ELSEVIER

European Journal of Pharmacology 355 (1998) 33–39

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Chronic infusion of adrenomedullin reduces pulmonary hypertension and lessens right ventricular hypertrophy in rats administered monocrotaline

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Received 5 February 1998; revised 22 June 1998; accepted 23 June 1998

Abstract

A novel vasorelaxant peptide, adrenomedullin, its messenger ribonucleic acid (mRNA), and the mRNA for its receptor are highly expressed in the lung, suggesting that adrenomedullin may play a role in the regulation of the pulmonary circulation. We investigated whether the chronic infusion of rat adrenomedullin would affect pulmonary hypertension and right ventricular hypertrophy produced by the administration of monocrotaline. Four-week-old male Wistar rats received a single subcutaneous injection of 60 mg/kg monocrotaline and were then chronically and subcutaneously infused with rat adrenomedullin (PH + AM group, $n = 8$) or saline (PH group, $n = 10$) by an osmotic minipump for a period of 21 days. Plasma levels of adrenomedullin were significantly higher in the PH vs. the control group. The chronic infusion of adrenomedullin in rats with pulmonary hypertension increased the plasma levels of adrenomedullin to a value 94% greater than that of the control group and 55% greater than that of the untreated PH group. Chronic infusion of adrenomedullin significantly lessened the increase in right ventricular systolic pressure and the ratio of right ventricular weight to body weight seen after monocrotaline treatment. Histological examination revealed that adrenomedullin also attenuated the medial thickening of the pulmonary artery. These results suggest that chronic infusion of adrenomedullin attenuates the pulmonary hypertension and right ventricular hypertrophy seen in rats treated with monocrotaline.

Keywords: Adrenomedullin; Pulmonary hypertension; Pulmonary circulation; Ventricular hypertrophy, right; Monocrotaline

1. Introduction

Adrenomedullin, a potent vasorelaxant peptide, was isolated from pheochromocytoma tissue (Kitamura et al., 1993a). Human adrenomedullin messenger ribonucleic acid (mRNA) is highly expressed in several other normal tissues including lung (Kitamura et al., 1993b). The concentration of adrenomedullin in the lung is several-fold higher than that in the cardiac ventricle and the kidney (Ichiki et al., 1994; Sakata et al., 1994). In addition, binding sites for adrenomedullin are abundant in rat lung (Owji et al., 1995), and the mRNA for the adrenomedullin receptor is highly expressed in lung tissue (Kapas et al., 1995). The presence of adrenomedullin receptors in the lung make it likely that adrenomedullin may act as an autocrine and paracrine factor.

It has been previously reported that adrenomedullin injections decrease pulmonary vascular resistance in a dose-dependent manner (Lippton et al., 1994; DeWitt et al., 1994) under conditions of elevated pulmonary vascular tone (Lippton et al., 1994). With regard to the mechanism for pulmonary vasorelaxation by adrenomedullin, Nossaman et al. (1996) previously reported that the pulmonary vasodilator response to adrenomedullin is dependent on the release of nitric oxide from the endothelium in the rat, but that the response to adrenomedullin is endothelium independent in the pulmonary vascular bed of the cat. In addition, Champion et al. (1997) demonstrated that adrenomedullin dilates the hindlimb vascular bed of the cat

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0014-2999/98/$19.00

by an adenosine 3′,5′-cyclic monophospate-dependent mechanism. Thus, these findings suggest that the mechanisms by which adrenomedullin dilates the vascular bed are not same in different vessels or in different species. However, plasma adrenomedullin levels previously have been reported to be elevated in patients with primary and secondary pulmonary hypertension and to correlate with pulmonary arterial pressure, suggesting the involvement of adrenomedullin in the regulation of the pulmonary circulation (Yoshibayashi et al., 1997; Nishikimi et al., 1997a).

The administration of monocrotaline to rats induces vascular endothelial cell damage, medial thickening of the muscular pulmonary arteries, and neomuscularization of the nonmuscular distal arteries, leading to pulmonary hypertension and right ventricular hypertrophy (Meyrick et al., 1980; Ghodsi and Will, 1981). A previous report demonstrated that the concentrations of adrenomedullin in the right ventricle and plasma, and the expression of adrenomedullin mRNA in the right ventricle were significantly higher in monocrotaline-treated rats than in controls (Shimokubo et al., 1995). While it has been suggested that adrenomedullin could counteract monocrotaline-induced pulmonary hypertension, it has not been investigated whether adrenomedullin can inhibit the progression of pulmonary hypertension and right ventricular hypertrophy in such a model at pathophysiological levels.

The purpose of the present study was to study whether adrenomedullin in pathophysiological concentrations attenuates the development and progression of the cardiopulmonary changes in rats induced by monocrotaline. To accomplish this, we measured plasma adrenomedullin levels in monocrotaline-treated rats, control animals and those given monocrotaline and receiving a chronic infusion of adrenomedullin. We also measured and compared cardiovascular parameters and the degree of medial thickening of the small pulmonary arteries these groups of animals.

2. Materials and methods

The study was performed in accordance with the guidelines of the Animal Care Committee of the National Cardiovascular Center Research Institute.

2.1. Animals and experimental design

Four-week-old male Wistar rats, weighing 100 to 120 g, were used ($n = 31$). Monocrotaline (Sigma) was dissolved in 1 M HCl, neutralized with 0.5 M NaOH, and then diluted with distilled water to pH 7.0. Experimental animals were given a single subcutaneous injection of 60 mg/kg monocrotaline while control animals were injected subcutaneously with 0.9% saline. Synthetic rat adrenomedullin was obtained from the Peptide Institute (Osaka, Japan). The experimental rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and subcutaneously implanted with osmotic minipumps (model 2 ML4, Alza; Palo Alto, CA) filled with synthetic rat adrenomedullin dissolved in 0.9% saline. The pumps were designed to release 200 ng/h of the peptide for 21 days. They were positioned in a pocket constructed in the subcutaneous tissue just below the sub-scapular region. For the control group, 0.9% saline was infused from the minipumps in a similar manner.

The animals were randomly divided into three groups. In the PH group, 10 rats were given a single injection of monocrotaline followed by a chronic infusion of saline. In the PH + AM group, 8 rats were given a single injection of monocrotaline followed by a chronic infusion of rat adrenomedullin. In the control group, 13 rats were given a single injection of saline followed by a chronic infusion of saline.

Hemodynamic studies were performed 21 days postoperatively as previously reported (Nishikimi et al., 1995). Animals were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and a catheter (PE-10 fused to PE-50) filled with heparin-saline solution was inserted into the thoracic aorta of each animal through the right carotid artery to measure aortic pressure. A similar catheter was inserted into the right ventricle through the right jugular vein to measure right ventricular pressure. Mean arterial pressure and right ventricular systolic pressure were also recorded. Blood (1.5 ml) was then collected from the carotid arterial catheter for measuring plasma adrenomedullin. The rats were then killed and their hearts and lungs were excised. Each heart was divided into the right ventricle, the left ventricular free wall, the septum, the right atrium, and the left atrium, and each portion was separately weighed. We also calculated the ratio of right ventricular weight to body weight (RV/BW) and the ratio of left ventricular weight to body weight (LV/BW) as indices of ventricular hypertrophy.

2.2. Radioimmunoassay

Each blood sample was placed in a chilled tube containing aprotinin (70 μg/ml) and EDTA (1.5 mg/ml), and then centrifuged at $3000 \times g$ for 10 min at 4°C. Radioimmunoassay for rat adrenomedullin was performed as reported before (Sakata et al., 1994) using antiserum against human adrenomedullin-(40-52)NH_2, since rat adrenomedullin was found to have 100% crossreactivity with it. Rat calcitonin gene-related peptide (CGRP) and amylin showed no crossreactivity with the antiserum, although they have a slight homology in amino acid residues with rat adrenomedullin. The minipumps were removed at the time of euthanasia and weighed in order to confirm that their contents were delivered and that adrenomedullin was not degraded by the end of the study. The infusate samples

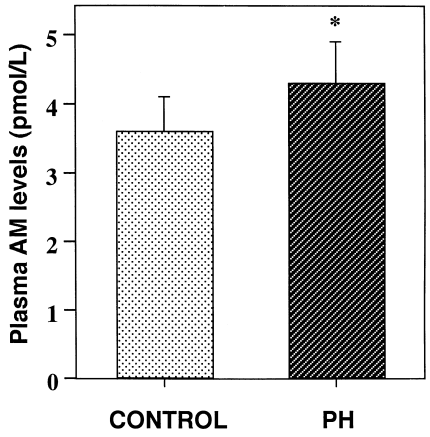

Fig. 1. Plasma adrenomedullin (AM) levels in the control and pulmonary hypertension (PH) groups 21 days after saline administration. Values are means ± S.D.. * $P < 0.05$ vs. control.

were stored at −80°C until later radioimmunoassay for adrenomedullin measurement.

2.3. Histological examination

The lungs were excised and immersed in neutralized formalin for histological examination. Paraffin sections of 4-μm thickness from the middle region of the left and right lungs were stained with elastin van Gieson stain and examined under light microscopy. Statistical analysis of the medial wall thickness of the arteries was performed as described by Ono and Voelkel (1991). In brief, the external diameter and the medial wall thickness were measured in 30 muscular arteries (ranging in size from 25 to 50, and 51 to 100 μm in external diameter) per lung section. For each artery, the medial wall thickness was expressed as follows: % wall thickness = [(medial thickness × 2)/external diameter] × 100. One lung section was obtained from each rat.

2.4. Statistics

All values are expressed as means ± S.D. Statistical comparisons between more than three groups were made by using analysis of variance and the Scheffe test for multiple comparisons. Comparisons between two groups were made by using an unpaired Student's *t*-test. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Plasma adrenomedullin concentrations

Plasma adrenomedullin levels were significantly higher in the PH group than in the control group (Fig. 1). Chronic infusion of rat adrenomedullin significantly increased the plasma adrenomedullin levels more than those of the control group and the PH group (6.8 ± 1.1 vs. 3.5 ± 0.8 or 4.4 ± 0.6 pmol/l, $P < 0.001$, respectively).

3.2. Effects of chronic adrenomedullin infusion on hemodynamics, cardiac hypertrophy, and alteration of lung vascular morphology in monocrotaline-treated rats

There were no significant differences in the heart rate between the three groups at 21 days after injection of monocrotaline (Fig. 2A). Mean arterial pressure in the PH group was significantly lower than that in the control group. While chronic adrenomedullin infusion decreased mean arterial pressure by 3% in the PH + AM group compared with the PH group, the difference did not reach

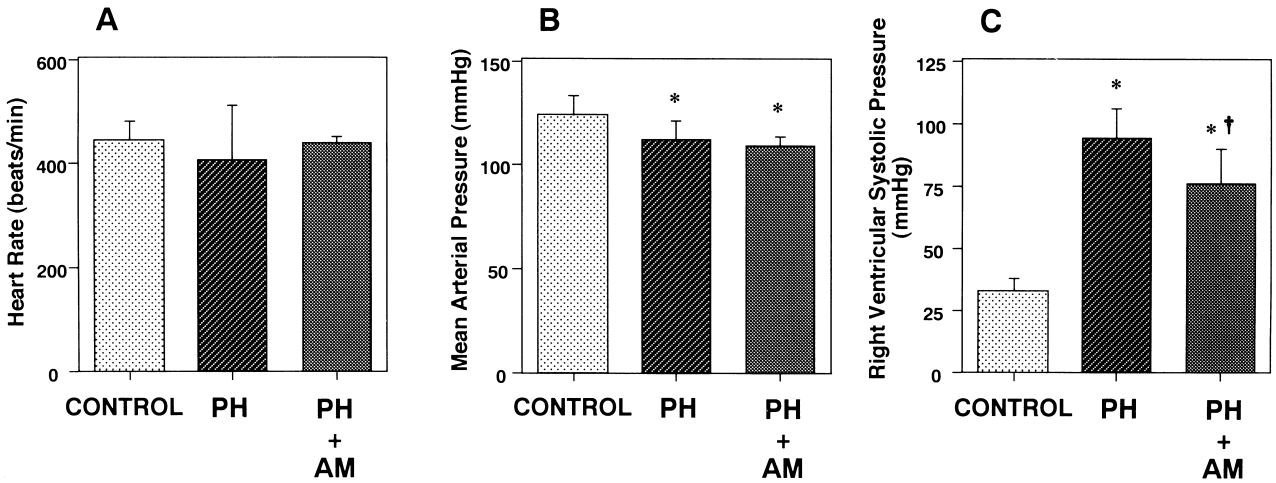

Fig. 2. Effects of chronic adrenomedullin (AM) infusion on heart rate (A), mean arterial pressure (B), and right ventricular systolic pressure (C) in control and monocrotaline-treated (60 mg/kg) rats (PH). Values are means ± S.D.. * $P < 0.05$ vs. control; † $P < 0.05$ vs. PH group.

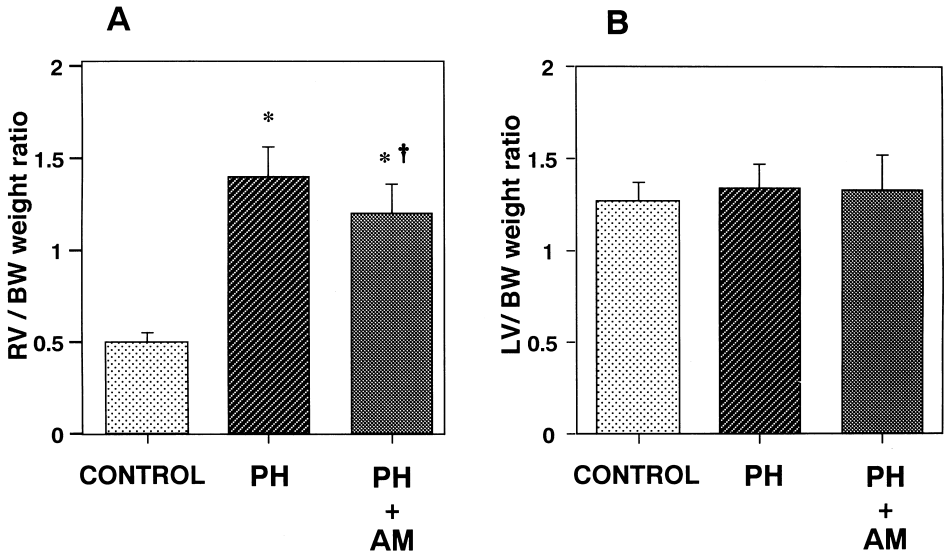

Fig. 3. Effects of chronic adrenomedullin (AM) infusion on the ratio right ventricular weight to body weight (RV/BW weight ratio, A) and the ratio of left ventricular weight to body weight (LV/BW weight ratio, B) in control and monocrotaline-treated rats (PH). Values are means ± S.D. * $P < 0.05$ vs. control; †$P < 0.05$ vs. PH group.

statistical significance. (Fig. 2B). In control and non-treated PH, there was a significant correlation between right ventricular systolic pressure and RV/BW ($r = 0.95$, $P < 0.001$), suggesting that right ventricular pressure is an indicator of pulmonary hypertension. Chronic infusion of adrenomedullin significantly attenuated the increase of both the right ventricular systolic pressure and the RV/BW seen in the PH group (Fig. 2C and Fig. 3A). The LV/BW ratio was comparable in the three groups (Fig. 3B). However, the septum/BW ratio was greater in the PH and PH + AM groups than in the control group (0.61 ± 0.06 and 0.59 ± 0.05 vs. 0.49 ± 0.07, $P < 0.01$, respectively). Histological examination revealed that chronic adrenomedullin infusion also attenuated the increase in wall thickness seen in the small pulmonary arteries of the PH group (Fig. 4A,B).

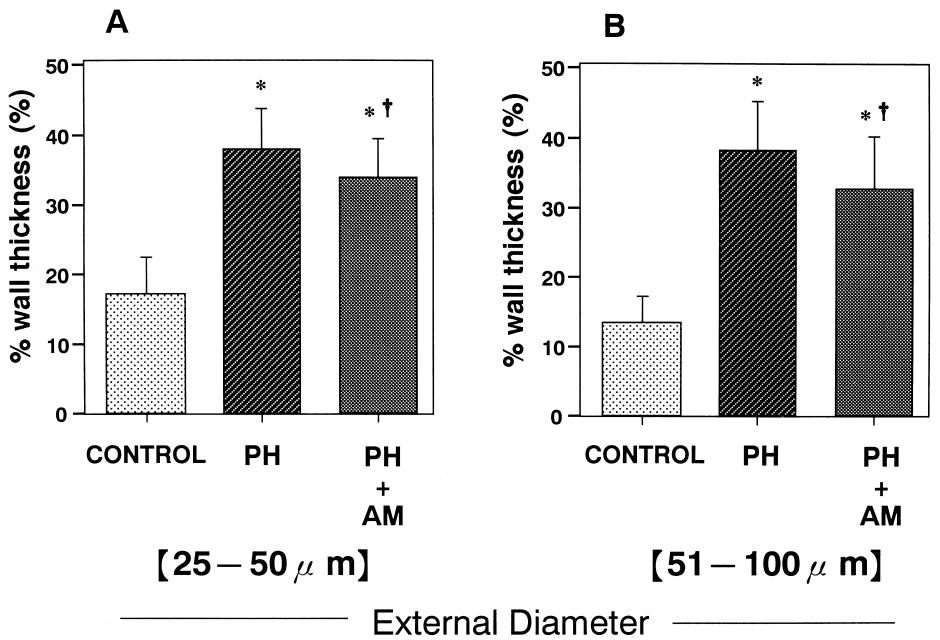

Fig. 4. Effects of chronic adrenomedullin (AM) infusion on the % wall thickness of small pulmonary arteries in control and monocrotaline-treated rats (PH). The external diameter and medial wall thickness were measured in 30 muscular arteries ranging in the size from 25 to 50 μm (A) and 51 to 100 μm (B). Values are means ± S.D. * $P < 0.05$ vs. control; †$P < 0.05$ vs. PH group.

3.3. Adrenomedullin concentrations of solution in osmotic minipumps after 21-day administration

The osmotic minipumps originally contained 74.6 ng/μl of synthetic rat adrenomedullin. After 21 days, the mean adrenomedullin concentration of the infusate in the spent minipumps was 74.4 ± 10.5 ng/μl ($n = 8$), suggesting that the adrenomedullin had been not degraded.

4. Discussion

To investigate whether chronic infusion of rat adrenomedullin attenuates the progressive pulmonary hypertension, right ventricular hypertrophy and pulmonary arterial medial thickening seen in monocrotaline-treated rats, we measured right ventricular systolic pressure, RV/BW and % wall thickness in these animals and compared these values with those obtained from normal control and monocrotaline-treated control animals. Chronic infusion of adrenomedullin significantly attenuated the increase in the right ventricular systolic pressure and the RV/BW ratio. Histological examination revealed that adrenomedullin also attenuated the increased wall thickness seen in the small pulmonary arteries. The present study demonstrates for the first time that chronically infused adrenomedullin has an effect on the response to monocrotaline in the pulmonary circulation.

The plasma adrenomedullin levels were significantly increased in the monocrotaline-treated rats compared with control rats in the present study. High levels of plasma adrenomedullin have been reported in patients with primary and secondary pulmonary hypertension (Yoshibayashi et al., 1997; Nishikimi et al., 1997a). Increased adrenomedullin levels were reported to be about 1.5- to 2.0-fold higher than the levels in normal controls, comparable to the levels seen in adrenomedullin-infused PH rats. Shimokubo et al. (1995) reported that the expression of adrenomedullin mRNA in the right ventricle was increased in monocrotaline-treated rats. An increased expression of adrenomedullin mRNA has also been reported in the ventricle of rats with chronic heart failure induced by aortocaval fistula (Nishikimi et al., 1997b). Patients with chronic heart failure show an increased cardiac secretion of adrenomedullin (Jougasaki et al., 1996). All of these findings suggest that increased cardiac synthesis and secretion of adrenomedullin may contribute to an increase in plasma adrenomedullin levels.

In the present study, there was no significant difference in mean arterial pressure between the PH and the PH + AM groups. Lippton et al. (1994) reported that adrenomedullin reduces pulmonary arterial pressure only under conditions of elevated pulmonary vasomotor tone. They also demonstrated that adrenomedullin, at a dose which dose not reduce systemic arterial pressure, significantly reduces pulmonary arterial pressure, suggesting that a low dose of adrenomedullin has a selective effect on the pulmonary circulation under these conditions. Our results may be consistent with these findings. However, the previous report demonstrated that chronic infusion of human adrenomedullin, at the same dose as in the present study, significantly reduced mean arterial pressure in normotensive rats (Khan et al., 1997). With regard to the mechanism of the reduction of mean arterial pressure in the PH group, a decreased cardiac output in association with right ventricular dysfunction induced by increased pulmonary arterial resistance may play an important role. Adrenomedullin attenuated the pulmonary hypertension probably due to the reduction in the increased pulmonary vascular resistance, resulting in the preservation of right ventricular function and cardiac output. Thus, the reduction of mean arterial pressure might be canceled by the preservation of cardiac output in the PH + AM group. Although the exact reason for the selective effect on the pulmonary circulation is unclear, these results suggest that adrenomedullin may play a pathophysiological role in monocrotaline-induced pulmonary hypertension.

With regard to the mechanism for attenuation of pulmonary hypertension by adrenomedullin, the vasodilator action of adrenomedullin in the pulmonary resistance vessels (Shirai et al., 1997) should be considered first. However, the pulmonary vasodilator response to adrenomedullin has been reported to be partly mediated by the release of nitric oxide in rats (Nossaman et al., 1996) and endogenous nitric oxide generation has been reported to be inhibited in rats with monocrotaline-induced pulmonary hypertension (Mathew et al., 1995). Thus, the effects of chronic adrenomedullin infusion in the present study may not be due to the vasodilator action of adrenomedullin only. Previous reports showed that endothelin-1 receptor antagonist significantly inhibited the progression of pulmonary hypertension, right ventricular hypertrophy and pulmonary arterial medial thickening (Miyauchi et al., 1993; Hill et al., 1997), and prevented the endothelial metabolic dysfunction (Prie et al., 1997) in rats with monocrotaline-induced pulmonary hypertension. These results suggested that endogenous endothelin-1 contributes to the progression of cardiopulmonary alterations in monocrotaline-treated rats. Adrenomedullin has been reported to inhibit smooth muscle proliferation (Kano et al., 1996) and the production of endothelin-1, a potent vasoconstrictor with a mitogenic effect on smooth muscle cells (Kohno et al., 1995), suggesting that a reciprocal relationship may exist between adrenomedullin and endothelin-1 in the control of vascular tone and smooth muscle cell pathophysiology. Taken together, these findings suggest that adrenomedullin attenuates the progression of pulmonary hypertension by not only its vasodilator action but also its anti-proliferative effects and its inhibition of the production of endothelin-1. Further studies are required to clarify the exact cellular

mechanism for the attenuation of pulmonary hypertension by adrenomedullin.

Adrenomedullin exhibits some sequence similarity to CGRP (Kitamura et al., 1993b). Zhao et al. (1996) previously reported that CGRP-(8-37), in a dose that inhibited the hypotensive effect of CGRP in the isolated perfused rat lung, also significantly attenuated the response to adrenomedullin, suggesting that the hypotensive effects of adrenomedullin in the rat pulmonary circulation may be in part mediated by CGRP receptors. The concentrations of adrenomedullin (Shimokubo et al., 1995) and CGRP (O'Neill et al., 1991) in the lungs decreased in rats with monocrotaline-induced pulmonary hypertension, but this could be attributed to dilution by an increased mass of tissue. However, the density of binding sites for both adrenomedullin and CGRP was increased in lung membranes from rats with hypoxia-induced pulmonary hypertension (Zhao et al., 1996). Consistent with the increased expression of functional receptors, the hypotensive effect of adrenomedullin and CGRP was greater in the isolated perfused lung from hypoxic rats compared with normal rats. These results suggested that adrenomedullin may counteract pulmonary hypertension in cooperation with CGRP.

In the present study, we evaluated the effect of chronic adrenomedullin infusion on mean arterial pressure, right ventricular systolic pressure, RV/BW and % wall thickness of small pulmonary arteries in monocrotaline-treated rats, but we did not evaluate the influence of adrenomedullin on blood flow and cardiac function. Adrenomedullin has recently been reported to increase blood flow in the lungs (He et al., 1995) and to have a negative inotropic effect (Ikenouchi et al., 1997). Therefore, we cannot exclude the possibility that chronic adrenomedullin infusion may affect the systemic and pulmonary circulation by its effect on blood flow and cardiac function.

In conclusion, the present study demonstrated for the first time that chronic infusion of rat adrenomedullin attenuates the pulmonary hypertension, the right ventricular hypertrophy, and the pulmonary arterial medial thickening seen in response to monocrotaline treatment. This attenuation was observed at plasma adrenomedullin concentrations that were pathophysiological. The data suggest that adrenomedullin participates in the regulation of pulmonary vascular tone in rats administered monocrotaline.

Acknowledgements

This work was supported in part by Special Coordination Funds for Promoting Science and Technology (Encouragement System of COE) from the Science and Technology Agency of Japan, the Ministry of Health and Welfare, and the Human Science Foundation of Japan. We thank Yoko Saito for her technical assistance.

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