcholine, at the same concentration, caused a similar relaxing effect in both vessels. As shown in Fig. 3, no significant change in the vasorelaxing response to adrenomedullin was observed in the aorta at 3 or 4 weeks following monocrotaline injection although an apparent reduction is present at 4 weeks. The relaxation induced by acetylcholine in the aorta rings from 3-week monocro-

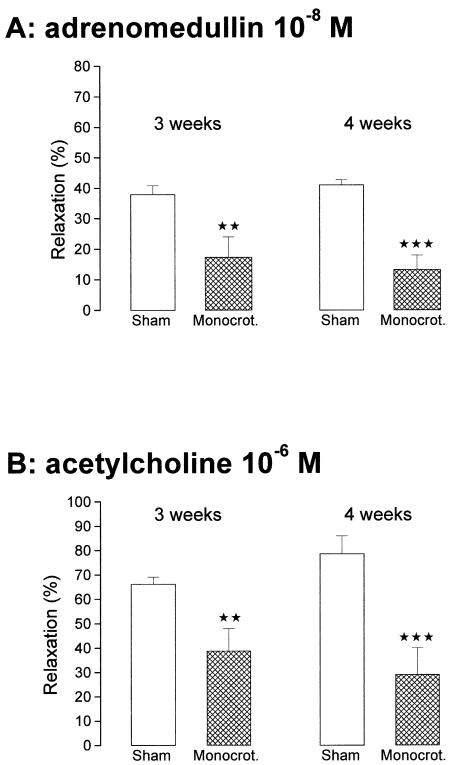

Fig. 2. Vasorelaxation induced by adrenomedullin (10^{-8} M, panel A) and acetylcholine (10^{-6} M, panel B) in rat isolated pulmonary artery: comparison between isolated vessels from sham rats and from 3- to 4-week monocrotaline-treated rats (60 mg/kg single injection). The results are expressed as a percentage of relaxation of the vessel ring precontracted with phenylephrine 10^{-7} M ($n = 5$–6 animals). $^{**}P < 0.01$; $^{***}P < 0.001$ vs. sham.

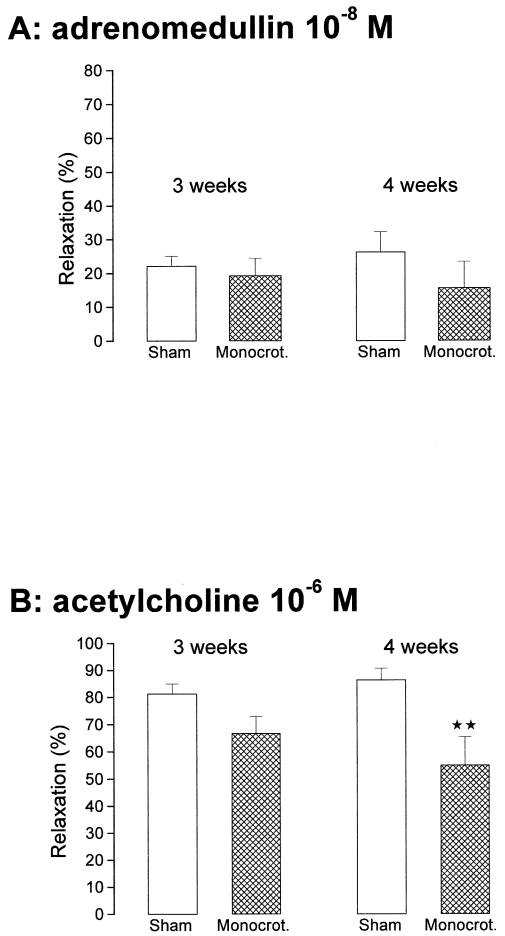

Fig. 3. Vasorelaxation induced by adrenomedullin (10^{-8} M, panel A) and acetylcholine (10^{-6} M, panel B) in rat isolated thoracic aorta: comparison between isolated vessels from sham rats and from 3- to 4-week monocrotaline-treated rats (60 mg/kg single injection). The results are expressed as a percentage of relaxation of the vessel ring precontracted with phenylephrine 10^{-7} M ($n = 5$–6 animals). $^{**}P < 0.01$ vs. sham.

taline-treated rats was not significantly different from that observed in sham-treated rats. In contrast, a moderate decrease was observed after 4 weeks (36.5% decrease, $P < 0.01$ vs. sham).

4. Discussion

This study shows that both adrenomedullin and acetylcholine induce endothelium-dependent relaxations in rat isolated aorta and pulmonary artery and that these effects are significantly reduced in a model of right ventricular hypertrophy and failure induced by monocrotaline. A severe impairment of vasorelaxing efficacy of adrenomedullin was observed after 3 weeks following monocrotaline treatment and was more pronounced after 4 weeks, consistent with a progressive pathological process. Finally, the loss of endothelial function was stronger in the pulmonary artery than in the aorta, suggesting a higher vulnerability of the pulmonary vascular bed than of the systemic circulation in this model (Nasa et al., 1996).

Monocrotaline has been shown to cause severe pulmonary arterial lesions specifically of the endothelial cells via a direct effect of its metabolite monocrotaline pyrrole, leading to apoptosis and altered cardiac and vascular responses (Brown et al., 1998; Thomas et al., 1998). Pulmonary hypertension and right ventricular hypertrophy are the main observed alterations which may ultimately result in severe heart failure. Concomitant morphological alterations such as progressive increases in the ratio of heart weight to body weight and in the right ventricular weight/heart weight ratio were shown in the 3- and 4-week monocrotaline-treated rats, respectively. Similar changes of body and heart weights have been previously reported to occur in parallel with a significant increase in adrenomedullin tissue level in the right ventricle of diseased rats (Shimokubo et al., 1995). In the present study, a significant elevation in plasma levels of endothelin-1 associated with the severity of the disease symptoms was also observed. Previously, a progressive decrease in rate–pressure product was demonstrated 3 and 4 weeks after monocrotaline treatment (-26%, $P < 0.05$ and -59%, $P < 0.001$, respectively, $n = 10$) but not after 2 weeks, reflecting an evolving hemodynamic dysfunction (Landais et al., 1998). Consistent with these data, an increased circulating level of endothelin-1 was reported in monocrotaline-rats early during the disease process, thus preceding the development of pulmonary hypertension and was suggested to contribute to the progression of right ventricular hypertrophy (Miyauchi et al., 1993).

Using this established model of pulmonary hypertension and right ventricular hypertrophy, alterations in the vasorelaxant properties of the new peptide adrenomedullin were evaluated in the main pulmonary artery and in the thoracic aorta of diseased rats. The primary result observed during the course of the disease was a dramatic impairment of the efficacy of adrenomedullin paralleled with the occurrence of cardiac hypertrophy and dysfunction in the monocrotaline-treated rats.

The endothelium-dependent mechanism of relaxation of adrenomedullin and acetylcholine in rat arterial rings was validated by chemical destruction of the endothelium with saponin pretreatment. The relaxation induced by adrenomedullin and acetylcholine in normal tissues was attenuated and the consequence on the vasodilatory action of adrenomedullin and acetylcholine was more prominent on aorta probably because the access of the luminal wall by saponin is easier on this vessel. The implication of the NO pathway in the adrenomedullin- and acetylcholine-induced relaxation was confirmed by the suppression of the adrenomedullin and acetylcholine relaxations of precontracted vessels by L-NAME. Similar observations have been reported by others in the rat aorta and pulmonary artery, or in canine arteries and veins, suggesting that the vascular endothelium plays a primary role in the relaxing effect of adrenomedullin (Nakamura et al., 1995; Matsunaga et al., 1996; Nossaman et al., 1996; Barber et al.,

1997). In agreement with the present results, the vasorelaxing effect of adrenomedullin has been proposed to occur primarily through specific activation of endothelial adrenomedullin binding sites, increasing the production of cAMP and the release of NO (Kato et al., 1995; Shimekake et al., 1995; Nandha et al., 1996).

Adrenomedullin was shown to produce a greater vasorelaxation of the pulmonary artery than of the aorta. A different degree of relaxation in response to adrenomedullin between both arterial vessels has been previously described in rat arteries although this discrepancy is less marked in the present study (Matsunaga et al., 1996). However, in both tissues, the vascular responses to adrenomedullin were markedly reduced by treatment with L-NAME or by chemical endothelial withdrawal, showing a clearcut involvement of the endothelium (Yang et al., 1996). Such a tissue specificity was not observed in response to acetylcholine, suggesting that the vasorelaxant effects of both agents may involve distinct effector pathways in the aorta. Alternatively, the difference in response could be related to different tissue receptor densities as previously proposed (Wanstall and Crilley, 1996).

In arteries of rats with monocrotaline-induced pulmonary hypertension, both adrenomedullin and acetylcholine exhibited an impairment of vasorelaxing potency. A previous study of the relaxing effects of adrenomedullin in phenylephrine-precontracted aorta from normal WKY and SHR rats did not show a significantly weaker response of aorta in pathological situation (Matsunaga et al., 1996). Based on the observation of a limited vasorelaxing effect of adrenomedullin in aorta and on the lack of difference in normal and pathological rats, it was proposed that the modulation of vasomotor tone through adrenomedullin receptors is operating mainly in peripheral arterioles (Matsunaga et al., 1996). The present study demonstrates, at least in the main pulmonary artery, a dramatic alteration of the endothelium-mediated effects of adrenomedullin in rats with pulmonary hypertension. Similar findings were obtained in a model of hypoxia-induced pulmonary hypertension in rat suggesting the presence of a functionally impaired endothelium (Wanstall and Crilley, 1996). The defect in endothelium-mediated control of the vasomotion has been described in various experimental animal models and in patients with heart failure (Wang et al., 1994; Nasa et al., 1996). Other responses to endothelium-dependent vasodilators such as acetylcholine but not to the smooth muscle dilator adenosine are markedly impaired, indicating a specific endothelium defect (Treasure and Alexander, 1993). The most plausible explanations for the loss of activity of adrenomedullin in such pathological situation include i) alterations of adrenomedullin receptors directly on the endothelial cell surface or a defect in their signal transduction and ii) functional abnormalities of the endothelium. In this study, a marked reduction in the vasodilator activity of adrenomedullin was observed predominantly in the pulmonary artery of monocrotaline-treated rats (more than 60% reduction 4 weeks after monocrotaline injection). Although the weak vasodilating effect of adrenomedullin in normal aorta may account for the lack of measurable changes in vessels from diseased animals, it is more likely that the endothelium shows greater alterations in the pulmonary artery than in the aorta in response to the development of pathology. Such an hypothesis was confirmed in these experiments using acetylcholine instead of adrenomedullin as relaxing agent which showed a moderate reduction of response in diseased aorta (4 weeks after treatment) as compared with sham tissues. Apart from a slower time course of the acetylcholine response in pulmonary artery from monocrotaline-treated rats, no alteration of acetylcholine potency was mentioned in previous studies, although the lack of detectable alteration was probably due to the higher concentration (10^{-5} M) of acetylcholine used (Wanstall and O'Donnell, 1990). Distinct alterations of pulmonary artery and aorta were also described in heart failure rats with chronic myocardial infarction. Although the results showed that both cGMP- and cAMP-mediated relaxations are altered in this model of failure, cGMP-dependent relaxation with acetylcholine was more markedly attenuated in pulmonary artery than in aorta (Nasa et al., 1996). In the present study, it is to be noticed that the pulmonary artery but not the aorta is hypertrophied in monocrotaline-treated rats, suggesting that a global vascular lesion (endothelium and vascular smooth muscle) may contribute to the impairment of relaxation. In this model, a 44% loss of relaxation induced by sodium nitroprusside (10^{-7} M) was reported in pulmonary artery but not in aorta from monocrotaline-treated rats. This result may be consistent with a marked decrease in basal cGMP and cAMP levels in vessels from heart failure rats (Nasa et al., 1996).

The molecular mechanism of the endothelium-dependent loss of adrenomedullin vasorelaxing action in heart failure is not yet clarified. To date, no study has investigated the potential changes in adrenomedullin receptor density during the development of experimental heart failure, neither in endothelial cells nor in myocytes. Several reports have proposed that the impairment of vascular function in heart failure was caused by a reduced endothelial production of NO (Katz et al., 1993; Drexler et al., 1994; Wang et al., 1994). Consistent with this observation, a decreased expression of the constitutive NO synthase was shown in the aortic wall of monocrotaline-treated rats (Comini et al., 1996). Such results give further support for the reduction of adrenomedullin potency in pathological vessels being caused by an impaired NO pathway. Interestingly, a recent study demonstrated a beneficial effect of chronic administration of adrenomedullin in the same model of monocrotaline-induced pulmonary hypertension (Yoshihara et al., 1998). Although the mechanism for the protective action of adrenomedullin is not fully elucidated, the authors suggested that adrenomedullin attenuates the progression of pulmonary hypertension by its vasodilator

action and also by inhibition of endothelin-1 production. The present study suggests that chronic administration of adrenomedullin is unlikely to afford a benefit through direct pulmonary vasodilation and that its main action could be related to a reduced endothelin-1 production (although the plasma endothelin-1 concentration was not evaluated in the study by Yoshihara et al. (1998)).

In conclusion, the present study showed that functional endothelial alterations of the pulmonary and aortic vessels are concomitant with the development of pulmonary hypertension which may ultimately lead to heart failure in monocrotaline-treated rats. As increased plasma levels of adrenomedullin have been shown in heart failure in both experimental and clinical reports (Jougasaki et al., 1995; Nishikimi et al., 1995; Shimokubo et al., 1995; Jougasaki et al., 1997), it may be hypothesized that a reduced sensitivity of the main arterial vessels to this new endogenous dilator agent may play a role in the overall impairment of cardiac function. Additional studies remain to be performed in other models of heart failure to determine the relative contribution of impaired adrenomedullin response to the severity of this endothelium defect during evolving pathology.

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